ISSN 2150-5349 (online)

World Journal of *Gastrointestinal Pharmacology and Therapeutics*

World J Gastrointest Pharmacol Ther 2016 February 6; 7(1): 1-170

Published by Baishideng Publishing Group Inc

WJGPT

World Journal of Gastrointestinal Pharmacology and Therapeutics

Contents

Quarterly Volume 7 Number 1 February 6, 2016

World Journal of Gastrointestinal Pharmacology and Therapeutics
Volume 7 Number 1 February 6, 2016

Contents

NAME OF JOURNAL
World Journal of Gastrointestinal Pharmacology and Therapeutics

ISSN
ISSN 2150-5349 (online)

LAUNCH DATE
May 6, 2010

FREQUENCY
Quarterly

World Journal of Gastrointestinal Pharmacology and Therapeutics
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Inc
8226 Regency Drive,
Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLICATION DATE
February 6, 2016

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/2150-5349/g_info_20100315084234.htm

ONLINE SUBMISSION
http://www.wjgnet.com/esps/

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i1.1

World J Gastrointest Pharmacol Ther 2016 February 6; 7(1): 1-4
ISSN 2150-5349 (online)

EDITORIAL

Proton pump inhibitor prescription abuse and sepsis in cirrhosis

Antonio Picardi, Umberto Vespasiani-Gentilucci

Antonio Picardi, Umberto Vespasiani-Gentilucci, Internal Medicine and Hepatology Unit, University Campus Bio-Medico, 00128 Rome, Italy

Author contributions: Picardi A wrote the manuscript; Vespasiani-Gentilucci U contributed a critical revision of the manuscript.

Conflict-of-interest statement: Both authors declare no conflicts of interest.

Correspondence to: Antonio Picardi, MD, PhD, Internal Medicine and Hepatology Unit, University Campus Bio-Medico, Via Alvaro del Portillo 200, 00128 Rome, Italy. a.picardi@unicampus.it
Telephone: +39-6-225411207
Fax: +39-6-225411944

Received: May 27, 2015
Peer-review started: May 30, 2015
First decision: August 14, 2015
Revised: September 8, 2015
Accepted: November 3, 2015
Article in press: November 4, 2015
Published online: February 6, 2016

Abstract

Proton pump inhibitors (PPIs) represent one of the most extensively prescribed classes of drugs in general and in patients with liver cirrhosis. Many prescriptions are made without a clear adherence to standard indications. As a class of ordinarily well tolerated drug, PPIs are not free of side-effects and concerns have been raised about a possible role for PPIs in predisposing patients to an increased risk of bacterial infections and sepsis. As evidences of different power are accumulating on this topic, prospective studies are needed to reach a more universal agreement, but definitely more attention is needed by prescribers in being more adherent to the few recognized indications for the use of PPIs, particularly in patients with liver cirrhosis. Otherwise, doctors could run the risk of being accused of "abused" prescription.

Key words: Proton pump inhibitors; Liver cirrhosis; Bacterial infection; Sepsis

Core tip: Many prescriptions of proton pump inhibitors (PPIs) are made without adhering to standard recognized indications. PPIs are ordinarily well tolerated but are not free of side-effects, and, in patients with liver cirrhosis, concerns are accruing on a possible role for PPIs in increasing the risk of infections and sepsis. As evidences of different power are accumulating, prospective studies are needed. However, prescribers should put definitely more attention in adhering to the recognized indications for the use of PPIs, especially in patients with cirrhosis. Otherwise, doctors could be responsible for "abuse" of prescription.

Picardi A, Vespasiani-Gentilucci U. Proton pump inhibitor prescription abuse and sepsis in cirrhosis. *World J Gastrointest Pharmacol Ther* 2016; 7(1): 1-4 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i1/1.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i1.1

UPPER DIGESTIVE BLEEDING DISORDERS IN CIRRHOSIS

Portal hypertension changes the clinical picture of patients with liver cirrhosis causing the most untamable complications that set up the clinical syndrome of advanced liver cirrhosis, such as ascites, porto-systemic encephalopathy, *etc*. Among those, upper gastrointestinal bleeding (UGIB) from esophageal varices is one of the most fearsome acute complications of liver cirrhosis, and constitutes one of the genuine medical emergencies. This is the reason why UGIB entails a prompt and appropriate management and every effort is attempted by clinicians and investigators to predict and, possibly, prevent its occurrence[1]. Mortality of UGIB can range from 2% to 15%, with re-bleeding rates as high as 10%-30% in the brief term[2,3]. Indeed, the underlying hepatic dysfunction in liver cirrhosis is associated with an impaired coagulation capacity, that further increases morbidity. Recently, mortality at 90 d after UGIB in liver cirrhosis was shown to be significantly increased included when bleeding was not due to esophageal varices[4].

PROTON PUMP INHIBITORS INDICATIONS AND SHORTCOMINGS

Incidentally, proton pump inhibitors (PPIs) are powerful agents for controlling gastric acid secretion and actually they changed the clinical scenario of peptic ulcer disease[5-8]. Thus, assuming a causative role for gastric acid production in any UGIB, a very extensive use of PPIs is made worldwide in very different clinical settings, ranging from peptic ulcer disease to general medicine and the more so in liver cirrhosis. Actually, acid suppressant drugs and especially PPIs represent the most frequently prescribed drugs in chronic among patients with liver cirrhosis, ranging from 25% to more than 40% of cirrhotic patients in various series. They are often prescribed on discharge also from specialized tertiary centers to cirrhotic patients who were not assuming ulcer healing drugs on hospital admission and who lack any proper current indication[9,10]. Indeed, the only recognized indications for the use of PPIs - at term or in chronic - include very few items, namely: Peptic ulcer disease, gastroesophageal reflux disease (GERD), non-variceal UGIB, and bleeding prophylaxis in selected users of nonsteroidal anti-inflammatory drugs (NSAIDs)[11].

Even if usually well tolerated[12], PPIs are not avoid of class specific systemic side effects- incidence on the order of 1% to 5% - that include headache, diarrhea, constipation, nausea, and rash. Recently, they also surged as culprit of important drug-drug interactions that could reduce the efficacy of life saving drugs[8]. Secondary side effects after long term use of PPIs include osteoporosis, infections (as will be discussed further on) and the formation of gastric polyps/carcinoids[8,12].

IS THERE A ROLE FOR PPI IN PREVENTING UPPER DIGESTIVE BLEEDING ACCORDING TO GUIDELINES?

If we look at the case of patients with liver cirrhosis, few and scant specific indications exist for the use of PPIs. Benefits are documented only for their use in active bleeding from varices - starting before and continuing after endoscopic band ligation or sclerotherapy - and merely if associated with the mainstay of medical treatment of bleeding in cirrhosis with vasoconstrictors and antibiotics[13-15]. The efficacy of PPIs after endoscopic treatment for the prevention of re-bleeding seems to be linked with the reduction of the dimensions of post-ligation ulcers[15], then with a use limited to a short period of time (2-3 wk after band ligation). Conversely, manifestations of portal hypertension like congestive gastropathy and not actively bleeding esophageal or gastric varices are at risk for an inappropriate use of PPIs[16]. Definitely, PPIs have been shown recently to be not effective in the primary prevention of UGIB in cirrhotic patients[17]. Whatsoever, more than 60% of patients with liver cirrhosis have been found to have no documented indications for the use of a PPI in different published series[10,14,16-18], in contrast with the low overall prevalence of peptic disease in patients with cirrhosis that ranges from 5% to 20% in different populations, and reaches a maximum of 28% in the most severe patients with decompensated cirrhosis[14].

CONCERNS AND WARNINGS: DO PPIS INCREASE THE RISK OF INFECTION/SEPSIS?

Starting from the early 80's, evidences are accumulating on an increased risk of infections in patients who are persistently assuming acid suppressant drugs in different clinical settings in the general population[19-23], but also in the more definite population of patients with liver cirrhosis. In these patients any intercurrent infection is at higher risk of evolution into sepsis or severe sepsis, by the addition of any other organ dysfunction (mainly the kidney), the deterioration of the same liver function and of the overall prognosis[24-26]. Pneumonia, *Clostridium difficile* (*C. difficile*) and other infectious gastroenteritis or cholangitis have been extensively studied, where the administration of PPIs also changed the pattern of etiologic agents over time[23,27]. The mechanisms involved in shaping such predisposition to infection and sepsis are supposed to be related, on the one hand, to the loss of the modulating effect of the acidic gastric secretion on the gut microbiota (favoring small intestinal bacterial overgrowth: SIBO). On the

other hand, to a possible direct immunosuppressive effect of PPIs making patients prone to bacterial colonization and translocation[23,28,29]. Whatever the underlying mechanistic reason, with the exception of a recent metanalysis[12], recent prospective papers have shown that PPIs render the cirrhotic patients prone to infections and sepsis[18,23,30-35], with a class specific effect independent from acidic secretion control. In facts, anti-H2 agents had no influence on the risk of infection when compared to PPIs[25].

Indeed, in another recent prospective paper, the use of a PPI was documented in a rough 80% of patients with cirrhosis, and in 50% of those patients the prescription was fixed merely to control generic abdominal symptoms. Finally, in this population the use of PPIs was associated with a trend toward more infections in PPI users compared to non-users, and resulted independently associated with increased overall mortality compared with cirrhotic patients who were not assuming PPIs[36].

CONCLUSION

In conclusion, we need to keep in mind - and maybe re-discover - the few unquestionable and appropriate indications for the use of PPIs, especially when dealing with the very fragile population of patients with liver cirrhosis (mainly if advanced cirrhosis). This not only to reduce and control the pharmaceutical expenditure, whoever is the payer, but also in the respect of the rules of good medical practice regarding adherence to guidelines and recognized indications. Cirrhosis constitutively exposes patients to develop more severe infections with systemic involvement and other organ dysfunction, compared to other patients. Otherwise, patients with cirrhosis have a basally reduced gastric acid secretion and are exposed to many drug-drug interactions. Declining to adhere to the universally recognized indications for the use of such an extensively prescribed class of drugs could actually expose many doctors at the fault of "abuse" in drug prescription!

REFERENCES

1 **Pezzulo G**, Kruger D. Assessing upper gastrointestinal bleeding in adults. *JAAPA* 2014; **27**: 19-25 [PMID: 25102077 DOI: 10.1097/01.JAA.0000453234.32378.cb]

2 **Carbonell N**, Pauwels A, Serfaty L, Fourdan O, Lévy VG, Poupon R. Improved survival after variceal bleeding in patients with cirrhosis over the past two decades. *Hepatology* 2004; **40**: 652-659 [PMID: 15349904 DOI: 10.1002/hep.20339]

3 **Cheng DW**, Lu YW, Teller T, Sekhon HK, Wu BU. A modified Glasgow Blatchford Score improves risk stratification in upper gastrointestinal bleed: a prospective comparison of scoring systems. *Aliment Pharmacol Ther* 2012; **36**: 782-789 [PMID: 22928529 DOI: 10.1111/apt.12029]

4 **Holland-Bill L**, Christiansen CF, Gammelager H, Mortensen RN, Pedersen L, Sørensen HT. Chronic liver disease and 90-day mortality in 21,359 patients following peptic ulcer bleeding--a Nationwide Cohort Study. *Aliment Pharmacol Ther* 2015; **41**: 564-572 [PMID: 25588862 DOI: 10.1111/apt.13073]

5 **Holt S**, Howden CW. Omeprazole. Overview and opinion. *Dig Dis Sci* 1991; **36**: 385-393 [PMID: 2007354]

6 **Leontiadis GI**, Sharma VK, Howden CW. WITHDRAWN: Proton pump inhibitor treatment for acute peptic ulcer bleeding. *Cochrane Database Syst Rev* 2010; **(5)**: CD002094 [PMID: 20464720 DOI: 10.1002/14651858.CD002094.pub4]

7 **Ramakrishnan K**, Salinas RC. Peptic ulcer disease. *Am Fam Physician* 2007; **76**: 1005-1012 [PMID: 17956071]

8 **Chubineh S**, Birk J. Proton pump inhibitors: the good, the bad, and the unwanted. *South Med J* 2012; **105**: 613-618 [PMID: 23128806 DOI: 10.1097/SMJ.0b013e31826efbea]

9 **Lewis JH**, Stine JG. Review article: prescribing medications in patients with cirrhosis - a practical guide. *Aliment Pharmacol Ther* 2013; **37**: 1132-1156 [PMID: 23638982 DOI: 10.1111/apt.12324]

10 **Lucena MI**, Andrade RJ, Tognoni G, Hidalgo R, De La Cuesta FS. Multicenter hospital study on prescribing patterns for prophylaxis and treatment of complications of cirrhosis. *Eur J Clin Pharmacol* 2002; **58**: 435-440 [PMID: 12242604 DOI: 10.1007/s00228-002-0474-1]

11 **Barrison AF**, Jarboe LA, Weinberg BM, Nimmagadda K, Sullivan LM, Wolfe MM. Patterns of proton pump inhibitor use in clinical practice. *Am J Med* 2001; **111**: 469-473 [PMID: 11690573 DOI: 10.1016/S0002-9343(01)00901-9]

12 **Attwood SE**, Ell C, Galmiche JP, Fiocca R, Hatlebakk JG, Hasselgren B, Långström G, Jahreskog M, Eklund S, Lind T, Lundell L. Long-term safety of proton pump inhibitor therapy assessed under controlled, randomised clinical trial conditions: data from the SOPRAN and LOTUS studies. *Aliment Pharmacol Ther* 2015; **41**: 1162-1174 [PMID: 25858519 DOI: 10.1111/apt.13194]

13 **Garcia-Tsao G**, Bosch J. Management of varices and variceal hemorrhage in cirrhosis. *N Engl J Med* 2010; **362**: 823-832 [PMID: 20200386 DOI: 10.1056/NEJMra0901512]

14 **Lodato F**, Azzaroli F, Di Girolamo M, Feletti V, Cecinato P, Lisotti A, Festi D, Roda E, Mazzella G. Proton pump inhibitors in cirrhosis: tradition or evidence based practice? *World J Gastroenterol* 2008; **14**: 2980-2985 [PMID: 18494046]

15 **Shaheen NJ**, Stuart E, Schmitz SM, Mitchell KL, Fried MW, Zacks S, Russo MW, Galanko J, Shrestha R. Pantoprazole reduces the size of postbanding ulcers after variceal band ligation: a randomized, controlled trial. *Hepatology* 2005; **41**: 588-594 [PMID: 15726658 DOI: 10.1002/hep.20593]

16 **Chavez-Tapia NC**, Tellez-Avila FI, Garcia-Leiva J, Valdovinos MA. Use and overuse of proton pump inhibitors in cirrhotic patients. *Med Sci Monit* 2008; **14**: CR468-CR472 [PMID: 18758417]

17 **Garcia-Saenz-de-Sicilia M**, Sanchez-Avila F, Chavez-Tapia NC, Lopez-Arce G, Garcia-Osogobio S, Ruiz-Cordero R, Tellez-Avila FI. PPIs are not associated with a lower incidence of portal-hypertension-related bleeding in cirrhosis. *World J Gastroenterol* 2010; **16**: 5869-5873 [PMID: 21155009 DOI: 10.3748/wjg.v16.i46.5869]

18 **Goel GA**, Deshpande A, Lopez R, Hall GS, van Duin D, Carey WD. Increased rate of spontaneous bacterial peritonitis among cirrhotic patients receiving pharmacologic acid suppression. *Clin Gastroenterol Hepatol* 2012; **10**: 422-427 [PMID: 22155557 DOI: 10.1016/j.cgh.2011.11.019]

19 **du Moulin GC**, Paterson DG, Hedley-Whyte J, Lisbon A. Aspiration of gastric bacteria in antacid-treated patients: a frequent cause of postoperative colonisation of the airway. *Lancet* 1982; **1**: 242-245 [PMID: 6120273 DOI: 10.1016/S0140-6736(82)90974-6]

20 **Aseeri M**, Schroeder T, Kramer J, Zackula R. Gastric acid suppression by proton pump inhibitors as a risk factor for clostridium difficile-associated diarrhea in hospitalized patients. *Am J Gastroenterol* 2008; **103**: 2308-2313 [PMID: 18702653 DOI: 10.1111/j.1572-0241.2008.01975.x]

21 **Bavishi C**, Dupont HL. Systematic review: the use of proton pump inhibitors and increased susceptibility to enteric infection. *Aliment Pharmacol Ther* 2011; **34**: 1269-1281 [PMID: 21999643 DOI: 10.1111/j.1365-2036.2011.04874.x]

22 **Dalton BR**, Lye-Maccannell T, Henderson EA, Maccannell DR,

Louie TJ. Proton pump inhibitors increase significantly the risk of Clostridium difficile infection in a low-endemicity, non-outbreak hospital setting. *Aliment Pharmacol Ther* 2009; **29**: 626-634 [PMID: 19183143 DOI: 10.1111/j.1365-2036.2008.03924.x]

23 **Wang YP**, Liu CJ, Chen TJ, Lin YT, Fung CP. Proton pump inhibitor use significantly increases the risk of cryptogenic liver abscess: a population-based study. *Aliment Pharmacol Ther* 2015; **41**: 1175-1181 [PMID: 25871643 DOI: 10.1111/apt.13203]

24 **Bajaj JS**, Zadvornova Y, Heuman DM, Hafeezullah M, Hoffmann RG, Sanyal AJ, Saeian K. Association of proton pump inhibitor therapy with spontaneous bacterial peritonitis in cirrhotic patients with ascites. *Am J Gastroenterol* 2009; **104**: 1130-1134 [PMID: 19337238 DOI: 10.1038/ajg.2009.80]

25 **Bajaj JS**, Ratliff SM, Heuman DM, Lapane KL. Proton pump inhibitors are associated with a high rate of serious infections in veterans with decompensated cirrhosis. *Aliment Pharmacol Ther* 2012; **36**: 866-874 [PMID: 22966967 DOI: 10.1111/apt.12045]

26 **Bajaj JS**, O'Leary JG, Reddy KR, Wong F, Biggins SW, Patton H, Fallon MB, Garcia-Tsao G, Maliakkal B, Malik R, Subramanian RM, Thacker LR, Kamath PS. Survival in infection-related acute-on-chronic liver failure is defined by extrahepatic organ failures. *Hepatology* 2014; **60**: 250-256 [PMID: 24677131 DOI: 10.1002/hep.27077]

27 **Schneider J**, Weidner W, Hapfelmeier A, Wantia N, Feihl S, Schmid RM, Neu B, von Delius S, Algül H, Huber W, Weber A. The use of proton pump inhibitors and the spectrum and number of biliary pathogens in patients with acute cholangitis. *Aliment Pharmacol Ther* 2014; **39**: 1194-1203 [PMID: 24628434 DOI: 10.1111/apt.12694]

28 **Kedika RR**, Souza RF, Spechler SJ. Potential anti-inflammatory effects of proton pump inhibitors: a review and discussion of the clinical implications. *Dig Dis Sci* 2009; **54**: 2312-2317 [PMID: 19714466 DOI: 10.1007/s10620-009-0951-9]

29 **Lombardo L**, Foti M, Ruggia O, Chiecchio A. Increased incidence of small intestinal bacterial overgrowth during proton pump inhibitor therapy. *Clin Gastroenterol Hepatol* 2010; **8**: 504-508 [PMID: 20060064 DOI: 10.1016/j.cgh.2009.12.022]

30 **Sargenti K**, Prytz H, Strand A, Nilsson E, Kalaitzakis E. Healthcare-associated and nosocomial bacterial infections in cirrhosis: predictors and impact on outcome. *Liver Int* 2015; **35**: 391-400 [PMID: 25039438 DOI: 10.1111/liv.12625]

31 **Merli M**, Lucidi C, Di Gregorio V, Giannelli V, Giusto M, Ceccarelli G, Riggio O, Venditti M. The chronic use of beta-blockers and proton pump inhibitors may affect the rate of bacterial infections in cirrhosis. *Liver Int* 2015; **35**: 362-369 [PMID: 24836902 DOI: 10.1111/liv.12593]

32 **Deshpande A**, Pasupuleti V, Thota P, Pant C, Mapara S, Hassan S, Rolston DD, Sferra TJ, Hernandez AV. Acid-suppressive therapy is associated with spontaneous bacterial peritonitis in cirrhotic patients: a meta-analysis. *J Gastroenterol Hepatol* 2013; **28**: 235-242 [PMID: 23190338 DOI: 10.1111/jgh.12065]

33 **Min YW**, Lim KS, Min BH, Gwak GY, Paik YH, Choi MS, Lee JH, Kim JJ, Koh KC, Paik SW, Yoo BC, Rhee PL. Proton pump inhibitor use significantly increases the risk of spontaneous bacterial peritonitis in 1965 patients with cirrhosis and ascites: a propensity score matched cohort study. *Aliment Pharmacol Ther* 2014; **40**: 695-704 [PMID: 25078671 DOI: 10.1111/apt.12875]

34 **Kwon JH**, Koh SJ, Kim W, Jung YJ, Kim JW, Kim BG, Lee KL, Im JP, Kim YJ, Kim JS, Yoon JH, Lee HS, Jung HC. Mortality associated with proton pump inhibitors in cirrhotic patients with spontaneous bacterial peritonitis. *J Gastroenterol Hepatol* 2014; **29**: 775-781 [PMID: 24219827 DOI: 10.1111/jgh.12426]

35 **de Vos M**, De Vroey B, Garcia BG, Roy C, Kidd F, Henrion J, Deltenre P. Role of proton pump inhibitors in the occurrence and the prognosis of spontaneous bacterial peritonitis in cirrhotic patients with ascites. *Liver Int* 2013; **33**: 1316-1323 [PMID: 23730823 DOI: 10.1111/liv.12210]

36 **Dultz G**, Piiper A, Zeuzem S, Kronenberger B, Waidmann O. Proton pump inhibitor treatment is associated with the severity of liver disease and increased mortality in patients with cirrhosis. *Aliment Pharmacol Ther* 2015; **41**: 459-466 [PMID: 25523381 DOI: 10.1111/apt.13061]

P- Reviewer: Abdel-Salam OME **S- Editor**: Tian YL **L- Editor**: A
E- Editor: Wang CH

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i1.5

World J Gastrointest Pharmacol Ther 2016 February 6; 7(1): 5-20
ISSN 2150-5349 (online)

TOPIC HIGHLIGHT

2016 Inflammatory Bowel Disease: Global view

Pharmacological- and non-pharmacological therapeutic approaches in inflammatory bowel disease in adults

Gerda C Leitner, Harald Vogelsang

Gerda C Leitner, University Clinic for Blood Group Serology and Transfusion Medicine, Medical University, 1090 Vienna, Austria

Harald Vogelsang, University Clinic of Gastroenterology, 3rd Internal Medicine, Medical University, 1090 Vienna, Austria

Author contributions: Leitner GC collected the data and wrote the paper; Vogelsang H gave scientific support and revised the paper.

Conflict-of-interest statement: Authors declare no conflict of interests' for this article.

Correspondence to: Gerda C Leitner, MD, University Clinic for Blood Group Serology and Transfusion Medicine, Medical University, Waehringer Guertel 18-20, 1090 Vienna, Austria. gerda.leitner@meduniwien.ac.at
Telephone: +43-1-4040053080
Fax: +43-1-4040075070

Received: April 29, 2015
Peer-review started: May 7, 2015
First decision: August 4, 2015
Revised: December 14, 2015
Accepted: January 8, 2016
Article in press: January 11, 2016
Published online: February 6, 2016

Abstract

Inflammatory bowel diseases (IBDs) are a group of chronic inflammatory conditions mainly of the colon and small intestine. Crohn's disease (CD) and ulcerative colitis (UC) are the most frequent types of IBD. IBD is a complex disease which arises as a result of the interaction of environmental, genetic and immunological factors. It is increasingly thought that alterations of immunological reactions of the patients to their own enterable bacteria (microfilm) may contribute to inflammation. It is characterized by mucosal and sub mucosal inflammation, perpetuated by infiltration of activated leukocytes. CD may affect the whole gastrointestinal tract while UC only attacks the large intestine. The therapeutic goal is to achieve a steroid-free long lasting remission in both entities. UC has the possibility to be cured by a total colectomy, while CD never can be cured by any operation. A lifelong intake of drugs is mostly necessary and essential. Medical treatment of IBD has to be individualized to each patient and usually starts with anti-inflammatory drugs. The choice what kind of drugs and what route administered (oral, rectal, intravenous) depends on factors including the type, the localization, and severity of the patient's disease. IBD may require immune-suppression to control symptoms such as prednisolone, thiopurines, calcineurin or sometimes folic acid inhibitors or biologics like TNF-α inhibitors or anti-integrin antibodies. For both types of disease (CD, UC) the same drugs are available but they differ in their preference in efficacy between CD and UC as 5-aminosalicylic acid for UC or budesonide for ileocecal CD. As therapeutic alternative the main mediators of the disease, namely the activated pro-inflammatory cytokine producing leukocytes can be selectively removed *via* two apheresis systems (Adacolumn and Cellsorba) in steroid-refractory or dependent cases. Extracorporeal photopheresis results in an increase of regulatory B cells, regulatory $CD8^+$ T cells and T-regs Type 1. Both types of apheresis were able to induce clinical remission and mucosal healing accompanied by tapering of steroids.

Key words: Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Extracorporeal treatment; Step up/top down; Psychological aspects in inflammatory bowel disease; Current medical treatment of inflammatory bowel disease; Future therapeutical strategies

Core tip: This review describes current and future therapeutic strategies in Crohn's disease and ulcerative colitis and outlines the most important publications in this field. It comprises surgical, medical and extracorporeal treatment options. All described treatment options are carefully reviewed regarding therapeutic effects and side effects. Extracorporeal treatment options are a potent measure to withdraw patients from steroids. Standard treatment as well as innovative therapeutic approaches, like autologous stem cell transplantation are addressed, which revealed promising results in therapy refractory patients.

Leitner GC, Vogelsang H. Pharmacological- and non-pharmacological therapeutic approaches in inflammatory bowel disease in adults. *World J Gastrointest Pharmacol Ther* 2016; 7(1): 5-20 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i1/5.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i1.5

INTRODUCTION

Inflammatory bowel diseases (IBDs) are a group of inflammatory conditions of the colon and small intestine. Crohn's disease (CD) and ulcerative colitis (UC) are the leading entities in IBD. While UC primarily affects the colon, CD can be related to the whole gut. The disease was named after gastroenterologist Burrill Bernard Crohn who described a series of patients with inflammation of the terminal ileum of the small intestine, the area most commonly affected by the illness, in 1932, together with two other colleagues at Mount Sinai Hospital in New York[1]. We will never know who described UC for the 1st time although the disease was primarily referred to by name in 1859 by Sir Samuel Wilkes. Among his major discoveries, Wilks recognized UC, differentiating it from bacterial dysentery. His work was confirmed by Sir Arthur Hirst 1931[2]. IBD is a complex disease which arises as a result of the interaction of genetic dispositions, environment and alterations in the function of the immune system[3]. It is also increasingly thought that altered immunological reactions to patient's own enteral bacteria may contribute to inflammatory gut diseases[4]. IBD affected individuals have been found to have 30%-50% reduced biodiversity of commensalism bacteria such as a decrease in Firmicutes (namely Lachnospiraceae) and Bacteroidetes[5]. Further evidence of the role of gut flora in the cause of IBD-besides animal and *in vitro* studies - is that IBD affected individuals are more likely to have been prescribed antibiotics in the 2-5 year period before their diagnosis than unaffected individuals[6,7].

Table 1 Complications[11]

	Crohn's disease		Ulcerative colitis	
	Females	Males	Females	Males
Primary sclerosing cholangitis	0.3%	0.4%	3.2%	0.9%
Ankylosing spondylitis	0.7%	2.7%	1.0%	3.0%
Pyoderma gangrenosum	1.2%	1.3%	0.8%	1.5%
Erythema nodosum	1.9%	0.6%	0.8%	0.7%

The enteral bacteria can be altered by environmental factors, such as diets or oral medications (antibiotics or oral iron preparations)[8].

Genetics

There is strong evidence to suggest a genetic basis for IBD, including familial clustering and racial and ethnic differences in risk for IBD. Ten to 20% of affected individuals will have family history of IBD, with the highest risk among first-degree relatives. A strong association between HLA B27 and ankylosing spondylitis is known since the early 1970s which is also classified as extra intestinal complication in patients with IBD (Table 1)[9-11]. The genetic contribution is poorly understood and seems to arise from the small contribution of dozens of genes. In 2012, 163 IBD susceptibility loci were confirmed which means that 163 different alleles may increase the susceptibility to the disease. These 163 loci explain from 8.2% to a 13.6% of variance in CD and 4.1% to 7.5% in UC. The 163 loci were related to 300 known genes. The most well-known and frequent gene associated with CD is the NOD2/CARD15 gene[12-14].

Environmental factors

There is evidence that IBD is primarily a disease of the developed countries. The rise in certain regions (*i.e.*, India, China) parallelizes the industrialization of these countries. It seems likely that environmental factors may also influence the normal intestinal commensal flora and thus trigger an inappropriate mucosal immune response.

A number of environmental risk factors have been explored, including smoking, appendectomy, oral contraceptives, diet, breastfeeding, infections/vaccinations, antibiotics, and childhood hygiene. However, most of these identified risk factors have demonstrated inconsistent findings so that further investigations are warranted. Smoking and infections in childhood may trigger IBD especially CD[15-18].

Immune system

The intestinal immune system defends against pathogens and entry of excessive intestinal microbes;

Table 2 Sensitivity and specificity of atypical perinuclear anti-neutrophil cytoplasmic antibody/anti-Saccharomyces cerevisiae antibody combinations for ulcerative colitis and Crohn's disease in patients with inflammatory bowel disease[24]

Marker	UC		CD	
	Sensitivity	Specificity	Sensitivity	Specificity
pANCA+/ASCA-	51%	94%	-	-
pANCA-/ASCA+	-	-	55%	93%

pANCA: Atypical perinuclear anti-neutrophil cytoplasmic antibody; ASCA: Anti-Saccharomyces cerevisiae antibody; CD: Crohn's disease; UC: Ulcerative colitis.

simultaneously, a state of immune tolerance to resident intestinal microbes must be maintained. Perturbation of this balance is associated with intestinal inflammation in various mouse models and is thought to predispose humans to IBD. The immune system continuously monitors resident microbiota and utilizes constitutive antimicrobial mechanisms to maintain immune homeostasis. There is increasing evidence that intestinal microbes influence host immune development, immune responses, and susceptibility to human diseases such as IBD[19].

An imbalanced intestinal immune defense and intestinal immune tolerance is one of the risks for developing IBD. A numerous of inflammatory and anti-inflammatory cytokines as well as immune active cells, like T-and B-cells, T-regs are involved in this intact mechanism. Although UC and CD can usually be differentiated on the basis of clinical, radiographic, endoscopic, and histological findings, these conditions can be difficult to distinguish in about 10% to 15% of IBD patients[20]. Numerous studies have investigated the utility of 2 serologic markers in differentiating between UC and CD: Atypical perinuclear anti-neutrophil cytoplasmic antibody (pANCA) and anti-Saccharomyces cerevisiae antibody (ASCA). Unlike the pANCA or cytoplasmic ANCA found in vasculitis, the IBD-associated pANCA has an "atypical" perinuclear staining pattern. This atypical pANCA is detected in about 40% to 80% of UC patients but only in 5% to 25% of CD patients[21,22]. ASCA, on the other hand, is detected in 40% to 68% of CD patients[21,22] but only in about 6% to 12% of UC patients[20,23]. Table 2, based on a meta-analysis of 60 studies comprising 7860 IBD patients, summarizes the sensitivity and specificity of pANCA/ASCA combinations for UC and CD[22]. The diagnostic and therapeutic value of serological markers (more than pANCA/ASCA) was reviewed by Andrea T Kuna[24] in 2013. Due to the lack of sensitivity, serological markers were not advised for their use in the diagnosis of IBD but rather in differentiating CD from UC. The most important clinical utility of serological markers could be in stratifying patients according to risk for aggressive disease phenotype or postoperative complications. At the current time, there is no usefulness of serological markers in monitoring the treatment of IBD patients[24].

Calprotectin level in feces, produced from granulocytes, is a useful marker to measure disease activity and can predict disease recurrence. It is more precise than the common used markers like CRP and ESR[25].

Laboratory findings

Beside chemical parameters like increase of unspecific inflammatory markers as CRP (activity marker) and erythrocyte sedimentation rate, iron deficiency and anemia in different severities, there is also found a distinguished pattern of specific cytokines.

In IBD, increased amounts of soluble and membrane-bound TNF are produced by various immune and stromal cell populations, such as macrophages, dendritic cells (DCs), effector T cells, adipocytes and fibroblasts. TNF has been shown to exert various pro-inflammatory functions in the inflamed mucosa in IBD. In particular, TNF induces hypervascularization and angiogenesis, augments pro-inflammatory cytokine production by macrophages and T cells. TNF-specific antibodies may alleviate disease by simultaneously suppressing several pro-inflammatory pathways in patients with IBD. There are many proteins involved in gastrointestinal angiogenesis, including pro-inflammatory cytokines, vascular growth factors, and adhesion molecules[26].

A promising approach in getting more insight in the pathophysiology of IBD may be the development of new biomarkers. The techniques available for biomarkers development are genomics and proteomics. In the future it is expected that all these biomarkers will be implemented in an integrated molecular diagnostic and prognostic approach[27].

The heterogeneous nature of IBD implicates heterogeneous therapeutic strategies. Acute flares as well as the chronic status are treated with a variety of medications. Current therapies include the use of corticosteroids, anti-inflammatories, immune suppressive drugs, antibiotics and biologicals.

CD

The active disease is categorized into mild-, moderate- and severe localized ileocaecal disease, colonic disease, extensive small bowel disease and esophageal and gastroduodenal disease. It is graded by the Crohn's Disease Activity Index (CDAI) which ranges between 150 and 450 points and more for heavily active disease and acute flare, established in 1978[28]. This calculator is primary a research tool, but is increasingly used to define responses and remissions (< 150 pt)[29]. Besides CDAI, there is a variety of scoring systems which partly are very time consuming and require compliance of the patients (exception: Bradshaw index). Thus their use is rather limited to clinical trials (Tables 3 and 4)[30,31].

CD is a chronic disease (as well as UC) and the

Table 3 Crohn's disease activity index scoring system

Clinical or laboratory variable	Multiplication factor
Number of liquid or soft stools each day for seven days	× 2
Abdominal pain (graded from 0-3 on severity) each day for seven days	× 5
General wellbeing, subjectively assessed from 0 (well) to 4 (terrible) each day for seven days	× 7
Presence of complications[1]	× 20
Taking Lomotil or opiates for diarrhea	× 30
Presence of an abdominal mass (0 as none, 2 as questionable, 5 as definite)	× 10
Hematocrit of < 47% in men and < 42% in women	× 6
Percentage deviation from standard weight	× 1

[1]One point each is added for each set of complications.

Table 4 Harvey-Bradshaw index

General well-being	Very well	Below average	Poor	Very poor	Terrible
	0	1	2	3	4
Abdominal pain	None	Mild	Moderate	Severe	
	0	1	2	3	
# liquid stools/d	#	#	#	#	#
Abdominal mass	None	Dubious	Definite	Tender	
	0	1	2	3	

A score of less than 5 is generally considered to represent clinical remission.

therapeutic goal is to achieve sustained, steroid-free remission. Medical therapy is considered to be the treatment modality of choice for most patients. According to the severity of CD current therapy strategies include nutritional approaches, anti-inflammatory drugs, immunosuppression, chemotherapy and biologicals. The therapeutic decision is influenced by the extent of severity, the presence of septic complications and extra-intestinal manifestations. The surgical management is reserved for individuals who fail medical treatment or develop potentially life-threatening complications. The surgical management has changed substantially during the last 10 years. Surgical treatment of CD is solely symptomatic. In addition, medical therapy always precedes surgery and almost always continues afterwards. The indications for surgical treatment are failure of medical treatment and progressive complications (*e.g.*, fistula, abscess, obstruction)[32]. Hulten described 1988 disease recurrence of about 50% within 10 years post operation[33]. Bernell described a significantly higher relative risk for recurrence after first resection in CD in women and when the small bowel or the continuous ileocolonic was affected[34,35].

When operating on advanced CD, usually associated with abscess or fistula, a significantly higher complication rate (49%) was reported than after surgery for otherwise uncomplicated CD (12% complication rate)[36].

Postoperative medical treatment can hardly prevent recurrence of CD, therefore an aggressive medical intervention is recommended when objective signs of active disease are found in endoscopy or X ray[37]. There is still a need for preventive strategies[37]. A recent investigation postulated that TNF-α blockers are most effective in treatment and prevention of postoperative disease recurrence[38-41]. Currently there is no consent among the experts concerning the optimal time between TNF-α blocker treatment and surgery[42].

Recommended by the ECCO guidelines 2010[42] surgery should be considered as a primary treatment option in selected cases as localized ileocaecal disease with obstruction but without inflammation[19,43-45]. Patients with a maximum of 40 cm affected bowel, obstructions and clinical symptoms who failed to respond to steroids (CDAI > 220) will also require surgery during the course of disease[46-49]. In general the methods depend on the extent, the localization and severity of morphological complications (ECCO 2010)[42].

Medical treatment of active CD should always be balanced between the potential of the chosen drugs and their side effects. It has to take the state of the disease (*e.g.*, relapse, steroid-refractoriness, quality of life) the severity and extra-intestinal manifestations (Table 2) into account.

A nutritional approach inclusive probiotics was shown to be less effective in these patients even with a mild pattern of disease[50,51]. Neither Omega-3 fatty acid diet, nor probiotics nor nutritional supplementation were convincing in modulating the disease positively[52-54], although a recent study showed a disease controlling effect from whey and soy proteins especially when the patients were treated with TNF-α antibodies and azathioprine[55]. A randomized controlled trial conducted by Takagi has shown the effectiveness of half elemental diet in maintenance therapy in selected patients[56].

However a benefit in reducing pain can be attributed to n-3 unsaturated fatty acids[57]. Thus nutritional therapy as supplementation to medical treatment may be helpful in induction and maintenance of remission or controlling symptoms especially in children[50,56].

It is important to be aware that a considerable portion of patients suffer only from a mild type of CD and need no medical therapy as pointed out in a systematic review of clinical trials by Su *et al*[58]. In general the initial therapy should consist of steroids as immune-modulating agent and mesalazine as antiphlogistic medication. Mesalazine is an amino-derivative of salicylic acid [5-amino-salicylic-acid (5-ASA)]. Beside antiphlogistic activity, which is due to a suppressive effect on pro-inflammatory cytokines (IL1, TNF-α) by inhibition of interleukin-1 stimulated Re1A phosphorylation[59] and by inhibiting macrophage chemotaxis, 5-ASA is also thought to be an antioxidant and traps free radicals which are found to be present in CD[60]. The therapeutic effect of 5-ASA in mild to moderate CD is discussed controversially in the

literature. Tromm found no difference in the efficacy to induce remission in moderate CD of 5-ASA compared to budesonide. Remission rate for budesonide was 69% *vs* 62% for 5-ASA[61]. These data have to compare with a previous meta-analysis which showed 5-ASA no more effective than placebo[62]. The minimal efficient dose is 4 g/d. High dose (6 g/d) for active CD is currently under investigation[42]. One medication of choice to induce remission in mild to moderate CD is budesonide, a synthetic glucocorticoid with limited systemic bioavailability due to extensive first-pass hepatic metabolism. It is effective for induction of remission and causes almost no side effects due to its low bioavailability. It seems to be superior to 5-ASA in moderate disease[63]. Both are also applied as topical treatment in mild types of disease. The systemic administration of corticosteroids/prednisolone is of course much more effective in induction of clinical remission[64,65], but commonly causes more side effects than budesonide[63,64,66]. Two recent studies support this observation even in high dose 5-ASA therapy[67]. The risk to develop Cushing syndrome due to systemic steroid therapy is known at a daily dose of 7.5 mg prednisolone. Therefore, disease control under dose reduction or discontinuation of steroids should be achieved, especially as steroids commonly fail to maintain clinical remission in the majority of patients with active disease[68]. Thus the early onset of the monoclonal antibody anti TNF-α may help to achieve clinical remission even in steroid free or steroid naive conditions[69]. TNF-α is a cell signaling protein which is involved in systemic inflammation.

It is produced mainly by activated macrophages[70]. Antibodies to tumor necrosis factor (anti TNF-α) are highly efficient immune-suppressive drugs. TNF-α inhibitors offer a targeted strategy that contrasts with the nonspecific immune-suppressive agents traditionally used to treat most inflammatory diseases. Anti TNF-α suppresses immune responses in CD by binding to membrane-bound and soluble TNF (mTNF)[71]. Several trials prove the efficacy of Anti TNF-α in achieving clinical remission[72-74]. A recent study, conducted by a Danish group, confirmed the results from previous investigations. Among 492 patients with CD and 267 patients with UC, 74%/13%/14% and 65%/12%/24% were responders, partial responders and non-responders to anti-TNF therapy, respectively[75]. Atreya R developed a method to predict the response rate of this therapy, in brief: Topical antibody administration in 25 patients with CD led to detection of intestinal membrane-bound TNF (mTNF) immune cells during confocal laser endomicroscopy. Patients with high numbers of mTNF cells showed significantly higher short-term response rates (92%) at week 12 upon subsequent anti-TNF therapy as compared to patients with low amounts of mTNF cells (15%). These data indicate that molecular imaging with fluorescent antibodies has the potential to predict therapeutic responses to biological treatment and can be used for personalized medicine in CD and other autoimmune or inflammatory disorders[76]. Due to their mode of action TNF-α-blockers may cause expected and paradoxical side effects, like "*de novo* psoriasis", described by Joyau *et al*[77]. In general patients treated with TNF-α-blockers are at increased risk to develop life threatening opportunistic infections[78-80]. There are also reports of developing rare white blood cell cancer (hepatosplenic T-cell lymphoma) in combination with thiopurines[81].

The disease modulating relevance of antibiotics is restricted to their use in septic complications. The efficacy of metronidazole and ciprofloxacin alone is similar to mesalazine (5-ASA), but inferior to steroids[82,83]. A meta-analysis of 6 trials showed no convincing positive influence on the disease[82-84].

Thiopurines are purine antimetabolites which are widely used in the treatment of autoimmune disorders (*e.g.*, CD, rheumatoid arthritis), and organ transplant recipients[85]. The leading substances are azothioprine (AZA) and mercaptopurine (6-MP). Azathioprine acts as a prodrug for mercaptopurine, inhibiting an enzyme required for the synthesis of DNA. Thus, it most strongly affects proliferating cells, such as the T- and B-cells of the immune system[86,87]. The main adverse effects of thiopurines are bone marrow suppression, hepatotoxicity and pancreatitis and may result in a withdrawal of this drug[86,88] AZA was shown to be efficient in inducing and maintaining remission after tapering of steroids. It is used in the management of moderately to severely or chronically active CD and in corticosteroid-dependent patients[89,90]. Azathioprine treatment is associated with an increased risk of lymphoma, but it is unclear if this is due to the drug or to a predisposition related to CD[91,92]. A recent study provides evidence that In CD, treatment with azathioprine shortly after diagnosis was no more likely to result in corticosteroid-free remission than standard care or placebo[93,94]. No such investigations were performed for 6-MP but as 6-MP is a metabolite of AZA it is considered equivalent. Thioguanine, a 3rd related drug may be an alternative in patients, who are refractory or intolerant to AZA and 6-MP (occurs in up to 15% of long term exposed patients)[92,95]. Patients who relapse under thiopurine therapy should have their dose optimized. Also a switch to TNF-α blockers or MTX should be considered. A long term combination of AZA/6-MP and TNF-α blockers should be avoided in young male patients due to the risk of hepatosplenic T-cell lymphoma[43].

Methotrexate (MTX) is a folic acid inhibitor and thus interferes with cell-growth[96]. It is used predominantly for immunosuppression in autoimmune diseases and as chemotherapy. In CD it has - to date - remained in treatment algorithms as a salvage therapy for patients who have failed to respond or became intolerant to azathioprine[97]. However, its use is not so common in the clinical routine for CD but MTX seems to be sufficient in maintenance therapy. Recently a user's

guide was published which favors the safe and efficient use of MTX in several clinical conditions of CD and UC. The authors Swaminath *et al*[97] provided an ease to use algorithm. MTX is largely used as a second line therapy after AZA failure.

Cyclosporin A (CSA), tacrolimus and mycophenolat-mofetil currently play only a minor role CD as evidence for induction and maintenance of remission is lacking[42].

Current treatment algorithm for CD is displayed in the Guidelines for the Management of CD by Ye *et al*[98] and IBD Study Group of the Korean Association for the Study of the Intestinal Diseases.

UC

UC is graded into 4 disease activities (mild, moderate, sever and remission) and divided into 3 different distribution patterns (proctitis, left-sided, pancolitis).

The severity of the disease is classified by a clinical activity index (CAI)-Rachmilewitz index or Mayo score including stool frequency, rectal bleeding, the endoscopic activity of the colon, and a physician rating of disease activity. Each of these items is given a number from 0 to 3, with 3 being the highest rating for disease activity[99]. A drop to less than 2 points is defined as a clinical relevant remission[99].

The therapeutic goal in UC is to induce steroid-free clinical long-term remission or to increase intervals of acute flare[100]. A cancer surveillance in UC patients is strongly recommended as these individuals have an elevated risk to develop a colon cancer within 10 years. Advanced endoscopic and imaging techniques are warranted to optimize the diagnosis as cancer in UC is a clear indication for surgery[100,101].

Therapeutic management of active UC

The choice of the appropriate medication depends on severity, the previous course (relapsed or persistent active disease) and the localization of the disease. The topical use of 5-ASA (mesalazine) is still the treatment of choice for proctitis or left sided mild to moderate disease or topical steroids although topical steroids (budesonide) were found to be less effective than topical mesalazine[102]. The systemic use of aminosalicyl-derivatives is additionally recommended in more extensive or severe cases[100]. Mesalazine is comparable with newer substances (balsalazide) in its efficacy to induce remission and is better tolerated[103-105]. As previously described two studies have shown that patients treated with oral mesalazine 4 g/d and 1 g/d mesalazine enema experienced a shorter time to resolution of rectal bleeding than those treated with oral therapy alone (P = 0.0025)[106,107]. A smaller study of patients with frequently relapsing disease found that dose escalation of oral mesalazine combined with the addition of topical 5-ASA significantly reduced the number of disease recurrences and courses of steroids (P < 0.0001)[108]. Patients on high dose sulfasalazine (prodrug of mesalazine) require folic supplementation (1 mg/d) to maintain normal cell division. This is of specific importance for patients who receive additionally MTX[96]. Steroids have been a mainstay of UC therapy for many years, based on a thoroughly established efficacy profile for the induction of remission[109,110]. For severe UC, and in patients refractory to 5-ASA, the need for systemic steroids is a general knowledge[100,111-113]. The combination of oral steroids and 5-ASA in escalating doses in case of treatment failure with 5-ASA alone is strongly recommended[109]. Steroids should be tapered as soon as possible as they usually fail to maintain remission and have a problematic safety profile[42,100,114,115]. A therapeutic challenge in UC (similar to CD) is the steroid-dependent or steroid-refractory patient. In this setting additional immune-modulating therapy with thiopurines is recommended[100,111,116-118]. In contrast to CD calcineurin-inhibitors as CSA or tacrolimus play a disease modulating role in UC. Due to the binding-mechanism to calcineurin, transcriptions for pro-inflammatory cytokines IL-2 and TNF-α are inhibited. Side effects as renal impairment or hypomagnesaemia are common in about 50% of the patients, but the main concerns are opportunistic infections. Arts found an incidence of 3.5% (3/86) to die of such infections[119].

Thiopurines [azathioprine (AZA)/mercaptopurine (6-MP)] have limited utility in the acute setting of UC, but are recommended in the maintenance therapy of UC and have a steroid sparing effect[100,111]. CSA in combination with thiopurines are effective to induce remission and avoid colectomy in patients at risk[120]. The treatment with TNF-α blockers (infliximab, adalimumab) is discussed controversial in the literature. Two large well conducted placebo-controlled trials described the induction and maintenance of steroid-free remission in about 26%[121] and 13%[122] at 12 mo of enrolled patients. These data could not be confirmed by a real life observation[111,123]. Only a short term clinical response was observed. A recently presented study suggested a benefit from a combination therapy of TNF-α blockers and thiopurines in early stage of active UC. A steroid free response was seen in 40% of patients at week 16[124,125]. The major drawbacks of TNF-α blockers are already discussed in CD section. Although luminal bacteria are thought to play a major role in pathogenesis of IBD the use of *antibiotics* in UC is also restricted to the therapy of complications due to infections[114].

Surgical interventions

The cumulative risk for colectomy in relation to time of diagnosis has been reported as 13.1% in 5 years[126]. A recent population based UC study observed the global risk for colectomy by 8.7% over 10 years[100]. About 27% of patients with severe UC require colectomy[114,126]. Early surgery, within 3 mo of diagnosis is increasingly performed in patient >

65 years but should not be favored as older patients (> 50 years) have a reduced risk to need colectomy during their course of disease[127-129]. They are more likely to respond to pharmacologicals than younger people[130]. Emergency colectomy or ileostomy due to toxic megacolon, bleeding or perforation is associated with a complication risk or death of 5%[131].

Elective surgery is indicated in chronic therapy refractory cases or when signs of dysplasia are found. Here the common surgery therapy is total proctocolectomy with ileal-pouch anal anastomosis (IPAA)[100,111]. This surgical intervention may have a high curative potential in UC but high rates (up to 20%) of postoperative complications are still emerging problems[100,132-134].

High dose steroids should be weaned before surgery because they are a risk factor for complications[111]. Neither thiopurines nor calcineurin inhibitors seem to increase the risk of postoperative complications while biologicals may be associated with a higher complication rate[111].

Maintenance therapy

Generally maintenance therapy is recommended for all patients. The goal is to keep a steroid free clinical and endoscopic remission. The medication of choice again is 5-ASA (masalazine) topically and/or orally depending on the site and severity of inflammation as long term treatment since this may reduce the risk of colon cancer[100,111]. In steroid dependent patients thiopurines (AZA) are steroid sparing medications. MTX is generally not recommended for UC as the beneficial effect is not proven, although in selected patients (intolerant to the other immunosuppressants) MTX was seen to be effective[100,111,114,135,136]. A large retrospective cohort study of 91 patients reported that one-third of patients were successfully weaned off steroids with MTX therapy. MTX may be considered in the long-term management of patients with UC on steroids[137]. TNF-α blockers are recommended in patients who were treated with this medication initially and achieved remission[121].

A detailed figure of current treatment algorithm for UC is published by Meier and Sturm[100] in *World J Gastroenterology* 2011.

NON-PHARMOCOLOGICAL OPTIONS IN IBD

Granulocyte monocyte apheresis

A special challenge in this setting is the steroid refractory and steroid dependent type of IBD as well as azathioprine-intolerant or - resistant patients. These patients are at high risk to undergo surgery. Thus a Japanese group developed a non-pharmacological therapeutic alternative to conventional therapy. It is an apheresis system, which removes activated monocytes/macrophages (one of the main disease mediators), from the patient's blood circulation (GMA = granulocyte monocyte apheresis). Currently two systems are available, the Adacolumn® system which is approved by the Japanese Ministry of Health and Welfare and is CE marked in Japan and Europe by the TUV since 1999[138,139], and the Cellsorba® System. It is also approved by the Japanese Ministry of Health and Welfare since 1989[140]. Both systems are defined to selectively remove activated WBC, especially granulocytes, monocytes and macrophages (source of TNF-α) from the patients' blood circulation without compromising patients' peripheral blood counts[141]. With the Cellsorba® System additionally activated platelets are removed. The action is based on columns (cellulose-acetate beads in Adasystem and polyester fibers, respectively in Cellsorba) in both systems. The severity of the disease (CD, UC) and mucosal damage correlate with the excess of mucosal granulocyte infiltration[142-144]. Both systems have a great immune-modulating effect. The exact mode of action is not yet sufficiently understood, but certainly, a modulation of the immune system takes place[145]. As a result, less pro-inflammatory cytokines are released. Furthermore, the production of interleukin-1-receptor-antagonist with its anti-inflammatory property is increased and the apoptosis of granulocytes boosted. The decreased LECAM-1-expression on leucocytes impedes the leukotaxis to the inflamed tissue and CD10-negative immature granulocytes appear in the peripheral blood[141,146,147]. Another effect to be mentioned is the removal of the peripheral DCs[148] and the leachate of regulatory T-cells (T-regs)[30,149,150]. Pro-inflammatory cytokines decrease while anti-inflammatory cytokines increase[151]. The induction of clinical and morphological remission of IBD can be explained by this mechanism of action.

Most clinical trials are conducted using the Adacolumn® system for GMA in both, UC and CD whereby UC is the leading entity. The best responders seem to be steroid naive patients followed by steroid dependent patients or steroid refractory patients with a short history of disease[152,153]. A recent study tried to define predictive factors to identify patients with UC who are likely to respond to GMA[154]. In this trial 43 patients with active UC were enrolled. Best responders to GMA were those treated immediately (< 49.5 d) after relapse. Also an initial low WBC count (< 10 G/L) was predictive for a good response. They conclude that in these selected patients GMA is efficient as mono-therapy inducing remission (defined by CAI). Sacco investigated 118 steroid dependent or refractory patients (UC *n* = 83, CD *n* = 35). GMA was efficient in inducing and maintaining remission during a follow up of 12 mo, irrespective the entity or the steroid status (UC: CAI = 6; CD: CDAI < 150)[155].

Also a combination therapy (thiopurines and GMA) promises a rapid induced high remission rate in patients with active early diagnosed CD[156]. In this study 22 steroid and biological naïve patients were

treated with thiopurines and GMA. The rate of mucosal healing was 50% after 1 year.

A meta-analysis by Yoshino *et al*[157] 2014 revealed that intensive granulocyte and monocyte adsorption apheresis is a safe and effective treatment with higher rates of clinical remission and response for UC compared with corticosteroids.

GMA treatment is known to have an excellent safety profile. Almost no side effects occur[153,158]. Up to now no treatment had to be discontinued because of adverse events[158]. There is a strong recommendation to introduce GMA at an early state of disease before patients develop extensive mucosal damage and become dependent or refractory to drugs (similar to a TNF-α therapy)[159].

There is still a controversy regarding the optimal treatment schedule. Five sessions in 5 wk *vs* 10 sessions in 8 wk was shown to be similar efficient[160]. Therefore Vecchi recommended in his review the perpetuation of the traditional treatment schedule of 5 sessions in 5 wk (1/wk) as it is seems not to be inferior to the more intensive version, more convenient to the patients and more cost effective[158].

Still more randomized sham controlled double blind trials with a long term surveillance to evaluate the long term outcome, treatment schedules and cost effectiveness (equipment, pharmacological therapy, avoidance of surgical intervention) are required, although several reports attest comparable costs to conventional therapy as the good safety profile should comprise higher costs[161].

According to current data the American Society for Apheresis (ASFA) assigned 2013 GMA in UC to category Ⅱ/Ⅲ with a recommendation level of Grade 1B/2B (strong to weak recommendation, moderate quality evidence) and the CD to category Ⅲ, recommendation 1B (strong, but randomized controlled trials with important limitations)[162].

ASFA defines its categories as below

Cat Ⅱ: Disorders for which apheresis is accepted as second-line therapy; either as a stand alone treatment or in conjunction with other modes of treatment[162].

Cat Ⅲ: Optimum role of apheresis therapy is not established. Decision making should be individualized.

Centrifugal lymphocytapheresis

In the 80ties Bicks *et al*[163] described successful treatment of active CD by centrifugal lymphocytapheresis (CLA) and lymphoplasmapheresis[164]. Nowadays these treatment options are replaced by selective adsorption systems (Adacolumn and Cellsorba).

Extracorporealphotopheresis

Two uncontrolled case series have been published suggesting that extracorporealphotopheresis (ECP) can promote remission for a proportion of patients in the category of steroid and/or immunosuppressant intolerant or refractory CD[165,166].

The domain of ECP is T-cell mediated diseases, like T-cell lymphoma or acute or chronic graft *vs* host disease (GvHD). The mode of action is, like for all extracorporeal treatments, elusive. The mechanism of this treatment is likely due to the induction of anticlonotypic immunity directed against pathogenic clones of T lymphocytes. Treatment induces apoptotic death of pathogenic T-cells, and it is postulated that activation of antigen-presenting cells has important effects in this process[167]. More recently, it has been suggested that ECP may induce Ag-specific immune-modulation *via* regulatory T-cells, which could explain its efficacy in immune-mediated diseases and lack of toxicities. The frequency of T-regs was significantly increased in the blood of ECP-treated patients[168]. *In vitro* these cells exerted suppressive activity and showed features of T-regs. The best-characterized subtypes of T-regs are those expressing CD4 and CD25[149,150]. It is also suggested that ECP induces IL-10 producing regulatory B cells, regulatory $CD8^+$ T cells and IL-10 producing T-regs Type 1[167,169,170]. IL-10, also known as cytokine-synthesis inhibitory factor, is a potent anti-inflammatory cytokine[171]. Abreu *et al*[165] observed in 28 patients with moderate-to-severely active CD (mean baseline CDAI 324) who were refractory to or intolerant of immunosuppressants and/or anti-TNF agents that ECP was well tolerated and induced clinical response (50%) and remission (25%) in patients with CD[165]. Reinisch confirmed these results in 31 CD patients, ECP permitted reduction or discontinuation in steroid dependent or - refractory patients[166].

As sham controlled trials are still missing ASFA recommends assigned ECP in CD to category Ⅲ with a recommendation level 2[162].

Currently ECP plays a minor role in the treatment of CD (weak recommendation due to low quality of evidence).

FUTURE ASPECTS IN THE MANAGEMENT OF IBD

CD and UC are complex disorders which need complex therapeutic strategies and a continuous development of treatment managements, new drugs and alternative measures. Despite the high prevalence of mental health co-morbidities in IBD, psychological illness remains largely under treated, with studies showing that 60% of IBD patients experiencing mental health problems do not receive adequate help. Therapeutic approaches must always be chosen in agreement with the patients to increase the patients' compliance[172].

Due to the complexity of the disease a collaboration of medical specialists is necessary to cover all complications and to improve treatment success[173].

A new scoring system for CD, the Lémann score,

is developed to allow a better identification of patients with severe epithelial damage and those with rapid progression of damage. This system monitors the cumulative damage. It measures cumulative structural bowel progression at a specific time point, based on medical and disease history, by endoscopy and other imaging methods[173,174]. This instrument can also be used to assess the effect of various medical therapies on the progression of bowel damage.

The big hope is set on advances in biologicals with different mechanism of action (*i.e.*, anti TNF vaccination, TNF gene silencing and TNF neutralizing nanobodies) so that in case of treatment failure other biologicals or even combinations are available[175]. As mentioned earlier (in the CD section), laboratory assays to identify TNF-α blocker responders and non- responders would be very helpful in treatment optimization[176]. Further drugs targeting other pro-inflammatory cytokines are in evaluation[175]. Another therapeutic approach is given by vedolizumab, a biological which targets cell adhesion molecules (CAMs), a monoclonal antibody that inhibits mucosal leukocyte infiltration[177]. In contrast to natalizumab (α_4-integrin-inhibitor, Tysabri®) vedolizumab ($\alpha_4\beta_7$ integrin-inhibitor, Entyvio®) modulates the adaptive immune system without systemic side effects[178].

Enteric bacteria, viruses or fungi may induce IBD, thus fecal microbiota transplantation or fecal bacteriotherapy (from a healthy individual) is also a therapeutic option in IBD[179]. Autologous hematopoietic stem cell transplantation (aHSCT) has been used as a treatment for severe, active and therapy-refractory autoimmune diseases for more than 13 years[180,181]. In 2012 the EBMT published guidelines for the selection of patients with autoimmune diseases[182]. Among disorders like multiple sclerosis, systemic lupus erythematosus, myasthenia gravis also severe active CD refractory to conventional therapy has been proposed as a potential indication for aHSCT[180]. Meanwhile several studies were conducted, which revealed promising results. The majority of so treated patients showed clinical and endoscopic remission within 6 mo, and remained free of medical therapy for at least one year. In relapsing patients a disease control with low dose steroids and immune-suppressive therapy was achieved[183,184].

Another point in therapeutic care in IBD is a harmonization between different countries and centers. Two retrospective cross sectional studies from 2009 describe country and care-setting specific therapeutic variations. The impact on outcome is not yet clear[185,186].

Step up and top down strategy

The current clinical practice and recommended treatment for CD is the "step-up" approach which refers to a sequential treatment strategy that often begins with a less potent and less toxic treatment strategy, such as topical steroids or aminosalicylates with escalation to the highly effective but potentially more toxic treatment strategies[42,187]. The top down strategy refers to the use highly effective but potentially more toxic treatment strategies early in the course of a chronic illness to prevent disease progression and achieve remission[188].

The introduction of biologic therapy, and particularly the use of anti TNF-α therapy, has provided a powerful tool in the treatment and management of IBD. The prevention of structural damage by achievement of "mucosal healing", however, is associated with the more "aggressive" treatment and an earlier use of immune-suppressants and biologicals[189]. A recent study from D'Haens[190] has provided evidence suggesting that reversing the treatment paradigm from a "step-up" to a "top-down" approach may positively alter the natural course of this illness by mucosal healing[188,190]. Several further studies show, that the onset of biologicals in the early course of disease results in better mucosal healing, earlier tapering of steroids, less complications and less need for surgery. Also the incidence of relapse is reduced[191]. On the other hand one must be aware, that the early use of biologicals, especially TNF-α blockers, may result in an increased risk of complications, particularly server life threatening infections. LIN described that about 30% of patients might be over-treated by the top down strategy[188]. Thus it is certainly a crucial point to identify high-risk patients who clearly would benefit from the early use of a more aggressive treatment so that the expected benefit outweighs increased risk for probably severe side effects.

In UC conventional treatment strategies (step-up) are still favored as aminosalycylate derivatives (5-ASA) seem to prevent cancer, the most important complication in UC. Therefore, at present, there is little rationale for a top-down approach to managing UC. Although, Sandborn[192] did not exclude that a top down approach for a selected subgroup of patients with UC (patients at high risk to develop complicated or therapy refractory disease) may profit from the administration of biologicals or other immune-suppressants at early stage of the disease.

CONCLUSION

Up to now there is no cure for IBD. This review describes shortly current and advanced therapeutic options for patients with IBD. They all have limitations due to side effects, refractoriness or unresponsiveness of the patients due to known and unknown causes. There are still a number of individuals in whom the current strategies are insufficient in controlling symptoms. Further studies are in progress to develop new therapeutic options or to improve those already in use in order to achieve durable remission in the majority of patients. The future strategy aims at early hard therapy of patients at risk for severe course

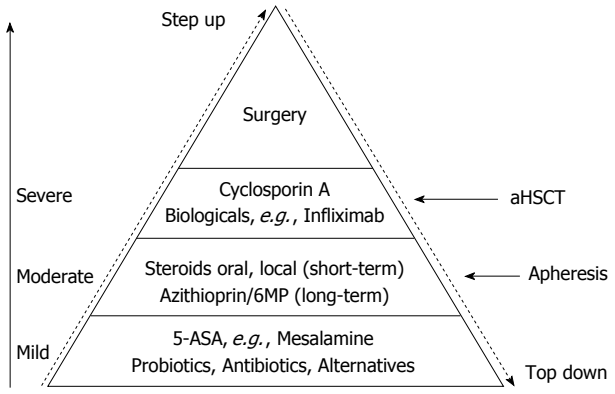

Figure 1 Therapeutic approaches in inflammatory bowel disease. 5-ASA: 5-amino-salicylate-acid; aHSCT: Autologous stem cell transplantation.

of IBD- even with combined immunosuppression, if necessary. After achieving complete remission-endoscopic and biologic (comprising normal stool calprotectin) - tapering of immunosuppression might be possible. A lot of new pharmacological and immune modifying therapies are currently studied in phase Ⅱ and Ⅲ studies in IBD and are the hope for therapy refractory patients. Even for patients with short bowel syndrome a new therapeutic approach with a GLP-2 analog - teduglutide - is on the market and fighting for coverage by insurance companies[193]. Patients with malnutrition and weight loss of more than 5% within 3 mo should be treated with appropriate medication but also with oral nutritional therapy to avoid further complications like opportunistic infections, long hospitalization and higher mortality. Serum levels of Vit B12, folic acid, Vit D and zinc should be monitored carefully[194]. Figure 1 comprises current therapeutic options in IBD including alternative strategies, like extracorporeal techniques and autologous stem cell transplantation.

At present, clinical manifestations are the most useful way to make therapeutic decisions.

REFERENCES

1 **Crohn BB**, Ginzburg L, Oppenheimer GD. Regional ileitis: a pathologic and clinical entity. 1932. *Mt Sinai J Med* 2000; **67**: 263-268 [PMID: 10828911]

2 **Crohn BB**. An historic note on ulcerative colitis. *Gastroenterology* 1962; **42**: 366-367 [PMID: 13882293]

3 **Khor B**, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. *Nature* 2011; **474**: 307-317 [PMID: 21677747 DOI: 10.1038/nature10209]

4 **Mukhopadhya I**, Hansen R, El-Omar EM, Hold GL. IBD-what role do Proteobacteria play? *Nat Rev Gastroenterol Hepatol* 2012; **9**: 219-230 [PMID: 22349170 DOI: 10.1038/nrgastro.2012.14]

5 **Devkota S**, Wang Y, Musch MW, Leone V, Fehlner-Peach H, Nadimpalli A, Antonopoulos DA, Jabri B, Chang EB. Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10-/- mice. *Nature* 2012; **487**: 104-108 [PMID: 22722865 DOI: 10.1038/nature11225]

6 **Aroniadis OC**, Brandt LJ. Fecal microbiota transplantation: past, present and future. *Curr Opin Gastroenterol* 2013; **29**: 79-84 [PMID: 23041678 DOI: 10.1097/MOG.0b013e32835a4b3e]

7 **Bernstein CN**. Antibiotic use and the risk of Crohn's disease. *Gastroenterol Hepatol* (N Y) 2013; **9**: 393-395 [PMID: 23935547]

8 **Kotanko P**, Carter M, Levin NW. Intestinal bacterial microflora--a potential source of chronic inflammation in patients with chronic kidney disease. *Nephrol Dial Transplant* 2006; **21**: 2057-2060 [PMID: 16762961 DOI: 10.1093/ndt/gfl281]

9 **Brewerton DA**, Hart FD, Nicholls A, Caffrey M, James DC, Sturrock RD. Ankylosing spondylitis and HL-A 27. *Lancet* 1973; **1**: 904-907 [PMID: 4123836]

10 **Schlosstein L**, Terasaki PI, Bluestone R, Pearson CM. High association of an HL-A antigen, W27, with ankylosing spondylitis. *N Engl J Med* 1973; **288**: 704-706 [PMID: 4688372 DOI: 10.1056/NEJM197304052881403]

11 **Greenstein AJ**, Janowitz HD, Sachar DB. The extra-intestinal complications of Crohn's disease and ulcerative colitis: a study of 700 patients. *Medicine* (Baltimore) 1976; **55**: 401-412 [PMID: 957999]

12 **Gaya DR**, Russell RK, Nimmo ER, Satsangi J. New genes in inflammatory bowel disease: lessons for complex diseases? *Lancet* 2006; **367**: 1271-1284 [PMID: 16631883 DOI: 10.1016/S0140-6736(06)68345-1]

13 **Jess T**, Riis L, Jespersgaard C, Hougs L, Andersen PS, Orholm MK, Binder V, Munkholm P. Disease concordance, zygosity, and NOD2/CARD15 status: follow-up of a population-based cohort of Danish twins with inflammatory bowel disease. *Am J Gastroenterol* 2005; **100**: 2486-2492 [PMID: 16279904 DOI: 10.1111/j.1572-0241.2005.00224.x]

14 **Jostins L**, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY, Lee JC, Schumm LP, Sharma Y, Anderson CA, Essers J, Mitrovic M, Ning K, Cleynen I, Theatre E, Spain SL, Raychaudhuri S, Goyette P, Wei Z, Abraham C, Achkar JP, Ahmad T, Amininejad L, Ananthakrishnan AN, Andersen V, Andrews JM, Baidoo L, Balschun T, Bampton PA, Bitton A, Boucher G, Brand S, Büning C, Cohain A, Cichon S, D'Amato M, De Jong D, Devaney KL, Dubinsky M, Edwards C, Ellinghaus D, Ferguson LR, Franchimont D, Fransen K, Gearry R, Georges M, Gieger C, Glas J, Haritunians T, Hart A, Hawkey C, Hedl M, Hu X, Karlsen TH, Kupcinskas L, Kugathasan S, Latiano A, Laukens D, Lawrance IC, Lees CW, Louis E, Mahy G, Mansfield J, Morgan AR, Mowat C, Newman W, Palmieri O, Ponsioen CY, Potocnik U, Prescott NJ, Regueiro M, Rotter JI, Russell RK, Sanderson JD, Sans M, Satsangi J, Schreiber S, Simms LA, Sventoraityte J, Targan SR, Taylor KD, Tremelling M, Verspaget HW, De Vos M, Wijmenga C, Wilson DC, Winkelmann J, Xavier RJ, Zeissig S, Zhang B, Zhang CK, Zhao H, Silverberg MS, Annese V, Hakonarson H, Brant SR, Radford-Smith G, Mathew CG, Rioux JD, Schadt EE, Daly MJ, Franke A, Parkes M, Vermeire S, Barrett JC, Cho JH. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* 2012; **491**: 119-124 [PMID: 23128233 DOI: 10.1038/nature11582]

15 **Danese S**, Sans M, Fiocchi C. Inflammatory bowel disease: the role of environmental factors. *Autoimmun Rev* 2004; **3**: 394-400 [PMID: 15288007 DOI: 10.1016/j.autrev.2004.03.002]

16 **Mikhailov TA**, Furner SE. Breastfeeding and genetic factors in the etiology of inflammatory bowel disease in children. *World J Gastroenterol* 2009; **15**: 270-279 [PMID: 19140226]

17 **Molodecky NA**, Kaplan GG. Environmental risk factors for inflammatory bowel disease. *Gastroenterol Hepatol* (N Y) 2010; **6**: 339-346 [PMID: 20567592]

18 **Ng SC**, Woodrow S, Patel N, Subhani J, Harbord M. Role of genetic and environmental factors in British twins with inflammatory bowel disease. *Inflamm Bowel Dis* 2012; **18**: 725-736 [PMID: 21557397 DOI: 10.1002/ibd.21747]

19 **Abraham C**, Medzhitov R. Interactions between the host innate immune system and microbes in inflammatory bowel disease. *Gastroenterology* 2011; **140**: 1729-1737 [PMID: 21530739 DOI: 10.1053/j.gastro.2011.02.012]

20 **Savige J**, Dimech W, Fritzler M, Goeken J, Hagen EC, Jennette JC, McEvoy R, Pusey C, Pollock W, Trevisin M, Wiik A, Wong R. Addendum to the International Consensus Statement on testing and reporting of antineutrophil cytoplasmic antibodies. Quality control

guidelines, comments, and recommendations for testing in other autoimmune diseases. *Am J Clin Pathol* 2003; **120**: 312-318 [PMID: 14502794 DOI: 10.1309/WAEP-ADW0-K4LP-UHFN]
21 **Bossuyt X**. Serologic markers in inflammatory bowel disease. *Clin Chem* 2006; **52**: 171-181 [PMID: 16339302 DOI: 10.1373/clinchem.2005.058560]
22 **Reese GE**, Constantinides VA, Simillis C, Darzi AW, Orchard TR, Fazio VW, Tekkis PP. Diagnostic precision of anti-Saccharomyces cerevisiae antibodies and perinuclear antineutrophil cytoplasmic antibodies in inflammatory bowel disease. *Am J Gastroenterol* 2006; **101**: 2410-2422 [PMID: 16952282 DOI: 10.1111/j.1572-0241.2006.00840.x]
23 **Seibold F**, Stich O, Hufnagl R, Kamil S, Scheurlen M. Anti-Saccharomyces cerevisiae antibodies in inflammatory bowel disease: a family study. *Scand J Gastroenterol* 2001; **36**: 196-201 [PMID: 11252413]
24 **Kuna AT**. Serological markers of inflammatory bowel disease. *Biochem Med* (Zagreb) 2013; **23**: 28-42 [PMID: 23457764]
25 **Xiang JY**, Ouyang Q, Li GD, Xiao NP. Clinical value of fecal calprotectin in determining disease activity of ulcerative colitis. *World J Gastroenterol* 2008; **14**: 53-57 [PMID: 18176961]
26 **Eder P**, Korybalska K, Linke K, Witowski J. Angiogenesis-related proteins--their role in the pathogenesis and treatment of inflammatory bowel disease. *Curr Protein Pept Sci* 2015; **16**: 249-258 [PMID: 25707471]
27 **Cioffi M**, Rosa AD, Serao R, Picone I, Vietri MT. Laboratory markers in ulcerative colitis: Current insights and future advances. *World J Gastrointest Pathophysiol* 2015; **6**: 13-22 [PMID: 25685607 DOI: 10.4291/wjgp.v6.i1.13]
28 **Best WR**, Becktel JM, Singleton JW, Kern F. Development of a Crohn's disease activity index. National Cooperative Crohn's Disease Study. *Gastroenterology* 1976; **70**: 439-444 [PMID: 1248701]
29 **Irvine EJ**, Feagan B, Rochon J, Archambault A, Fedorak RN, Groll A, Kinnear D, Saibil F, McDonald JW. Quality of life: a valid and reliable measure of therapeutic efficacy in the treatment of inflammatory bowel disease. Canadian Crohn's Relapse Prevention Trial Study Group. *Gastroenterology* 1994; **106**: 287-296 [PMID: 8299896]
30 **C Leitner G**, Worel N, Vogelsang H. Selective Granulocyte and Monocyte Apheresis as a Non-Pharmacological Option for Patients with Inflammatory Bowel Disease. *Transfus Med Hemother* 2012; **39**: 246-252 [PMID: 22969694]
31 **Sostegni R**, Daperno M, Scaglione N, Lavagna A, Rocca R, Pera A. Review article: Crohn's disease: monitoring disease activity. *Aliment Pharmacol Ther* 2003; **17** Suppl 2: 11-17 [PMID: 12786607]
32 **Malafosse M**. [Crohn's disease: current surgical treatment]. *Bull Acad Natl Med* 2007; **191**: 1143-1156; discussion 1157-1158 [PMID: 18402169]
33 **Hultén L**. Surgical treatment of Crohn's disease of the small bowel or ileocecum. *World J Surg* 1988; **12**: 180-185 [PMID: 3293325]
34 **Bernell O**, Lapidus A, Hellers G. Risk factors for surgery and recurrence in 907 patients with primary ileocaecal Crohn's disease. *Br J Surg* 2000; **87**: 1697-1701 [PMID: 11122187 DOI: 10.1046/j.1365-2168.2000.01589.x]
35 **Bernell O**, Lapidus A, Hellers G. Risk factors for surgery and postoperative recurrence in Crohn's disease. *Ann Surg* 2000; **231**: 38-45 [PMID: 10636100]
36 **Strong SA**. Surgical treatment of inflammatory bowel disease. *Curr Opin Gastroenterol* 2002; **18**: 441-446 [PMID: 17033319]
37 **Rutgeerts P**. Review article: recurrence of Crohn's disease after surgery - the need for treatment of new lesions. *Aliment Pharmacol Ther* 2006; **24** Suppl 3: 29-32 [PMID: 16961741 DOI: 10.1111/j.1365-2036.2006.03056.x]
38 **Singh S**, Garg SK, Pardi DS, Wang Z, Murad MH, Loftus EV. Comparative efficacy of pharmacologic interventions in preventing relapse of Crohn's disease after surgery: a systematic review and network meta-analysis. *Gastroenterology* 2015; **148**: 64-76.e2; quiz e14 [PMID: 25263803 DOI: 10.1053/j.gastro.2014.09.031]
39 **Sorrentino D**. State-of-the-art medical prevention of postoperative recurrence of Crohn's disease. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 413-422 [PMID: 23648935 DOI: 10.1038/nrgastro.2013.69]
40 **Sorrentino D**, Fogel S, Van den Bogaerde J. Surgery for Crohn's disease and anti-TNF agents: the changing scenario. *Expert Rev Gastroenterol Hepatol* 2013; **7**: 689-700 [PMID: 24161133 DOI: 10.1586/17474124.2013.842895]
41 **Vuitton L**, Koch S, Peyrin-Biroulet L. Preventing postoperative recurrence in Crohn's disease: what does the future hold? *Drugs* 2013; **73**: 1749-1759 [PMID: 24132799 DOI: 10.1007/s40265-013-0128-x]
42 **Dignass A**, Van Assche G, Lindsay JO, Lémann M, Söderholm J, Colombel JF, Danese S, D'Hoore A, Gassull M, Gomollón F, Hommes DW, Michetti P, O'Morain C, Oresland T, Windsor A, Stange EF, Travis SP. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Current management. *J Crohns Colitis* 2010; **4**: 28-62 [PMID: 21122489 DOI: 10.1016/j.crohns.2009.12.002]
43 **Solem CA**, Loftus EV, Tremaine WJ, Harmsen WS, Zinsmeister AR, Sandborn WJ. Correlation of C-reactive protein with clinical, endoscopic, histologic, and radiographic activity in inflammatory bowel disease. *Inflamm Bowel Dis* 2005; **11**: 707-712 [PMID: 16043984]
44 **Tromm A**, Tromm CD, Hüppe D, Schwegler U, Krieg M, May B. Evaluation of different laboratory tests and activity indices reflecting the inflammatory activity of Crohn's disease. *Scand J Gastroenterol* 1992; **27**: 774-778 [PMID: 1411285]
45 **Vermeire S**, Van Assche G, Rutgeerts P. The role of C-reactive protein as an inflammatory marker in gastrointestinal diseases. *Nat Clin Pract Gastroenterol Hepatol* 2005; **2**: 580-586 [PMID: 16327837 DOI: 10.1038/ncpgasthep0359]
46 **Graadal O**, Nygaard K. [Crohn disease. Long-term effects of surgical treatment]. *Tidsskr Nor Laegeforen* 1994; **114**: 1603-1605 [PMID: 8079260]
47 **Kim NK**, Senagore AJ, Luchtefeld MA, MacKeigan JM, Mazier WP, Belknap K, Chen SH. Long-term outcome after ileocecal resection for Crohn's disease. *Am Surg* 1997; **63**: 627-633 [PMID: 9202538]
48 **Nordgren SR**, Fasth SB, Oresland TO, Hultén LA. Long-term follow-up in Crohn's disease. Mortality, morbidity, and functional status. *Scand J Gastroenterol* 1994; **29**: 1122-1128 [PMID: 7886401]
49 **Weston LA**, Roberts PL, Schoetz DJ, Coller JA, Murray JJ, Rusin LC. Ileocolic resection for acute presentation of Crohn's disease of the ileum. *Dis Colon Rectum* 1996; **39**: 841-846 [PMID: 8756837]
50 **Donnellan CF**, Yann LH, Lal S. Nutritional management of Crohn's disease. *Therap Adv Gastroenterol* 2013; **6**: 231-242 [PMID: 23634187 DOI: 10.1177/1756283X13477715]
51 **Sheil B**, Shanahan F, O'Mahony L. Probiotic effects on inflammatory bowel disease. *J Nutr* 2007; **137**: 819S-824S [PMID: 17311981]
52 **Feagan BG**, Sandborn WJ, Mittmann U, Bar-Meir S, D'Haens G, Bradette M, Cohen A, Dallaire C, Ponich TP, McDonald JW, Hébuterne X, Paré P, Klvana P, Niv Y, Ardizzone S, Alexeeva O, Rostom A, Kiudelis G, Spleiss J, Gilgen D, Vandervoort MK, Wong CJ, Zou GY, Donner A, Rutgeerts P. Omega-3 free fatty acids for the maintenance of remission in Crohn disease: the EPIC Randomized Controlled Trials. *JAMA* 2008; **299**: 1690-1697 [PMID: 18398081 DOI: 10.1001/jama.299.14.1690]
53 **Rolfe VE**, Fortun PJ, Hawkey CJ, Bath-Hextall F. Probiotics for maintenance of remission in Crohn's disease. *Cochrane Database Syst Rev* 2006; **(4)**: CD004826 [PMID: 17054217 DOI: 10.1002/14651858.CD004826.pub2]
54 **Verma S**, Kirkwood B, Brown S, Giaffer MH. Oral nutritional supplementation is effective in the maintenance of remission in Crohn's disease. *Dig Liver Dis* 2000; **32**: 769-774 [PMID: 11215556]
55 **Machado JF**, Oya V, Coy CS, Morcillo AM, Severino SD, Wu C, Sgarbieri VC, Vilela MM. Whey and soy protein supplements changes body composition in patients with Crohn's disease undergoing azathioprine and anti-TNF-alpha therapy. *Nutr Hosp* 2015; **31**: 1603-1610 [PMID: 25795947 DOI: 10.3305/nh.2015.31.4.8362]

56 **Takagi S**, Utsunomiya K, Kuriyama S, Yokoyama H, Takahashi S, Iwabuchi M, Takahashi H, Takahashi S, Kinouchi Y, Hiwatashi N, Funayama Y, Sasaki I, Tsuji I, Shimosegawa T. Effectiveness of an 'half elemental diet' as maintenance therapy for Crohn's disease: A randomized-controlled trial. *Aliment Pharmacol Ther* 2006; **24**: 1333-1340 [PMID: 17059514 DOI: 10.1111/j.1365-2036.2006.03120.x]

57 **Tokuyama S**, Nakamoto K. Unsaturated fatty acids and pain. *Biol Pharm Bull* 2011; **34**: 1174-1178 [PMID: 21804202]

58 **Su C**, Lichtenstein GR, Krok K, Brensinger CM, Lewis JD. A meta-analysis of the placebo rates of remission and response in clinical trials of active Crohn's disease. *Gastroenterology* 2004; **126**: 1257-1269 [PMID: 15131785]

59 **Egan LJ**, Mays DC, Huntoon CJ, Bell MP, Pike MG, Sandborn WJ, Lipsky JJ, McKean DJ. Inhibition of interleukin-1-stimulated NF-kappaB RelA/p65 phosphorylation by mesalamine is accompanied by decreased transcriptional activity. *J Biol Chem* 1999; **274**: 26448-26453 [PMID: 10473604]

60 **Langmead L**, Rampton DS. Review article: herbal treatment in gastrointestinal and liver disease--benefits and dangers. *Aliment Pharmacol Ther* 2001; **15**: 1239-1252 [PMID: 11552894]

61 **Tromm A**, Bunganič I, Tomsová E, Tulassay Z, Lukáš M, Kykal J, Bátovský M, Fixa B, Gabalec L, Safadi R, Kramm HJ, Altorjay I, Löhr H, Koutroubakis I, Bar-Meir S, Stimac D, Schäffeler E, Glasmacher C, Dilger K, Mohrbacher R, Greinwald R. Budesonide 9 mg is at least as effective as mesalamine 4.5 g in patients with mildly to moderately active Crohn's disease. *Gastroenterology* 2011; **140**: 425-434.e1; quiz e13-14 [PMID: 21070781 DOI: 10.1053/j.gastro.2010.11.004]

62 **Hanauer SB**. Review article: aminosalicylates in inflammatory bowel disease. *Aliment Pharmacol Ther* 2004; **20** Suppl 4: 60-65 [PMID: 15352896 DOI: 10.1111/j.1365-2036.2004.02048.x]

63 **Seow CH**, Benchimol EI, Griffiths AM, Otley AR, Steinhart AH. Budesonide for induction of remission in Crohn's disease. *Cochrane Database Syst Rev* 2008; **(3)**: CD000296 [PMID: 18646064 DOI: 10.1002/14651858.CD000296.pub3]

64 **Otley A**, Steinhart AH. Budesonide for induction of remission in Crohn's disease. *Cochrane Database Syst Rev* 2005; **(4)**: CD000296 [PMID: 16235274 DOI: 10.1002/14651858.CD000296.pub2]

65 **Silverman J**, Otley A. Budesonide in the treatment of inflammatory bowel disease. *Expert Rev Clin Immunol* 2011; **7**: 419-428 [PMID: 21790284 DOI: 10.1586/eci.11.34]

66 **Campieri M**, Ferguson A, Doe W, Persson T, Nilsson LG. Oral budesonide is as effective as oral prednisolone in active Crohn's disease. The Global Budesonide Study Group. *Gut* 1997; **41**: 209-214 [PMID: 9301500]

67 **Benchimol EI**, Seow CH, Steinhart AH, Griffiths AM. Traditional corticosteroids for induction of remission in Crohn's disease. *Cochrane Database Syst Rev* 2008; **(2)**: CD006792 [PMID: 18425970 DOI: 10.1002/14651858.CD006792.pub2]

68 **Ho GT**, Chiam P, Drummond H, Loane J, Arnott ID, Satsangi J. The efficacy of corticosteroid therapy in inflammatory bowel disease: analysis of a 5-year UK inception cohort. *Aliment Pharmacol Ther* 2006; **24**: 319-330 [PMID: 16842459 DOI: 10.1111/j.1365-2036.2006.02974.x]

69 **D'Haens G**. Anti-TNF treatment in Crohn's disease: toward tailored therapy? *Am J Gastroenterol* 2010; **105**: 1140-1141 [PMID: 20445512 DOI: 10.1038/ajg.2010.15]

70 **Locksley RM**, Killeen N, Lenardo MJ. The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell* 2001; **104**: 487-501 [PMID: 11239407]

71 **Wong M**, Ziring D, Korin Y, Desai S, Kim S, Lin J, Gjertson D, Braun J, Reed E, Singh RR. TNFalpha blockade in human diseases: mechanisms and future directions. *Clin Immunol* 2008; **126**: 121-136 [PMID: 17916444 DOI: 10.1016/j.clim.2007.08.013]

72 **Behm BW**, Bickston SJ. Tumor necrosis factor-alpha antibody for maintenance of remission in Crohn's disease. *Cochrane Database Syst Rev* 2008; **(1)**: CD006893 [PMID: 18254120 DOI: 10.1002/14651858.CD006893]

73 **Peyrin-Biroulet L**. [Anti-TNF therapy and Crohn's disease]. *Gastroenterol Clin Biol* 2008; **32**: 478-481 [PMID: 18439777 DOI: 10.1016/j.gcb.2008.03.004]

74 **Peyrin-Biroulet L**, Oussalah A, Boucekkine T, Bigard MA. TNF antagonists in the treatment of inflammatory bowel disease: results of a survey of gastroenterologists in the French region of Lorraine. *Gastroenterol Clin Biol* 2009; **33**: 23-30 [PMID: 19118965 DOI: 10.1016/j.gcb.2008.07.012]

75 **Bank S**, Andersen PS, Burisch J, Pedersen N, Roug S, Galsgaard J, Turino SY, Brodersen JB, Rashid S, Avlund S, Olesen TB, Green A, Hoffmann HJ, Thomsen MK, Thomsen VØ, Nexø BA, Vogel U, Andersen V. Effectiveness of anti-tumour necrosis factor-α therapy in Danish patients with inflammatory bowel diseases. *Dan Med J* 2015; **62**: pii: A4994 [PMID: 25748864]

76 **Atreya R**, Neumann H, Neufert C, Waldner MJ, Billmeier U, Zopf Y, Willma M, App C, Münster T, Kessler H, Maas S, Gebhardt B, Heimke-Brinck R, Reuter E, Dörje F, Rau TT, Uter W, Wang TD, Kiesslich R, Vieth M, Hannappel E, Neurath MF. In vivo imaging using fluorescent antibodies to tumor necrosis factor predicts therapeutic response in Crohn's disease. *Nat Med* 2014; **20**: 313-318 [PMID: 24562382 DOI: 10.1038/nm.3462]

77 **Joyau C**, Veyrac G, Dixneuf V, Jolliet P. Anti-tumour necrosis factor alpha therapy and increased risk of de novo psoriasis: is it really a paradoxical side effect? *Clin Exp Rheumatol* 2012; **30**: 700-706 [PMID: 22935567]

78 **Deepak P**, Stobaugh DJ, Ehrenpreis ED. Infectious complications of TNF-α inhibitor monotherapy versus combination therapy with immunomodulators in inflammatory bowel disease: analysis of the Food and Drug Administration Adverse Event Reporting System. *J Gastrointestin Liver Dis* 2013; **22**: 269-276 [PMID: 24078983]

79 **Salmon-Ceron D**, Tubach F, Lortholary O, Chosidow O, Bretagne S, Nicolas N, Cuillerier E, Fautrel B, Michelet C, Morel J, Puéchal X, Wendling D, Lemann M, Ravaud P, Mariette X. Drug-specific risk of non-tuberculosis opportunistic infections in patients receiving anti-TNF therapy reported to the 3-year prospective French RATIO registry. *Ann Rheum Dis* 2011; **70**: 616-623 [PMID: 21177290 DOI: 10.1136/ard.2010.137422]

80 **Winthrop KL**. Risk and prevention of tuberculosis and other serious opportunistic infections associated with the inhibition of tumor necrosis factor. *Nat Clin Pract Rheumatol* 2006; **2**: 602-610 [PMID: 17075599 DOI: 10.1038/ncprheum0336]

81 **Mackey AC**, Green L, Liang LC, Dinndorf P, Avigan M. Hepatosplenic T cell lymphoma associated with infliximab use in young patients treated for inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 2007; **44**: 265-267 [PMID: 17255842 DOI: 10.1097/MPG.0b013e31802f6424]

82 **Colombel JF**, Lémann M, Cassagnou M, Bouhnik Y, Duclos B, Dupas JL, Notteghem B, Mary JY. A controlled trial comparing ciprofloxacin with mesalazine for the treatment of active Crohn's disease. Groupe d'Etudes Thérapeutiques des Affections Inflammatoires Digestives (GETAID). *Am J Gastroenterol* 1999; **94**: 674-678 [PMID: 10086650 DOI: 10.1111/j.1572-0241.1999.935_q.x]

83 **Ursing B**, Alm T, Bárány F, Bergelin I, Ganrot-Norlin K, Hoevels J, Huitfeldt B, Järnerot G, Krause U, Krook A, Lindström B, Nordle O, Rosén A. A comparative study of metronidazole and sulfasalazine for active Crohn's disease: the cooperative Crohn's disease study in Sweden. II. Result. *Gastroenterology* 1982; **83**: 550-562 [PMID: 6124474]

84 **Borgaonkar MR**, MacIntosh DG, Fardy JM. A meta-analysis of antimycobacterial therapy for Crohn's disease. *Am J Gastroenterol* 2000; **95**: 725-729 [PMID: 10710065 DOI: 10.1111/j.1572-0241.2000.01842.x]

85 **Sahasranaman S**, Howard D, Roy S. Clinical pharmacology and pharmacogenetics of thiopurines. *Eur J Clin Pharmacol* 2008; **64**: 753-767 [PMID: 18506437 DOI: 10.1007/s00228-008-0478-6]

86 **Konidari A**, Matary WE. Use of thiopurines in inflammatory bowel disease: Safety issues. *World J Gastrointest Pharmacol Ther* 2014; **5**: 63-76 [PMID: 24868487 DOI: 10.4292/wjgpt.v5.i2.63]

87 **Maltzman JS**, Koretzky GA. Azathioprine: old drug, new actions. *J Clin Invest* 2003; **111**: 1122-1124 [PMID: 12697731 DOI: 10.1172/JCI18384]

88 **Mazor Y**, Koifman E, Elkin H, Chowers Y, Krivoy N, Karban A,

Efrati E. Risk factors for serious adverse effects of thiopurines in patients with Crohn's disease. *Curr Drug Saf* 2013; **8**: 181-185 [PMID: 23845145]

89 **Biancone L**, Tosti C, Fina D, Fantini M, De Nigris F, Geremia A, Pallone F. Review article: maintenance treatment of Crohn's disease. *Aliment Pharmacol Ther* 2003; **17** Suppl 2: 31-37 [PMID: 12786610]

90 **Sandborn WJ**. Azathioprine: state of the art in inflammatory bowel disease. *Scand J Gastroenterol Suppl* 1998; **225**: 92-99 [PMID: 9515759]

91 **Kandiel A**, Fraser AG, Korelitz BI, Brensinger C, Lewis JD. Increased risk of lymphoma among inflammatory bowel disease patients treated with azathioprine and 6-mercaptopurine. *Gut* 2005; **54**: 1121-1125 [PMID: 16009685 DOI: 10.1136/gut.2004.049460]

92 **Neurath M**. Thiopurines in IBD: What Is Their Mechanism of Action? *Gastroenterol Hepatol* (N Y) 2010; **6**: 435-436 [PMID: 20827366]

93 **Cosnes J**, Bourrier A, Laharie D, Nahon S, Bouhnik Y, Carbonnel F, Allez M, Dupas JL, Reimund JM, Savoye G, Jouet P, Moreau J, Mary JY, Colombel JF. Early administration of azathioprine vs conventional management of Crohn's Disease: a randomized controlled trial. *Gastroenterology* 2013; **145**: 758-765.e2; quiz e14-15 [PMID: 23644079 DOI: 10.1053/j.gastro.2013.04.048]

94 **Cosnes J**, Seksik P. Early azathioprine in Crohn's disease. *Inflamm Bowel Dis* 2013; **19**: 674-675 [PMID: 23429451 DOI: 10.1097/MIB.0b013e318281d697]

95 **Derijks LJ**, Gilissen LP, de Boer NK, Mulder CJ. 6-Thioguanine-related hepatotoxicity in patients with inflammatory bowel disease: dose or level dependent? *J Hepatol* 2006; **44**: 821-822 [PMID: 16487623 DOI: 10.1016/j.jhep.2005.11.049]

96 **Rossi S**. Australian medicines handbook 2013. Australian: Australian Medicines Handbook Pty Ltd, 2013

97 **Swaminath A**, Taunk R, Lawlor G. Use of methotrexate in inflammatory bowel disease in 2014: A User's Guide. *World J Gastrointest Pharmacol Ther* 2014; **5**: 113-121 [PMID: 25133040 DOI: 10.4292/wjgpt.v5.i3.113]

98 **Ye BD**, Yang SK, Shin SJ, Lee KM, Jang BI, Cheon JH, Choi CH, Kim YH, Lee H. Guidelines for the management of Crohn's disease. *Korean J Gastroenterol* 2012; **59**: 141-179 [PMID: 22387837]

99 **Schoepfer AM**, Beglinger C, Straumann A, Trummler M, Renzulli P, Seibold F. Ulcerative colitis: correlation of the Rachmilewitz endoscopic activity index with fecal calprotectin, clinical activity, C-reactive protein, and blood leukocytes. *Inflamm Bowel Dis* 2009; **15**: 1851-1858 [PMID: 19462421 DOI: 10.1002/ibd.20986]

100 **Meier J**, Sturm A. Current treatment of ulcerative colitis. *World J Gastroenterol* 2011; **17**: 3204-3212 [PMID: 21912469 DOI: 10.3748/wjg.v17.i27.3204]

101 **Matsumoto T**, Kudo T, Jo Y, Esaki M, Yao T, Iida M. Magnifying colonoscopy with narrow band imaging system for the diagnosis of dysplasia in ulcerative colitis: a pilot study. *Gastrointest Endosc* 2007; **66**: 957-965 [PMID: 17826773 DOI: 10.1016/j.gie.2007.04.014]

102 **Cottone M**, Renna S, Modesto I, Orlando A. Is 5-ASA still the treatment of choice for ulcerative colitis? *Curr Drug Targets* 2011; **12**: 1396-1405 [PMID: 21466493]

103 **Ford AC**, Khan KJ, Achkar JP, Moayyedi P. Efficacy of oral vs. topical, or combined oral and topical 5-aminosalicylates, in Ulcerative Colitis: systematic review and meta-analysis. *Am J Gastroenterol* 2012; **107**: 167-176; author reply 177 [PMID: 22108446 DOI: 10.1038/ajg.2011.410]

104 **Kamm MA**, Sandborn WJ, Gassull M, Schreiber S, Jackowski L, Butler T, Lyne A, Stephenson D, Palmen M, Joseph RE. Once-daily, high-concentration MMX mesalamine in active ulcerative colitis. *Gastroenterology* 2007; **132**: 66-75; quiz 432-433 [PMID: 17241860 DOI: 10.1053/j.gastro.2006.10.011]

105 **Sutherland L**, Macdonald JK. Oral 5-aminosalicylic acid for induction of remission in ulcerative colitis. *Cochrane Database Syst Rev* 2006; **(2)**: CD000543 [PMID: 16625536 DOI: 10.1002/14651858.CD000543.pub2]

106 **Marteau P**, Probert CS, Lindgren S, Gassul M, Tan TG, Dignass A, Befrits R, Midhagen G, Rademaker J, Foldager M. Combined oral and enema treatment with Pentasa (mesalazine) is superior to oral therapy alone in patients with extensive mild/moderate active ulcerative colitis: a randomised, double blind, placebo controlled study. *Gut* 2005; **54**: 960-965 [PMID: 15951542 DOI: 10.1136/gut.2004.060103]

107 **Probert CS**, Dignass AU, Lindgren S, Oudkerk Pool M, Marteau P. Combined oral and rectal mesalazine for the treatment of mild-to-moderately active ulcerative colitis: rapid symptom resolution and improvements in quality of life. *J Crohns Colitis* 2014; **8**: 200-207 [PMID: 24012063 DOI: 10.1016/j.crohns.2013.08.007]

108 **Frieri G**, Pimpo M, Galletti B, Palumbo G, Corrao G, Latella G, Chiaramonte M, Caprilli R. Long-term oral plus topical mesalazine in frequently relapsing ulcerative colitis. *Dig Liver Dis* 2005; **37**: 92-96 [PMID: 15733520 DOI: 10.1016/j.dld.2004.09.017]

109 **Probert C**. Steroids and 5-aminosalicylic acids in moderate ulcerative colitis: addressing the dilemma. *Therap Adv Gastroenterol* 2013; **6**: 33-38 [PMID: 23320048 DOI: 10.1177/1756283X12461395]

110 **Truelove SC**, Witts LJ. Cortisone in ulcerative colitis; final report on a therapeutic trial. *Br Med J* 1955; **2**: 1041-1048 [PMID: 13260656]

111 **Dignass A**, Lindsay JO, Sturm A, Windsor A, Colombel JF, Allez M, D'Haens G, D'Hoore A, Mantzaris G, Novacek G, Oresland T, Reinisch W, Sans M, Stange E, Vermeire S, Travis S, Van Assche G. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 2: current management. *J Crohns Colitis* 2012; **6**: 991-1030 [PMID: 23040451 DOI: 10.1016/j.crohns.2012.09.002]

112 **Regueiro M**, Loftus EV, Steinhart AH, Cohen RD. Medical management of left-sided ulcerative colitis and ulcerative proctitis: critical evaluation of therapeutic trials. *Inflamm Bowel Dis* 2006; **12**: 979-994 [PMID: 17012969 DOI: 10.1097/01.mib.0000231495.92013.5e]

113 **Regueiro M**, Loftus EV, Steinhart AH, Cohen RD. Clinical guidelines for the medical management of left-sided ulcerative colitis and ulcerative proctitis: summary statement. *Inflamm Bowel Dis* 2006; **12**: 972-978 [PMID: 17012968 DOI: 10.1097/01.mib.0000231496.92013.85]

114 **Garud S**, Peppercorn MA. Ulcerative colitis: current treatment strategies and future prospects. *Therap Adv Gastroenterol* 2009; **2**: 99-108 [PMID: 21180538 DOI: 10.1177/1756283X09102329]

115 **Kornbluth A**, Sachar DB. Ulcerative colitis practice guidelines in adults: American College Of Gastroenterology, Practice Parameters Committee. *Am J Gastroenterol* 2010; **105**: 501-23; quiz 524 [PMID: 20068560 DOI: 10.1038/ajg.2009.727]

116 **Ghosh S**, Chaudhary R, Carpani M, Playford RJ. Is thiopurine therapy in ulcerative colitis as effective as in Crohn's disease? *Gut* 2006; **55**: 6-8 [PMID: 16344570 DOI: 10.1136/gut.2005.074401]

117 **Gisbert JP**, Linares PM, McNicholl AG, Maté J, Gomollón F. Meta-analysis: the efficacy of azathioprine and mercaptopurine in ulcerative colitis. *Aliment Pharmacol Ther* 2009; **30**: 126-137 [PMID: 19392869 DOI: 10.1111/j.1365-2036.2009.04023.x]

118 **Gisbert JP**, Luna M, Maté J, González-Guijarro L, Cara C, Pajares JM. Choice of azathioprine or 6-mercaptopurine dose based on thiopurine methyltransferase (TPMT) activity to avoid myelosuppression. A prospective study. *Hepatogastroenterology* 2006; **53**: 399-404 [PMID: 16795981]

119 **Arts J**, D'Haens G, Zeegers M, Van Assche G, Hiele M, D'Hoore A, Penninckx F, Vermeire S, Rutgeerts P. Long-term outcome of treatment with intravenous cyclosporin in patients with severe ulcerative colitis. *Inflamm Bowel Dis* 2004; **10**: 73-78 [PMID: 15168804]

120 **Cheifetz AS**, Stern J, Garud S, Goldstein E, Malter L, Moss AC, Present DH. Cyclosporine is safe and effective in patients with severe ulcerative colitis. *J Clin Gastroenterol* 2011; **45**: 107-112 [PMID: 20679905 DOI: 10.1097/MCG.0b013e3181e883dd]

121 **Rutgeerts P**, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J, Travers S, Rachmilewitz D, Hanauer SB, Lichtenstein GR, de Villiers WJ, Present D, Sands BE, Colombel JF. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2005; **353**: 2462-2476 [PMID: 16339095 DOI: 10.1056/NEJMoa050516]

122 **Sandborn WJ**, Van Asche GA, Reinisch W, Colombel JF, D'Haens GR, Wolf DC, Kron M, Tighe MB, Lazar A, Thakkar R. Induction and maintenance of clinical remission by adalimumab in patients with moderate-to-severe ulcerative colitis. *Gastroenterology* 2011; **140** Suppl 1: S123-S124 [DOI: 10.1016/S0016-5085(11)60502-7]

123 **Gies N**, Kroeker KI, Wong K, Fedorak RN. Treatment of ulcerative colitis with adalimumab or infliximab: long-term follow-up of a single-centre cohort. *Aliment Pharmacol Ther* 2010; **32**: 522-528 [PMID: 20500733 DOI: 10.1111/j.1365-2036.2010.04380.x]

124 **Panaccione R**, Loftus EV, Binion D, McHugh K, Alam S, Chen N, Guerette B, Mulani P, Chao J. Efficacy and safety of adalimumab in Canadian patients with moderate to severe Crohn's disease: results of the Adalimumab in Canadian SubjeCts with ModErate to Severe Crohn's DiseaSe (ACCESS) trial. *Can J Gastroenterol* 2011; **25**: 419-425 [PMID: 21912766]

125 **Sokol H**, Seksik P, Carrat F, Nion-Larmurier I, Vienne A, Beaugerie L, Cosnes J. Usefulness of co-treatment with immunomodulators in patients with inflammatory bowel disease treated with scheduled infliximab maintenance therapy. *Gut* 2010; **59**: 1363-1368 [PMID: 20587545 DOI: 10.1136/gut.2010.212712]

126 **Marchioni Beery R**, Kane S. Current approaches to the management of new-onset ulcerative colitis. *Clin Exp Gastroenterol* 2014; **7**: 111-132 [PMID: 24872716 DOI: 10.2147/CEG.S35942]

127 **Chapman JR**, Larson DW, Wolff BG, Dozois EJ, Cima RR, Pemberton JH, Crownhart BS, Larson DR. Ileal pouch-anal anastomosis: does age at the time of surgery affect outcome? *Arch Surg* 2005; **140**: 534-539; discussion 539-540 [PMID: 15967900 DOI: 10.1001/archsurg.140.6.534]

128 **Pinto RA**, Canedo J, Murad-Regadas S, Regadas SF, Weiss EG, Wexner SD. Ileal pouch-anal anastomosis in elderly patients: is there a difference in morbidity compared with younger patients? *Colorectal Dis* 2011; **13**: 177-183 [PMID: 19878516 DOI: 10.1111/j.1463-1318.2009.02097.x]

129 **Solberg IC**, Lygren I, Jahnsen J, Aadland E, Høie O, Cvancarova M, Bernklev T, Henriksen M, Sauar J, Vatn MH, Moum B. Clinical course during the first 10 years of ulcerative colitis: results from a population-based inception cohort (IBSEN Study). *Scand J Gastroenterol* 2009; **44**: 431-440 [PMID: 19101844 DOI: 10.1080/00365520802600961]

130 **Sanjay Bandyopadhyay K**. Ulcerative Colitis: Current management strategy. *Medicine Update* 2010; **20**: 497-509

131 **Bernstein CN**, Ng SC, Lakatos PL, Moum B, Loftus EV. A review of mortality and surgery in ulcerative colitis: milestones of the seriousness of the disease. *Inflamm Bowel Dis* 2013; **19**: 2001-2010 [PMID: 23624887 DOI: 10.1097/MIB.0b013e318281f3bb]

132 **Hueting WE**, Buskens E, van der Tweel I, Gooszen HG, van Laarhoven CJ. Results and complications after ileal pouch anal anastomosis: a meta-analysis of 43 observational studies comprising 9,317 patients. *Dig Surg* 2005; **22**: 69-79 [PMID: 15838175 DOI: 10.1159/000085356]

133 **Loftus EV**, Delgado DJ, Friedman HS, Sandborn WJ. Colectomy and the incidence of postsurgical complications among ulcerative colitis patients with private health insurance in the United States. *Am J Gastroenterol* 2008; **103**: 1737-1745 [PMID: 18564126 DOI: 10.1111/j.1572-0241.2008.01867.x]

134 **Waljee A**, Waljee J, Morris AM, Higgins PD. Threefold increased risk of infertility: a meta-analysis of infertility after ileal pouch anal anastomosis in ulcerative colitis. *Gut* 2006; **55**: 1575-1580 [PMID: 16772310 DOI: 10.1136/gut.2005.090316]

135 **Oren R**, Arber N, Odes S, Moshkowitz M, Keter D, Pomeranz I, Ron Y, Reisfeld I, Broide E, Lavy A, Fich A, Eliakim R, Patz J, Bardan E, Villa Y, Gilat T. Methotrexate in chronic active ulcerative colitis: a double-blind, randomized, Israeli multicenter trial. *Gastroenterology* 1996; **110**: 1416-1421 [PMID: 8613046]

136 **Paoluzi OA**, Pica R, Marcheggiano A, Crispino P, Iacopini F, Iannoni C, Rivera M, Paoluzi P. Azathioprine or methotrexate in the treatment of patients with steroid-dependent or steroid-resistant ulcerative colitis: results of an open-label study on efficacy and tolerability in inducing and maintaining remission. *Aliment Pharmacol Ther* 2002; **16**: 1751-1759 [PMID: 12269968]

137 **Khan N**, Abbas AM, Moehlen M, Balart L. Methotrexate in ulcerative colitis: a nationwide retrospective cohort from the Veterans Affairs Health Care System. *Inflamm Bowel Dis* 2013; **19**: 1379-1383 [PMID: 23542534 DOI: 10.1097/MIB.0b013e31828133e8]

138 **Sands BE**, Sandborn WJ, Feagan B, Löfberg R, Hibi T, Wang T, Gustofson LM, Wong CJ, Vandervoort MK, Hanauer S. A randomized, double-blind, sham-controlled study of granulocyte/monocyte apheresis for active ulcerative colitis. *Gastroenterology* 2008; **135**: 400-409 [PMID: 18602921]

139 **Saniabadi AR**, Hanai H, Suzuki Y, Ohmori T, Sawada K, Yoshimura N, Saito Y, Takeda Y, Umemura K, Kondo K, Ikeda Y, Fukunaga K, Nakashima M, Beretta A, Bjarnason I, Lofberg R. Adacolumn for selective leukocytapheresis as a non-pharmacological treatment for patients with disorders of the immune system: an adjunct or an alternative to drug therapy? *J Clin Apher* 2005; **20**: 171-184 [PMID: 15892107 DOI: 10.1002/jca.20046]

140 **Shibata H**, Kuriyama T, Yamawaki N. Cellsorba. *Ther Apher Dial* 2003; **7**: 44-47 [PMID: 12921114]

141 **Kashiwagi N**, Sugimura K, Koiwai H, Yamamoto H, Yoshikawa T, Saniabadi AR, Adachi M, Shimoyama T. Immunomodulatory effects of granulocyte and monocyte adsorption apheresis as a treatment for patients with ulcerative colitis. *Dig Dis Sci* 2002; **47**: 1334-1341 [PMID: 12064810]

142 **Grisham MB**, Yamada T. Neutrophils, nitrogen oxides, and inflammatory bowel disease. *Ann N Y Acad Sci* 1992; **664**: 103-115 [PMID: 1456643]

143 **Grisham MB**, Granger N. Mechanisms of neutrophil-mediated tissue injury. In: MacDermott RP, Stenson WF, editors. Inflammatory Bowel Disease. New York, Elsevier, 1992: 225-239

144 **Sandborn WJ**. Preliminary data on the use of apheresis in inflammatory bowel disease. *Inflamm Bowel Dis* 2006; **12** Suppl 1: S15-S21 [PMID: 16378006]

145 **Hanai H**, Takeda Y, Eberhardson M, Gruber R, Saniabadi AR, Winqvist O, Lofberg R. The mode of actions of the Adacolumn therapeutic leucocytapheresis in patients with inflammatory bowel disease: a concise review. *Clin Exp Immunol* 2011; **163**: 50-58 [PMID: 21078086 DOI: 10.1111/j.1365-2249.2010.04279.x]

146 **Kashiwagi N**, Nakano M, Saniabadi AR, Adachi M, Yoshikawa T. Anti-inflammatory effect of granulocyte and monocyte adsorption apheresis in a rabbit model of immune arthritis. *Inflammation* 2002; **26**: 199-205 [PMID: 12184634]

147 **Kuijpers TW**, Tool AT, van der Schoot CE, Ginsel LA, Onderwater JJ, Roos D, Verhoeven AJ. Membrane surface antigen expression on neutrophils: a reappraisal of the use of surface markers for neutrophil activation. *Blood* 1991; **78**: 1105-1111 [PMID: 1907873]

148 **Waitz G**, Petermann S, Liebe S, Emmrich J, Ramlow W. Reduction of dendritic cells by granulocyte and monocyte adsorption apheresis in patients with ulcerative colitis. *Dig Dis Sci* 2008; **53**: 2507-2515 [PMID: 18253828 DOI: 10.1007/s10620-007-0168-8]

149 **Cuadrado E**. Granulocyte/monocyte apheresis as immunotherapic tool: cellular adsorption and immune modulation. *Autoimmun Rev* 2009; **8**: 292-296 [PMID: 18804557]

150 **Cuadrado E**, Alonso M, de Juan MD, Echaniz P, Arenas JI. Regulatory T cells in patients with inflammatory bowel diseases treated with adacolumn granulocytapheresis. *World J Gastroenterol* 2008; **14**: 1521-1527 [PMID: 18330941]

151 **Toya Y**, Chiba T, Mizutani T, Sato K, Kasugai S, Matsuda N, Orikasa S, Shibata S, Abiko Y, Akasaka R, Yokoyama N, Oana S, Hirota S, Endo M, Suzuki K. The effect of granulocyte and monocyte adsorptive apheresis on serum cytokine levels in patients with ulcerative colitis. *Cytokine* 2013; **62**: 146-150 [PMID: 23465691 DOI: 10.1016/j.cyto.2013.01.019]

152 **Hanai H**. Positions of selective leukocytapheresis in the medical therapy of ulcerative colitis. *World J Gastroenterol* 2006; **12**: 7568-7577 [PMID: 17171783]

153 **Yamamoto T**, Umegae S, Matsumoto K. Safety and clinical efficacy of granulocyte and monocyte adsorptive apheresis therapy for ulcerative colitis. *World J Gastroenterol* 2006; **12**: 520-525 [PMID: 16489663]

154 **Yokoyama Y**, Kawai M, Fukunaga K, Kamikozuru K, Nagase K,

Nogami K, Kono T, Ohda Y, Iimuro M, Hida N, Nakamura S, Miwa H, Matsumoto T. Looking for predictive factors of clinical response to adsorptive granulocyte and monocyte apheresis in patients with ulcerative colitis: markers of response to GMA. *BMC Gastroenterol* 2013; **13**: 27 [PMID: 23399416 DOI: 10.1186/1471-230X-13-27]

155 **Sacco R**, Tanaka T, Yamamoto T, Bresci G, Saniabadi AR. Adacolumn leucocytapheresis for ulcerative colitis: clinical and endoscopic features of responders and unresponders. *Expert Rev Gastroenterol Hepatol* 2015; **9**: 327-333 [PMID: 25160857 DOI: 10.1586/17474124.2014.953060]

156 **Fukuchi T**, Nakase H, Ubukata S, Matsuura M, Yoshino T, Toyonaga T, Shimazu K, Koga H, Yamashita H, Ito D, Ashida K. Therapeutic effect of intensive granulocyte and monocyte adsorption apheresis combined with thiopurines for steroid- and biologics-naïve Japanese patients with early-diagnosed Crohn's disease. *BMC Gastroenterol* 2013; **13**: 124 [PMID: 25015328 DOI: 10.1186/1471-230X-14-124]

157 **Yoshino T**, Nakase H, Minami N, Yamada S, Matsuura M, Yazumi S, Chiba T. Efficacy and safety of granulocyte and monocyte adsorption apheresis for ulcerative colitis: a meta-analysis. *Dig Liver Dis* 2014; **46**: 219-226 [PMID: 24268950 DOI: 10.1016/j.dld.2013.10.011]

158 **Vecchi M**, Vernia P, Riegler G, D'Incà R, Annese V, Bagnoli S. Therapeutic landscape for ulcerative colitis: where is the Adacolumn(®) system and where should it be? *Clin Exp Gastroenterol* 2013; **6**: 1-7 [PMID: 23323022 DOI: 10.2147/CEG.S33275]

159 **Saniabadi AR**, Tanaka T, Ohmori T, Sawada K, Yamamoto T, Hanai H. Treating inflammatory bowel disease by adsorptive leucocytapheresis: a desire to treat without drugs. *World J Gastroenterol* 2014; **20**: 9699-9715 [PMID: 25110409 DOI: 10.3748/wjg.v20.i29.9699]

160 **Dignass AU**, Eriksson A, Kilander A, Pukitis A, Rhodes JM, Vavricka S. Clinical trial: five or ten cycles of granulocyte-monocyte apheresis show equivalent efficacy and safety in ulcerative colitis. *Aliment Pharmacol Ther* 2010; **31**: 1286-1295 [PMID: 20331579]

161 **Tominaga K**, Nakano M, Hoshino M, Kanke K, Hiraishi H. Efficacy, safety and cost analyses in ulcerative colitis patients undergoing granulocyte and monocyte adsorption or receiving prednisolone. *BMC Gastroenterol* 2013; **13**: 41 [PMID: 23452668 DOI: 10.1186/1471-230X-13-41]

162 **Szczepiorkowski ZM**, Winters JL, Bandarenko N, Kim HC, Linenberger ML, Marques MB, Sarode R, Schwartz J, Weinstein R, Shaz BH. Guidelines on the use of therapeutic apheresis in clinical practice--evidence-based approach from the Apheresis Applications Committee of the American Society for Apheresis. *J Clin Apher* 2010; **25**: 83-177 [PMID: 20568098 DOI: 10.1002/jca.20240]

163 **Bicks RO**, Groshart KD. The current status of T-lymphocyte apheresis (TLA) treatment of Crohn's disease. *J Clin Gastroenterol* 1989; **11**: 136-138 [PMID: 2786898]

164 **Matsumoto T**, Fukunaga K, Kamikozuru K, Tozawa K, Yokoyama Y, Kusaka T, Onishi K, Miwa H, Nakamura S. Cytapheresis as a Non-Pharmacological Therapy for Inflammatory Bowel Disease. *Transfus Med Hemother* 2008; **35**: 18-23 [PMID: 21547106 DOI: 10.1159/000111763]

165 **Abreu MT**, von Tirpitz C, Hardi R, Kaatz M, Van Assche G, Rutgeerts P, Bisaccia E, Goerdt S, Hanauer S, Knobler R, Mannon P, Mayer L, Ochsenkuhn T, Sandborn WJ, Parenti D, Lee K, Reinisch W. Extracorporeal photopheresis for the treatment of refractory Crohn's disease: results of an open-label pilot study. *Inflamm Bowel Dis* 2009; **15**: 829-836 [PMID: 19130617 DOI: 10.1002/ibd.20833]

166 **Reinisch W**, Knobler R, Rutgeerts PJ, Ochsenkühn T, Anderson F, von Tirpitz C, Kaatz M, Janneke van der Woude C, Parenti D, Mannon PJ. Extracorporeal photopheresis (ECP) in patients with steroid-dependent Crohn's disease: an open-label, multicenter, prospective trial. *Inflamm Bowel Dis* 2013; **19**: 293-300 [PMID: 22573600 DOI: 10.1002/ibd.23012]

167 **Oliven A**, Shechter Y. Extracorporeal photopheresis: a review. *Blood Rev* 2001; **15**: 103-108 [PMID: 11409910 DOI: 10.1054/blre.2001.0155]

168 **Di Biaso I**, Di Maio L, Bugarin C, Gaipa G, Dander E, Balduzzi A, Parma M, D'Amico G, Perseghin P, Biondi A, Biagi E. Regulatory T cells and extracorporeal photochemotherapy: correlation with clinical response and decreased frequency of proinflammatory T cells. *Transplantation* 2009; **87**: 1422-1425 [PMID: 19424046 DOI: 10.1097/TP.0b013e3181a27a5d]

169 **Maeda A**, Schwarz A, Bullinger A, Morita A, Peritt D, Schwarz T. Experimental extracorporeal photopheresis inhibits the sensitization and effector phases of contact hypersensitivity via two mechanisms: generation of IL-10 and induction of regulatory T cells. *J Immunol* 2008; **181**: 5956-5962 [PMID: 18941184]

170 **Worel N**, Leitner G. Clinical Results of Extracorporeal Photopheresis. *Transfus Med Hemother* 2012; **39**: 254-262 [PMID: 22969695]

171 **Mosser DM**, Zhang X. Interleukin-10: new perspectives on an old cytokine. *Immunol Rev* 2008; **226**: 205-218 [PMID: 19161426 DOI: 10.1111/j.1600-065X.2008.00706.x]

172 **Simon R**, Mikocka-Walus AA. Psychological Aspects of Inflammatory Bowel Disease: A biopsychosocial approac. London; New York: Routledge, 2014

173 **Ghosh S**, Pariente B, Mould DR, Schreiber S, Petersson J, Hommes D. New tools and approaches for improved management of inflammatory bowel diseases. *J Crohns Colitis* 2014; **8**: 1246-1253 [PMID: 24742735 DOI: 10.1016/j.crohns.2014.02.026]

174 **Pariente B**, Cosnes J, Danese S, Sandborn WJ, Lewin M, Fletcher JG, Chowers Y, D'Haens G, Feagan BG, Hibi T, Hommes DW, Irvine EJ, Kamm MA, Loftus EV, Louis E, Michetti P, Munkholm P, Oresland T, Panés J, Peyrin-Biroulet L, Reinisch W, Sands BE, Schoelmerich J, Schreiber S, Tilg H, Travis S, van Assche G, Vecchi M, Mary JY, Colombel JF, Lémann M. Development of the Crohn's disease digestive damage score, the Lémann score. *Inflamm Bowel Dis* 2011; **17**: 1415-1422 [PMID: 21560202 DOI: 10.1002/ibd.21506]

175 **D'Haens GR**, Sartor RB, Silverberg MS, Petersson J, Rutgeerts P. Future directions in inflammatory bowel disease management. *J Crohns Colitis* 2014; **8**: 726-734 [PMID: 24742736 DOI: 10.1016/j.crohns.2014.02.025]

176 **Bendtzen K**, Ainsworth M, Steenholdt C, Thomsen OØ, Brynskov J. Individual medicine in inflammatory bowel disease: monitoring bioavailability, pharmacokinetics and immunogenicity of anti-tumour necrosis factor-alpha antibodies. *Scand J Gastroenterol* 2009; **44**: 774-781 [PMID: 19140087 DOI: 10.1080/00365520802699278]

177 **McLean LP**, Shea-Donohue T, Cross RK. Vedolizumab for the treatment of ulcerative colitis and Crohn's disease. *Immunotherapy* 2012; **4**: 883-898 [PMID: 23046232 DOI: 10.2217/imt.12.85]

178 **Krupka N**, Baumgart DC. Designing biologic selectivity for inflammatory bowel disease--role of vedolizumab. *Drug Des Devel Ther* 2015; **9**: 147-154 [PMID: 25552903 DOI: 10.2147/DDDT.S50348]

179 **Anderson JL**, Edney RJ, Whelan K. Systematic review: faecal microbiota transplantation in the management of inflammatory bowel disease. *Aliment Pharmacol Ther* 2012; **36**: 503-516 [PMID: 22827693 DOI: 10.1111/j.1365-2036.2012.05220.x]

180 **Hügle T**, Daikeler T. Stem cell transplantation for autoimmune diseases. *Haematologica* 2010; **95**: 185-188 [PMID: 20139390 DOI: 10.3324/haematol.2009.017038]

181 **Sales-Campos H**, Basso PJ, Alves VB, Fonseca MT, Bonfá G, Nardini V, Cardoso CR. Classical and recent advances in the treatment of inflammatory bowel diseases. *Braz J Med Biol Res* 2015; **48**: 96-107 [PMID: 25466162 DOI: 10.1590/1414-431X20143774]

182 **Snowden JA**, Saccardi R, Allez M, Ardizzone S, Arnold R, Cervera R, Denton C, Hawkey C, Labopin M, Mancardi G, Martin R, Moore JJ, Passweg J, Peters C, Rabusin M, Rovira M, van Laar JM, Farge D. Haematopoietic SCT in severe autoimmune diseases: updated guidelines of the European Group for Blood and Marrow Transplantation. *Bone Marrow Transplant* 2012; **47**: 770-790 [PMID: 22002489 DOI: 10.1038/bmt.2011.185]

183 **Burt RK**, Craig RM, Milanetti F, Quigley K, Gozdziak P, Bucha J, Testori A, Halverson A, Verda L, de Villiers WJ, Jovanovic B, Oyama Y. Autologous nonmyeloablative hematopoietic stem cell transplantation in patients with severe anti-TNF refractory Crohn disease: long-term follow-up. *Blood* 2010; **116**: 6123-6132 [PMID:

20837778 DOI: 10.1182/blood-2010-06-292391]

184 **Hasselblatt P**, Drognitz K, Potthoff K, Bertz H, Kruis W, Schmidt C, Stallmach A, Schmitt-Graeff A, Finke J, Kreisel W. Remission of refractory Crohn's disease by high-dose cyclophosphamide and autologous peripheral blood stem cell transplantation. *Aliment Pharmacol Ther* 2012; **36**: 725-735 [PMID: 22937722 DOI: 10.1111/apt.12032]

185 **Cassinotti A**, Keshav S, Ardizzone S, Mortensen N, Sampietro G, Fociani P, Duca P, George B, Lazzaroni M, Manes G, Warren B, Foschi D, Vago G, Porro GB, Travis S. IBD care in Europe: A comparative audit of the inpatient management of Crohn's disease and ulcerative colitis using the national UK IBD audit tool. *J Crohns Colitis* 2009; **3**: 291-301 [PMID: 21172290 DOI: 10.1016/j.crohns.2009.08.002]

186 **Colletti RB**, Baldassano RN, Milov DE, Margolis PA, Bousvaros A, Crandall WV, Crissinger KD, D'Amico MA, Day AS, Denson LA, Dubinsky M, Ebach DR, Hoffenberg EJ, Kader HA, Keljo DJ, Leibowitz IH, Mamula P, Pfefferkorn MD, Qureshi MA. Variation in care in pediatric Crohn disease. *J Pediatr Gastroenterol Nutr* 2009; **49**: 297-303 [PMID: 19590456 DOI: 10.1097/MPG.0b013e3181919695]

187 **Panaccione R**, Ghosh S. Optimal use of biologics in the management of Crohn's disease. *Therap Adv Gastroenterol* 2010; **3**: 179-189 [PMID: 21180600 DOI: 10.1177/1756283X09357579]

188 **Lin MV**, Blonski W, Lichtenstein GR. What is the optimal therapy for Crohn's disease: step-up or top-down? *Expert Rev Gastroenterol Hepatol* 2010; **4**: 167-180 [PMID: 20350264 DOI: 10.1586/egh.10.4]

189 **Rogler G**. Top-down or step-up treatment in Crohn's disease? *Dig Dis* 2013; **31**: 83-90 [PMID: 23797128 DOI: 10.1159/000347190]

190 **D'Haens GR**. Top-down therapy for IBD: rationale and requisite evidence. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 86-92 [PMID: 20134490 DOI: 10.1038/nrgastro.2009.222]

191 **Chen QQ**, Yan L, Wan J. Select a suitable treatment strategy for Crohn's disease: step-up or top-down. *EXCLI J* 2014; **13**: 111-122 [PMID: 26417246]

192 **Sandborn WJ**. Step-up versus top-down therapy in the treatment of ulcerative colitis. *Gastroenterol Hepatol* (N Y) 2007; **3**: 16-17 [PMID: 21960772]

193 **Jeppesen PB**, Pertkiewicz M, Messing B, Iyer K, Seidner DL, O'keefe SJ, Forbes A, Heinze H, Joelsson B. Teduglutide reduces need for parenteral support among patients with short bowel syndrome with intestinal failure. *Gastroenterology* 2012; **143**: 1473-1481.e3 [PMID: 22982184 DOI: 10.1053/j.gastro.2012.09.007]

194 **Fuchssteiner H**, Nigl K, Mayer A, Kristensen B, Platzer R, Brunner B, Weiß I, Haas T, Benedikt M, Gröchenig HP, Eisenberger A, Hillebrand P, Reinisch W, Vogelsang H. [Nutrition and IBD-Consensus of the Austrian Working Group of IBD (Inflammatory Bowel Diseases) of the ÖGGH]. *Z Gastroenterol* 2014; **52**: 376-386 [PMID: 24718944 DOI: 10.1055/s-0034-1366252]

P- Reviewer: Tomizawa M **S- Editor**: Ji FF **L- Editor**: A
E- Editor: Wang CH

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i1.21

World J Gastrointest Pharmacol Ther 2016 February 6; 7(1): 21-32
ISSN 2150-5349 (online)

REVIEW

Recent discoveries and emerging therapeutics in eosinophilic esophagitis

Aakash Goyal, Edaire Cheng

Aakash Goyal, Department of Pediatrics, Division of Gastroenterology, Children's Health Children's Medical Center and University of Texas Southwestern Medical Center, Dallas, TX 75390-9063, United States

Edaire Cheng, Department of Pediatrics and Internal Medicine, Division of Gastroenterology, Children's Health Children's Medical Center and University of Texas Southwestern Medical Center, Dallas, TX 75390-9063, United States

Author contributions: All authors equally contributed to this paper with conception and drafting; Cheng E was responsible for critical revision, editing, and final approval of the final version.

Supported by The National Institutes of Health (K08-DK099383 to Cheng E); NASPGHAN Foundation/AstraZeneca Award (Cheng E); and AGA Research Scholar Award (Cheng E).

Conflict-of-interest statement: No conflicts of interest exist for any of the authors.

Correspondence to: Edaire Cheng, MD, Assistant Professor, Department of Pediatrics and Internal Medicine, Division of Gastroenterology, Children's Health Children's Medical Center and University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390-9063, United States. edaire.cheng@utsouthwestern.edu
Telephone: +1-214-8570404
Fax: +1-214-6482096

Received: August 8, 2015
Peer-review started: August 11, 2015
First decision: September 18, 2015
Revised: October 13, 2015
Accepted: December 9, 2015

Article in press: December 11, 2015
Published online: February 6, 2016

Abstract

Eosinophilic esophagitis (EoE) is an allergy-mediated disease culminating in severe eosinophilic inflammation and dysfunction of the esophagus. This chronic disorder of the esophagus causes significant morbidity, poor quality of life, and complications involving fibrosis and esophageal remodeling. Overlapping features between EoE and gastroesophageal reflux disease (GERD) pose great challenges to differentiating the two conditions, although the two disorders are not mutually exclusive. Recent findings suggest that the confounding condition proton pump inhibitor - responsive esophageal eosinophilia (PPI-REE) is likely a subset of EoE. Since PPIs have therapeutic properties that can benefit EoE, PPIs should be considered as a therapeutic option for EoE rather than a diagnostic screen to differentiate GERD, PPI-REE, and EoE. Other current treatments include dietary therapy, corticosteroids, and dilation. Immunomodulators and biologic agents might have therapeutic value, and larger trials are needed to assess efficacy and safety. Understanding the pathophysiology of EoE is critical to the development of novel therapeutics.

Key words: Eosinophilic esophagitis; Interleukin-5; Proton pump inhibitors; Proton pump inhibitor-responsive esophageal eosinophilia; Gastroesophageal reflux disease; Eotaxin-3

Core tip: In this review, we will discuss recent challenges and discoveries in eosinophilic esophagitis (EoE). While current treatment options are limited, mainly dietary therapy and steroids, we will highlight emerging therapeutics targeting pathogenic mechanisms of the

disease. Although EoE is an allergy-mediated disease, the overlapping features of EoE and gastroesophageal reflux disease (GERD) present a diagnostic quandary in distinguishing the two disorders. EoE and GERD are not mutually exclusive and might share a complex relationship. We will review how proton pump inhibitor (PPI)s might exert therapeutic effects in EoE, and why a PPI response does not provide clear diagnostic distinction between EoE and GERD.

Goyal A, Cheng E. Recent discoveries and emerging therapeutics in eosinophilic esophagitis. *World J Gastrointest Pharmacol Ther* 2016; 7(1): 21-32 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i1/21.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i1.21

INTRODUCTION

Eosinophilic esophagitis (EoE) is allergy-mediated clinicopathologic entity in which eosinophils infiltrate into the esophagus resulting in esophageal inflammation, fibrosis and dysfunction[1]. EoE commonly causes symptoms of heartburn and dysphagia, and if left untreated, will likely progress to esophageal remodeling and stricture formation[2,3]. EoE was first recognized almost 20 years ago as a distinct entity[4,5]. Since several clinical and histological features of gastroesophageal reflux disease (GERD) and EoE overlap, patients with esophageal eosinophilia consistent with EoE were often diagnosed as GERD prior to recognizing EoE as a distinct entity[6]. EoE is relatively a newly recognized disease. Much of EoE pathophysiology is unclear, and several research efforts are dedicated to elucidating the relationships between host immune system, environmental factors, and genetic factors. Understanding and identifying pathogenic targets may lead to therapeutic advances. Currently, only off-label use of drugs and therapies are available for the treatment and management of EoE. Each therapy has benefits and challenges. Several therapies, including immunomodulators and biologic agents, will need further studies to determine safety and efficacy. In this review, we will discuss challenges in EoE diagnosis, new discoveries in pathophysiology, and emerging therapeutics.

EOE CLINICAL FEATURES AND DIAGNOSIS

EoE is a clinicopathologic disorder that requires both clinical and/or endoscopic features of esophageal dysfunction and histologic features of esophageal eosinophilia. EoE in children generally causes symptoms of nausea, emesis, abdominal pain, and failure to thrive. Adolescents and adults are more likely to present with dysphagia, food impaction, heartburn, and strictures[1]. The esophageal tissue remodeling rising from unabated inflammation has profound impact on disease activity such as dysphagia and stricture formation[7,8].

The endoscopic findings include white mucosal plaques, linear furrowing, esophageal trachealization (concentric rings), esophageal narrowing, stricture and mucosal tearing[3]. However, the endoscopic features alone are not sufficient to confirm diagnosis of EoE[9]. It is estimated that 10% of adults with EoE have normal endoscopy results[10]. The EoE endoscopic reference score EREFS is a new classification system recently developed to describe endoscopic findings and disease severity in patients with EoE[11]. The EREFS scores endoscopic features of EoE (exudates, rings, edema, furrows, and strictures) providing a validated outcome measure that will be critical in upcoming clinical trials.

The current consensus guidelines for diagnosis of EoE recommend $\geqslant$ 15 eosinophils per high-power field on at least one esophageal biopsy specimen, without increase in eosinophils in stomach and duodenum[1]. Biopsy specimens should be obtained from both the proximal and distal aspects of the esophagus during diagnostic and surveillance endoscopy[1]. Proper tissue sampling from the esophagus is a current challenge. EoE is a transmural disease involving all layers of the esophagus, however mucosal pinch biopsies often only attains the superficial epithelial layer. In addition, eosinophilic infiltration can be patchy, so biopsy collected from one site may not be sufficient for diagnosis[12]. Thus, current guidelines suggest taking 2-4 biopsies from the proximal and distal esophagus[13]. However, Nielsen *et al*[14] recently examined biopsy fragments in 102 adult EoE cases and determined that a minimum of 4 biopsy fragments from the mid and/or proximal esophagus submitted in separate containers would optimize diagnostic yield for EoE.

The main diagnostic conundrum is distinguishing EoE from GERD. However, GERD and EoE are not mutually exclusive disorders, and might share a complex relationship[15]. Firstly, GERD can have mild esophageal eosinophilia. Reflux-induced inflammation can involve eosinophil trafficking. Secondly, EoE might predispose to GERD. EoE inflammation and remodeling conceivably can alter esophageal motility, delay acid reflux clearance, and compromise the lower esophageal sphincter. Disrupted barrier function and increased permeability due to EoE inflammation might leave the epithelium hypersensitive to acid reflux injury as described in EoE patients[16]. Alternatively, GERD might perpetuate EoE. Gastric reflux induces mediators in the epithelium that can exacerbate activation of immune cells that promote allergic inflammation[17]. Disrupted barrier function due to acid-related injury might increase mucosal permeability to allergic antigens perpetuating allergic inflammation.

Early experts proposed using a PPI trial to dis-

tinguish GERD from EoE, assuming that PPIs only exert antisecretory, acid-suppressive effects, and therefore only GERD can respond to PPIs[18]. However, this assumption was called into question as reports of patients with PPI-responsive esophageal eosinophilia (PPI-REE) emerged[1,19-22]. PPI-REE patients have clinical, endoscopic, and histological findings consistent with EoE yet achieve clinical and histologic remission after PPI therapy. Prospective studies estimated that 33%-74% of patients with esophageal eosinophilia respond to PPI therapy[19-22]. Furthermore, there are multiple mechanisms whereby EoE patients might benefit from, not only acid-suppressive effects, but also anti-inflammatory effects of PPIs, as discussed later in this review[15]. Lastly, recent genetic transcriptome analysis of PPI-REE patients and EoE patients revealed remarkable molecular signature overlap suggesting that PPI-REE might indeed be a subset of EoE or represent a similar allergy-mediated process that responds to PPI effects[23].

EOE PATHOPHYSIOLOGY OVERVIEW

An understanding of EoE pathophysiology is essential in order to identify therapeutic targets and develop treatment options for the disease. The pathophysiology of EoE seems to involve disturbances in allergen exposures, the epithelial barrier, immune effector cells, and inflammatory cytokines. Current and investigational therapies are directed to these areas. In EoE, allergen (food and/or aeroallergens) permeate the epithelial barrier and initiate a T-helper type 2 (Th2) inflammatory reaction where activated Th2 lymphocytes increase tissue levels of Th2 cytokines, such as interleukin (IL)-5, IL-13, and IL-4[24,25]. These Th2 cytokines are responsible for driving eosinophil recruitment and activation. Eotaxin-3 is a potent eosinophil chemoattractant, highly regulated by Th2 cytokines IL-13 and IL-4, and is a signature gene for EoE[26]. Upon activation, eosinophils can secrete a variety of pro-inflammatory cytokines and chemokines or degranulate, releasing preformed granules containing cationic and cytotoxic proteins that are injurious to the tissue. While EoE involves an eosinophil-predominant inflammation, there is evidence to suggest that other immune cells, such as mast cells, basophils, and invariant natural killer T cells also mediate inflammation[27]. The ongoing chronic inflammation drives fibrogenesis and remodeling in the deeper layers of esophagus[8,28]. Next, we will highlight current and investigational therapies, therapeutic targets, and their relationship to EoE pathophysiology.

TARGETING ALLERGENS

Diet

First and foremost, EoE is an allergy-driven disease. A definitive link between EoE and food allergens was recognized after children with EoE achieved disease remission on an elemental diet and subsequently disease recrudescence following food reintroduction[29]. Therefore, dietary avoidance is, not only logical, but also one of the most effective treatment options for EoE. There are three types of dietary approaches: (1) elemental diets with an amino acid-based liquid formula[30]; (2) directed elimination diets based on allergy test results[31]; and (3) non-directed, empirical elimination diets[32]. A recent meta-analysis revealed that elemental diets, non-directed diets, and allergy test-directed diets had efficacy rates of 91%, 72%, and 46%, respectively in adults with EoE[33].

Strict elemental diets have been very effective in inducing remission in 88% to 96% of children[30,32,34] and 72% of adults with EoE[35]. The advantages of an elemental diet include rapid remission, balanced nutrition, and no dietary contamination. The disadvantages of this approach are poor palatability, poor patient adherence, probable enteral feeding tube placement, and cost-prohibitive elemental formulas[36].

Directed elimination diets are based on allergy test results. The most common allergy tests used are radioallergosorbent (RAST), skin prick tests (SPT) and atopy patch test (APT). Elimination diets directed by allergy testing achieved complete clinical and histological remission in 78% of pediatric subjects and only 26% of adult subjects with EoE[31,37]. The prospect of eliminating only 1-2 foods based on allergy tests is appealing and practical to patients. However, allergy testing can be time-consuming, expensive, and limited by false-positives rates. Currently, atopy patch testing is not standardized.

The empiric elimination diet or six food elimination diet (SFED) excludes the most common allergenic foods (milk, wheat, egg, soy, nuts and fish) and successfully improved histology and alleviated symptoms in 74% of pediatric patients with EoE[32]. The process of reintroducing these foods identified milk as the most likely offending agent, followed by wheat, egg and soy in children[38]. SFED demonstrated 70% efficacy in adult patients with EoE, where wheat and milk were the most common offending foods[39]. SFED achieves better efficacy without needing allergy testing while still allowing a variety of foods in the patient's diet. However, SFED entails stepwise reintroduction of foods with multiple follow-up endoscopies, which makes the cumbersome process unappealing. In addition, the diet imposes risk of nutritional deficiencies, and each re-introduction step poses a risk for disease relapse by re-introducing a potential offending food. Overall, while any dietary therapy can be extremely effective in EoE, dietary guidance such as a registered dietician can safeguard from the pitfalls of patient adherence, contamination, and nutritional deficiencies.

Anti-IgE therapy

There might be a relationship between EoE and IgE-

mediated food allergy. Traditionally, IgE-mediated hypersensitivity requires Th2 cells to signal B cell class switching to generate antigen-specific IgE. Cross-linking of allergen antigen, IgE, and Fc receptors on mast cells or basophils activate the release of inflammatory mediators such as histamine, tryptase, and leukotrienes. In EoE patients, the prevalence of IgE-mediated food allergies is about 15%-43%[1]. Food-specific IgE has been detected by skin prick test with more success in children than adults[37,40]. IgE-bearing mast cells and B cells are detected at elevated levels in the esophageal biopsies of EoE patients[41].

Omalizumab is an anti-IgE monoclonal antibody used to control asthma in severely allergic asthmatic patients. Results of an open labeled study, where the majority of the EoE subjects were adolescents, showed significant reduction in esophageal tissue IgE levels. Fifteen subjects were administered omalizumab for 3 mo. Thirty-three percent of the subjects demonstrated complete clinical and histological remission. The responders had low peripheral blood absolute eosinophil counts, suggesting that perhaps in a subset of EoE patients, IgE might play a role in the pathophysiology[42]. However, EoE might not be entirely dependent on IgE-mediated inflammation, since IgE-deficient mice continued to develop esophageal inflammation[43,44]. Furthermore, in a prospective trial, omalizumab was ineffective in reducing symptoms and esophageal eosinophilia in adults with EoE compared to placebo[45,46]. Thus, strategies to stratify and identify potential candidates for anti-IgE therapy have been proposed and will require larger clinical trials.

TARGETING THE ESOPHAGEAL EPITHELIAL BARRIER

In health, the esophagus has a stratified epithelium forming a barrier from luminal contents including food allergens, aeroallergens, bacteria, and gastric acid refluxate. Disturbances in the epithelial barrier might allow allergens to enter the esophageal epithelium initiating or perpetuating an allergic inflammation. Histological findings of spongiosis or dilated intercellular spaces in active EoE implicate some impairment in epithelial barrier[1]. Mucosal integrity, based on intraluminal impedance measurements, was compromised in EoE patients[47]. Measurements of permeability and transepithelial electrical resistance on esophageal tissue biopsies also indicate barrier disturbances[48]. Expression of cell junction and adhesion proteins (E-cadherin, claudin-1, zonula occludens-3, and desmoglein-1)[48-50] and epithelial differentiation genes (involucrin, small proline-rich protein, and filaggrin) were downregulated[51]. Restoring epithelial barrier function might be an appropriate therapeutic target. Treatment with high-dose esomeprazole improved mucosal integrity in PPI-REE patients[52]. Currently, investigators are examining the effect of sucralfate slurry on dilated intercellular spaces, tight junctions, mucosal impedance and mucosal activity in patient with EoE (NCT02353078 www.clinicaltrials.gov). Sucralfate is a medication originally developed to treat mucosal ulceration due to acid-peptic diseases. While the exact mechanism of the drug is unknown, binding to and protection of exposed eroded areas, increased prostaglandin production, improved vascular flow, and increased mucus production are all proposed mechanisms.

TARGETING IMMUNE CELLS (EOSINOPHILS, TH2 LYMPHOCYTES, AND MAST CELLS)

Topical corticosteroids

Corticosteroids have pleiotropic effects on immune cells, esophageal cells, and mediators relevant to EoE pathogenesis. After steroid therapy, eosinophils from EoE patients had decreased surface marker CD18 which might impair eosinophil cell adhesion[53]. The elevated numbers of CD3+, CD4+, and CD8+ T cells in the esophageal mucosa of EoE patients were decreased after steroid treatment[54,55]. Mast cell associated genes were downregulated after steroid therapy in EoE patients[56]. Furthermore, steroid therapy reversed IL-13-induced gene transcriptome[57], attenuated IL-5 gene expression[58], and modulated transforming growth factor (TGF)β1 expression and SMAD 2/3 phosphorylation in the esophagus[59].

It is clear from several recent meta-analyses and systematic reviews of randomized controlled trials that topical steroid therapy significantly reduces esophageal eosinophilia in EoE[60-62]. However, there was no clear trend in symptom response, and this may be due to the lack of validated patient reported outcome measures during those trials[19,21,63-67]. In children, both fluticasone[63] and oral viscous budesonide (OVB)[66] have demonstrated histological remission after 3 mo of intervention in double blind randomized placebo controlled trials which correlated to symptom improvement. Subepithelial fibrosis was seen to improve with OVB therapy. In adults, fluticasone induced histological remission in 62% of adults, however the response was not accompanied by a relief of symptomatic dysphagia[64]. Long-term data on budesonide as a maintenance therapy was assimilated by Straumann *et al*[68]. Patients who took low dose swallowed budesonide for 50 wk achieved partial remission (*i.e.,* reduced eosinophilia) compared to placebo. In addition, mucosal remodeling was attenuated in the treatment group without signs of epithelial atrophy[68]. Overall, the disease typically relapses within 2-9 mo after discontinuation of steroids[34,69,70].

Swallowed fluticasone propionate and oral viscous budesonide have both been commonly used as topical

applications of steroids in EoE. With fluticasone, patients are instructed to puff the inhaler into the mouth. The patients hold their breath, instead of inhaling, and swallow the aerosolized medication directly. With OVB, patients are directed to mix the contents of the budesonide respules (0.5 mg/2 mL) with sucralose to create a slurry; although other various viscous agents such as honey, apple sauce, amino acid-based semisolid, and food thickeners have been used successfully[71]. Currently, the American College of Gastroenterology (ACG) guidelines recommend Fluticasone 880-1760 mcg/d in a divided dose for adults and 88-440 mcg/d for children. The dosage of OVB is 1 mg/d for children and 2 mg/d in divided doses for adults. The recommended duration is 8 wk for topical steroid therapy[13].

Other formulations of topical steroids are currently investigated. A recent randomized controlled trial demonstrated that an effervescent tablet of budesonide was comparable to viscous budesonide in remission rates and the preferred choice by patients[72]. Two more trials will be examining the efficacy and tolerability of effervescent budesonide over placebo (NCT02434029 and NCT02493335, www.clinicaltrials.gov).

Topical steroids are generally well tolerated, with the rare exception of esophageal candidiasis. In addition, topical steroid did not suppress adrenal function in EoE pediatric patients during 8-43 wk of therapy[73]. Nevertheless, ciclesonide, another corticosteroid with lower systemic bioavailability and favorable safety profile, has been proposed and used successfully in a few pediatric cases of EoE[74,75].

Immunomodulators

Systemic steroid, like prednisone, was one of the earliest pharmacologic agents used in EoE[76], however a 40% rate of systemic adverse effects has essentially reserved the drug for only severe refractory cases[70]. Yet, in the event of severe cases, steroid sparing agents such as azathioprine and 6-mercaptopurine have also been proposed. In a case series, 3 EoE patients were treated with azathioprine or 6-mercaptopurine and achieve histological remission[77]. Patients were successfully tapered off of steroids; however, the disease relapsed after the immunomodulators were discontinued. Therefore, this study showed that immunosuppressive therapy was necessary to maintain disease control.

CRTH2 antagonist

Chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2) is expressed by Th2 lymphocytes, eosinophils, and basophils[78]. Therefore, CRTH2 is an appealing target for Th2-type inflammatory disorders. CRTH2 antagonists interfere with the prostaglandin pathway, blocking the prostaglandin D2 receptor, subsequently preventing the activation and the recruitment of the CRTH2-expressing inflammatory cells. OC000459, a CRTH2 antagonist, is a promising new drug for the treatment of allergic diseases because it is selective and is orally bioavailable. A randomized controlled trial demonstrated a modest improvement in eosinophilic inflammation and clinical symptoms in 26 severe, steroid-refractory EoE adults compared to placebo[79]. The 8 wk drug therapy was well-tolerated. Further studies are needed to determine if the drug can achieve greater improvement in moderately-active EoE patients.

Mast cell stabilizers

Mast cells release inflammatory mediators (TGFβ1, IL-4, IL-13, leukotrienes, and tryptase), and several human and animal EoE studies suggest that mast cells might contribute independently or in tandem with eosinophils to esophageal inflammation[56,80-84]. The mast cell mediator prostaglandin D2 induces eosinophil trafficking in an EoE guinea pig model[84]. Tryptase and IgE immunostaining confirmed that IgE-bearing mast cells are increased in the esophageal mucosa[85] and smooth muscle layer of EoE patients[81]. The expression of TGFβ1 suggests that mast cells might mediate esophageal contraction and remodeling. Unfortunately, earlier small case series demonstrated unsuccessful results with mast cell stabilizer cromolyn sodium[1,34]. There is a double-blind, randomized controlled study examining the safety and efficacy of cromolyn sodium for the treatment of EoE (NCT02371941 www.clinicaltrials.gov).

TARGETING INFLAMMATORY MEDIATORS

IL-5

IL-5 promotes eosinophil proliferation in the bone marrow and primes eosinophils for cytokine stimulation. EoE murine studies established that eosinophil trafficking is IL-5-dependent[86] and drives esophageal remodeling and fibrosis[87-89]. Reslizumab, a humanized monoclonal antibody to IL-5, neutralizes circulating IL-5 by preventing it from binding to its receptor which is expressed by several cells, including eosinophils. A randomized controlled trial conducted in children and adolescents did not show statistically significant symptomatic improvement with reslizumab compared to placebo, but did show significant improvement in esophageal eosinophilia compared to placebo[90]. Mepolizumab is another monoclonal antibody to IL-5. In a randomized controlled trial with adult EoE patients, intraepithelial eosinophil numbers decreased in esophageal tissues. The expression of molecules associated with esophageal remodeling was reversed. However, there was minimal improvement in the clinical symptoms[91]. In addition, a mepolizumab study in pediatric subjects with EoE demonstrated significantly fewer mast cells, IL-9 cells, and mast cell-eosinophil couplets in responders[92].

IL-13

IL-13 appears to activate the local tissue inflammatory response in Th2-associated diseases. The EoE gene transcriptome analysis discovered many IL-13-inducible genes responsible for pathogenesis[26,57]. Elevated IL-13 mRNA levels are detected in esophageal biopsies from EoE patients[41,57]. IL-13 decreases esophageal epithelial cell differentiation, a process that may be critical for maintaining the barrier function of the esophageal mucosa[51]. Additionally, IL-13 mediates eotaxin-1, eotaxin-2, and eotaxin-3 expression *via* STAT6 in esophageal epithelial cells from mice[93] and humans[51,57,94]. Eotaxin-3 is the highest upregulated gene in the EoE transcriptome[26]. Mice with genetic deletion of the eotaxin-3 receptor were protected from allergen-induced EoE[26]. Animal studies confirmed that IL-13 facilitates eosinophil recruitment[95] and induces features of esophageal remodeling[96]. A randomized controlled trial tested QAX576, a monoclonal antibody against IL-13, with promising results[97]. QAX576 was well-tolerated, although treated subjects did not meet the primary endpoint. Intraepithelial eosinophils were reduced by 60%, and there was some symptom improvement. Most strikingly, genetic markers of EoE inflammation, including eotaxin-3, periostin, mast cells markers, and barrier function, were modified after treatment. This study provides proof-of-principal that the biology of a human Th2-driven disease at the molecular level in a relevant tissue can be altered by a specific anti-IL-13 antibody. Presently, a double-blind, randomized controlled trial will evaluate the efficacy and safety of the anti-IL-13 monoclonal antibody RPC4046 in EoE adults (NCT02098473 www.clinicaltrials.gov).

IL-4

IL-4 expression is higher in EoE patients compare to control cases[25,98]. While IL-4 and IL-13 often share similar downstream effects, such as driving eotaxin-3 expression in esophageal epithelial cells, the role of IL-4 has not been clearly delineated in EoE[94,99]. In other allergic disorders, IL-4 induces naïve T cells to differentiate to Th2 cells. IL-4 also facilitates B cell class switching to IgE. Dupilumab, a human monoclonal antibody that blocks the IL-4 receptor α subunit, is therapeutically effective in patients with asthma and elevated eosinophil levels[100]. The blockade Th2-mediated inflammation by dupilumab was also seen in atopic dermatitis[101]. Currently, a randomized controlled trial is underway to study dupilumab in adults with active EoE. The study will assess the clinical efficacy of repeat subcutaneous doses of dupilumab to relieve symptoms in adult patients with active, moderate to severe EoE (NCT02379052 www.clinicaltrials.gov).

TGFβ1

Fibrogenesis is part of normal repair response to epithelial injury, in which fibroblasts synthesize extracellular matrix proteins such as collagen, fibronectin, and tenascin-C for wound healing[28]. A chronic inflammatory disorder such as EoE will progress to fibrostenotic complications[2,3], such as food impactions, fibrotic strictures, esophageal narrowing, mucosal tears, and transmural perforations[1,8,102]. TGFβ1 is a well-known fibrogenic factor in many fibrotic diseases. It is produced by eosinophils, mast cells, and other inflammatory cells, and is directly involved in esophageal fibrous remodeling in both pediatric and adult patients[103,104]. Mast cells infiltrating the esophageal smooth muscle layer of EoE patients express TGFβ1[81]. Silencing TGFβ1 molecular targets such as phospholamban[105] and Smad3[106] can diminish smooth muscle cell contraction and abrogated fibrosis and angiogenesis in mice. Angiotensin Ⅱ receptor blockers inhibit TGFβ, and have been studied in connective tissue diseases such as Marfan's syndrome where there is excessive TGFβ production[107]. An open-label trial of losartan, an angiotensin Ⅱ receptor blocker, in EoE subjects with or without a connective tissue disorder is underway. The trial will measure histologic improvement, symptomatic improvement, reduction in TGFβ, and drug safety (NCT01808196 www.clinicaltrials.gov).

Tumor necrosis factor

Tumor necrosis factor (TNF)α is a prominent inflammatory mediator in many chronic inflammatory diseases, such as Crohns disease. Not surprisingly, TNFα is found to be upregulated and highly expressed by esophageal epithelial cells in patients with EoE[24,57]. Evidence of TNFα signaling, including NFκB subunits p50 and p65, are detected in EoE and might play a role in angiogensis[108]. Infliximab, a chimeric IgG1 monoclonal antibody, is a potent inhibitor of TNFα. Thus far, a case series of 3 adults with corticosteroid-dependent EoE demonstrated variable histological and symptom response with two 5 mg/kg doses given every two weeks[109]. Although infliximab was well-tolerated in these cases, further studies are warranted to establish efficacy.

Leukotriene

Leukotrienes, in particular, cysteinyl-leukotrienes (LTC_4, LTD_4, and LTE_4) are potent lipid mediators synthesized from arachidonic acid *via* the 5-lipoxygenase pathway in immune cells. Cysteinyl-leukotrienes are best known for their pathophysiologic role in asthma, and many of their effects are mediated through their receptor $CysLT_1$ which are expressed on eosinophils, basophils, mast cells, T cells, airway smooth muscle cells, bronchial fibroblasts, and vascular endothelial cells. Montelukast is a $CysLT_1$ receptor antagonist that is effective in asthma treatment. Similarities in pathophysiology between asthma and EoE prompted trials of montelukast in EoE. In a case series of 8 pediatric EoE patients, 6 had symptomatic relief with

montelukast[110]. Similarly, another 3 out of 8 pediatric EoE patients reported symptomatic response, but histological response to montelukast could not be verified[111]. In a prospective study, montelukast failed to maintain steroid-induced remission in 11 adult EoE subjects[112]. Currently, there is a randomized controlled study evaluating clinical effectiveness of montelukast compared to placebo on prevention of dysphagia and food impaction in EoE subjects (NCT00511316, www.clinicaltrials.gov). The study will also examine tolerability and safety of the drug. Another study will compare response to treatment of EoE with montelukast compared to topical fluticasone therapy (NCT01702701, www.clinicaltrials.gov).

PPI THERAPY FOR EOE

As mentioned above, PPI-REE patients might be a subset of EoE patients. There are multiple conceivable mechanisms whereby EoE patients might benefit from PPI-induced acid suppression[15]. Reducing acid exposure might ameliorate inflammatory cytokines and pain related to acid-induced injury. In addition, acid reflux might exacerbate esophageal epithelial permeability facilitating allergen entry in EoE. Indeed, mucosal integrity, determined by electrical tissue impedance and transepithelial electrical resistance, was impaired in both EoE and PPI-REE patients[52]. High-dose PPI therapy improved mucosal integrity in the PPI-REE patients.

EoE patients might benefit from PPIs through mechanisms that are not related to acid suppression. PPIs can inhibit Th2 cytokine-induced eotaxin-3 secretion in esophageal epithelial cell, thereby potentially reducing eosinophil recruitment[94,99]. Eotaxin-3 expression by epithelial cells in the esophageal biopsies of children with esophageal eosinophilia was examined before and after PPI therapy[113]. With PPI therapy, subjects achieved a decrease in eotaxin-3 expression in the proximal esophagus, where gastroesophageal reflux is unlikely to reach, suggesting that anti-inflammatory effects might be the predominant therapeutic effect[113]. Other anti-inflammatory effects of PPIs include inhibition of immune cell functions, anti-oxidant properties, minimizing cell adhesion molecules, and decreasing inflammatory cytokines[15]. Finally, EoE transcriptome expression (an array of genes associated with eosinophilia, mastocytosis, tissue remodeling, and impaired barrier function) reversed in PPI-REE patients after PPI therapy substantiating that PPIs have therapeutic properties that target an allergic inflammation[23].

Overall, PPIs have multiple effects that might benefit EoE patients. The diagnosis of EoE should be based on the conceptual definition that the patient has an "immune/antigen-mediated" disease. Thus, for any patient who has esophageal symptoms and esophageal eosinophilia, a clinical and/or histological response to PPIs does not necessarily implicate GERD as the sole diagnosis and does not rule out EoE. Using a trial of PPI therapy as a diagnostic screen should be done with a caveat in mind. Instead, high-dose PPI therapy should be considered as a therapeutic option rather than a diagnostic screen for EoE.

DILATION

As previously mentioned, medical therapies can attenuate esophageal inflammation, ideally preventing further fibrosis and remodeling. The ability for any of these therapies to reverse long-term fibrosis and remodeling still remains to be substantiated. Although medical therapy is a logical first approach, clinicians might have to resort to dilating high-grade fibrostenotic lesions to provide symptomatic relief[114]. High complication rates with esophageal dilation procedures in EoE patients were initial concerns, but a recent meta-analysis reports complication (perforation, haemorrhage and chest pain requiring hospitalization) rate of < 1% at medical centers experienced with the EoE population[115]. Although dilation does not address histological inflammation[116], the procedure is 75% effective in improving short term clinical symptoms[115]. However, without medical therapy to abate the inflammation, EoE patients will require repeat dilation procedures as fibrostenotic lesions recur. Recently, a randomized blinded controlled trial evaluated response to dilation as an early treatment strategy in adults with dysphagia (without severe strictures) and esophageal eosinophilia[114]. However, the subjects treated with medications (PPI and fluticasone) and randomized to dilation procedures did not have any better dysphagia score outcomes compared to subjects treated with medical therapy alone. Thus, dilation should probably be considered an adjuvant therapy to a long-standing medical therapy.

CONCLUSION

EoE is a chronic inflammatory disease that can lead to fibrosis and remodeling and requires long-term treatment. EoE treatment goals include symptom resolution, induction and maintenance of disease remission, prevention of fibrostenotic complication, maintenance of quality of life, and minimizing adverse effect from medical therapies. Current and emerging medical therapies are designed to interrupt the inflammatory cascade in EoE. Esophageal dilation disrupts fibrotic strictures, providing symptomatic relief, but does not address the underlying inflammatory process. Distinguishing GERD and EoE remains a challenge. However, a response to PPI therapy does not provide diagnostic utility since EoE patients may benefit from both acid-suppressive and anti-inflammatory effects of PPIs. Therefore, PPIs should be considered as a potential therapeutic agent for EoE rather than a diagnostic screen. Much of EoE pathophysiology and natural progression still needs to

be explored to identify novel targets for therapy.

REFERENCES

1 **Liacouras CA**, Furuta GT, Hirano I, Atkins D, Attwood SE, Bonis PA, Burks AW, Chehade M, Collins MH, Dellon ES, Dohil R, Falk GW, Gonsalves N, Gupta SK, Katzka DA, Lucendo AJ, Markowitz JE, Noel RJ, Odze RD, Putnam PE, Richter JE, Romero Y, Ruchelli E, Sampson HA, Schoepfer A, Shaheen NJ, Sicherer SH, Spechler S, Spergel JM, Straumann A, Wershil BK, Rothenberg ME, Aceves SS. Eosinophilic esophagitis: updated consensus recommendations for children and adults. *J Allergy Clin Immunol* 2011; **128**: 3-20.e6; quiz 21-22 [PMID: 21477849 DOI: 10.1016/j.jaci.2011.02.040]

2 **Dellon ES**, Kim HP, Sperry SL, Rybnicek DA, Woosley JT, Shaheen NJ. A phenotypic analysis shows that eosinophilic esophagitis is a progressive fibrostenotic disease. *Gastrointest Endosc* 2014; **79**: 577-85.e4 [PMID: 24275329 DOI: 10.1016/j.gie.2013.10.027]

3 **Schoepfer AM**, Safroneeva E, Bussmann C, Kuchen T, Portmann S, Simon HU, Straumann A. Delay in diagnosis of eosinophilic esophagitis increases risk for stricture formation in a time-dependent manner. *Gastroenterology* 2013; **145**: 1230-6.e1-2 [PMID: 23954315 DOI: 10.1053/j.gastro.2013.08.015]

4 **Attwood SE**, Smyrk TC, Demeester TR, Jones JB. Esophageal eosinophilia with dysphagia. A distinct clinicopathologic syndrome. *Dig Dis Sci* 1993; **38**: 109-116 [PMID: 8420741]

5 **Straumann A**, Spichtin HP, Bernoulli R, Loosli J, Vögtlin J. [Idiopathic eosinophilic esophagitis: a frequently overlooked disease with typical clinical aspects and discrete endoscopic findings]. *Schweiz Med Wochenschr* 1994; **124**: 1419-1429 [PMID: 7939509]

6 **DeBrosse CW**, Collins MH, Buckmeier Butz BK, Allen CL, King EC, Assa'ad AH, Abonia JP, Putnam PE, Rothenberg ME, Franciosi JP. Identification, epidemiology, and chronicity of pediatric esophageal eosinophilia, 1982-1999. *J Allergy Clin Immunol* 2010; **126**: 112-119 [PMID: 20620567 DOI: 10.1016/j.jaci.2010.05.027]

7 **Aceves SS**, Ackerman SJ. Relationships between eosinophilic inflammation, tissue remodeling, and fibrosis in eosinophilic esophagitis. *Immunol Allergy Clin North Am* 2009; **29**: 197-211, xiii-xiv [PMID: 19141355 DOI: 10.1016/j.iac.2008.10.003]

8 **Hirano I**, Aceves SS. Clinical implications and pathogenesis of esophageal remodeling in eosinophilic esophagitis. *Gastroenterol Clin North Am* 2014; **43**: 297-316 [PMID: 24813517 DOI: 10.1016/j.gtc.2014.02.015]

9 **Kim HP**, Vance RB, Shaheen NJ, Dellon ES. The prevalence and diagnostic utility of endoscopic features of eosinophilic esophagitis: a meta-analysis. *Clin Gastroenterol Hepatol* 2012; **10**: 988-996.e5 [PMID: 22610003 DOI: 10.1016/j.cgh.2012.04.019]

10 **Prasad GA**, Talley NJ, Romero Y, Arora AS, Kryzer LA, Smyrk TC, Alexander JA. Prevalence and predictive factors of eosinophilic esophagitis in patients presenting with dysphagia: a prospective study. *Am J Gastroenterol* 2007; **102**: 2627-2632 [PMID: 17764492 DOI: 10.1111/j.1572-0241.2007.01512.x]

11 **Hirano I**, Moy N, Heckman MG, Thomas CS, Gonsalves N, Achem SR. Endoscopic assessment of the oesophageal features of eosinophilic oesophagitis: validation of a novel classification and grading system. *Gut* 2013; **62**: 489-495 [PMID: 22619364 DOI: 10.1136/gutjnl-2011-301817]

12 **Saffari H**, Peterson KA, Fang JC, Teman C, Gleich GJ, Pease LF. Patchy eosinophil distributions in an esophagectomy specimen from a patient with eosinophilic esophagitis: Implications for endoscopic biopsy. *J Allergy Clin Immunol* 2012; **130**: 798-800 [PMID: 22502795 DOI: 10.1016/j.jaci.2012.03.009]

13 **Dellon ES**, Gonsalves N, Hirano I, Furuta GT, Liacouras CA, Katzka DA. ACG clinical guideline: Evidenced based approach to the diagnosis and management of esophageal eosinophilia and eosinophilic esophagitis (EoE). *Am J Gastroenterol* 2013; **108**: 679-692; quiz 693 [PMID: 23567357 DOI: 10.1038/ajg.2013.71]

14 **Nielsen JA**, Lager DJ, Lewin M, Rendon G, Roberts CA. The optimal number of biopsy fragments to establish a morphologic diagnosis of eosinophilic esophagitis. *Am J Gastroenterol* 2014; **109**: 515-520 [PMID: 24445569 DOI: 10.1038/ajg.2013.463]

15 **Cheng E**, Souza RF, Spechler SJ. Eosinophilic esophagitis: interactions with gastroesophageal reflux disease. *Gastroenterol Clin North Am* 2014; **43**: 243-256 [PMID: 24813513 DOI: 10.1016/j.gtc.2014.02.004]

16 **Krarup AL**, Villadsen GE, Mejlgaard E, Olesen SS, Drewes AM, Funch-Jensen P. Acid hypersensitivity in patients with eosinophilic oesophagitis. *Scand J Gastroenterol* 2010; **45**: 273-281 [PMID: 20001646 DOI: 10.3109/00365520903469931]

17 **Souza RF**, Huo X, Mittal V, Schuler CM, Carmack SW, Zhang HY, Zhang X, Yu C, Hormi-Carver K, Genta RM, Spechler SJ. Gastroesophageal reflux might cause esophagitis through a cytokine-mediated mechanism rather than caustic acid injury. *Gastroenterology* 2009; **137**: 1776-1784 [PMID: 19660463 DOI: 10.1053/j.gastro.2009.07.055]

18 **Furuta GT**, Liacouras CA, Collins MH, Gupta SK, Justinich C, Putnam PE, Bonis P, Hassall E, Straumann A, Rothenberg ME. Eosinophilic esophagitis in children and adults: a systematic review and consensus recommendations for diagnosis and treatment. *Gastroenterology* 2007; **133**: 1342-1363 [PMID: 17919504 DOI: 10.1053/j.gastro.2007.08.017]

19 **Peterson KA**, Thomas KL, Hilden K, Emerson LL, Wills JC, Fang JC. Comparison of esomeprazole to aerosolized, swallowed fluticasone for eosinophilic esophagitis. *Dig Dis Sci* 2010; **55**: 1313-1319 [PMID: 19533356 DOI: 10.1007/s10620-009-0859-4]

20 **Molina-Infante J**, Ferrando-Lamana L, Ripoll C, Hernandez-Alonso M, Mateos JM, Fernandez-Bermejo M, Dueñas C, Fernandez-Gonzalez N, Quintana EM, Gonzalez-Nuñez MA. Esophageal eosinophilic infiltration responds to proton pump inhibition in most adults. *Clin Gastroenterol Hepatol* 2011; **9**: 110-117 [PMID: 20920599 DOI: 10.1016/j.cgh.2010.09.019]

21 **Moawad FJ**, Veerappan GR, Dias JA, Baker TP, Maydonovitch CL, Wong RK. Randomized controlled trial comparing aerosolized swallowed fluticasone to esomeprazole for esophageal eosinophilia. *Am J Gastroenterol* 2013; **108**: 366-372 [PMID: 23399553 DOI: 10.1038/ajg.2012.443]

22 **Dellon ES**, Speck O, Woodward K, Gebhart JH, Madanick RD, Levinson S, Fritchie KJ, Woosley JT, Shaheen NJ. Clinical and endoscopic characteristics do not reliably differentiate PPI-responsive esophageal eosinophilia and eosinophilic esophagitis in patients undergoing upper endoscopy: a prospective cohort study. *Am J Gastroenterol* 2013; **108**: 1854-1860 [PMID: 24145677 DOI: 10.1038/ajg.2013.363]

23 **Wen T**, Dellon ES, Moawad FJ, Furuta GT, Aceves SS, Rothenberg ME. Transcriptome analysis of proton pump inhibitor-responsive esophageal eosinophilia reveals proton pump inhibitor-reversible allergic inflammation. *J Allergy Clin Immunol* 2015; **135**: 187-197 [PMID: 25441638 DOI: 10.1016/j.jaci.2014.08.043]

24 **Straumann A**, Bauer M, Fischer B, Blaser K, Simon HU. Idiopathic eosinophilic esophagitis is associated with a T(H)2-type allergic inflammatory response. *J Allergy Clin Immunol* 2001; **108**: 954-961 [PMID: 11742273 DOI: 10.1067/mai.2001.119917]

25 **Straumann A**, Kristl J, Conus S, Vassina E, Spichtin HP, Beglinger C, Simon HU. Cytokine expression in healthy and inflamed mucosa: probing the role of eosinophils in the digestive tract. *Inflamm Bowel Dis* 2005; **11**: 720-726 [PMID: 16043986]

26 **Blanchard C**, Wang N, Stringer KF, Mishra A, Fulkerson PC, Abonia JP, Jameson SC, Kirby C, Konikoff MR, Collins MH, Cohen MB, Akers R, Hogan SP, Assa'ad AH, Putnam PE, Aronow BJ, Rothenberg ME. Eotaxin-3 and a uniquely conserved gene-expression profile in eosinophilic esophagitis. *J Clin Invest* 2006; **116**: 536-547 [PMID: 16453027 DOI: 10.1172/JCI26679]

27 **Cheng E**. Translating new developments in eosinophilic esophagitis pathogenesis into clinical practice. *Curr Treat Options Gastroenterol* 2015; **13**: 30-46 [PMID: 25598233 DOI: 10.1007/s11938-014-0041-8]

28 **Cheng E**, Souza RF, Spechler SJ. Tissue remodeling in eosinophilic esophagitis. *Am J Physiol Gastrointest Liver Physiol*

2012; **303**: G1175-G1187 [PMID: 23019192 DOI: 10.1152/ajpgi.00313.2012]
29 **Kelly KJ**, Lazenby AJ, Rowe PC, Yardley JH, Perman JA, Sampson HA. Eosinophilic esophagitis attributed to gastroesophageal reflux: improvement with an amino acid-based formula. *Gastroenterology* 1995; **109**: 1503-1512 [PMID: 7557132]
30 **Markowitz JE**, Spergel JM, Ruchelli E, Liacouras CA. Elemental diet is an effective treatment for eosinophilic esophagitis in children and adolescents. *Am J Gastroenterol* 2003; **98**: 777-782 [PMID: 12738455 DOI: 10.1111/j.1572-0241.2003.07390.x]
31 **Spergel JM**, Andrews T, Brown-Whitehorn TF, Beausoleil JL, Liacouras CA. Treatment of eosinophilic esophagitis with specific food elimination diet directed by a combination of skin prick and patch tests. *Ann Allergy Asthma Immunol* 2005; **95**: 336-343 [PMID: 16279563 DOI: 10.1016/S1081-1206(10)61151-9]
32 **Kagalwalla AF**, Sentongo TA, Ritz S, Hess T, Nelson SP, Emerick KM, Melin-Aldana H, Li BU. Effect of six-food elimination diet on clinical and histologic outcomes in eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2006; **4**: 1097-1102 [PMID: 16860614 DOI: 10.1016/j.cgh.2006.05.026]
33 **Arias A**, González-Cervera J, Tenias JM, Lucendo AJ. Efficacy of dietary interventions for inducing histologic remission in patients with eosinophilic esophagitis: a systematic review and meta-analysis. *Gastroenterology* 2014; **146**: 1639-1648 [PMID: 24534634 DOI: 10.1053/j.gastro.2014.02.006]
34 **Liacouras CA**, Spergel JM, Ruchelli E, Verma R, Mascarenhas M, Semeao E, Flick J, Kelly J, Brown-Whitehorn T, Mamula P, Markowitz JE. Eosinophilic esophagitis: a 10-year experience in 381 children. *Clin Gastroenterol Hepatol* 2005; **3**: 1198-1206 [PMID: 16361045]
35 **Peterson KA**, Byrne KR, Vinson LA, Ying J, Boynton KK, Fang JC, Gleich GJ, Adler DG, Clayton F. Elemental diet induces histologic response in adult eosinophilic esophagitis. *Am J Gastroenterol* 2013; **108**: 759-766 [PMID: 23381017 DOI: 10.1038/ajg.2012.468]
36 **Dellon ES**, Liacouras CA. Advances in clinical management of eosinophilic esophagitis. *Gastroenterology* 2014; **147**: 1238-1254 [PMID: 25109885 DOI: 10.1053/j.gastro.2014.07.055]
37 **Molina-Infante J**, Martin-Noguerol E, Alvarado-Arenas M, Porcel-Carreño SL, Jimenez-Timon S, Hernandez-Arbeiza FJ. Selective elimination diet based on skin testing has suboptimal efficacy for adult eosinophilic esophagitis. *J Allergy Clin Immunol* 2012; **130**: 1200-1202 [PMID: 22867695 DOI: 10.1016/j.jaci.2012.06.027]
38 **Kagalwalla AF**, Shah A, Li BU, Sentongo TA, Ritz S, Manuel-Rubio M, Jacques K, Wang D, Melin-Aldana H, Nelson SP. Identification of specific foods responsible for inflammation in children with eosinophilic esophagitis successfully treated with empiric elimination diet. *J Pediatr Gastroenterol Nutr* 2011; **53**: 145-149 [PMID: 21788754 DOI: 10.1097/MPG.0b013e31821cf503]
39 **Gonsalves N**, Yang GY, Doerfler B, Ritz S, Ditto AM, Hirano I. Elimination diet effectively treats eosinophilic esophagitis in adults; food reintroduction identifies causative factors. *Gastroenterology* 2012; **142**: 1451-1459.e1; quiz e14-15 [PMID: 22391333 DOI: 10.1053/j.gastro.2012.03.001]
40 **Spergel JM**, Brown-Whitehorn TF, Cianferoni A, Shuker M, Wang ML, Verma R, Liacouras CA. Identification of causative foods in children with eosinophilic esophagitis treated with an elimination diet. *J Allergy Clin Immunol* 2012; **130**: 461-467.e5 [PMID: 22743304 DOI: 10.1016/j.jaci.2012.05.021]
41 **Vicario M**, Blanchard C, Stringer KF, Collins MH, Mingler MK, Ahrens A, Putnam PE, Abonia JP, Santos J, Rothenberg ME. Local B cells and IgE production in the oesophageal mucosa in eosinophilic oesophagitis. *Gut* 2010; **59**: 12-20 [PMID: 19528036 DOI: 10.1136/gut.2009.178020]
42 **Loizou D**, Enav B, Komlodi-Pasztor E, Hider P, Kim-Chang J, Noonan L, Taber T, Kaushal S, Limgala R, Brown M, Gupta R, Balba N, Goker-Alpan O, Khojah A, Alpan O. A pilot study of omalizumab in eosinophilic esophagitis. *PLoS One* 2015; **10**: e0113483 [PMID: 25789989 DOI: 10.1371/journal.pone.0113483]
43 **Noti M**, Wojno ED, Kim BS, Siracusa MC, Giacomin PR, Nair MG, Benitez AJ, Ruymann KR, Muir AB, Hill DA, Chikwava KR, Moghaddam AE, Sattentau QJ, Alex A, Zhou C, Yearley JH, Menard-Katcher P, Kubo M, Obata-Ninomiya K, Karasuyama H, Comeau MR, Brown-Whitehorn T, de Waal Malefyt R, Sleiman PM, Hakonarson H, Cianferoni A, Falk GW, Wang ML, Spergel JM, Artis D. Thymic stromal lymphopoietin-elicited basophil responses promote eosinophilic esophagitis. *Nat Med* 2013; **19**: 1005-1013 [PMID: 23872715 DOI: 10.1038/nm.3281]
44 **Mishra A**, Schlotman J, Wang M, Rothenberg ME. Critical role for adaptive T cell immunity in experimental eosinophilic esophagitis in mice. *J Leukoc Biol* 2007; **81**: 916-924 [PMID: 17194734 DOI: 10.1189/jlb.1106653]
45 **Clayton F**, Fang JC, Gleich GJ, Lucendo AJ, Olalla JM, Vinson LA, Lowichik A, Chen X, Emerson L, Cox K, O'Gorman MA, Peterson KA. Eosinophilic esophagitis in adults is associated with IgG4 and not mediated by IgE. *Gastroenterology* 2014; **147**: 602-609 [PMID: 24907494 DOI: 10.1053/j.gastro.2014.05.036]
46 **Rocha R**, Vitor AB, Trindade E, Lima R, Tavares M, Lopes J, Dias JA. Omalizumab in the treatment of eosinophilic esophagitis and food allergy. *Eur J Pediatr* 2011; **170**: 1471-1474 [PMID: 21809010 DOI: 10.1007/s00431-011-1540-4]
47 **van Rhijn BD**, Kessing BF, Smout AJ, Bredenoord AJ. Oesophageal baseline impedance values are decreased in patients with eosinophilic oesophagitis. *United European Gastroenterol J* 2013; **1**: 242-248 [PMID: 24917968 DOI: 10.1177/2050640613496411]
48 **Sherrill JD**, Kc K, Wu D, Djukic Z, Caldwell JM, Stucke EM, Kemme KA, Costello MS, Mingler MK, Blanchard C, Collins MH, Abonia JP, Putnam PE, Dellon ES, Orlando RC, Hogan SP, Rothenberg ME. Desmoglein-1 regulates esophageal epithelial barrier function and immune responses in eosinophilic esophagitis. *Mucosal Immunol* 2014; **7**: 718-729 [PMID: 24220297 DOI: 10.1038/mi.2013.90]
49 **Abdulnour-Nakhoul SM**, Al-Tawil Y, Gyftopoulos AA, Brown KL, Hansen M, Butcher KF, Eidelwein AP, Noel RA, Rabon E, Posta A, Nakhoul NL. Alterations in junctional proteins, inflammatory mediators and extracellular matrix molecules in eosinophilic esophagitis. *Clin Immunol* 2013; **148**: 265-278 [PMID: 23792687 DOI: 10.1016/j.clim.2013.05.004]
50 **Katzka DA**, Tadi R, Smyrk TC, Katarya E, Sharma A, Geno DM, Camilleri M, Iyer PG, Alexander JA, Buttar NS. Effects of topical steroids on tight junction proteins and spongiosis in esophageal epithelia of patients with eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2014; **12**: 1824-1829.e1 [PMID: 24681080 DOI: 10.1016/j.cgh.2014.02.039]
51 **Blanchard C**, Stucke EM, Burwinkel K, Caldwell JM, Collins MH, Ahrens A, Buckmeier BK, Jameson SC, Greenberg A, Kaul A, Franciosi JP, Kushner JP, Martin LJ, Putnam PE, Abonia JP, Wells SI, Rothenberg ME. Coordinate interaction between IL-13 and epithelial differentiation cluster genes in eosinophilic esophagitis. *J Immunol* 2010; **184**: 4033-4041 [PMID: 20208004 DOI: 10.4049/jimmunol.0903069]
52 **van Rhijn BD**, Weijenborg PW, Verheij J, van den Bergh Weerman MA, Verseijden C, van den Wijngaard RM, de Jonge WJ, Smout AJ, Bredenoord AJ. Proton pump inhibitors partially restore mucosal integrity in patients with proton pump inhibitor-responsive esophageal eosinophilia but not eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2014; **12**: 1815-23.e2 [PMID: 24657840 DOI: 10.1016/j.cgh.2014.02.037]
53 **Lingblom C**, Bergquist H, Johnsson M, Sundström P, Quiding-Järbrink M, Bove M, Wennerås C. Topical corticosteroids do not revert the activated phenotype of eosinophils in eosinophilic esophagitis but decrease surface levels of CD18 resulting in diminished adherence to ICAM-1, ICAM-2, and endothelial cells. *Inflammation* 2014; **37**: 1932-1944 [PMID: 24870064 DOI: 10.1007/s10753-014-9926-x]
54 **Lucendo AJ**, Navarro M, Comas C, Pascual JM, Burgos E, Santamaría L, Larrauri J. Immunophenotypic characterization and quantification of the epithelial inflammatory infiltrate in

eosinophilic esophagitis through stereology: an analysis of the cellular mechanisms of the disease and the immunologic capacity of the esophagus. *Am J Surg Pathol* 2007; **31**: 598-606 [PMID: 17414108 DOI: 10.1097/01.pas.0000213392.49698.8c]

55 **Teitelbaum JE**, Fox VL, Twarog FJ, Nurko S, Antonioli D, Gleich G, Badizadegan K, Furuta GT. Eosinophilic esophagitis in children: immunopathological analysis and response to fluticasone propionate. *Gastroenterology* 2002; **122**: 1216-1225 [PMID: 11984507]

56 **Hsu Blatman KS**, Gonsalves N, Hirano I, Bryce PJ. Expression of mast cell-associated genes is upregulated in adult eosinophilic esophagitis and responds to steroid or dietary therapy. *J Allergy Clin Immunol* 2011; **127**: 1307-1308.e3 [PMID: 21333344 DOI: 10.1016/j.jaci.2010.12.1118]

57 **Blanchard C**, Mingler MK, Vicario M, Abonia JP, Wu YY, Lu TX, Collins MH, Putnam PE, Wells SI, Rothenberg ME. IL-13 involvement in eosinophilic esophagitis: transcriptome analysis and reversibility with glucocorticoids. *J Allergy Clin Immunol* 2007; **120**: 1292-1300 [PMID: 18073124 DOI: 10.1016/j.jaci.2007.10.024]

58 **Lucendo AJ**, De Rezende L, Comas C, Caballero T, Bellón T. Treatment with topical steroids downregulates IL-5, eotaxin-1/CCL11, and eotaxin-3/CCL26 gene expression in eosinophilic esophagitis. *Am J Gastroenterol* 2008; **103**: 2184-2193 [PMID: 18844613 DOI: 10.1111/j.1572-0241.2008.01937.x]

59 **Aceves SS**, Newbury RO, Chen D, Mueller J, Dohil R, Hoffman H, Bastian JF, Broide DH. Resolution of remodeling in eosinophilic esophagitis correlates with epithelial response to topical corticosteroids. *Allergy* 2010; **65**: 109-116 [PMID: 19796194 DOI: 10.1111/j.1398-9995.2009.02142.x]

60 **Chuang MY**, Chinnaratha MA, Hancock DG, Woodman R, Wong GR, Cock C, Fraser RJ. Topical Steroid Therapy for the Treatment of Eosinophilic Esophagitis (EoE): A Systematic Review and Meta-Analysis. *Clin Transl Gastroenterol* 2015; **6**: e82 [PMID: 25809314 DOI: 10.1038/ctg.2015.9]

61 **Tan ND**, Xiao YL, Chen MH. Steroids therapy for eosinophilic esophagitis: Systematic review and meta-analysis. *J Dig Dis* 2015; **16**: 431-442 [PMID: 26058809 DOI: 10.1111/1751-2980.12265]

62 **Sawas T**, Dhalla S, Sayyar M, Pasricha PJ, Hernaez R. Systematic review with meta-analysis: pharmacological interventions for eosinophilic oesophagitis. *Aliment Pharmacol Ther* 2015; **41**: 797-806 [PMID: 25728929 DOI: 10.1111/apt.13147]

63 **Konikoff MR**, Noel RJ, Blanchard C, Kirby C, Jameson SC, Buckmeier BK, Akers R, Cohen MB, Collins MH, Assa'ad AH, Aceves SS, Putnam PE, Rothenberg ME. A randomized, double-blind, placebo-controlled trial of fluticasone propionate for pediatric eosinophilic esophagitis. *Gastroenterology* 2006; **131**: 1381-1391 [PMID: 17101314 DOI: 10.1053/j.gastro.2006.08.033]

64 **Alexander JA**, Jung KW, Arora AS, Enders F, Katzka DA, Kephardt GM, Kita H, Kryzer LA, Romero Y, Smyrk TC, Talley NJ. Swallowed fluticasone improves histologic but not symptomatic response of adults with eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2012; **10**: 742-749.e1 [PMID: 22475741 DOI: 10.1016/j.cgh.2012.03.018]

65 **Butz BK**, Wen T, Gleich GJ, Furuta GT, Spergel J, King E, Kramer RE, Collins MH, Stucke E, Mangeot C, Jackson WD, O'Gorman M, Abonia JP, Pentiuk S, Putnam PE, Rothenberg ME. Efficacy, dose reduction, and resistance to high-dose fluticasone in patients with eosinophilic esophagitis. *Gastroenterology* 2014; **147**: 324-33.e5 [PMID: 24768678 DOI: 10.1053/j.gastro.2014.04.019]

66 **Dohil R**, Newbury R, Fox L, Bastian J, Aceves S. Oral viscous budesonide is effective in children with eosinophilic esophagitis in a randomized, placebo-controlled trial. *Gastroenterology* 2010; **139**: 418-429 [PMID: 20457157 DOI: 10.1053/j.gastro.2010.05.001]

67 **Straumann A**, Conus S, Degen L, Felder S, Kummer M, Engel H, Bussmann C, Beglinger C, Schoepfer A, Simon HU. Budesonide is effective in adolescent and adult patients with active eosinophilic esophagitis. *Gastroenterology* 2010; **139**: 1526-1537, 1537.e1 [PMID: 20682320 DOI: 10.1053/j.gastro.2010.07.048]

68 **Straumann A**, Conus S, Degen L, Frei C, Bussmann C, Beglinger C, Schoepfer A, Simon HU. Long-term budesonide maintenance treatment is partially effective for patients with eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2011; **9**: 400-409.e1 [PMID: 21277394 DOI: 10.1016/j.cgh.2011.01.017]

69 **Helou EF**, Simonson J, Arora AS. 3-yr-follow-up of topical corticosteroid treatment for eosinophilic esophagitis in adults. *Am J Gastroenterol* 2008; **103**: 2194-2199 [PMID: 18637093 DOI: 10.1111/j.1572-0241.2008.01989.x]

70 **Schaefer ET**, Fitzgerald JF, Molleston JP, Croffie JM, Pfefferkorn MD, Corkins MR, Lim JD, Steiner SJ, Gupta SK. Comparison of oral prednisone and topical fluticasone in the treatment of eosinophilic esophagitis: a randomized trial in children. *Clin Gastroenterol Hepatol* 2008; **6**: 165-173 [PMID: 18237866 DOI: 10.1016/j.cgh.2007.11.008]

71 **Contreras EM**, Gupta SK. Steroids in pediatric eosinophilic esophagitis. *Gastroenterol Clin North Am* 2014; **43**: 345-356 [PMID: 24813520 DOI: 10.1016/j.gtc.2014.02.008]

72 **Miehlke S**, Hruz P, Vieth M, Bussmann C, von Arnim U, Bajbouj M, Schlag C, Madisch A, Fibbe C, Wittenburg H, Allescher HD, Reinshagen M, Schubert S, Tack J, Muller M, Krummenerl P, Arts J, Mueller R, Dilger K, Greinwald R, Straumann A. A randomised, double-blind trial comparing budesonide formulations and dosages for short-term treatment of eosinophilic oesophagitis. *Gut* 2015; Epub ahead of print [PMID: 25792708 DOI: 10.1136/gutjnl-2014-308815]

73 **Philla KQ**, Min SB, Hefner JN, Howard RS, Reinhardt BJ, Nazareno LG, Vogt KS. Swallowed glucocorticoid therapy for eosinophilic esophagitis in children does not suppress adrenal function. *J Pediatr Endocrinol Metab* 2015; **28**: 1101-1106 [PMID: 26024243 DOI: 10.1515/jpem-2014-0260]

74 **Schroeder S**, Fleischer DM, Masterson JC, Gelfand E, Furuta GT, Atkins D. Successful treatment of eosinophilic esophagitis with ciclesonide. *J Allergy Clin Immunol* 2012; **129**: 1419-1421 [PMID: 22480537 DOI: 10.1016/j.jaci.2012.03.007]

75 **Lee JJ**, Fried AJ, Hait E, Yen EH, Perkins JM, Rubinstein E. Topical inhaled ciclesonide for treatment of eosinophilic esophagitis. *J Allergy Clin Immunol* 2012; **130**: 1011; author reply 1011-1012 [PMID: 23021144 DOI: 10.1016/j.jaci.2012.06.053]

76 **Liacouras CA**, Wenner WJ, Brown K, Ruchelli E. Primary eosinophilic esophagitis in children: successful treatment with oral corticosteroids. *J Pediatr Gastroenterol Nutr* 1998; **26**: 380-385 [PMID: 9552132]

77 **Netzer P**, Gschossmann JM, Straumann A, Sendensky A, Weimann R, Schoepfer AM. Corticosteroid-dependent eosinophilic oesophagitis: azathioprine and 6-mercaptopurine can induce and maintain long-term remission. *Eur J Gastroenterol Hepatol* 2007; **19**: 865-869 [PMID: 17873610 DOI: 10.1097/MEG.0b013e32825a6ab4]

78 **Pettipher R**, Vinall SL, Xue L, Speight G, Townsend ER, Gazi L, Whelan CJ, Armer RE, Payton MA, Hunter MG. Pharmacologic profile of OC000459, a potent, selective, and orally active D prostanoid receptor 2 antagonist that inhibits mast cell-dependent activation of T helper 2 lymphocytes and eosinophils. *J Pharmacol Exp Ther* 2012; **340**: 473-482 [PMID: 22106101 DOI: 10.1124/jpet.111.187203]

79 **Straumann A**, Hoesli S, Bussmann Ch, Stuck M, Perkins M, Collins LP, Payton M, Pettipher R, Hunter M, Steiner J, Simon HU. Anti-eosinophil activity and clinical efficacy of the CRTH2 antagonist OC000459 in eosinophilic esophagitis. *Allergy* 2013; **68**: 375-385 [PMID: 23379537 DOI: 10.1111/all.12096]

80 **Abonia JP**, Blanchard C, Butz BB, Rainey HF, Collins MH, Stringer K, Putnam PE, Rothenberg ME. Involvement of mast cells in eosinophilic esophagitis. *J Allergy Clin Immunol* 2010; **126**: 140-149 [PMID: 20538331 DOI: 10.1016/j.jaci.2010.04.009]

81 **Aceves SS**, Chen D, Newbury RO, Dohil R, Bastian JF, Broide DH. Mast cells infiltrate the esophageal smooth muscle in patients with eosinophilic esophagitis, express TGF-β1, and increase esophageal smooth muscle contraction. *J Allergy Clin Immunol* 2010; **126**: 1198-1204.e4 [PMID: 21047675 DOI: 10.1016/j.jaci.2010.08.050]

82 **Chehade M**, Sampson HA, Morotti RA, Magid MS. Esophageal subepithelial fibrosis in children with eosinophilic esophagitis. *J Pediatr Gastroenterol Nutr* 2007; **45**: 319-328 [PMID: 17873744 DOI: 10.1097/MPG.0b013e31806ab384]

83 **Niranjan R**, Mavi P, Rayapudi M, Dynda S, Mishra A. Pathogenic role of mast cells in experimental eosinophilic esophagitis. *Am J Physiol Gastrointest Liver Physiol* 2013; **304**: G1087-G1094 [PMID: 23599040 DOI: 10.1152/ajpgi.00070.2013]

84 **Zhang S**, Wu X, Yu S. Prostaglandin D2 receptor D-type prostanoid receptor 2 mediates eosinophil trafficking into the esophagus. *Dis Esophagus* 2014; **27**: 601-606 [PMID: 24165271 DOI: 10.1111/dote.12118]

85 **Mulder DJ**, Mak N, Hurlbut DJ, Justinich CJ. Atopic and non-atopic eosinophilic oesophagitis are distinguished by immunoglobulin E-bearing intraepithelial mast cells. *Histopathology* 2012; **61**: 810-822 [PMID: 22978418 DOI: 10.1111/j.1365-2559.2012.4303.x]

86 **Mishra A**, Hogan SP, Brandt EB, Rothenberg ME. IL-5 promotes eosinophil trafficking to the esophagus. *J Immunol* 2002; **168**: 2464-2469 [PMID: 11859139]

87 **Mavi P**, Rajavelu P, Rayapudi M, Paul RJ, Mishra A. Esophageal functional impairments in experimental eosinophilic esophagitis. *Am J Physiol Gastrointest Liver Physiol* 2012; **302**: G1347-G1355 [PMID: 22361731 DOI: 10.1152/ajpgi.00013.2012]

88 **Mishra A**, Wang M, Pemmaraju VR, Collins MH, Fulkerson PC, Abonia JP, Blanchard C, Putnam PE, Rothenberg ME. Esophageal remodeling develops as a consequence of tissue specific IL-5-induced eosinophilia. *Gastroenterology* 2008; **134**: 204-214 [PMID: 18166354 DOI: 10.1053/j.gastro.2007.10.002]

89 **Masterson JC**, McNamee EN, Hosford L, Capocelli KE, Ruybal J, Fillon SA, Doyle AD, Eltzschig HK, Rustgi AK, Protheroe CA, Lee NA, Lee JJ, Furuta GT. Local hypersensitivity reaction in transgenic mice with squamous epithelial IL-5 overexpression provides a novel model of eosinophilic oesophagitis. *Gut* 2014; **63**: 43-53 [PMID: 23161496 DOI: 10.1136/gutjnl-2012-303631]

90 **Spergel JM**, Rothenberg ME, Collins MH, Furuta GT, Markowitz JE, Fuchs G, O'Gorman MA, Abonia JP, Young J, Henkel T, Wilkins HJ, Liacouras CA. Reslizumab in children and adolescents with eosinophilic esophagitis: results of a double-blind, randomized, placebo-controlled trial. *J Allergy Clin Immunol* 2012; **129**: 456-463, 463.e1-3 [PMID: 22206777 DOI: 10.1016/j.jaci.2011.11.044]

91 **Straumann A**, Conus S, Grzonka P, Kita H, Kephart G, Bussmann C, Beglinger C, Smith DA, Patel J, Byrne M, Simon HU. Anti-interleukin-5 antibody treatment (mepolizumab) in active eosinophilic oesophagitis: a randomised, placebo-controlled, double-blind trial. *Gut* 2010; **59**: 21-30 [PMID: 19828470 DOI: 10.1136/gut.2009.178558]

92 **Otani IM**, Anilkumar AA, Newbury RO, Bhagat M, Beppu LY, Dohil R, Broide DH, Aceves SS. Anti-IL-5 therapy reduces mast cell and IL-9 cell numbers in pediatric patients with eosinophilic esophagitis. *J Allergy Clin Immunol* 2013; **131**: 1576-1582 [PMID: 23623266 DOI: 10.1016/j.jaci.2013.02.042]

93 **Neilsen CV**, Bryce PJ. Interleukin-13 directly promotes oesophagus production of CCL11 and CCL24 and the migration of eosinophils. *Clin Exp Allergy* 2010; **40**: 427-434 [PMID: 20030665 DOI: 10.1111/j.1365-2222.2009.03419.x]

94 **Cheng E**, Zhang X, Huo X, Yu C, Zhang Q, Wang DH, Spechler SJ, Souza RF. Omeprazole blocks eotaxin-3 expression by oesophageal squamous cells from patients with eosinophilic oesophagitis and GORD. *Gut* 2013; **62**: 824-832 [PMID: 22580413 DOI: 10.1136/gutjnl-2012-302250]

95 **Mishra A**, Rothenberg ME. Intratracheal IL-13 induces eosinophilic esophagitis by an IL-5, eotaxin-1, and STAT6-dependent mechanism. *Gastroenterology* 2003; **125**: 1419-1427 [PMID: 14598258]

96 **Zuo L**, Fulkerson PC, Finkelman FD, Mingler M, Fischetti CA, Blanchard C, Rothenberg ME. IL-13 induces esophageal remodeling and gene expression by an eosinophil-independent, IL-13R alpha 2-inhibited pathway. *J Immunol* 2010; **185**: 660-669 [PMID: 20543112 DOI: 10.4049/jimmunol.1000471]

97 **Rothenberg ME**, Wen T, Greenberg A, Alpan O, Enav B, Hirano I, Nadeau K, Kaiser S, Peters T, Perez A, Jones I, Arm JP, Strieter RM, Sabo R, Gunawardena KA. Intravenous anti-IL-13 mAb QAX576 for the treatment of eosinophilic esophagitis. *J Allergy Clin Immunol* 2015; **135**: 500-507 [PMID: 25226850 DOI: 10.1016/j.jaci.2014.07.049]

98 **Blanchard C**, Stucke EM, Rodriguez-Jimenez B, Burwinkel K, Collins MH, Ahrens A, Alexander ES, Butz BK, Jameson SC, Kaul A, Franciosi JP, Kushner JP, Putnam PE, Abonia JP, Rothenberg ME. A striking local esophageal cytokine expression profile in eosinophilic esophagitis. *J Allergy Clin Immunol* 2011; **127**: 208-217, 217.e1-7 [PMID: 21211656 DOI: 10.1016/j.jaci.2010.10.039]

99 **Zhang X**, Cheng E, Huo X, Yu C, Zhang Q, Pham TH, Wang DH, Spechler SJ, Souza RF. Omeprazole blocks STAT6 binding to the eotaxin-3 promoter in eosinophilic esophagitis cells. *PLoS One* 2012; **7**: e50037 [PMID: 23185525 DOI: 10.1371/journal.pone.0050037]

100 **Wenzel S**, Ford L, Pearlman D, Spector S, Sher L, Skobieranda F, Wang L, Kirkesseli S, Rocklin R, Bock B, Hamilton J, Ming JE, Radin A, Stahl N, Yancopoulos GD, Graham N, Pirozzi G. Dupilumab in persistent asthma with elevated eosinophil levels. *N Engl J Med* 2013; **368**: 2455-2466 [PMID: 23688323 DOI: 10.1056/NEJMoa1304048]

101 **Beck LA**, Thaçi D, Hamilton JD, Graham NM, Bieber T, Rocklin R, Ming JE, Ren H, Kao R, Simpson E, Ardeleanu M, Weinstein SP, Pirozzi G, Guttman-Yassky E, Suárez-Fariñas M, Hager MD, Stahl N, Yancopoulos GD, Radin AR. Dupilumab treatment in adults with moderate-to-severe atopic dermatitis. *N Engl J Med* 2014; **371**: 130-139 [PMID: 25006719 DOI: 10.1056/NEJMoa1314768]

102 **Fontillón M**, Lucendo AJ. Transmural eosinophilic infiltration and fibrosis in a patient with non-traumatic Boerhaave's syndrome due to eosinophilic esophagitis. *Am J Gastroenterol* 2012; **107**: 1762 [PMID: 23160304 DOI: 10.1038/ajg.2012.226]

103 **Aceves SS**, Newbury RO, Dohil R, Bastian JF, Broide DH. Esophageal remodeling in pediatric eosinophilic esophagitis. *J Allergy Clin Immunol* 2007; **119**: 206-212 [PMID: 17208603 DOI: 10.1016/j.jaci.2006.10.016]

104 **Lucendo AJ**, Arias A, De Rezende LC, Yagüe-Compadre JL, Mota-Huertas T, González-Castillo S, Cuesta RA, Tenias JM, Bellón T. Subepithelial collagen deposition, profibrogenic cytokine gene expression, and changes after prolonged fluticasone propionate treatment in adult eosinophilic esophagitis: a prospective study. *J Allergy Clin Immunol* 2011; **128**: 1037-1046 [PMID: 21880354 DOI: 10.1016/j.jaci.2011.08.007]

105 **Beppu LY**, Anilkumar AA, Newbury RO, Dohil R, Broide DH, Aceves SS. TGF-β1-induced phospholamban expression alters esophageal smooth muscle cell contraction in patients with eosinophilic esophagitis. *J Allergy Clin Immunol* 2014; **134**: 1100-1107.e4 [PMID: 24835503 DOI: 10.1016/j.jaci.2014.04.004]

106 **Cho JY**, Doshi A, Rosenthal P, Beppu A, Miller M, Aceves S, Broide D. Smad3-deficient mice have reduced esophageal fibrosis and angiogenesis in a model of egg-induced eosinophilic esophagitis. *J Pediatr Gastroenterol Nutr* 2014; **59**: 10-16 [PMID: 24590208 DOI: 10.1097/MPG.0000000000000343]

107 **Brooke BS**, Habashi JP, Judge DP, Patel N, Loeys B, Dietz HC. Angiotensin II blockade and aortic-root dilation in Marfan's syndrome. *N Engl J Med* 2008; **358**: 2787-2795 [PMID: 18579813 DOI: 10.1056/NEJMoa0706585]

108 **Persad R**, Huynh HQ, Hao L, Ha JR, Sergi C, Srivastava R, Persad S. Angiogenic remodeling in pediatric EoE is associated with increased levels of VEGF-A, angiogenin, IL-8, and activation of the TNF-α-NFκB pathway. *J Pediatr Gastroenterol Nutr* 2012; **55**: 251-260 [PMID: 22331014 DOI: 10.1097/MPG.0b013e31824b6391]

109 **Straumann A**, Bussmann C, Conus S, Beglinger C, Simon HU. Anti-TNF-alpha (infliximab) therapy for severe adult eosinophilic esophagitis. *J Allergy Clin Immunol* 2008; **122**: 425-427 [PMID: 18678345 DOI: 10.1016/j.jaci.2008.06.012]

110 **Attwood SE**, Lewis CJ, Bronder CS, Morris CD, Armstrong GR, Whittam J. Eosinophilic oesophagitis: a novel treatment using

Montelukast. *Gut* 2003; **52**: 181-185 [PMID: 12524397]
111 **Stumphy J**, Al-Zubeidi D, Guerin L, Mitros F, Rahhal R. Observations on use of montelukast in pediatric eosinophilic esophagitis: insights for the future. *Dis Esophagus* 2011; **24**: 229-234 [PMID: 21073625 DOI: 10.1111/j.1442-2050.2010.01134.x]
112 **Lucendo AJ**, De Rezende LC, Jiménez-Contreras S, Yagüe-Compadre JL, González-Cervera J, Mota-Huertas T, Guagnozzi D, Angueira T, González-Castillo S, Arias A. Montelukast was inefficient in maintaining steroid-induced remission in adult eosinophilic esophagitis. *Dig Dis Sci* 2011; **56**: 3551-3558 [PMID: 21674173 DOI: 10.1007/s10620-011-1775-y]
113 **Park JY**, Zhang X, Nguyen N, Souza RF, Spechler SJ, Cheng E. Proton pump inhibitors decrease eotaxin-3 expression in the proximal esophagus of children with esophageal eosinophilia. *PLoS One* 2014; **9**: e101391 [PMID: 24988451 DOI: 10.1371/journal.pone.0101391]
114 **Kavitt RT**, Ates F, Slaughter JC, Higginbotham T, Shepherd BD, Sumner EL, Vaezi MF. Randomized controlled trial comparing esophageal dilation to no dilation among adults with esophageal eosinophilia and dysphagia. *Dis Esophagus* 2015; Epub ahead of print [PMID: 26228516 DOI: 10.1111/dote.12398]
115 **Moawad FJ**, Cheatham JG, DeZee KJ. Meta-analysis: the safety and efficacy of dilation in eosinophilic oesophagitis. *Aliment Pharmacol Ther* 2013; **38**: 713-720 [PMID: 23915046 DOI: 10.1111/apt.12438]
116 **Schoepfer AM**, Gonsalves N, Bussmann C, Conus S, Simon HU, Straumann A, Hirano I. Esophageal dilation in eosinophilic esophagitis: effectiveness, safety, and impact on the underlying inflammation. *Am J Gastroenterol* 2010; **105**: 1062-1070 [PMID: 19935783 DOI: 10.1038/ajg.2009.657]

P- Reviewer: Gara N, Septer S **S- Editor**: Qiu S **L- Editor**: A
E- Editor: Wang CH

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i1.33

World J Gastrointest Pharmacol Ther 2016 February 6; 7(1): 33-40
ISSN 2150-5349 (online)

REVIEW

Hepatitis C virus: A time for decisions. Who should be treated and when?

Bashar M Attar, David H Van Thiel

Bashar M Attar, Cook County Health and Hospitals System, Rush University Medical Center, Chicago, IL 60612, United States

David H Van Thiel, Advanced Liver and Gastrointestinal Disease Center, Berwyn, IL 60402, United States

Author contributions: Both the authors contribute to collect of data, synthesis, and write of the manuscript.

Conflict-of-interest statement: The authors declare no conflicts of interest regarding this manuscript.

Correspondence to: Bashar M Attar, MD, PhD, MPH, Cook County Health and Hospitals System, Rush University Medical Center, 1901 West Harrison Street, Chicago, IL 60612, United States. battar@rush.edu
Telephone: +1-312-8647213
Fax: +1-312-8649214

Received: June 24, 2015
Peer-review started: June 24, 2015
First decision: August 3, 2015
Revised: September 16, 2015
Accepted: November 13, 2015
Article in press: November 17, 2015
Published online: February 6, 2016

Abstract

Cirrhosis is the most important risk factor for hepatocellular carcinoma (HCC) regardless of the etiology of cirrhosis. Compared to individuals who are anti-hepatitis C virus (HCV) seronegative, anti-HCV seropositive individuals have a greater mortality from both hepatic as well as nonhepatic disease processes. The aim of this paper is do describe the burden of HCV infection and consider treatment strategies to reduce HCV-related morbidity and mortality. The newly developed direct acting antiviral (DAA) therapies are associated with greater rates of drug compliance, fewer adverse effects, and appear not to be limited by the presence of a variety of factors that adversely affect the outcome of interferon-based therapies. Because of the cost of the current DAA, their use has been severely rationed by insurers as well as state and federal agencies to those with advanced fibrotic liver disease (Metavir fibrosis stage F3-F4). The rationale for such rationing is that many of those recognized as having the disease progress slowly over many years and will not develop advanced liver disease manifested as chronic hepatitis C, cirrhosis, and experience any of the multiple complications of liver disease to include HCC. This mitigation has a short sided view of the cost of treatment of hepatitis C related disease processes and ignores the long-term expenses of hepatitis C treatment consisting of the cost of treatment of hepatitis C, the management of cirrhosis with or without decompensation as well as the cost of treatment of HCC and liver transplantation. We believe that treatment should include all HCV infected patients including those with stage F0-F2 fibrosis with or without evidence of coexisting liver disease. Specifically, interferon (IFN)-free regimens with the current effective DAAs without liver staging requirements and including those without evidence of hepatic diseases but having recognized extrahepatic manifestations of HCV infection is projected to be the most cost-effective approach for treating HCV in all of its varied presentations. Early rather than later therapy of HCV infected individuals would be even more efficacious than waiting particularly if it includes all cases from F0-F4 hepatic disease. Timely therapy will reduce the number of individuals developing advanced

liver disease, reduce the cost of treating these cases and more importantly, reduce the lifetime cost of treatment of those with any form of HCV related disease as well as HCV associated all - cause mortality. Importantly, HCV treatment regimens without any restrictions would result in a substantial reduction in health care expenditure and simultaneously reduce the number of infected individuals who are infecting others.

Key words: Hepatitis C virus; Direct acting antivirals; Cirrhosis of the liver; Timing of treatment

Core tip: This study presents the burden of hepatitis C virus (HCV) infection. Current guidelines limit treatment to those with advanced liver disease (Metavir F-3 or F-4 fibrosis). This represents a small fraction of those infected having the worse prognosis. They are unlikely to infect others. In contrast, the much larger group F-0 to F-2 is the vectors for additional infections. The plague of HCV can only be eliminated if the larger groups that infect others are treated. The cost of treating this larger population is expensive but much less expensive than treating only those with advanced fibrosis in the long run.

Attar BM, Van Thiel DH. Hepatitis C virus: A time for decisions. Who should be treated and when? *World J Gastrointest Pharmacol Ther* 2016; 7(1): 33-40 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i1/33.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i1.33

INTRODUCTION

Chronic hepatitis C virus (HCV) infection is an important cause of advanced liver disease and liver-related deaths. The aim of this document is to describe the burden of HCV infection and consider treatment strategies to reduce HCV-related morbidity and mortality[1-5].

It is estimated that the incidence of hepatocellular carcinoma (HCC) in Europe and United States will peak at 2020 at which there will be 78000 new HCC cases in Europe and 27000 in the United States[6]. Cirrhosis is the most important risk factor for HCC regardless of the etiology and cirrhosis occurs in the background of 90% of cases of HCC[6].

These figures probably under estimate the actual prevalence of the disease HCV as they are based upon data that excludes groups at recognized highest risks for the infection. Despite these limitations relative to the current estimates of the disease prevalence, it is well recognized that 50%-85% of the patients infected with HCV and manifest a hepatic disease process develop a chronic hepatitis and 20%-25% of these cases progress to cirrhosis with 20% of this latter group progressing further to HCC[7-9].

RISK OF HCV INFECTION AND NEED FOR SCREENING

HCV infection has an increasing HCV-related mortality from 1.09 to 2.40 per 100000 person years in the United States from 1995 to 2004[10]. The predicted mortality of HCV related disease over a 20-year period is expected to continue to rise as more and more individuals, who are currently infected, will have their disease for many more years. As a result, the healthcare burden in direct and indirect costs related to HCV infection will continue to rise in the foreseeable future[10]. The detection of HCV RNA in serum identifies active cases manifested by replication of the virus. Lee *et al*[10] reported that 52%-80% of serum samples seropositive for anti-HCV have been reported to have detectable serum levels of HCV RNA. Importantly, anti-HCV seropositive individuals with detectable serum HCV RNA have an increased risk of dying from all causes, whereas the risk for anti-HCV seropositives with negative HCV RNA is similar to that of HCV seronegative individuals[9,10]. Indeed, 2394 deaths occurred in HCV positive individuals during an average follow-up period of 16.2 years. Compared to individuals, who are anti-HCV seronegative, anti-HCV seropositive individuals have a greater mortality from both hepatic as well as nonhepatic disease processes. The multivariate-adjusted hazard ratio [95% confidence interval (CI)] of 1.89 (1.66-2.15) for all causes of death in HCV seropositive individuals and 12.48 (9.34-16.66) for hepatic diseases, 1.35 (1.15-1.57) for extrahepatic diseases, 1.50 (1.10-2.03) for circulatory diseases, 2.77 (1.49-5.15) for nephritis, nephrotic syndrome, and nephrosis, 4.08 (1.38-12.08) for esophageal cancer, 8.22 (1.36-49.66) for thyroid cancer, and 4.19 (1.18-14.94) for prostate cancer. Thus, the presence of HCV seropositivity increases the risk of death from a wide array of extrahepatic disease processes. Moreover, anti-HCV seropositives with detectable HCV RNA levels have a significantly greater mortality risk for death due to both hepatic and extrahepatic diseases processes than do individuals who are anti-HCV seropositives but who are HCV RNA negative. These data imply that individuals with chronic hepatitis C having an active infection manifested by HCV-RNA positivity should benefit from antiviral treatment to reduce both their overall mortality as well as hepatic disease mortality risk[10].

Recently, the Center for Disease Control (CDC) has identified individuals born between 1945 and 1965 as well as veterans, males, people in low income groups, prisoners, those in various institutions, and African American as well as the Latino populations as being at higher risk for a HCV infection[11]. As the majority of infected individuals have little or no symptoms, they may never know that they are infected despite the

Table 1 Individuals at high risk for hepatitis C virus infection

Individuals working in emergency departments
Anesthesiologists
First responders
Fire
Police
Ambulance attendants
Individuals undergoing chronic hemodialysis
Healthcare workers including employees in dialysis center
Institutional residents (prisons, individuals with physical, mental, and developmental abnormalities)
Individuals born between 1945-1965
Those receiving blood or blood products before 1992
Intravenous drug abusers
Presence of human immunodeficiency virus infection or individuals with high risk sexual behaviors

fact that 75%-80% of them may develop a lifelong chronic infection that adversely affects their life quality as well as their longevity. Individuals in this latter group also include those who received plasma or blood transfusions prior to 1992, hemophiliacs, individuals on hemodialysis, organ transplant recipients, those who experience needle sticks as a result of illicit drug use or an occupational exposures and possibly those infected as a result of tattoos or the use of unsterile equipment for body piercing, children born of a hepatitis C positive mothers and those who practice unprotected or high risk sex with multiple partners (Table 1). Most importantly, these asymptomatic individuals can unknowingly transmit the disease to others, thereby perpetuating the disease process in society at large[11,12].

THERAPY AND ERADICATION OF HCV

Historically, the available therapeutic agents utilized for the treatment of chronic hepatitis C (interferon-based therapies) have had only limited success at the elimination of the disease with efficacy rates ranging between 20% and 40% manifested as a sustained viral response (SVR) 6 mo after a presumed end of treatment (EOT) course of therapy[12]. In addition, these historical treatment regimens were expensive in terms of their direct and indirect costs, albeit less so than the new direct acting antiviral agents and had numerous adverse effect that limited their acceptability by individuals, who would have been considered as appropriate candidates for therapy. Moreover, the use of IFN-based therapies are contraindicated in individuals with a variety of autoimmune disease processes, those with a clinically significant depressive disorders, and those with advanced coronary artery or cerebrovascular disease. In addition, IFN-therapies have limited efficacy in individuals with different viral genotypes as well as specific genetic as well as phenotypic characteristics that include variant IL28B polymorphisms, obesity, diabetes mellitus, ethnicity and coinfection with either HBV or HIV[13,14].

Table 2 Goals of treatment of hepatitis C virus

Current goals of HCV treatment
Cure HCV infection in those infected with the virus
Reduce the downstream consequences of chronic hepatitis C
Prevent cirrhosis
Prevent decompensation of cirrhosis
Prevent hepatocellular carcinoma
Reduce the requirement for liver transplantation in individuals with chronic hepatitis C
Improve life quality of those with HCV
Reduction of all-cause as well as liver disease mortality
Ideal goals of HCV treatment:
Eliminate HCV disease in its all of varied manifestations (both hepatic and extrahepatic)
Reduce the number of individuals infected with minimal or no liver disease who are important transmitters of the virus within the population
Improve the life expectancy and quality of those infected with HCV regardless of the specific clinical presentation of their infection

HCV: Hepatitis C virus.

In contrast, the newly developed direct acting (DAA) antiviral therapies are administered orally and require less complex regimes. As a result, they are more readily acceptable. As a consequence of their enhanced acceptability and increased rate of drug compliance, they achieve a significantly greater efficacy rate, have fewer adverse effects and appear not to be limited by the presence of a variety of concurrent medical disease processes to include the aforementioned genetic and phenotypic characteristics that adversely affect the outcome of interferon-based therapies. It is expected that the newer 3rd generation DAAs soon to be approved by the Food and Drug Administration (FDA) are even more efficacious and are effective across all genotypes as compared to the current 2nd generation DAA agents (Table 2)[15,16].

By increasing the sustained virological response (SVR) to 90% or more from 2016 onward the number of treated cases in Belgium has been estimated to increase from 710 to 2050 in 2030 resulting in a reduction of the number of cases with cirrhosis, decompensated cirrhosis and HCC disease process which have high direct and indirect costs of care[17]. The new DAAs are reported to be most efficacious as compared to historical regimens with interferon when applied to F2-F4 cases. To obtain comparable outcomes with all cases ranging from those with F0-F4 fibrosis, 50% more cases would have to be treated, a number which would appear to be achievable with the greater acceptability and reduced frequency of adverse events associated with the newer agents. Additionally, a two-year delay in access to the DAAs has been estimated to increase HCV related morbidity and mortality by 15%. These data suggest that early rather than later therapy of HCV infected individuals would be even more efficacious than waiting particularly if it

Table 3 Factors potentially contributing to fibrosis progression in individuals with chronic hepatitis C virus

Established factors[1]	More recently identified risk factors
Duration of HCV infection	Patient age at time of diagnosis
Older age at infection	Genotype 3 infection
Male gender	Insulin resistance
Presence of baseline fibrosis	Gene polymorphisms involved in inflammation and iron metabolism
HIV coinfection[1]/CD4 count < 200 cells/mL	Human leukocyte antigen DRB1*1201-3 allele
Long term alcohol consumption (> 20-50 g/d)	Latin ethnicity
HBV coinfection	Daily cannabis use
Metabolic syndrome (steatosis, insulin resistance, type 2 diabetes)	

[1]HCV viral load and mode of infection are not associated with faster fibrosis progression. HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; HBV: Hepatitis B virus.

includes all cases from F0-F4 hepatic disease[17].

van der Meer *et al*[18] have shown in an international, multicenter, long-term follow-up study from 5 large tertiary care hospitals in Canada and Europe consisting of 530 patients with chronic HCV infection, who started an interferon-based treatment regimen between 1990 and 2003, that the 10-year cumulative incidence rate of liver-related mortality or transplantation was 1.9% (95%CI: 0.0%-4.1%) with a prior SVR following treatment and 27.4% (95%CI: 22.0%-32.8%) without a SVR (P < 001). Thus, in patients with chronic HCV infection and advanced hepatic fibrosis, a sustained virological response to interferon-based treatment is associated with a lower all-cause mortality rate and obviously a substantial reduction in overall direct and indirect costs of healthcare.

Molnar *et al*[19] reported an association between HCV infection and the progression of chronic kidney disease. HCV infection was associated with a 2.2 fold increase in mortality, a 98% higher hazard of development of end-stage kidney, and a 15% worsening of renal function in a large cohort of United States veterans. In addition to death related to hepatocellular cancer, all-cause mortality increased with HCV infection was attributed in part to association with extrahepatic manifestations of HCV such as cryoglobulinemia, lymphoma, glomerlulonephritides, as well as rheumatologic, hematologic, and dermatologic disorders.

Simmons *et al*[20] reported in a meta-analysis and systematic review of 31 studies that achieving SVR in individuals with chronic HCV. After adjustment for potential confounding factors, the results of the pooled HR analysis revealed a decreased risk of all-cause mortality by approximately 50%, 74%, and 79% in the general populations, cirrhotic patients, and coinfected (HCV/HIV) individuals respectively.

Sievert *et al*[21] described three different treatment scenarios based upon the anticipated introduction of DAA regimens have been estimated to reduce the overall HCV disease burden. Scenario 1 evaluated the impact of increased treatment efficacy alone estimated to be 80%-90% by 2016. Scenario 2 evaluated the increased expected efficacy as well as the increase in numbers of individuals expected to be treated from a value of 2550 to 13500 by 2018 without any treatment restrictions. Scenario 3 considered the same increases in efficiency and number expected to be treated limited to those with fibrotic disease ⩾ F3 during the period of 2015-2017. The authors estimated that 233490 people with chronic HCV infection. This group has included 13850 individuals with cirrhosis, 590 with HCC and 530 with liver-related deaths. Scenario 1 would result in a modest reduction in disease burden (4% decreases in HCC, decompensated cirrhosis, and liver deaths) and the overall costs related to these diseases. Scenario 3 had the greatest impact on disease burden projected at a 50% decrease in HCC, decompensated cirrhosis, and liver deaths and overall healthcare costs. Scenario 2 had only a slightly lower impact than did Scenario 3.

These data suggest that treatment regimens without any restrictions would result in a substantial reduction in health care costs and simultaneously reduce the number of infected individuals infected who can infect others (Table 3)[22,23].

The development of the second-generation protease inhibitors (PIs) had a higher antiviral efficiency as a result of their plurigenotypic range but also as they were more convenient to administer and were associated with fewer side effects[24]. The NS5B inhibitors include nucleoside/nucleotide inhibitors (NIs) and non-nucleotide inhibitors (NNIs). NIs have even higher efficacy rates and even more useful as they can be used across all genotypes. Sofosbuvir has highly potent antiviral activity across all genotypes when used in association with pegylated interferon and ribavirin (PR). NS5A inhibitors (NS5A) also have potent antiviral activity and when used in combination with protease inhibitors are reported to achieve a SVR in GT-1b prior null responders to a prior interferon-based regimen. Several additional studies have demonstrated that interferon (IFN)-free regimens with DAA agent combinations achieve even higher rates of SVR in naïve as well as treatment-experienced GT-1 patients, who have failed prior interferon based treatment regimes. Moreover, quadruple regimens with peginterferon plus ribavirin (PR) achieve a SVR in almost all GT-1 null responders. The development of pan-genotypic direct-acting antiviral agents (NIs or NS5A.I) will allow additional new combinations with or without PR that are expected to increase the rate of SVRs for all patient populations regardless of genotype and those with cirrhosis[24].

COST OF HCV TREATMENT AND THE NEED FOR TIMELY THERAPY

In contrast to the recommendation for screening for

Table 4 Extrahepatic manifestations associated with hepatitis C virus infection

Neuropsychiatric	Ocular
Depression	Corneal ulcer
Cerebral vasculitis	Uveitis
Endocrine	Autoimmune phenomena
hypothyroidism	CREST syndrome
Diabetes mellitus	Thyroiditis/hypothyroidism
Thyroiditis	Sicca syndrome
Neuromuscular	Renal
Weakness/myalgia	Membranous glomerulonephritis
Peripheral neuropathy	Nephrotic syndrome
Arthritis/arthralgia	Cryoglobulinemia related glomerulonephritis
Vascular	Hematologic
Necrotizing vasculitis	Aplastic anemia
Polyarteritis nodosa	Thrombocytopenia
Cryoglobulinemia	Non-Hodgkin's B cell lymphoma
Dermatologic	
Porphyria cutanea tarda	
Lichen planus	
Cutaneous necrotizing vasculitis	
Livedo reticularis	

HCV and the subsequent recognition of cases, the use of DAA therapy has been severely rationed by insurers as well as state and federal agencies. The cost of these drugs can be effectively reduced by an increase of the use of these agents to include all those patients infected with HCV rather than just those with advanced hepatic fibrosis[25,26]. The rationale for such rationing is that many of those recognized as having the disease will progress slowly over many years, many identified cases will not develop advanced liver disease manifested as advanced chronic hepatitis and cirrhosis (F3-4 cases) and experience any of the multiple complications of their liver disease requiring specific treatment[27-31].

This reasoning fails to recognize the non-hepatic consequences of hepatitis C infection and the adverse effects of these non-hepatic diseases on patient's quality of life. This represents a short sided view of the cost of treatment of hepatitis C related disease (infections) and ignores the long-term costs of hepatitis C treatment consisting of the cost of treatment of cirrhosis, the cost of treatment of decompensated cirrhosis as well as the cost of treatment of HCC as well as the cost of liver transplantation and its long-term follow up. These costs far exceed the costs related to the treatment of hepatitis C before any of these complications occurs. In addition, this reasoning ignores the fact that hepatitis C infection is not limited to the liver *per se* and also includes a wide range of extra hepatic disease processes that occur in the absence of clinical liver disease and have extensive direct and indirect costs of their own (Table 4)[30-35]. These diseases affect adversely the individual's life quality and potentially longevity[36-41]. Most importantly, the exclusion of cases with recognized hepatic disease ranging from those with F0 to F2 and those with extra hepatic disease processes fails to recognize that these individuals are the principal vectors for new cases of HCV infection[42-47]. Their treatment would be expected to greatly reduce the numbers of newly infected cases to include those with and without recognized hepatic disease and potentially eliminate the disease in the population at large[48-50].

The rationing of therapy to those with advanced liver disease, also calls into question the ethical consequences of the recommendations of the CDC and other health related organizations and societies to screen individuals for the disease if no treatment is to be made available to those identified as having the disease. To do so under these circumstances only produces anguish and inappropriate fear in those identified as having the infection[51].

The alternative approach of recognizing those that have the infection and treating them before they develop clinically evident disease associated with the tremendous costs to society in terms of direct and indirect costs of health care as a result of hepatic as well as the many extra hepatic disease processes known to occur as a result of hepatitis C infection should result in major long term reductions in health care costs[52]. Moreover, by treating these larger populations, the number of individuals, who unknowingly infect others and perpetuate the infection in the population, would be reduced with even greater overall health care cost reductions. The institution of this alternative approach incorporating a much larger population of infected individuals would make it possible for a marked reduction in cost per unit pill or course of therapy while maintaining the overall profit for pharmaceutical companies, who have expended large amounts of money to bring the drugs to market[52,53].

Finally, at the day to day clinical level, treatment of patients with stages F0-F3 would be expected to be even more efficacious, be better tolerated with fewer cases dropping out of therapy than what would occur by delaying, treatment until more advanced stages of liver disease (cirrhosis, hepatic cancer, liver transplant) or not providing treatment at all[54].

Younossi *et al*[55] administered a questionnaire to 1923 individuals with chronic hepatitis C, genotype-1, who were enrolled in the ION trials and received HCV treatment of combination of ledipasvir and sofosbuvir (LDV/Sof) with a SVR-12 rate of 93.21%.Reduced work productivity secondary to absenteeism and presenteesim impairments dropped after achieving SVR-12 which would result in a productivity loss saving of 2.7 billion over one-year.

Tandon *et al*[56] using a health insurance claims database from January 2001 to March 2012, compared a total of 1017 patients, who completed interferon therapy and 953 patients, who discontinued

therapy. Both resource utilization and healthcare cost statistically significant lower cost allocation of 3687 and 1644 dollars for all-causes and CHC-related healthcare costs, respectively, relative to those who discontinued therapy.

CONCLUSION

Many patients achieve a SVR with PEG-IFN containing therapies. The continued improvements in the ability to obtain a SVR (expected cure) of HCV have been made within the past several years. The principal reason to utilize DAAs is to avoid the side effects of IFN which enhances acceptability, compliance and efficacy of treatment. The enhanced efficacy of these agents and the shorter duration of therapy are additional benefits.

Secondly, considerable increases in the burden of HCV-related advanced liver disease and its complications are expected to be seen in the United States utilizing current treatment regimens. The introduction of improved DAA regimens with enhanced efficacy and a non-restricted requirement for treatment should result in an even greater impact on the total health care costs and reduce the life-long costs of HCV disease management costs. A combination of increased treatment efficacy and greater utilization by treating all presentations of all the disease to include not only those with evident hepatic disease but also those without evidence of liver disease should result in major reductions in the lifetime costs of HCV related disease costs.

Finally, treating all HCV infected patients to include those with and without hepatic disease with DAA regimens will reduce the number of individuals developing advanced liver disease, reduce the cost of treating these cases and more importantly, reduce the cost of treatment of those with any form of HCV related disease to include not only those with F0-F2 fibrosis of the liver but also those with extra hepatic disease related to HCV infection with or without evidence for coexistent liver disease. Specifically, IFN-free regimens without liver staging requirements and including those without evidence of hepatic diseases but having recognized extrahepatic manifestations of HCV infection is projected to be the most cost-effective approach for treating HCV in all of its varied presentations. Therefore, treatment regimens without any restrictions would result in a substantial reduction in health care expenditure and simultaneously reduce the number of infected Individuals who are infected can infect others.

REFERENCES

1 **Boccaccio V**, Bruno S. Management of HCV patients with cirrhosis with direct acting antivirals. *Liver Int* 2014; **34** Suppl 1: 38-45 [PMID: 24373077 DOI: 10.1111/liv.12391]

2 **Bourlière M**, Wendt A, Fontaine H, Hézode C, Pol S, Bronowicki JP. How to optimize HCV therapy in genotype 1 patients with cirrhosis. *Liver Int* 2013; **33** Suppl 1: 46-55 [PMID: 23286846 DOI: 10.1111/liv.12067]

3 **Shiffman ML**, Benhamou Y. Patients with HCV and F1 and F2 fibrosis stage: treat now or wait? *Liver Int* 2013; **33** Suppl 1: 105-110 [PMID: 23286853 DOI: 10.1111/liv.12066]

4 **Afdhal NH**, Zeuzem S, Schooley RT, Thomas DL, Ward JW, Litwin AH, Razavi H, Castera L, Poynard T, Muir A, Mehta SH, Dee L, Graham C, Church DR, Talal AH, Sulkowski MS, Jacobson IM. The new paradigm of hepatitis C therapy: integration of oral therapies into best practices. *J Viral Hepat* 2013; **20**: 745-760 [PMID: 24168254 DOI: 10.1111/jvh.12173]

5 **Oramasionwu CU**, Moore HN, Toliver JC. Barriers to hepatitis C antiviral therapy in HIV/HCV co-infected patients in the United States: a review. *AIDS Patient Care STDS* 2014; **28**: 228-239 [PMID: 24738846 DOI: 10.1089/apc.2014.0033]

6 **Flores A**, Marrero JA. Emerging trends in hepatocellular carcinoma: focus on diagnosis and therapeutics. *Clin Med Insights Oncol* 2014; **8**: 71-76 [PMID: 24899827 DOI: 10.4137/CMO.S9926]

7 **Shiffman ML**. Hepatitis C virus therapy in the direct acting antiviral era. *Curr Opin Gastroenterol* 2014; **30**: 217-222 [PMID: 24625897 DOI: 10.1097/MOG.0000000000000062]

8 **Sebastiani G**, Gkouvatsos K, Pantopoulos K. Chronic hepatitis C and liver fibrosis. *World J Gastroenterol* 2014; **20**: 11033-11053 [PMID: 25170193 DOI: 10.3748/wjg.v20.i32.11033]

9 **Maan R**, van der Meer AJ, Hansen BE, Feld JJ, Wedemeyer H, Dufour JF, Zangneh HF, Lammert F, Manns MP, Zeuzem S, Janssen HL, de Knegt RJ, Veldt BJ. Effect of thrombocytopenia on treatment tolerability and outcome in patients with chronic HCV infection and advanced hepatic fibrosis. *J Hepatol* 2014; **61**: 482-491 [PMID: 24780302 DOI: 10.1016/j.jhep.2014.04.021]

10 **Lee MH**, Yang HI, Lu SN, Jen CL, You SL, Wang LY, Wang CH, Chen WJ, Chen CJ. Chronic hepatitis C virus infection increases mortality from hepatic and extrahepatic diseases: a community-based long-term prospective study. *J Infect Dis* 2012; **206**: 469-477 [PMID: 22811301 DOI: 10.1002/ijc.28753]

11 **Ward JW**. The epidemiology of chronic hepatitis C and one-time hepatitis C virus testing of persons born during 1945 to 1965 in the United States. *Clin Liver Dis* 2013; **17**: 1-11 [PMID: 23177279 DOI: 10.1016/j.cld.2012.09.011]

12 **Muir AJ**. The rapid evolution of treatment strategies for hepatitis C. *Am J Gastroenterol* 2014; **109**: 628-635; quiz 636 [PMID: 24732866 DOI: 10.1038/ajg.2014.66]

13 **Kohli A**, Shaffer A, Sherman A, Kottilil S. Treatment of hepatitis C: a systematic review. *JAMA* 2014; **312**: 631-640 [PMID: 25117132 DOI: 10.1001/jama.2014.7085]

14 **Feeney ER**, Chung RT. Antiviral treatment of hepatitis C. *BMJ* 2014; **348**: g3308 [PMID: 25002352 DOI: 10.1136/bmj.g3308]

15 **Pawlotsky JM**. New hepatitis C therapies: the toolbox, strategies, and challenges. *Gastroenterology* 2014; **146**: 1176-1192 [PMID: 24631495 DOI: 10.1053/j.gastro.2014.03.003]

16 **Kabiri M**, Jazwinski AB, Roberts MS, Schaefer AJ, Chhatwal J. The changing burden of hepatitis C virus infection in the United States: model-based predictions. *Ann Intern Med* 2014; **161**: 170-180 [PMID: 25089861 DOI: 10.7326/M14-0095]

17 **Stärkel P**, Vandijck D, Laleman W, Van Damme P, Moreno C, Blach S, Razavi H, Van Vlierberghe H. The Disease Burden of Hepatitis C in Belgium : An update of a realistic disease control strategy. *Acta Gastroenterol Belg* 2015; **78**: 228-232 [PMID: 26151693]

18 **van der Meer AJ**, Veldt BJ, Feld JJ, Wedemeyer H, Dufour JF, Lammert F, Duarte-Rojo A, Heathcote EJ, Manns MP, Kuske L, Zeuzem S, Hofmann WP, de Knegt RJ, Hansen BE, Janssen HL. Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. *JAMA* 2012; **308**: 2584-2593 [PMID: 23268517 DOI: 10.1001/jama.2012.144878]

19 **Molnar MZ**, Alhourani HM, Wall BM, Lu JL, Streja E, Kalantar-Zadeh K, Kovesdy CP. Association of hepatitis C viral infection with incidence and progression of chronic kidney disease in a large cohort of US veterans. *Hepatology* 2015; **61**: 1495-1502 [PMID: 25529816

DOI: 10.1002/hep.27664]
20 **Simmons B**, Saleem J, Heath K, Cooke GS, Hill A. Long-Term Treatment Outcomes of Patients Infected With Hepatitis C Virus: A Systematic Review and Meta-analysis of the Survival Benefit of Achieving a Sustained Virological Response. *Clin Infect Dis* 2015; **61**: 730-740 [PMID: 25987643 DOI: 10.1093/cid/civ396]
21 **Sievert W**, Razavi H, Estes C, Thompson AJ, Zekry A, Roberts SK, Dore GJ. Enhanced antiviral treatment efficacy and uptake in preventing the rising burden of hepatitis C-related liver disease and costs in Australia. *J Gastroenterol Hepatol* 2014; **29** Suppl 1: 1-9 [PMID: 25055928 DOI: 10.1111/jgh.12677]
22 **Massard J**, Ratziu V, Thabut D, Moussalli J, Lebray P, Benhamou Y, Poynard T. Natural history and predictors of disease severity in chronic hepatitis C. *J Hepatol* 2006; **44**: S19-S24 [PMID: 16356583]
23 **Poynard T**, Ratziu V, Charlotte F, Goodman Z, McHutchison J, Albrecht J. Rates and risk factors of liver fibrosis progression in patients with chronic hepatitis c. *J Hepatol* 2001; **34**: 730-739 [PMID: 11434620]
24 **Wendt A**, Adhoute X, Castellani P, Oules V, Ansaldi C, Benali S, Bourlière M. Chronic hepatitis C: future treatment. *Clin Pharmacol* 2014; **6**: 1-17 [PMID: 24470777 DOI: 10.2147/CPAA.S30338]
25 **Hagan LM**, Sulkowski MS, Schinazi RF. Cost analysis of sofosbuvir/ribavirin versus sofosbuvir/simeprevir for genotype 1 hepatitis C virus in interferon-ineligible/intolerant individuals. *Hepatology* 2014; **60**: 37-45 [PMID: 24677184 DOI: 10.1002/hep.27151]
26 **Myers RP**, Krajden M, Bilodeau M, Kaita K, Marotta P, Peltekian K, Ramji A, Estes C, Razavi H, Sherman M. Burden of disease and cost of chronic hepatitis C infection in Canada. *Can J Gastroenterol Hepatol* 2014; **28**: 243-250 [PMID: 24839620]
27 **Vandijck D**, Moreno C, Stärkel P, Van Damme P, Van Vlierberghe H, Hindman SJ, Razavi H, Laleman W. Current and future health and economic impact of hepatitis C in Belgium. *Acta Gastroenterol Belg* 2014; **77**: 285-290 [PMID: 25090835]
28 **Razavi H**, Elkhoury AC, Elbasha E, Estes C, Pasini K, Poynard T, Kumar R. Chronic hepatitis C virus (HCV) disease burden and cost in the United States. *Hepatology* 2013; **57**: 2164-2170 [PMID: 23280550 DOI: 10.1002/hep.26218]
29 **Chan K**, Lai MN, Groessl EJ, Hanchate AD, Wong JB, Clark JA, Asch SM, Gifford AL, Ho SB. Cost effectiveness of direct-acting antiviral therapy for treatment-naive patients with chronic HCV genotype 1 infection in the veterans health administration. *Clin Gastroenterol Hepatol* 2013; **11**: 1503-1510 [PMID: 23707354 DOI: 10.1016/j.cgh.2013.05.014]
30 **Backx M**, Lewszuk A, White JR, Cole J, Sreedharan A, van Sanden S, Diels J, Lawson A, Neal KR, Wiselka MJ, Ito T, Irving WL. The cost of treatment failure: resource use and costs incurred by hepatitis C virus genotype 1-infected patients who do or do not achieve sustained virological response to therapy. *J Viral Hepat* 2014; **21**: 208-215 [PMID: 24438682 DOI: 10.1111/jvh.12132]
31 **Hagan LM**, Yang Z, Ehteshami M, Schinazi RF. All-oral, interferon-free treatment for chronic hepatitis C: cost-effectiveness analyses. *J Viral Hepat* 2013; **20**: 847-857 [PMID: 24304454 DOI: 10.1111/jvh.12111]
32 **Jacobson IM**, Cacoub P, Dal Maso L, Harrison SA, Younossi ZM. Manifestations of chronic hepatitis C virus infection beyond the liver. *Clin Gastroenterol Hepatol* 2010; **8**: 1017-1029 [PMID: 20870037 DOI: 10.1016/j.cgh.2010.08.026]
33 **Hajarizadeh B**, Grebely J, Dore GJ. Epidemiology and natural history of HCV infection. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 553-562 [PMID: 23817321 DOI: 10.1038/nrgastro.2013.107]
34 **Chou R**, Hartung D, Rahman B, Wasson N, Cottrell EB, Fu R. Comparative effectiveness of antiviral treatment for hepatitis C virus infection in adults: a systematic review. *Ann Intern Med* 2013; **158**: 114-123 [PMID: 23437439]
35 **Wei L**, Lok AS. Impact of new hepatitis C treatments in different regions of the world. *Gastroenterology* 2014; **146**: 1145-1150.e1-4 [PMID: 24662488 DOI: 10.1053/j.gastro.2014.03.008]
36 **Carreño V**, Bartolomé J, Castillo I, Quiroga JA. New perspectives in occult hepatitis C virus infection. *World J Gastroenterol* 2012; **18**: 2887-2894 [PMID: 22736911 DOI: 10.3748/wjg.v18.i23.2887]
37 **Castillo I**, Rodríguez-Iñigo E, Bartolomé J, de Lucas S, Ortíz-Movilla N, López-Alcorocho JM, Pardo M, Carreño V. Hepatitis C virus replicates in peripheral blood mononuclear cells of patients with occult hepatitis C virus infection. *Gut* 2005; **54**: 682-685 [PMID: 15831916]
38 **Roque-Afonso AM**, Ducoulombier D, Di Liberto G, Kara R, Gigou M, Dussaix E, Samuel D, Féray C. Compartmentalization of hepatitis C virus genotypes between plasma and peripheral blood mononuclear cells. *J Virol* 2005; **79**: 6349-6357 [PMID: 15858018]
39 **Roque-Cuéllar MC**, Sánchez B, García-Lozano JR, Praena-Fernández JM, Márquez-Galán JL, Núñez-Roldán A, Aguilar-Reina J. Hepatitis C virus-specific cellular immune responses in sustained virological responders with viral persistence in peripheral blood mononuclear cells. *Liver Int* 2014; **34**: e80-e88 [PMID: 24127783 DOI: 10.1111/liv.12320]
40 **Blackard JT**, Kemmer N, Sherman KE. Extrahepatic replication of HCV: insights into clinical manifestations and biological consequences. *Hepatology* 2006; **44**: 15-22 [PMID: 16799966]
41 **Quiroga JA**, Llorente S, Castillo I, Rodríguez-Iñigo E, Pardo M, Carreño V. Cellular immune responses associated with occult hepatitis C virus infection of the liver. *J Virol* 2006; **80**: 10972-10979 [PMID: 17071928]
42 **Carreño V**, Pardo M, López-Alcorocho JM, Rodríguez-Iñigo E, Bartolomé J, Castillo I. Detection of hepatitis C virus (HCV) RNA in the liver of healthy, anti-HCV antibody-positive, serum HCV RNA-negative patients with normal alanine aminotransferase levels. *J Infect Dis* 2006; **194**: 53-60 [PMID: 16741882]
43 **Sagnelli E**, Sagnelli C, Pisaturo M, Coppola N. Hepatic flares in chronic hepatitis C: spontaneous exacerbation vs hepatotropic viruses superinfection. *World J Gastroenterol* 2014; **20**: 6707-6715 [PMID: 24944463 DOI: 10.3748/wjg.v20.i22.6707]
44 **Richiardi L**, De Marco L, Gillio-Tos A, Merletti F, Fiano V, Palli D, Masala G, Agnoli C, Tagliabue G, Panico S, Mattiello A, Tumino R, Frasca G, Vineis P, Sacerdote C. Persistent infection by HCV and EBV in peripheral blood mononuclear cells and risk of non-Hodgkin's lymphoma. *Cancer Epidemiol* 2010; **34**: 709-712 [PMID: 20709616 DOI: 10.1016/j.canep.2010.07.014]
45 **Tillmann HL**. Hepatitis C virus core antigen testing: role in diagnosis, disease monitoring and treatment. *World J Gastroenterol* 2014; **20**: 6701-6706 [PMID: 24944462 DOI: 10.3748/wjg.v20.i22.6701]
46 **Choi YS**, Lee JE, Nam SJ, Park JT, Kim HS, Choi KH, Kim BS, Shin EC. Two distinct functional patterns of hepatitis C Virus (HCV)-specific T cell responses in seronegative, aviremic patients. *PLoS One* 2013; **8**: e62319 [PMID: 23638039 DOI: 10.1371/journal.pone.0062319]
47 **Falcón V**, Acosta-Rivero N, Shibayama M. Evidences of hepatitis C virus replication in hepatocytes and peripheral blood mononuclear cells from patients negative for viral RNA in serum. *Am J Infect Dis* 2005; **1**: 34-42
48 **Zignego AL**, Giannini C, Monti M, Gragnani L. Hepatitis C virus lymphotropism: lessons from a decade of studies. *Dig Liver Dis* 2007; **39** Suppl 1: S38-S45 [PMID: 17936221]
49 **Natarajan V**, Kottilil S, Hazen A, Adelsberger J, Murphy AA, Polis MA, Kovacs JA. HCV in peripheral blood mononuclear cells are predominantly carried on the surface of cells in HIV/HCV co-infected individuals. *J Med Virol* 2010; **82**: 2032-2037 [PMID: 20981790 DOI: 10.1002/jmv.21906]
50 **Angulo J**, Pino K, Pavez C, Biel F, Labbé P, Miquel JF, Soza A, López-Lastra M. Genetic variations in host IL28B links to the detection of peripheral blood mononuclear cells-associated hepatitis C virus RNA in chronically infected patients. *J Viral Hepat* 2013; **20**: 263-272 [PMID: 23490371 DOI: 10.1111/jvh.12076]
51 **Slomski A**. WHO issues guidelines on HCV amid drug cost controversy. *JAMA* 2014; **311**: 2262-2263 [PMID: 24915242 DOI: 10.1001/jama.2014.5277]
52 **van der Meer AJ**, Hansen BE, Fattovich G, Feld JJ, Wedemeyer H, Dufour JF, Lammert F, Duarte-Rojo A, Manns MP, Ieluzzi D,

Zeuzem S, Hofmann WP, de Knegt RJ, Veldt BJ, Janssen HL. Reliable prediction of clinical outcome in patients with chronic HCV infection and compensated advanced hepatic fibrosis: a validated model using objective and readily available clinical parameters. *Gut* 2015; **64**: 322-331 [PMID: 24815676 DOI: 10.1136/gutjnl-2013-305357]

53 **van der Meer AJ**, Veldt BJ, Feld JJ, Wedemeyer H, Dufour JF, Lammert F, Duarte-Rojo A, Manns MP, Zeuzem S, Hofmann WP, de Knegt RJ, Hansen BE, Janssen HL. The number needed to treat to prevent mortality and cirrhosis-related complications among patients with cirrhosis and HCV genotype 1 infection. *J Viral Hepat* 2014; **21**: 568-577 [PMID: 24118177 DOI: 10.1111/jvh.12185]

54 **Shiffman ML**, Benhamou Y. HCV F1/F2 patients: treat now or continue to wait. *Liver Int* 2014; **34** Suppl 1: 79-84 [PMID: 24373082 DOI: 10.1111/liv.12408]

55 **Younossi ZM**, Jiang Y, Smith NJ, Stepanova M, Beckerman R. Ledipasvir/sofosbuvir regimens for chronic hepatitis C infection: Insights from a work productivity economic model from the United States. *Hepatology* 2015; **61**: 1471-1478 [PMID: 25706754 DOI: 10.1002/hep.27757]

56 **Tandon N**, Balart LA, Laliberté F, Pilon D, Lefebvre P, Germain G, Prabhakar A. Impact of completing chronic hepatitis C (CHC) treatment on post-therapy healthcare cost. *J Med Econ* 2014; **17**: 862-871 [PMID: 25215925 DOI: 10.3111/13696998.2014.964720]

P- Reviewer: Enomoto H, Kaya M, Waisberg J **S- Editor**: Qiu S
L- Editor: A **E- Editor**: Wang CH

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i1.41

World J Gastrointest Pharmacol Ther 2016 February 6; 7(1): 41-50
ISSN 2150-5349 (online)

REVIEW

Emerging role of novel biomarkers in the diagnosis of inflammatory bowel disease

Anet A Soubières, Andrew Poullis

Anet A Soubières, Andrew Poullis, Department of Gastroenterology, St George's Hospital, University of London, London SW17 0QT, United Kingdom

Author contributions: Soubières AA wrote the paper; Poullis A supervised and reviewed the paper as senior author.

Conflict-of-interest statement: Authors declare no conflict of interests for this article.

Correspondence to: Dr. Anet A Soubières, Department of Gastroenterology, St George's Hospital, University of London, Blackshaw Road, Tooting, London SW17 0QT, United Kingdom. anet.soubieres@gmail.com
Telephone: +44-208-7253811
Fax: +44-208-7253520

Received: April 28, 2015
Peer-review started: May 7, 2015
First decision: September 14, 2015
Revised: October 6, 2015
Accepted: November 10, 2015
Article in press: November 11, 2015
Published online: February 6, 2016

Abstract

There is currently no gold standard test for the diagnosis of inflammatory bowel disease (IBD). Physicians must rely on a number of diagnostic tools including clinical and endoscopic evaluation as well as histologic, serologic and radiologic assessment. The real difficulty for physicians in both primary and secondary care is differentiating between patients suffering from functional symptoms and those with true underlying IBD. Alongside this, there is always concern regarding the possibility of a missed, or delayed diagnosis of ulcerative colitis (UC) or Crohn's disease. Even once the diagnosis of IBD has been made, there is often uncertainty in distinguishing between cases of UC or Crohn's. As a consequence, in cases of incorrect diagnosis, optimal treatment and management may be adversely affected. Endoscopic evaluation can be uncomfortable and inconvenient for patients. It carries significant risks including perforation and in terms of monetary cost, is expensive. The use of biomarkers to help in the diagnosis and differentiation of IBD has been increasing over time. However, there is not yet one biomarker, which is sensitive of specific enough to be used alone in diagnosing IBD. Current serum testing includes C-reactive protein and erythrocyte sedimentation rate, which are cheap, reliable but non-specific and thus not ideal. Stool based testing such as faecal calprotectin is a much more specific tool and is currently in widespread clinical use. Non-invasive sampling is of the greatest clinical value and with the recent advances in metabolomics, genetics and proteomics, there are now more tools available to develop sensitive and specific biomarkers to diagnose and differentiate between IBD. Many of these new advances are only in early stages of development but show great promise for future clinical use.

Key words: Biomarkers; Inflammatory bowel disease; Ulcerative colitis; Crohn's disease; Indeterminate colitis

Core tip: There is no gold standard test in the diagnosis of inflammatory bowel disease (IBD). Physicians must take into account clinical, endoscopic, and radiologic

as well as serologic and histologic evidence in order to correctly diagnose their patients. Endoscopic evaluation is not only expensive, but is uncomfortable for patients and not without significant risk such as perforation. The use of biomarkers to help in the diagnosis and sub classification of IBD is an expanding area. In this review we touch on those non-invasive markers currently in clinical use before focusing on those more novel tests, with the potential to be highly useful in both diagnosis and differentiation of IBD.

Soubières AA, Poullis A. Emerging role of novel biomarkers in the diagnosis of inflammatory bowel disease. *World J Gastrointest Pharmacol Ther* 2016; 7(1): 41-50 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i1/41.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i1.41

INTRODUCTION

The European evidence-based Consensus on the diagnosis and management of inflammatory bowel disease (IBD) states that diagnosis should rely on physicians taking into account a number of factors including clinical and endoscopic evaluation as well as histologic, serologic and radiologic assessment[1,2]. There is no gold standard diagnostic tool.

Abdominal pain with, or without a change in bowel habit is a common presenting symptom in primary care. A majority of these patients will be suffering from functional bowel disorders including functional dyspepsia and irritable bowel syndrome. Indeed, functional bowel disorders make up a significant proportion of referrals to gastroenterology outpatient clinics (up to 60%)[3].

The dilemma for physicians is distinguishing a patient with functional symptoms from one with an underlying diagnosis of IBD. Up to 50% of patients with a functional diagnosis are referred on for unnecessary endoscopic evaluation[3].

Conversely, there is also often a delay in diagnosis of cases of true Crohn's disease (CD) and ulcerative colitis (UC), (*i.e.,* time from onset of symptoms to diagnosis). This delay is more marked in the case of ileal CD[4].

Even once a diagnosis of IBD is made, there can still be uncertainty with regard to sub classification into either CD or UC. This is essential, as optimal treatment and management of both conditions is different.

Making this differential diagnosis between CD and UC can be difficult and around 10% of patients are labelled as having an indeterminate colitis (IC)[5].

It is thus clear that even with current available diagnostic tools, as physicians, we still struggle to make accurate diagnoses.

Any investigative test must be acceptable in terms of both cost and comfort to patients. Endoscopic evaluation is not only often uncomfortable as well as expensive, but can be related to significant risk, such as perforation. One recent French study found a rate of between 4.5 and 9.7 cases of perforation per 10000 patients[6].

Radiologic imaging, perhaps most useful in the investigation of small bowel pathology, also has its drawbacks with regard to inter and intra-observer variability, and obviously does not allow for histological sampling[7].

The use of biomarkers to aid the diagnosis of IBD is an ever-expanding investigative area.

A biomarker has been defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a [n]...intervention". Example: Cholesterol level[8].

As of yet, there is no one biomarker, which is sensitive or specific enough to make a confident diagnosis of IBD on its result alone. Many are indicative of systemic inflammation and so have limitations in their use.

This review will touch on those already well established in their use before focusing on more recent advances in the development of novel biomarkers for both the diagnosis and monitoring of IBD.

SCOPE OF THIS REVIEW

This review will focus on more recent advances in the development of novel biomarkers for both the diagnosis and monitoring of IBD.

A large number of biomarkers have been reported in the literature.

We have chosen to consider non-invasively obtained biomarkers, as those that are more acceptable to patients, and thus, most promising with regards to clinical utility.

LITERATURE SEARCH

This review of the English language literature on novel biomarkers in the diagnosis of IBD is based on papers contained within the PubMed database. Individual searches of the PubMed database were performed with the boolean operator AND, using the terms: "biomarker", "inflammatory bowel disease", "Crohn's disease", and "ulcerative colitis".

The abstracts were screened for eligibility and all relevant publications were requested as full-text articles. References used in requested papers were then checked for any further studies of potential interest.

BIOMARKERS IN WIDESPREAD USE

Blood based

C-reactive protein: C-reactive protein (CRP) is produced by hepatocytes in response to inflammation, stimulated by certain cytokines. In the case of active

IBD, these cytokines include tumour necrosis factor-alpha (TNF-α), interleukin (IL)-6 and IL-1β[9].

During active IBD, CRP may rise significantly. However, it is not specific and can go up in a variety of conditions including infection, autoimmune conditions, other inflammatory conditions, and malignancy as well as cell necrosis[10].

Elevations in CRP may vary from person to person depending on the individual's immune response; however, it has been shown that rises in CRP are more common in CD rather than UC. The reason for this is unclear, but may have to do with the deeper, more penetrating inflammation in CD compared with the superficial mucosal inflammation seen in UC. It has also been suggested that disease location, independent of severity may affect the level of rise in CRP[11].

In patients with known IBD, rises in CRP have been shown to correlate with active disease on colonoscopy and severe inflammation on histology, hence can be useful in distinguishing active from quiescent IBD[12].

Erythrocyte sedimentation rate: The erythrocyte sedimentation rate (ESR), like CRP is a measure of systemic inflammation and not entirely specific to IBD.

The test measures the distance that erythrocytes have fallen in 1 h in a vertical column of non-coagulated blood[13]. In comparison to CRP, ESR levels peak later and decrease at a slower rate. In view of this, ESR is better at monitoring disease activity/response to treatment after the first 24 h of onset whilst CRP may be more useful in the first 24 h.

ESR is still very commonly used in monitoring of IBD, despite it usefulness being quite limited. It is influenced by a number of factors including age, gender, anaemia, blood dyscrasias and pregnancy[14].

Yoon *et al*[15] found that with regard to correlation with endoscopic activity, both CRP and ESR levels correlated only modestly and that the low sensitivities for detecting endoscopic remission suggest that CRP or ESR alone is not sufficient to reflect endoscopic severity accurately.

Another, more recent meta-analysis found that no level of ESR was predictive of IBD. The highest predictive probability of IBD was reported as 1.6% at an ESR level of 200 mm/h[16].

Antineutrophil cytoplasmic antibodies: Antineutrophil cytoplasmic antibodies (ANCAs) are antibodies against granules of neutrophil cytoplasm. They are detected using indirect immunofluorescence (IIF) and show three main staining patterns: The cytoplasmic (cANCA), the speckled (sANCA) and the perinuclear (pANCA). Perinuclear ANCA (pANCA) has been shown to increase significantly in UC[17].

Joossens *et al*[18] found in their prospective follow-up study that 64% of UC patients were positive for pANCA [and anti-*Saccharomyces cerevisiae* antibody (ASCA) negative]. A further study calculated the rate of pANCA to be 55% in UC and 32% in healthy controls[19].

In UC, the presence of atypical pANCAs has been associated with resistance to treatment of left-sided disease and early surgery. This suggests a role in using the presence of pANCA to identify those UC patients who may require earlier intervention with immunomodulators[20].

ASCA: ASCA are antibodies for mannan in the cell wall of *Saccharomyces cerevisiae* (*S. cerevisiae*)[21].

In comparison to pANCA, which is found in higher titres in UC, high ASCA levels are more specific for CD. Using the combination test ASCA+/pANCA-, one meta-analysis of 60 studies looking at 7860 IBD patients and 3748 controls demonstrated the ability to differentiate adults with CD from those with UC with 55% sensitivity and 93% specificity[22]. Levels have also been associated with phenotypes corresponding to ileal disease, young age at onset, stricturing, as well as penetrating behavior and multiple bowel surgery[23].

Despite high specificity levels, the low sensitivity of ASCA/pANCA testing has prevented its routine clinical use in distinguishing between CD and UC.

Stool based

Faecal calprotectin: Calprotectin is a zinc and calcium binding protein belonging to the S100 family that is derived mostly from neutrophils and monocytes, and has also been detected in activated macrophages[24].

Calprotectin is found in serum, saliva, cerebrospinal fluid, urine and faeces[25]. It is an extremely stable protein, and can be found unaltered in stool samples left unprepared for longer than 7 d.

When the inflammatory process is triggered calprotectin is released due to degranulation of neutrophils, making it very specific for gastrointestinal inflammation[26].

Many studies in the literature have focused on faecal calprotectin (FCP) in terms of accuracy in diagnosis and monitoring of IBD. It has now become a widely used test since it was first described in 1980[27]. One meta-analysis calculated sensitivity and specificity of FCP of up to 95% and 91% respectively. In addition they showed that FCP outperformed other serological markers including CRP and ESR[28].

The National Institute for Health and Care Excellence (NICE) recommends the use of FCP as a diagnostic tool to help in the differential diagnosis of IBD and irritable bowel syndrome (IBS)[29]. When used in this way in both primary and secondary care, it may help reduce the number of referrals for unnecessary endoscopic evaluation. One meta-analysis of 13 studies concluded that FCP testing would result in a 67% reduction in the number of adults requiring endoscopy, but with a delayed diagnosis in 8% of adults because of false negative results[30]. One area of controversy surrounding FCP testing is the determination of an

appropriate cut-off value, above which the result is deemed as positive. In most centres, a relatively low level of 50 μg/g is used.

Pavlidis *et al*[31] looked at this issue in a cohort of adult patients undergoing faecal calprotectin testing in primary care. At a cut off of 50 μg/g, FCP testing had a negative predictive value (NPV) of 98% and positive predictive value (PPV) of 28%. Increasing the cut off value to 150 μg/g gave a very comparable negative NPV of 97%, but a much higher PPV of 71%.

Given these values, it was calculated that by increasing the cut off value to 150 μg/g, this would reduce colonoscopy and flexible sigmoidoscopy bookings by 10% at the cost of 4 missed cases of IBD (n = 686)[31].

Faecal lactoferrin: Lactoferrin is an iron-binding protein; it covers most mucosal surfaces. It is found within neutrophil granulocytes and becomes activated in acute inflammation[32]. Similar to faecal calprotectin, it is stable for up to 5 d in faeces. Levels of faecal lactoferrin increase significantly as neutrophils infiltrate the gastrointestinal tract[33]. Levels of faecal lactoferrin have been found to be significantly higher in active IBD than in inactive IBD, IBS and infectious bowel disease. One study reported the sensitivity and specificity of fecal lactoferrin as 92% and 88%, respectively, for UC, and 92% and 80%, respectively, for CD[34].

Sidhu *et al*[35] looked at the relationship between faecal lactoferrin levels in small bowel Crohn's in patients undergoing capsule endoscopy. They found positive predictive and negative predictive values of 100% and 83% respectively for faecal lactoferrin in the diagnosis of small bowel CD detected by capsule endoscopy.

Much like faecal calprotectin, faecal lactoferrin is a sensitive and specific marker in measuring IBD activity. It can help in discriminating between inflammatory and non-IBD as well allowing for the exclusion of IBS in the case of elevated levels.

Previously studied faecal biomarkers: Other faecal markers implemented in the diagnosis, assessment of severity and monitoring of response to therapy in IBD include neopterin and polymorphonuclear neutrophil (PMN)-elastase. Nancey *et al*[36] found faecal neopterin to correlate better with endoscopic activity compared with CRP. The authors also found neopterin to be as accurate as faecal calprotectin in the prediction and monitoring of severity of mucosal damage in IBD.

PMN-elastase has been shown to be able to differentiate active IBD from inactive IBD as well as from IBS, with a diagnostic accuracy of 74.1%, higher than that of CRP (64%)[37].

S100A12 is part of the calcium binding protein family (similar to FCP) and is a stimulator of pro-inflammatory mediators. It is also stable in room temperature for up to 7 d[38].

S100A12 has been shown to have sensitivity and specificity levels of up to 86% and 96% respectively, higher than FCP. It has also been shown to correlate better with intestinal inflammation in comparison to other biomarkers[39] as well as having the potential to be used in monitoring response to therapy[38].

However, despite its promise, S100A12 is not used routinely in practice, as more studies need to confirm its use in IBD evaluation.

Emerging novel blood based markers

Anti-outer membrane protein C: Anti-outer membrane protein C (anti-OmpC) is an antibody directed against the outer membrane porin C transport protein of *Escherichia coli*. Anti-OmpC has been reported in 55% of CD patients[40], whilst in UC and healthy controls, rates were insignificant.

It has been suggested that Anti-OmpC may be of value to aid diagnosis of ASCA negative CD patients. In those patients who are ASCA negative, the prevalence of anti-OmpC has been reported as 5%-15%[41].

Antibodies to flagellin: Identification of commensal bacterial proteins in colitic mice has found the dominant antigens to be flagellins. A strong immune response was seen in one particular flagellin, anti-CBir1. Percent of 50 patients with CD were found to have IgG reactivity to CBir1 in comparison to 6% of UC patients and 8% of healthy controls[42].

In atypical pANCA positive CD patients, 40%-44% have been found to be positive for anti-CBir1 in comparison to only 4% of atypical pANCA positive UC patients.

Thus, the detection of anti-CBir1 may help in the differentiation between atypical pANCA positive CD and UC patients, independently of ASCA[43].

In addition, anti-CBir1 antibody has been found to be associated with ileal involvement in CD patients independent of other serologic markers and has been suggested to predispose to stenosing and penetrating disease in CD[42].

More recently, Schoepfer *et al*[44] demonstrated reactivity towards two new anti-flagellins, anti-A4-Fla2 and anti-Fla-X in 59% and 57% of CD patients as compared to only 6% of UC patients, suggesting a possible role in distinguishing CD from UC.

Anti-I2 antibody: A fragment of bacterial DNA (I2), has been identified from lamina propria mononuclear cells in active CD and shown to be associated with *Pseudomonas fluorescens*[45].

Anti-I2 positivity has been reported as 30%-50% in CD, 2%-10% in UC, 36%-42% in indeterminate colitis and 4%-8% of healthy controls. Anti-I2 has also been found in patients with other inflammatory enteritis (19%)[40,46].

Anti-carbohydrate antibodies: Patients with CD have been found to express antibodies to cell wall carbohydrate epitopes found in different pathogenic

bacteria and fungi. These anti-glycan antibodies include anti-laminaribioside carbohydrate antibody (ALCA) (18%-38%), anti-chitobioside carbohydrate antibody (ACCA) (21%-36%), and anti- mannobioside carbohydrate antibody (AMCA) (28%). ALCA, ACCA and AMCA have been found in 18%-38%, 21%-36% and 28% of CD patients respectively[45-47].

Ferrante *et al*[48] found that patients with CD who were positive for at least one of ALCA, ACCA or gASCA (similar to ASCA) could be differentiated from UC patients with a 77% sensitivity and > 90% specificity. In the differentiation of CD patients from healthy controls however, the specificity fell to 70.3%.

Overall, the sensitivity of these anti-glycan antibodies has been found to be low by a number of studies, which is a limiting factor in their clinical use[48-53].

Pancreatic antibodies: Antigen-specific pancreatic antibodies (PABs) against exocrine pancreas have been found to be present in 20%-30% of patients with CD, but in less than 2%-9% of patients with UC, and can be found in very few patients with non-IBD related conditions[54,55].

The major zymogen glycoprotein 2 (MZGP2) has recently been identified as the primary autoantigen of PAB[56] and has prompted the development of techniques to allow for its identification in routine practice.

A study from Pavlidis *et al*[57] in 2014 assessed the clinical relevance of PABs by way of a novel ELISA technique in the largest IBD cohort tested in this way to date. They were able to confirm the high specificity of anti-MZGP2 antibodies for CD and their association with disease severity phenotypes. IgA anti-MZGP2 antibodies were more prevalent in CD patients with early disease onset (P = 0.011). In addition, anti-MZGP2 positive patients more frequently had extensive disease with ileal involvement. Patients with longer disease duration were more likely to have IgG anti-MZGP2 antibodies[57].

Alpha-1 antitrypsin and granulocyte colony-stimulating factor: Soendergaard *et al*[58] looked at serum samples from 65 patients with UC with varying disease activity and from 40 healthy controls. They measured levels of both alpha -1 antitrypsin (AAT) and granulocyte colony-stimulating factor (G-CSF).

AAT levels were able to differentiate between mild, moderate and severe UC, performing better than CRP.

In addition, the authors found that combination measurement of AAT and G-CSF in patients with diagnosed UC held enough statistical power to differentiate between patients with mild, moderate, and severe disease activity.

Genetics

In the recent past, a number of genome wide association studies (GWAS) have discovered a number of susceptibility loci in the investigation of UC and CD-specific genomic profiles.

Ellinghaus *et al*[59] found that variants in two genes, PRDM1 and NDP52 determined susceptibility to CD. PRDM1 was found adjacent to a CD interval identified in GWAS and encodes a transcription factor expressed by T and B cells. *NDP52* encodes a protein functioning in autophagy of intracellular bacteria and signaling molecules, supporting the role of autophagy in the pathogenesis of CD.

The IBD chip European project looked at a number of CD-single nucleotide polymorphisms to determine their influence on clinical course and phenotype of the disease. The *NOD2* gene was found to be the most important genetic factor, being an independent predictive factor for ileal location, stenosing and penetrating CD. It was also associated with a more complicated disease course and the need for surgery[60].

A further recent meta-analysis of CD and UC GWAS reported on significant findings from more than 75000 cases and controls. The authors identified 71 new associations increasing the total number of confirmed IBD susceptibility loci up to 163. They found that most loci contributed to both phenotypes. Interestingly, there was also considerable overlap between susceptibility loci for IBD and mycobacterial infection, suggesting pathways shared between host responses to mycobacteria and those predisposing to IBD[61].

Traditionally, CD has been associated with a Th1 cytokine profile, and UC with Th2 cytokines. However this concept has been since challenged by the discovery of Th17 cells and Treg cells. GWAS indicate that IL23R and five additional genes involved in Th17 differentiation (IL12B, JAK2, STAT3, CCR6 and TNFSF15) are associated with susceptibility to CD and partly also to UC[62].

In terms of the clinical application of genetics in the diagnosis of IBD, some focus has been made on identifying genetic markers from colonic tissue retrieved from endoscopic biopsy. von Stein *et al*[63] identified seven genes as differentially expressed in IBD, making it possible to discriminate between patients suffering from UC, CD, or IBS (P < 0.0001) using the clinical diagnosis as gold standard.

Much more recently, following on from this work, this same genetic panel was tested on biopsy material from 78 patients with a complicated course (38 probably UC, 18 CD, 22 IBDU). Testing led to a change of the primary diagnosis in a significant number of patients with the initial diagnosis of UC and CD and suggested a clinically probable diagnosis in most of the patients with IBDU and in those with an acute flare of colitis[64].

Epigenetics

Epigenetics describes gene-environment interactions affecting gene expression but with no changes in the

DNA sequence.

Micro-RNAs (miRs) are single-stranded noncoding RNAs, around 22 nucleotides in length that remain highly conserved throughout evolution[65]. Since they were first described in the 1990s, over 1600 miRs have been described in humans. miRs are transcribed by RNA polymerase into pre-miR, which is then processed in the nucleus and then cytoplasm. miRs regulate gene expression and thus a number of biological processes such as cell proliferation, differentiation and death. Changes in miR expression have been associated with a number of diseases including IBD[66].

Studies have looked at miR profiles in peripheral blood samples from patients with IBD *vs* controls and in CD patients *vs* UC patients. Several miRs have been found to be either up or down regulated. One paediatric study also found differentially expressed levels of certain miRs between serum samples from children with CD compared with healthy controls[67,68].

A recent paper from Schaefer *et al*[69] found CD was associated with altered expression of 6 miRNAs while UC was associated with 9 miRNAs in whole blood. They also found altered expression of different miRNAs in saliva from both UC and CD patients.

They suggest that there are specific miRNA expression patterns associated with UC *vs* CD, and hence that scrutinizing miRNA expression in saliva and blood samples may be beneficial in monitoring or diagnosing disease in IBD patients.

Metabolomics

Metabolomics refers to the study of the many small molecule metabolites present in biological samples, in order to determine the underlying fingerprint of specific cellular processes.

The current main technologies used for metabolomics include ^{1}H NMR spectroscopy, gas chromatography spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS). These techniques have the advantage of being extremely sensitive and of allowing experiments to be performed in a cost-effective high-throughput manner[70,71].

^{1}H NMR spectroscopy has so far been most widely used in studies on different biofluids from IBD patients. A number of studies have reported differences in metabolic profiles between IBD patients and healthy controls as well as between CD and UC[72,73].

These studies described have mainly focused on the detection of amino acids, TCA cycle intermediates, and on metabolites involved in fatty acid and purine metabolism.

Metabolites of gut bacteria have been detected in urine[74]. Any change in the gut microbiome, which has been shown to be important in the pathogenesis of IBD, may alter the urinary metabolic profile. Thus, urinary metabolites are an attractive option as potential biomarkers for IBD[75].

A study by Williams *et al*[76] looked at the urinary metabolic profiles of CD and UC patients using ^{1}H NMR spectroscopy. They found significant decreases in the levels of hippurate (a metabolite derived from microbiota) in IBD patients.

Other studies have also demonstrated low levels of hippurate in IBD patients using ^{1}H NMR spectroscopy and in addition, have been able to separate between IBD patients and healthy controls[72,77].

Studies have shown that metabolic profiling of serum and plasma by way of ^{1}H NMR spectroscopy is able to discriminate between UC and CD although less reliably than discrimination between UC/CD and healthy controls[72,73].

Further studies have found that profiling of amino acid and TCA cycle-related metabolites can distinguish reliably between UC and CD[78] and also that correlation of metabolic profiles of amino acids with disease activity, suggesting a role in monitoring of IBD[79].

The metabolic profiling of faecal extracts in IBD has shown significantly decreased levels of short chain fatty acids in comparison to healthy controls[80].

Profiling of the gut microbiota as well as the metabolites from faecal extracts may also give further indications to disturbances of gut bacteria in IBD and hence pathogenesis of the disease[81].

Another advance in the field of metabolomics and IBD is the use of breath testing as a potential biomarker.

A recent review by Kurada *et al*[82] found only 12 (small) studies in the literature, which evaluated the breath metabolome for diagnosis of IBD. In the case of diagnosis and differentiation of IBD, the volatile organic compounds (VOCs) measured in these studies included mainly pentane, ethane, propane, butane or nitric oxide (NO).

Dryahina *et al*[83] demonstrated elevated levels of pentane in IBD (CD > UC) compared to healthy controls, as did Pelli *et al*[84].

In addition, Pelli *et al*[84] also showed an association between both ethane and propane levels and IBD ($P \leqslant 0.001$ for both).

Exhaled NO has been shown to be higher in UC patients compared with CD[85].

With regard to disease activity, one study found a direct correlation between breath pentane levels and WBC scan uptake[86]. Ethane levels have also been shown to correlate with endoscopic activity of disease[87].

Although there have been some promising results from studies, breath analysis is not yet ready for clinical use. Further work is needed to determine the exact breath metabolome patterns in IBD.

Proteomics

Proteomics is a more recently advancing area in the identification of new biomarkers. It is based on the analysis of protein expression in healthy and diseased tissues and to carry out protein profiling.

Meuwis *et al*[88] looked at the sera of 120 patients (30 CD, 30 UC, 30 inflammatory controls and 30 healthy controls). They identified 4 proteins of acute phase inflammation (PF4, MRP8, FIBA and Hpα2).

A much more recent study looked at circulating protein biomarkers in the interleukin-10 knockout [IL-10(-/-)] mouse, a model that develops a time-dependent IBD-like disorder that predominates in the colon[89]. They identified a total of 15 different proteins to be differentially accumulated in serum samples from mid- to late-stage IL-10(-/-) mice compared to early non-inflamed IL-10(-/-) mice, suggesting a role for protein profiling in assessing severity and response to treatment.

CONCLUSION

There is a need for more accurate and cost effective biomarkers in the diagnosis and differentiation of IBD. Development of non-invasive biomarkers is paramount in order to be acceptable to patients and to avoid more invasive assessment, such as endoscopy, which is not without risk.

Current serum testing includes CRP and ESR, which are cheap, reliable but non-specific and thus not ideal. Stool based testing such as faecal calprotectin is a much more specific tool and has now a lot of positive evidence behind it to support its use clinically.

It should be highlighted that as of yet, and despite recent advances, there is no biomarker reliable enough to make a confident diagnosis of IBD without going on, in the case of a positive test, to perform confirmatory colonoscopy. Rather, these non-invasive tests are used currently as an adjuvant to endoscopic evaluation; and to avoid unnecessary procedures where a negative test would indicate no underlying inflammation and no pathology of any cause.

Non-invasive sampling is of the greatest clinical value and with the recent advances in metabolomics, genetics and proteomics, there are now more tools available to develop sensitive and specific biomarkers to diagnose and differentiate between IBD.

This review has touched on the great advances, which have been made in the ever-expanding area of biomarkers in IBD. However, more work is now required to help bring these new techniques into everyday clinical practice.

REFERENCES

1 **Van Assche G**, Dignass A, Panes J, Beaugerie L, Karagiannis J, Allez M, Ochsenkühn T, Orchard T, Rogler G, Louis E, Kupcinskas L, Mantzaris G, Travis S, Stange E. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Definitions and diagnosis. *J Crohns Colitis* 2010; **4**: 7-27 [PMID: 21122488 DOI: 10.1016/j.crohns.2009.12.003]

2 **Dignass A**, Eliakim R, Magro F, Maaser C, Chowers Y, Geboes K, Mantzaris G, Reinisch W, Colombel JF, Vermeire S, Travis S, Lindsay JO, Van Assche G. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 1: definitions and diagnosis. *J Crohns Colitis* 2012; **6**: 965-990 [PMID: 23040452 DOI: 10.1016/j.crohns.2012.09.003]

3 **British Society of Gastroenterology**. Chronic management: IBS/Functional. Available from: URL: http://www.bsg.org.uk/clinical/commissioning-report/ibs/functional-symptoms.html

4 **Vavricka SR**, Spigaglia SM, Rogler G, Pittet V, Michetti P, Felley C, Mottet C, Braegger CP, Rogler D, Straumann A, Bauerfeind P, Fried M, Schoepfer AM. Systematic evaluation of risk factors for diagnostic delay in inflammatory bowel disease. *Inflamm Bowel Dis* 2012; **18**: 496-505 [PMID: 21509908 DOI: 10.1002/ibd.21719]

5 **Tremaine WJ**. Is indeterminate colitis determinable? *Curr Gastroenterol Rep* 2012; **14**: 162-165 [PMID: 22314810 DOI: 10.1007/s11894-012-0244-x]

6 **Blotière PO**, Weill A, Ricordeau P, Alla F, Allemand H. Perforations and haemorrhages after colonoscopy in 2010: a study based on comprehensive French health insurance data (SNIIRAM). *Clin Res Hepatol Gastroenterol* 2014; **38**: 112-117 [PMID: 24268997 DOI: 10.1016/j.clinre.2013.10.005]

7 **Jensen MD**, Ormstrup T, Vagn-Hansen C, Østergaard L, Rafaelsen SR. Interobserver and intermodality agreement for detection of small bowel Crohn's disease with MR enterography and CT enterography. *Inflamm Bowel Dis* 2011; **17**: 1081-1088 [PMID: 21484959 DOI: 10.1002/ibd.21534]

8 **Biomarkers Definitions Working Group**. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther* 2001; **69**: 89-95 [PMID: 11240971 DOI: 10.1067/mcp.2001.113989]

9 **Darlington GJ**, Wilson DR, Lachman LB. Monocyte-conditioned medium, interleukin-1, and tumor necrosis factor stimulate the acute phase response in human hepatoma cells in vitro. *J Cell Biol* 1986; **103**: 787-793 [PMID: 3017995 DOI: 10.1083/jcb.103.3.787]

10 **Pepys MB**, Hirschfield GM. C-reactive protein: a critical update. *J Clin Invest* 2003; **111**: 1805-1812 [PMID: 12813013 DOI: 10.1172/JCI200318921]

11 **Florin TH**, Paterson EW, Fowler EV, Radford-Smith GL. Clinically active Crohn's disease in the presence of a low C-reactive protein. *Scand J Gastroenterol* 2006; **41**: 306-311 [PMID: 16497618 DOI: 10.1080/00365520500217118]

12 **Solem CA**, Loftus EV, Tremaine WJ, Harmsen WS, Zinsmeister AR, Sandborn WJ. Correlation of C-reactive protein with clinical, endoscopic, histologic, and radiographic activity in inflammatory bowel disease. *Inflamm Bowel Dis* 2005; **11**: 707-712 [PMID: 16043984 DOI: 10.1097/01.MIB.0000173271.18319.53]

13 **Gabay C**, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 1999; **340**: 448-454 [PMID: 9971870 DOI: 10.1056/NEJM199902113400607]

14 **Mendoza JL**, Abreu MT. Biological markers in inflammatory bowel disease: practical consideration for clinicians. *Gastroenterol Clin Biol* 2009; **33** Suppl 3: S158-S173 [PMID: 20117339 DOI: 10.1016/S0399-8320(09)73151-3]

15 **Yoon JY**, Park SJ, Hong SP, Kim TI, Kim WH, Cheon JH. Correlations of C-reactive protein levels and erythrocyte sedimentation rates with endoscopic activity indices in patients with ulcerative colitis. *Dig Dis Sci* 2014; **59**: 829-837 [PMID: 24352705 DOI: 10.1007/s10620-013-2907-3]

16 **Menees SB**, Powell C, Kurlander J, Goel A, Chey WD. A meta-analysis of the utility of C-reactive protein, erythrocyte sedimentation rate, fecal calprotectin, and fecal lactoferrin to exclude inflammatory bowel disease in adults with IBS. *Am J Gastroenterol* 2015; **110**: 444-454 [PMID: 25732419 DOI: 10.1038/ajg.2015.6]

17 **Prideaux L**, De Cruz P, Ng SC, Kamm MA. Serological antibodies in inflammatory bowel disease: a systematic review. *Inflamm Bowel Dis* 2012; **18**: 1340-1355 [PMID: 22069240 DOI: 10.1002/ibd.21903]

18 **Joossens S**, Reinisch W, Vermeire S, Sendid B, Poulain D, Peeters M, Geboes K, Bossuyt X, Vandewalle P, Oberhuber G, Vogelsang H, Rutgeerts P, Colombel JF. The value of serologic markers in indeterminate colitis: a prospective follow-up study. *Gastroenterology* 2002; **122**: 1242-1247 [PMID: 11984510 DOI: 10.1053/gast.2002.32980]

19 **Bernstein CN**, El-Gabalawy H, Sargent M, Landers C, Rawsthorne P, Elias B, Targan SR. Assessing inflammatory bowel disease-associated antibodies in Caucasian and First Nations cohorts. *Can J Gastroenterol* 2011; **25**: 269-273 [PMID: 21647462]

20 **Sandborn WJ**, Landers CJ, Tremaine WJ, Targan SR. Association of antineutrophil cytoplasmic antibodies with resistance to treatment of left-sided ulcerative colitis: results of a pilot study. *Mayo Clin Proc* 1996; **71**: 431-436 [PMID: 8628021 DOI: 10.4065/71.5.431]

21 **Sendid B**, Colombel JF, Jacquinot PM, Faille C, Fruit J, Cortot A, Lucidarme D, Camus D, Poulain D. Specific antibody response to oligomannosidic epitopes in Crohn's disease. *Clin Diagn Lab Immunol* 1996; **3**: 219-226 [PMID: 8991640]

22 **Reese GE**, Constantinides VA, Simillis C, Darzi AW, Orchard TR, Fazio VW, Tekkis PP. Diagnostic precision of anti-Saccharomyces cerevisiae antibodies and perinuclear antineutrophil cytoplasmic antibodies in inflammatory bowel disease. *Am J Gastroenterol* 2006; **101**: 2410-2422 [PMID: 16952282 DOI: 10.1111/j.1572-0241.2006.00840.x]

23 **Vandewalle-El Khoury P**, Colombel JF, Joossens S, Standaert-Vitse A, Collot M, Halfvarson J, Ayadi A, Landers CJ, Vermeire S, Rutgeerts P, Targan SR, Chamaillard M, Mallet JM, Sendid B, Poulain D. Detection of antisynthetic mannoside antibodies (ASigmaMA) reveals heterogeneity in the ASCA response of Crohn's disease patients and contributes to differential diagnosis, stratification, and prediction. *Am J Gastroenterol* 2008; **103**: 949-957 [PMID: 18047546 DOI: 10.1111/j.1572-0241.2007.01648.x]

24 **Poullis A**, Foster R, Mendall MA, Fagerhol MK. Emerging role of calprotectin in gastroenterology. *J Gastroenterol Hepatol* 2003; **18**: 756-762 [PMID: 12795745 DOI: 10.1046/j.1440-1746.2003.03014.x]

25 **Tibble JA**, Bjarnason I. Fecal calprotectin as an index of intestinal inflammation. *Drugs Today* (Barc) 2001; **37**: 85-96 [PMID: 12783101 DOI: 10.1358/dot.2001.37.2.614846]

26 **Boussac M**, Garin J. Calcium-dependent secretion in human neutrophils: a proteomic approach. *Electrophoresis* 2000; **21**: 665-672 [PMID: 10726775 DOI: 10.1002/(SICI)1522-2683(20000201)21:3]

27 **Fagerhol MK**, Dale I, Andersson T. A radioimmunoassay for a granulocyte protein as a marker in studies on the turnover of such cells. *Bull Eur Physiopathol Respir* 1980; **16** Suppl: 273-282 [PMID: 7225633]

28 **von Roon AC**, Karamountzos L, Purkayastha S, Reese GE, Darzi AW, Teare JP, Paraskeva P, Tekkis PP. Diagnostic precision of fecal calprotectin for inflammatory bowel disease and colorectal malignancy. *Am J Gastroenterol* 2007; **102**: 803-813 [PMID: 17324124 DOI: 10.1111/j.1572-0241.2007.01126.x]

29 **National Institute for Health and Care Excellence**. Faecal calprotectin diagnostic tests for inflammatory diseases of the bowel. *NICE Diagnostics Guidance* 2013; **11**: 1- 58

30 **van Rheenen PF**, Van de Vijver E, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis. *BMJ* 2010; **341**: c3369 [PMID: 20634346 DOI: 10.1136/bmj.c3369]

31 **Pavlidis P**, Chedgy FJ, Tibble JA. Diagnostic accuracy and clinical application of faecal calprotectin in adult patients presenting with gastrointestinal symptoms in primary care. *Scand J Gastroenterol* 2013; **48**: 1048-1054 [PMID: 23883068 DOI: 10.3109/00365521.2013.816771]

32 **Kane SV**, Sandborn WJ, Rufo PA, Zholudev A, Boone J, Lyerly D, Camilleri M, Hanauer SB. Fecal lactoferrin is a sensitive and specific marker in identifying intestinal inflammation. *Am J Gastroenterol* 2003; **98**: 1309-1314 [PMID: 12818275 DOI: 10.1111/j.1572-0241.2003.07458.x]

33 **Desai D**, Faubion WA, Sandborn WJ. Review article: biological activity markers in inflammatory bowel disease. *Aliment Pharmacol Ther* 2007; **25**: 247-255 [PMID: 17217454 DOI: 10.1111/j.1365-2036.2006.03184.x]

34 **Dai J**, Liu WZ, Zhao YP, Hu YB, Ge ZZ. Relationship between fecal lactoferrin and inflammatory bowel disease. *Scand J Gastroenterol* 2007; **42**: 1440-1444 [PMID: 17852860 DOI: 10.1080/00365520701427094]

35 **Sidhu R**, Sanders DS, Wilson P, Foye L, Morley S, McAlindon ME. Faecal lactoferrin, capsule endoscopy and Crohn's disease. Is there a three way relationship? A pilot study. *J Gastrointestin Liver Dis* 2010; **19**: 257-260 [PMID: 20922188]

36 **Nancey S**, Boschetti G, Moussata D, Cotte E, Peyras J, Cuerq C, Haybrard J, Charlois AL, Mialon A, Chauvenet M, Stroeymeyt K, Kaiserlian D, Drai J, Flourié B. Neopterin is a novel reliable fecal marker as accurate as calprotectin for predicting endoscopic disease activity in patients with inflammatory bowel diseases. *Inflamm Bowel Dis* 2013; **19**: 1043-1052 [PMID: 23511035 DOI: 10.1097/MIB.0b013e3182807577]

37 **Langhorst J**, Elsenbruch S, Koelzer J, Rueffer A, Michalsen A, Dobos GJ. Noninvasive markers in the assessment of intestinal inflammation in inflammatory bowel diseases: performance of fecal lactoferrin, calprotectin, and PMN-elastase, CRP, and clinical indices. *Am J Gastroenterol* 2008; **103**: 162-169 [PMID: 17916108 DOI: 10.1111/j.1572-0241.2007.01556.x]

38 **van de Logt F**, Day AS. S100A12: a noninvasive marker of inflammation in inflammatory bowel disease. *J Dig Dis* 2013; **14**: 62-67 [PMID: 23146044 DOI: 10.1111/1751-2980.12012]

39 **Kaiser T**, Langhorst J, Wittkowski H, Becker K, Friedrich AW, Rueffer A, Dobos GJ, Roth J, Foell D. Faecal S100A12 as a non-invasive marker distinguishing inflammatory bowel disease from irritable bowel syndrome. *Gut* 2007; **56**: 1706-1713 [PMID: 17675327 DOI: 10.1136/gut.2006.113431]

40 **Landers CJ**, Cohavy O, Misra R, Yang H, Lin YC, Braun J, Targan SR. Selected loss of tolerance evidenced by Crohn's disease-associated immune responses to auto- and microbial antigens. *Gastroenterology* 2002; **123**: 689-699 [PMID: 12198693 DOI: 10.1053/gast.2002.35379]

41 **Papp M**, Norman GL, Altorjay I, Lakatos PL. Utility of serological markers in inflammatory bowel diseases: gadget or magic? *World J Gastroenterol* 2007; **13**: 2028-2036 [PMID: 17465443 DOI: 10.3748/wjg.v13.i14.2028]

42 **Lodes MJ**, Cong Y, Elson CO, Mohamath R, Landers CJ, Targan SR, Fort M, Hershberg RM. Bacterial flagellin is a dominant antigen in Crohn disease. *J Clin Invest* 2004; **113**: 1296-1306 [PMID: 15124021 DOI: 10.1172/JCI200420295]

43 **Targan SR**, Landers CJ, Yang H, Lodes MJ, Cong Y, Papadakis KA, Vasiliauskas E, Elson CO, Hershberg RM. Antibodies to CBir1 flagellin define a unique response that is associated independently with complicated Crohn's disease. *Gastroenterology* 2005; **128**: 2020-2028 [PMID: 15940634 DOI: 10.1053/j.gastro.2005.03.046]

44 **Schoepfer AM**, Schaffer T, Mueller S, Flogerzi B, Vassella E, Seibold-Schmid B, Seibold F. Phenotypic associations of Crohn's disease with antibodies to flagellins A4-Fla2 and Fla-X, ASCA, p-ANCA, PAB, and NOD2 mutations in a Swiss Cohort. *Inflamm Bowel Dis* 2009; **15**: 1358-1367 [PMID: 19253375 DOI: 10.1002/ibd.20892]

45 **Wei B**, Huang T, Dalwadi H, Sutton CL, Bruckner D, Braun J. Pseudomonas fluorescens encodes the Crohn's disease-associated I2 sequence and T-cell superantigen. *Infect Immun* 2002; **70**: 6567-6575 [PMID: 12438326 DOI: 10.1128/IAI.70.12.6567-6575.2002]

46 **Bossuyt X**. Serologic markers in inflammatory bowel disease. *Clin Chem* 2006; **52**: 171-181 [PMID: 16339302 DOI: 10.1373/clinchem.2005.058560]

47 **Dotan I**, Fishman S, Dgani Y, Schwartz M, Karban A, Lerner A, Weishauss O, Spector L, Shtevi A, Altstock RT, Dotan N, Halpern Z. Antibodies against laminaribioside and chitobioside are novel serologic markers in Crohn's disease. *Gastroenterology* 2006; **131**: 366-378 [PMID: 16890590 DOI: 10.1053/j.gastro.2006.04.030]

48 **Ferrante M**, Henckaerts L, Joossens M, Pierik M, Joossens S, Dotan N, Norman GL, Altstock RT, Van Steen K, Rutgeerts P, Van Assche G, Vermeire S. New serological markers in inflammatory bowel disease are associated with complicated disease behaviour. *Gut* 2007; **56**: 1394-1403 [PMID: 17456509 DOI: 10.1136/gut.2006.108043]

49 **Simondi D**, Mengozzi G, Betteto S, Bonardi R, Ghignone RP, Fagoonee S, Pellicano R, Sguazzini C, Pagni R, Rizzetto M, Astegiano M. Antiglycan antibodies as serological markers in the differential diagnosis of inflammatory bowel disease. *Inflamm Bowel Dis* 2008; **14**: 645-651 [PMID: 18240283 DOI: 10.1002/ibd.20368]

50 **Papp M**, Altorjay I, Dotan N, Palatka K, Foldi I, Tumpek J, Sipka S, Udvardy M, Dinya T, Lakatos L, Kovacs A, Molnar T, Tulassay Z, Miheller P, Norman GL, Szamosi T, Papp J, Lakatos PL. New serological markers for inflammatory bowel disease are associated with earlier age at onset, complicated disease behavior, risk for surgery, and NOD2/CARD15 genotype in a Hungarian IBD cohort. *Am J Gastroenterol* 2008; **103**: 665-681 [PMID: 18047543 DOI: 10.1111/j.1572-0241.2007.01652.x]

51 **Lakatos PL**, Papp M, Rieder F. Serologic antiglycan antibodies in inflammatory bowel disease. *Am J Gastroenterol* 2011; **106**: 406-412 [PMID: 21245832]

52 **Seow CH**, Stempak JM, Xu W, Lan H, Griffiths AM, Greenberg GR, Steinhart AH, Dotan N, Silverberg MS. Novel anti-glycan antibodies related to inflammatory bowel disease diagnosis and phenotype. *Am J Gastroenterol* 2009; **104**: 1426-1434 [PMID: 19491856 DOI: 10.1038/ajg.2009.79]

53 **Rieder F**, Schleder S, Wolf A, Dirmeier A, Strauch U, Obermeier F, Lopez R, Spector L, Fire E, Yarden J, Rogler G, Dotan N, Klebl F. Association of the novel serologic anti-glycan antibodies anti-laminarin and anti-chitin with complicated Crohn's disease behavior. *Inflamm Bowel Dis* 2010; **16**: 263-274 [PMID: 19653286 DOI: 10.1002/ibd.21046]

54 **Klebl FH**, Bataille F, Huy C, Hofstädter F, Schölmerich J, Rogler G. Association of antibodies to exocrine pancreas with subtypes of Crohn's disease. *Eur J Gastroenterol Hepatol* 2005; **17**: 73-77 [PMID: 15647645]

55 **Joossens S**, Vermeire S, Van Steen K, Godefridis G, Claessens G, Pierik M, Vlietinck R, Aerts R, Rutgeerts P, Bossuyt X. Pancreatic autoantibodies in inflammatory bowel disease. *Inflamm Bowel Dis* 2004; **10**: 771-777 [PMID: 15626896]

56 **Roggenbuck D**, Hausdorf G, Martinez-Gamboa L, Reinhold D, Büttner T, Jungblut PR, Porstmann T, Laass MW, Henker J, Büning C, Feist E, Conrad K. Identification of GP2, the major zymogen granule membrane glycoprotein, as the autoantigen of pancreatic antibodies in Crohn's disease. *Gut* 2009; **58**: 1620-1628 [PMID: 19549613 DOI: 10.1136/gut.2008.162495]

57 **Pavlidis P**, Shums Z, Koutsoumpas AL, Milo J, Papp M, Umemura T, Lakatos PL, Smyk DS, Bogdanos DP, Forbes A, Norman GL. Diagnostic and clinical significance of Crohn's disease-specific anti-MZGP2 pancreatic antibodies by a novel ELISA. *Clin Chim Acta* 2015; **441**: 176-181 [PMID: 25512163 DOI: 10.1016/j.cca.2014.12.010]

58 **Soendergaard C**, Nielsen OH, Seidelin JB, Kvist PH, Bjerrum JT. Alpha-1 antitrypsin and granulocyte colony-stimulating factor as serum biomarkers of disease severity in ulcerative colitis. *Inflamm Bowel Dis* 2015; **21**: 1077-1088 [PMID: 25803506 DOI: 10.1097/MIB.0000000000000348]

59 **Ellinghaus D**, Zhang H, Zeissig S, Lipinski S, Till A, Jiang T, Stade B, Bromberg Y, Ellinghaus E, Keller A, Rivas MA, Skieceviciene J, Doncheva NT, Liu X, Liu Q, Jiang F, Forster M, Mayr G, Albrecht M, Häsler R, Boehm BO, Goodall J, Berzuini CR, Lee J, Andersen V, Vogel U, Kupcinskas L, Kayser M, Krawczak M, Nikolaus S, Weersma RK, Ponsioen CY, Sans M, Wijmenga C, Strachan DP, McArdle WL, Vermeire S, Rutgeerts P, Sanderson JD, Mathew CG, Vatn MH, Wang J, Nöthen MM, Duerr RH, Büning C, Brand S, Glas J, Winkelmann J, Illig T, Latiano A, Annese V, Halfvarson J, D'Amato M, Daly MJ, Nothnagel M, Karlsen TH, Subramani S, Rosenstiel P, Schreiber S, Parkes M, Franke A. Association between variants of PRDM1 and NDP52 and Crohn's disease, based on exome sequencing and functional studies. *Gastroenterology* 2013; **145**: 339-347 [PMID: 23624108 DOI: 10.1053/j.gastro.2013.04.040]

60 **Cleynen I**, González JR, Figueroa C, Franke A, McGovern D, Bortlík M, Crusius BJ, Vecchi M, Artieda M, Szczypiorska M, Bethge J, Arteta D, Ayala E, Danese S, van Hogezand RA, Panés J, Peña SA, Lukas M, Jewell DP, Schreiber S, Vermeire S, Sans M. Genetic factors conferring an increased susceptibility to develop Crohn's disease also influence disease phenotype: results from the IBDchip European Project. *Gut* 2013; **62**: 1556-1565 [PMID: 23263249 DOI: 10.1136/gutjnl-2011-300777]

61 **Jostins L,** Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY, Lee JC, Schumm LP, Sharma Y, Anderson CA, Essers J, Mitrovic M, Ning K, Cleynen I, Theatre E, Spain SL, Raychaudhuri S, Goyette P, Wei Z, Abraham C, Achkar JP, Ahmad T, Amininejad L, Ananthakrishnan AN, Andersen V, Andrews JM, Baidoo L, Balschun T, Bampton PA, Bitton A, Boucher G, Brand S, Büning C, Cohain A, Cichon S, D' Amato M, De Jong D, Devaney KL, Dubinsky M, Edwards C, Ellinghaus D, Ferguson LR, Franchimont D, Fransen K, Gearry R, Georges M, Gieger C, Glas J, Haritunians T, Hart A, Hawkey C, Hedl M, Hu X, Karlsen TH, Kupcinskas L, Kugathasan S, Latiano A, Laukens D, Lawrance IC, Lees CW, Louis E, Mahy G, Mansfield J, Morgan AR, Mowat C, Newman W, Palmieri O, Ponsioen CY, Potocnik U, Prescott NJ, Regueiro M, Rotter JI, Russell RK, Sanderson JD, Sans M, Satsangi J, Schreiber S, Simms LA, Sventoraityte J, Targan SR, Taylor KD, Tremelling M, Verspaget HW, De Vos M, Wijmenga C, Wilson DC, Winkelmann J, Xavier RJ, Zeissig S, Zhang B, Zhang CK, Zhao H; International IBD Genetics Consortium (IIBDGC), Silverberg MS, Annese V, Hakonarson H, Brant SR, Radford-Smith G, Mathew CG, Rioux JD, Schadt EE, Daly MJ, Franke A, Parkes M, Vermeire S, Barrett JC, Cho JH. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* 2012; **491**: 119-124 [PMID: 23128233 DOI: 10.1038/nature11582]

62 **Brand S**. Crohn's disease: Th1, Th17 or both? The change of a paradigm: new immunological and genetic insights implicate Th17 cells in the pathogenesis of Crohn's disease. *Gut* 2009; **58**: 1152-1167 [PMID: 19592695 DOI: 10.1136/gut.2008.163667]

63 **von Stein P**, Lofberg R, Kuznetsov NV, Gielen AW, Persson JO, Sundberg R, Hellstrom K, Eriksson A, Befrits R, Ost A, von Stein OD. Multigene analysis can discriminate between ulcerative colitis, Crohn's disease, and irritable bowel syndrome. *Gastroenterology* 2008; **134**: 1869-1881; quiz 2153-2154 [PMID: 18466904 DOI: 10.1053/j.gastro.2008.02.083]

64 **Janczewska I**, Kapraali M, Saboonchi F, Nekzada Q, Wessulv Å, Khoshkar J, Marouf F, Gorsetman J, Risberg D, Lissing M, Wirström G, Sandstedt B. Clinical application of the multigene analysis test in discriminating between ulcerative colitis and Crohn's disease: a retrospective study. *Scand J Gastroenterol* 2012; **47**: 162-169 [PMID: 22229803 DOI: 10.3109/00365521.2011.647065]

65 **O'Connell RM**, Rao DS, Chaudhuri AA, Baltimore D. Physiological and pathological roles for microRNAs in the immune system. *Nat Rev Immunol* 2010; **10**: 111-122 [PMID: 20098459 DOI: 10.1038/nri2708]

66 **Dalal SR**, Kwon JH. The Role of MicroRNA in Inflammatory Bowel Disease. *Gastroenterol Hepatol* (N Y) 2010; **6**: 714-722 [PMID: 21437020]

67 **Wu F**, Guo NJ, Tian H, Marohn M, Gearhart S, Bayless TM, Brant SR, Kwon JH. Peripheral blood microRNAs distinguish active ulcerative colitis and Crohn's disease. *Inflamm Bowel Dis* 2011; **17**: 241-250 [PMID: 20812331 DOI: 10.1002/ibd.21450]

68 **Zahm AM**, Thayu M, Hand NJ, Horner A, Leonard MB, Friedman JR. Circulating microRNA is a biomarker of pediatric Crohn disease. *J Pediatr Gastroenterol Nutr* 2011; **53**: 26-33 [PMID: 21546856 DOI: 10.1097/MPG.0b013e31822200c]

69 **Schaefer JS**, Attumi T, Opekun AR, Abraham B, Hou J, Shelby H, Graham DY, Streckfus C, Klein JR. MicroRNA signatures differentiate Crohn's disease from ulcerative colitis. *BMC Immunol* 2015; **16**: 5 [PMID: 25886994 DOI: 10.1186/s12865-015-0069-0]

70 **Jansson J**, Willing B, Lucio M, Fekete A, Dicksved J, Halfvarson J, Tysk C, Schmitt-Kopplin P. Metabolomics reveals metabolic biomarkers of Crohn's disease. *PLoS One* 2009; **4**: e6386 [PMID: 19636438 DOI: 10.1371/journal.pone.0006386]

71 **Zhang X**, Choi FF, Zhou Y, Leung FP, Tan S, Lin S, Xu H, Jia W, Sung JJ, Cai Z, Bian Z. Metabolite profiling of plasma and urine from rats with TNBS-induced acute colitis using UPLC-ESI-QTOF-MS-based metabonomics--a pilot study. *FEBS J* 2012; **279**: 2322-2338 [PMID: 22520047 DOI: 10.1111/j.1742-4658.2012.08612.x]

72 **Schicho R**, Shaykhutdinov R, Ngo J, Nazyrova A, Schneider C, Panaccione R, Kaplan GG, Vogel HJ, Storr M. Quantitative metabolomic profiling of serum, plasma, and urine by (1)H NMR spectroscopy discriminates between patients with inflammatory

bowel disease and healthy individuals. *J Proteome Res* 2012; **11**: 3344-3357 [PMID: 22574726 DOI: 10.1021/pr300139q]
73 **Williams HR**, Willsmore JD, Cox IJ, Walker DG, Cobbold JF, Taylor-Robinson SD, Orchard TR. Serum metabolic profiling in inflammatory bowel disease. *Dig Dis Sci* 2012; **57**: 2157-2165 [PMID: 22488632 DOI: 10.1007/s10620-012-2127-2]
74 **Ackerman AB**, Milde P. Naming acquired melanocytic nevi. Common and dysplastic, normal and atypical, or Unna, Miescher, Spitz, and Clark? *Am J Dermatopathol* 1992; **14**: 447-453 [PMID: 1415964]
75 **Takaishi H**, Matsuki T, Nakazawa A, Takada T, Kado S, Asahara T, Kamada N, Sakuraba A, Yajima T, Higuchi H, Inoue N, Ogata H, Iwao Y, Nomoto K, Tanaka R, Hibi T. Imbalance in intestinal microflora constitution could be involved in the pathogenesis of inflammatory bowel disease. *Int J Med Microbiol* 2008; **298**: 463-472 [PMID: 17897884]
76 **Williams HR**, Cox IJ, Walker DG, North BV, Patel VM, Marshall SE, Jewell DP, Ghosh S, Thomas HJ, Teare JP, Jakobovits S, Zeki S, Welsh KI, Taylor-Robinson SD, Orchard TR. Characterization of inflammatory bowel disease with urinary metabolic profiling. *Am J Gastroenterol* 2009; **104**: 1435-1444 [PMID: 19491857 DOI: 10.1038/ajg.2009.175]
77 **Stephens NS**, Siffledeen J, Su X, Murdoch TB, Fedorak RN, Slupsky CM. Urinary NMR metabolomic profiles discriminate inflammatory bowel disease from healthy. *J Crohns Colitis* 2013; **7**: e42-e48 [PMID: 22626506 DOI: 10.1016/j.crohns]
78 **Ooi M**, Nishiumi S, Yoshie T, Shiomi Y, Kohashi M, Fukunaga K, Nakamura S, Matsumoto T, Hatano N, Shinohara M, Irino Y, Takenawa T, Azuma T, Yoshida M. GC/MS-based profiling of amino acids and TCA cycle-related molecules in ulcerative colitis. *Inflamm Res* 2011; **60**: 831-840 [PMID: 21523508 DOI: 10.1007/s00011-011-0340-7]
79 **Hisamatsu T**, Okamoto S, Hashimoto M, Muramatsu T, Andou A, Uo M, Kitazume MT, Matsuoka K, Yajima T, Inoue N, Kanai T, Ogata H, Iwao Y, Yamakado M, Sakai R, Ono N, Ando T, Suzuki M, Hibi T. Novel, objective, multivariate biomarkers composed of plasma amino acid profiles for the diagnosis and assessment of inflammatory bowel disease. *PLoS One* 2012; **7**: e31131 [PMID: 22303484 DOI: 10.1371/journal.pone.0031131]
80 **Marchesi JR**, Holmes E, Khan F, Kochhar S, Scanlan P, Shanahan F, Wilson ID, Wang Y. Rapid and noninvasive metabonomic characterization of inflammatory bowel disease. *J Proteome Res* 2007; **6**: 546-551 [PMID: 17269711]
81 **Le Gall G**, Noor SO, Ridgway K, Scovell L, Jamieson C, Johnson IT, Colquhoun IJ, Kemsley EK, Narbad A. Metabolomics of fecal extracts detects altered metabolic activity of gut microbiota in ulcerative colitis and irritable bowel syndrome. *J Proteome Res* 2011; **10**: 4208-4218 [PMID: 21761941 DOI: 10.1021/pr2003598]
82 **Kurada S**, Alkhouri N, Fiocchi C, Dweik R, Rieder F. Review article: breath analysis in inflammatory bowel diseases. *Aliment Pharmacol Ther* 2015; **41**: 329-341 [PMID: 25523187 DOI: 10.1111/apt.13050]
83 **Dryahina K**, Španěl P, Pospíšilová V, Sovová K, Hrdlička L, Machková N, Lukáš M, Smith D. Quantification of pentane in exhaled breath, a potential biomarker of bowel disease, using selected ion flow tube mass spectrometry. *Rapid Commun Mass Spectrom* 2013; **27**: 1983-1992 [PMID: 23939966 DOI: 10.1002/rcm.6660]
84 **Pelli MA**, Trovarelli G, Capodicasa E, De Medio GE, Bassotti G. Breath alkanes determination in ulcerative colitis and Crohn's disease. *Dis Colon Rectum* 1999; **42**: 71-76 [PMID: 10211523]
85 **Koek GH**, Verleden GM, Evenepoel P, Rutgeerts P. Activity related increase of exhaled nitric oxide in Crohn's disease and ulcerative colitis: a manifestation of systemic involvement? *Respir Med* 2002; **96**: 530-535 [PMID: 12194639]
86 **Kokoszka J**, Nelson RL, Swedler WI, Skosey J, Abcarian H. Determination of inflammatory bowel disease activity by breath pentane analysis. *Dis Colon Rectum* 1993; **36**: 597-601 [PMID: 8500379]
87 **Sedghi S**, Keshavarzian A, Klamut M, Eiznhamer D, Zarling EJ. Elevated breath ethane levels in active ulcerative colitis: evidence for excessive lipid peroxidation. *Am J Gastroenterol* 1994; **89**: 2217-2221 [PMID: 7977245]
88 **Meuwis MA**, Fillet M, Geurts P, de Seny D, Lutteri L, Chapelle JP, Bours V, Wehenkel L, Belaiche J, Malaise M, Louis E, Merville MP. Biomarker discovery for inflammatory bowel disease, using proteomic serum profiling. *Biochem Pharmacol* 2007; **73**: 1422-1433 [PMID: 17258689]
89 **Viennois E**, Baker MT, Xiao B, Wang L, Laroui H, Merlin D. Longitudinal study of circulating protein biomarkers in inflammatory bowel disease. *J Proteomics* 2015; **112**: 166-179 [PMID: 25230104 DOI: 10.1016/j.jprot.2014.09.002]

P- Reviewer: Senates E, Tandon R **S- Editor**: Qi Y
L- Editor: A **E- Editor**: Wang CH

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i1.51

World J Gastrointest Pharmacol Ther 2016 February 6; 7(1): 51-65
ISSN 2150-5349 (online)

REVIEW

Elderly patients and inflammatory bowel disease

Danielle Nimmons, Jimmy K Limdi

Danielle Nimmons, Jimmy K Limdi, Inflammatory Bowel Diseases Section, the Pennine Acute Hospitals NHS Trust, Manchester BL97TD, United Kingdom

Jimmy K Limdi, Institute of Inflammation and Repair, Manchester Academic Health Sciences Centre, University of Manchester, Manchester M13 9PT, United Kingdom

Author contributions: Nimmons D and Limdi JK wrote the paper.

Conflict-of-interest statement: Authors declare no conflict of interests for this article.

Correspondence to: Jimmy K Limdi, FRCP (London and Edin), FEBGH, AGAF, FACG, Consultant Gastroenterologist and Clinical Lead (Inflammatory Bowel Diseases), Inflammatory Bowel Diseases Section, the Pennine Acute Hospitals NHS Trust, Manchester BL97TD, United Kingdom. jimmy.limdi@pat.nhs.uk
Telephone: +44-0161-7782642
Fax: +44-0161-7782659

Received: May 20, 2015
Peer-review started: June 9, 2015
First decision: August 31, 2015
Revised: November 27, 2015
Accepted: December 13, 2015
Article in press: December 14, 2015
Published online: February 6, 2016

Abstract

The incidence and prevalence of inflammatory bowel disease (IBD) is increasing globally. Coupled with an ageing population, the number of older patients with IBD is set to increase. The clinical features and therapeutic options in young and elderly patients are comparable but there are some significant differences. The wide differential diagnosis of IBD in elderly patients may result in a delay in diagnosis. The relative dearth of data specific to elderly IBD patients often resulting from their exclusion from pivotal clinical trials and the lack of consensus guidelines have made clinical decisions somewhat challenging. In addition, age specific concerns such as co-morbidity; locomotor and cognitive function, poly-pharmacy and its consequences need to be taken into account. In applying modern treatment paradigms to the elderly, the clinician must consider the potential for more pronounced adverse effects in this vulnerable group and set appropriate boundaries maximising benefit and minimising harm. Meanwhile, clinicians need to make personalised decisions but as evidence based as possible in the holistic, considered and optimal management of IBD in elderly patients. In this review we will cover the clinical features and therapeutic options of IBD in the elderly; as well as addressing common questions and challenges posed by its management.

Key words: Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Elderly; Therapy; Clinical features

Core tip: Inflammatory bowel disease can be misdiagnosed as its clinical features are similar in younger and elderly patients. Therapeutic regimes may be different with elderly patients being less likely to have immunosuppressant drugs and Anti-TNF's either driven by clinican or patient preference and disease related factors. Important factors such as polypharmacy and co-morbidity must also be considered when making clinical decisions. Finally, complications may be more common in the elderly. Further evidence through clinical trials and consensus guidelines are needed to

assist clinicians in making evidence based decisions in these patients.

Nimmons D, Limdi JK. Elderly patients and inflammatory bowel disease. *World J Gastrointest Pharmacol Ther* 2016; 7(1): 51-65 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i1/51.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i1.51

INTRODUCTION

The inflammatory bowel diseases (IBD) comprising Crohn's disease (CD) and ulcerative colitis (UC) are idiopathic diseases of the gastrointestinal tract characterized by a relapsing and remitting course[1]. The incidence and prevalence of IBD is increasing worldwide[2,3]. An official consensus estimated the proportion of elderly people at 3%-17.8% of the population[4]. Individuals aged over 65 years represent the fastest growing age group and is expected to increase by 31% during this decade in the United States[5]. The rising global incidence of IBD, its negligible impact on mortality and an ageing population will all contribute to increasing numbers of "elderly" patients with IBD.

The challenges posed by clinical co-morbidity, poly-pharmacy and drug interactions, likely mismatch between chronological and biological age (functional status) and social issues underpin the complexities involved in the management of the elderly patient with IBD. No consensus guidelines currently exist to guide the management of this vulnerable group, often limited by heterogenous populations studied with differential definitions of endpoints and based on 8-52 wk duration studies, which are not truly reflective of a lifelong disease. Patients over 65 years are often excluded from therapeutic trials[6] and in some the median age of participants has been in the 30's with few being elderly[7]. The paucity of clinical data compounded by the complexities in management emphasise the importance of an astute understanding of the available literature in compressing avoidable morbidity and achieving desired outcomes when treating elderly patients with IBD.

In this review we will address common questions and challenges posed by the management of elderly patients with IBD. As is widely accepted in the IBD literature, we have used age 60 years and above for this cohort of patients.

EPIDEMIOLOGY OF ELDERLY IBD

Although the peak incidence of IBD is between ages 20 to 39, a second peak is recognised between ages 50-70[8]. Individuals over the age of 60 contribute to 10%-15% of IBD diagnoses, compared to 5%-25% made in children or adolescents[8-11]. The incidence in the elderly decreases with increasing age, where 65% of patients are aged 60-70 years old, 25% aged 70-80 years and 10% are over 80 years[6]. Thus, it is important to recognise elderly patients with IBD as having either long-standing IBD or late-onset IBD, where a diagnosis is made at a later age.

The incidence of IBD is increasing world-wide although there is significant heterogeneity with some data coming from urban populations and others from large population-based registries[12]. Previous studies have also shown regional differences, with the incidence of CD and UC in the elderly being 4/100000 and 6-8/100000 respectively in the United States as compared to 8-10/100000 for UC and CD in Europe[13,14]. A large study investigating early-onset IBD from the EPIMAD registry in Northern France, suggests the percentage of late-onset IBD is on the rise at 5%-11%[15]. It is noteworthy that most epidemiological studies have been undertaken in Caucasian populations and in the "developed" world. Population ageing, however, is a global phenomenon and further epidemiological studies in the developing world including the elderly may help to unmask likely clues from the "exposome" in the aetiology of IBD[16].

CLINICAL PRESENTATION OF IBD IN THE ELDERLY

The clinical presentation is similar to that in younger individuals with some important differences (Table 1). The diagnosis may be delayed for reasons including access to specialist healthcare, disinclination to seek medical advice, an initial misdiagnosis compared to younger patients, and the higher prevalence of conditions mimicking IBD in the elderly[17]. Symptoms include weight loss, abdominal pain, anaemia and diarrhoea[17-19]. Elderly patients have a lower incidence of a family history of IBD and higher incidence of osteoporosis but the extent of extra-intestinal features is not significantly different to that in younger patients[17,18].

The diagnosis of CD may be delayed in older individuals with the mean time of diagnosis being 6.4 years compared to 2.4 years in younger people[17]. Elderly CD patients have more colonic involvement and inflammatory disease compared to younger patients with a lower frequency of fistula or strictures[17-21]. A change in disease behaviour is also less common in the elderly[21-23].

The first flare of UC may be more severe[7,24-26]. However, the clinical presentation of UC may be subtle with less bleeding, diarrhoea and abdominal pain[25]. Distal disease (proctitis and left-sided colitis) is more common[21,25-27]. In a World Gastroenterology Organisation survey, proctitis was observed in 42% of UC patients aged over 60 years as compared to 33% in those under 60 years[24]. In the EPIMAD registry,

Table 1 Phenotypic characteristics of inflammatory bowel disease in elderly-onset inflammatory bowel disease

	Crohn's disease	Ulcerative colitis
Location	Colonic or ileo-colonic	Left sided or extensive disease more common than isolated proctitis
Symptoms	Less bleeding and abdominal pain than younger patients	Less diarrhoea, abdominal pain and weight loss than younger patients
Disease behaviour	Inflammatory; less progression to penetrating and structuring disease	More likely to remain stable
First episode	More severe than in younger patients	More severe than in younger patients
Extra-intestinal manifestations	Less common than in younger patients	Less common than in younger patients
Family history	Less common	Less common
Cancer risk	Higher risk of non-Hodgkin lymphoma with thiopurines and of non-melanoma skin cancer with anti-TNF therapy	Higher risk of non-Hodgkin lymphoma with thiopurines and of non-melanoma skin cancer with anti-TNF therapy

45% had left-sided colitis, 29% had proctitis and 26% had extensive colitis[15]. Furthermore, disease location tended to remain stable with only 16% of patients at follow-up having proximal disease extension[15]. Studies have suggested that relapse in UC is less likely in elderly patients but when it does occur, it may be more severe[27-29]. The incidence of colectomy is higher in younger patients (1.9% *vs* 4.3% in older and younger patients)[21]. In the EPIMAD study only 16% of elderly onset UC had surgery 10 years after diagnosis[15].

Elderly patients tend to be hospitalised more often than younger patients. Ananthakrishnan *et al*[30] reported that 25% of all IBD-related hospital admissions in the United States were in patients aged over 65 years. These patients are more likely to be ill, more malnourished, anaemic and hypovolaemic with higher transfusion requirements, and longer post-operative hospital stay especially when they undergo surgery. The majority of the healthcare spend associated with IBD relates to the cost of hospitalisation and surgery[30-32]. Increased age is an independent risk factor for hospital fatality in these patients[30,33-35]. The worse outcomes in hospitalised elderly IBD patients, higher mortality and economic impact from health resource utilization underpin the need for further prospective research into the natural history and well-designed clinical trials for therapy in this population.

Finally, thrombotic complications are more common in elderly IBD patients potentially driven by a combination of disease related hypercoagulability, reduced mobility and dehydration, all of which are more common in the elderly[17,36]. In the Nationwide Inpatient Sample Cohort Study, the highest rates of venous thromboembolism were seen in elderly UC patients with a third aged 80 and above experiencing a venous thrombotic complication during hospitalization[30].

DIFFERENTIAL DIAGNOSIS

A wide range of conditions can mimic IBD often delaying a decisive diagnosis or leading to an erroneous diagnosis. Misdiagnosis may occur in up to 60% of elderly patients with IBD as compared to 15% of younger patients with a lag in diagnosis of up to 6 years[18]. Conditions commonly confused with IBD in this age group include complicated diverticular disease (diverticulitis and diverticular bleeding), ischaemic colitis, medication-associated diarrhoea (NSAIDs, antibiotics and others), infectious diarrhoea, radiation colopathy and microscopic colitis (Table 2).

Diverticular disease is present in 40%-60% of individuals aged 70 and above with diverticular bleeding being the most common complication to mimic IBD[37]. Abscess formation, perforation or indeed fistulisation may complicate differentiation between diverticultis and CD. Furthermore, diverticular colitis (also called segmental colitis associated with diverticula) can mimic distal colonic CD[38]. A recent Dutch study found that 8% of all IBD diagnoses had been inaccurate and were indeed segmental colitis associated with diverticular disease[39].

Radiation for gynaecological or prostate cancers and NSAID-induced ulcers with strictures or perforation can mimic or complicate IBD[28,40]. Some studies have reported relapses in patients with IBD with non-selective NSAID use but not with selective COX II inhibitors[41].

Ischaemic colitis may also occur with segmental involvement causing confusion with CD. Abrupt onset of pain with bloody diarrhoea is suggestive of this diagnosis. Endoscopy, histopathology and in some instances imaging make it possible to differentiate it from CD[42]. Brandt *et al*[43] found that nearly 50% of their patients had been misdiagnosed with IBD and actually had ischaemic colitis.

Infectious colitis with bloody diarrhoea may often be confused with UC and a broad differential diagnosis must be considered, particularly *Clostridium* difficile infection (CDI), *Shigella*, *Campylobacter*, *Salmonella* and *Escherichia coli* 0157:H7. The acute onset of symptoms and associated fever is characteristic and the diagnosis is often made by stool culture with rapid symptom resolution within 1-2 wk. Infections causing

Table 2 Differential diagnosis of inflammatory bowel disease

Disease	Clinical characteristics	Additional features
Segmental colitis associated with diverticulosis	Diarrhoea with bleeding Abdominal pain	Segmental peridiverticular distribution Rectum and proximal colon spared
Radiation colitis	Diarrhoea with bleeding and abdominal pain/cramps Proctitis (urgency and tenesmus) Symptoms often weeks to years after abdominal or pelvic radiation	Telangiectasia and fibrosis seen at histology
NSAID-induced colitis	Diarrhoea with recurrent abdominal pain Obstruction or perforation Iron deficiency anaemia	Lesions isolated Any part of intestine may be affected Diaphragm like small bowel strictures Exacerbate existing CD or UC
Ischaemic colitis	Sudden onset of abdominal pain Diarrhoea with bleeding	Segmental distribution of colitis Typically sigmoid/left sided colitis Rectum spared and abrupt cut off with non-involved segment
Infective colitis	Diarrhoea with bleeding Constitutional symptoms such as fever	Possible pseudomembranes with *Clostridium* difficile colitis Stool cultures usually diagnostic Rapid resolution with appropriate antibiotic therapy
Solitary rectal ulcer	Bleeding per rectum with straining	Mucosal thickening Crypt architectural distortion Collagen deposition and smooth muscle in lamina propria

NSAID: Nonsteroidal anti-inflammatory drug; CD: Crohn's disease; UC: Ulcerative colitis.

chronic diarrhoea include *Plesiomonas*, *Aeromonas* and *Yersinia*. *Yersinia* may cause terminal ileitis mimicking CD[44]. The rising incidence of CDI is of increasing concern, and is no longer regarded as a purely nosocomial infection but can also be community-acquired[45]. In older patients affected with a virulent strain this may have serious consequences with prolonged hospitalization, increased risk of surgery and greater mortality[45]. Specific risk factors for CDI in patients with IBD are colonic disease, corticosteroid therapy and the use of immunosuppressive drugs to control IBD[46]. Thus, CDI testing should be performed on all hospitalised patients with IBD, those experiencing a disease flare and those not responding to therapy[46].

MEDICAL THERAPY

General considerations

The principles of IBD treatment are the induction and maintenance of remission, prevent disease and treatment-related complications and to improve quality of life. Important considerations in choosing a therapeutic agent include location and severity of inflammation, disease behaviour (inflammatory, stricturing or fistulising), the presence of extra-intestinal manifestations and other clinical co-morbidities. In that respect, the therapy for IBD in the elderly is similar to that in younger patients but with some crucial considerations.

Elderly-onset IBD is usually not associated with disease progression[6,21-23]. Treatment paradigms have evolved often involving the use of highly potent immunosuppressive therapy (often in combination) earlier in the disease course in well selected patients, especially in CD[47,48]. The application of such treatment paradigms in the elderly, through extrapolation from clinical trial data must acknowledge important caveats discussed below.

Innate immune function declines with age. Ageing is associated with a reduction in Toll-like receptor 4-mediated pro-inflammatory cytokine production and mitogen-activated protein kinase expression[49-54]. Ageing also alters humoral immunity through decline in B cell precursors and consequent decline in immunoglobulins[55]. Malnutrition also accentuates decline in immune function[56,57].

Treatment of elderly IBD patients with immunosuppressive medication increases the risk of opportunistic infection and possibly even malignancy. The exclusion of patients older than 60 years from most therapeutic trials[6] and lack of drug efficacy trial data in older patients, coupled with a lack of clarity of appropriate clinical end points (objective *vs* symptom control) may limit evidence-based decision-making.

Polypharmacy is common in elderly patients with some studies showing that they may be on five medications on average and 25% regularly take more than six drugs[58,59]. Thus, choice of drug therapy must take into account clinical co-morbidity, drug interactions and also the impact of polypharmacy on treatment adherence, which itself will impact on clinical outcomes[60] (Table 3). Age-related conditions, home circumstances, the influence of impaired mobility or memory and consequent need for practical support require careful attention.

Aminosalicylates

5-aminosalicylates are foundational therapy for the induction and maintenance of remission in UC[61]. Their role in CD is conflicting though patients with Crohn's colitis may benefit[62]. Despite this, in the EPIMAD study

Table 3 Drug interactions of medications used in the treatment of inflammatory bowel disease relevant to elderly patients

IBD drug	Drug interaction
Aminosalicylates	Increase levels of thiopurine metabolite 6-TGN through weak TPMT inhibition
	Interact with warfarin and increase INR (particularly Olsalazine)
Metronidazole	Increases levels of: Simvastatin; Calcium channel blockers; sildenafil and lithium
	Antabuse (disulfuram) like reaction with ethanol
	Increased metabolism and consequent clearance when co-administered with phenytoin and phenobarbitone
	Potentiates Warfarin: May increase INR
Ciprofloxacin	NSAIDs: Risk of seizures may be increased
	Theophylline: Levels may increase
	Potentiates Warfarin: May increase INR
	Phenytoin: Levels of phenytoin may decrease
Corticosteroids	Antidiabetic agents: Hypoglycaemic effects may be decreased
	Calcium channel blockers: May increase corticosteroid levels
	Diuretics: Hypokalaemic effects increased
	Warfarin: May increase anticoagulant effects
Thiopurines	Allopurinol: Can lead to bone marrow toxicity
	Aminosalicylates: May lead to increased toxicity and cause leukopenia/myelosuppression
	Clotrimazole, angiotensin–converting enzyme inhibitors: increased risk of leucopenia
	Warfarin: Anticoagulant effect may decrease
Methotrexate	Loop diuretics: Can alter methotrexate concentrations and vice versa
	NSAIDs: Bone marrow suppression and gastrointestinal toxicity
	Penicillins: Increase methotrexate concentration
	Tetracyclines: Increase methotrexate toxicity
	Theophylline levels may be increased
Cyclosporine	Ciprofloxacin, gentamicin and vancomycin: Potentiate renal dysfunction
	Anti-inflammatory drugs and histamine-2 blockers: Potentiate renal dysfunction
	Azithromycin, clarithromycin: Increase cyclosporine levels
	Allopurinol: Increases cyclosporine levels
	Rifampicin: Decreases cyclosporine levels
	Phenytoin, phenobarbital and carbamazepine: Decrease levels of cyclosporine
	Grapefruit juice: Increases absorption of cyclosporine

IBD: Inflammatory bowel disease; NSAIDs: Nonsteroidal anti-inflammatory drugs; 6-TGN: 6-thioguanine nucleotide; TPMT: Thiopurine S-methyltransferase; INR: International normalised ratio.

nearly 80% of patients with late-onset CD were prescribed a 5-ASA, possibly a reflection of physician hesitancy with immunosuppressive therapy[15].

In UC, combined therapy with oral and topical 5-ASA is more effective than oral therapy alone[63,64]. Complex dosing regimens and polypharmacy may negatively influence compliance and once-daily dosing regimens may be preferable[65]. Adherence rates of 5-ASA's are 40%-60% based on self-reporting and urinary drug measurements[66]. Anal sphincter incompetence ranges between 10% to 25% in hospitalised patients and 4% in outpatients[67]. Difficulties with the use of topical therapy may arise from physical limitations with reduced retention of enema fluid in the presence of active inflammation. Reduction in volume of the enema or substitution with a corticosteroid foam preparation may circumvent this problem.

5-aminosalicylates are relatively safe and effective with reports of nephrotoxicity and interstitial nephritis being rare[61,68]. Interstitial nephritis, an idiosyncratic effect is unrelated to the duration or dose of 5-ASA. Common negative side effects include nausea, vomiting abdominal pain, headache and rash[64]. Paradoxical worsening of colitis can occur in just under 5% of patients, and improves after discontinuing the drug[69,70]. 5-aminosalicylates can increase levels of the thiopurine metabolitie 6-thioguanine[71-73]; interact with isoniazid[58], and warfarin (particularly olsalazine) when an increase in the international normalised ratio (INR) occurs[14,74].

Antibiotics

The role of antibiotics in the primary treatment of CD is debatable but they may be used in some patients with mild - moderate colonic CD, infectious complications of fistulising CD, pouchitis and as an adjunct to surgical drainage of CD related abscesses[75]. They do not have an important role in UC, but have been used in the expectant management of fulminant colitis[76,77]. Metronidazole and ciprofloxacin are commonly used in IBD, often in combination.

Adverse effects of metronidazole include nausea, a metallic taste and neuropathy with longer use[14,78,79]. Inhibition of cytochrome P450 may lead to increased levels of HMG-CoA reductase inhibitors such as simvastatin, sildenafil and calcium channel blockers[79]. Metronidazole may affect warfarin thus prolonging the INR[14,74,79]. Patients should be advised to avoid alcohol due to the well-recognised Antabuse (disulfuram) like effect. The metabolism of metronidazole is increased when used with phenytoin. Concomitant use may

increase the risk of lithium toxicity[79].

Ciprofloxacin decreases theophylline clearance and can cause central nervous system adverse effects including lowering of the seizure threshold; it may alter serum phenytoin levels and lead to an increased INR by increasing warfarin levels[80]. Elderly patients may be at particular risk of QT prolongation on ECG and Achilles tendon rupture[81]. The association of fluoroquinolones and Clostridium difficile colitis is of particular concern[82].

Corticosteroids

Corticosteroids are used to induce remission in UC with an inadequate response to 5-ASA, in acute severe UC and to induce but not maintain clinical remission in CD[83,84]. Elderly IBD patients with prolonged corticosteroid exposure are more likely to experience severe adverse events[85,86]. Dose related side effects were noted in 40% of elderly patients with long-term corticosteroid exposure and osteoporosis in 16% of patients[85]. Another study found osteoporotic-related fractures and osteonecrosis in 15% of elderly IBD patients[87]. Corticosteroids should be used in an appropriated manner in both dose and duration with careful contingency planning[88]. The wide prevalence of malabsorption and calcium and vitamin D deficiency in the elderly emphasises the importance of early and regular bone densitometry assessments. Bisphosphonates should be considered alongside vitamin D and calcium supplementation[89].

Other side effects of corticosteroids more pronounced in the elderly include altered mental state and depression[85,86], fluid retention, of particular significance in patients with underlying hypertension, congestive heart failure, diabetes and renal disease[85,86]. Older patients may also have ocular problems such as glaucoma, exacerbated by corticosteroids[85,86]. Corticosteroid clearance is decreased in the elderly[90]. The activity of drugs can be reduced, such as phenytoin, phenobarbital, ephedrine and rifampin due to an increase in corticosteroid metabolism[14]. Anticoagulant efficacy may also be affected and frequent checks of coagulation parameters are recommended[58].

Budesonide is recommended to induce remission in mild-to-moderate distal small bowel and right-sided colonic CD and affects bone metabolism less than conventional corticosteroids[90,91]. A novel formulation of Budesonide in a multi-matrix release formulation has been approved for use in mild-moderate extensive UC[91].

Immune modulator therapy

Immunomonthsdulator therapy may be indicated in elderly patients with aminosalicylate resistance or corticosteroid dependence for the maintenance of remission. Thiopurines (azathioprine and 6-mercaptopurine) are useful in the preservation of remission in CD and UC whereas intramuscular methotrexate has efficacy in moderate to severe CD[64,92]. Although the efficacy of immune modifying agents in elderly and younger IBD patients appears similar, the literature is disparate with one study showing no differences in uptake[93], and another demonstrating higher immunosuppressive therapy use secondary to corticosteroid dependence[94]. In contrast, in a retrospective study of 393 IBD patients aged over 65 years, a third were on long-term corticosteroids (treatment duration over 6 mo) with only 6% on thiopurines (azathioprine or 6-MP) and 1% on Methotrexate indicating underutilization[22].

Adverse events associated with thiopurine therapy include idiosyncratic reactions that develop in approximately 5% of patients and include: Fever, pancreatitis and hepatitis[95]. Leucopenia may occur at any time during therapy and is determined mainly by activity of the enzyme thiopurine methyltransferase (TPMT), which metabolises azathioprine to its metabolite 6-thioguanine. TPMT deficiency can result in serious leucopenia and mandates vigilant monitoring of blood counts and chemistry particularly as the incidence of serious infection in the elderly is greater[94-98]. TPMT testing (enzyme activity or genetic) prior to initiation of thiopurine therapy can identify those at risk of serious myelosuppression[99,100]. TPMT deficiency is not the only mechanistic explanation for thiopurine-induced myelosuppression and vigilant blood count monitoring is mandated in all patients[101]. Thiopurines are also associated with an increased risk of non-melanoma skin cancer and patients should be counselled regarding appropriated exposure to sunshine with adequate precaution using barrier sun creams and an annual dermatological assessment[102].

Bone marrow toxicity can occur when thiopurines are used with allopurinol. In such instances, the thiopurine dose should be reduced to 25% of the standard dose[73,103,104]. Using both immunomodulators and allopurinol also increases the incidence of infection in the elderly[103,104]. The risk of leucopenia is also increased when thiopurines are used along with with clotrimazole or angiotensin-converting enzyme inhibitors[103,104]. Thiopurine treatment increases the risk of non-Hodgkin's lymphoma, its duration, age and if used with TNF therapy[105-107]. The CESAME study identified older age, male sex and longer duration of disease as the main risk factors of developing lymphoma[107]. Of 23 patients diagnosed with incident lymphomas, 12 were aged 60 or above and the risk was further elevated in those on combined immunosuppressive therapy with thiopurine and anti-TNF agents[107].

Methotrexate

Methotrexate is used in the treatment of CD but consensus opinion does not currently recommend its use in UC[108,109]. Although it has not been studied specifically in the elderly there is experience with its use in older patients with psoriasis and rheumatoid

arthritis[110,111]. The efficacy is similar in younger and older patients although its metabolism through biliary and renal excretion may be affected by age[112].

NSAID's may inhibit renal excretion, increasing toxicity[112]. Gastrointestinal and haematological toxicity are also more likely in older patients[112]. Common side-effects may include nausea, fatigue, rash and stomatitis but also using folic acid supplements can prevent or reduce these[113]. Other clinically relevant drug interactions include inhibition of methotrexate absorption by tetracycline and reduction in renal clearance by penicillin. Methotrexate alters theophylline clearance[14]. Loop diuretics and methotrexate can alter concentrations of either drug[114]. Methotrexate does not appear to increase the risk of lymphoma[115].

Cyclosporine

Cyclosporine is sometimes used as rescue therapy in fulminant colitis but with no definite superiority over infliximab its use in modern IBD therapy is likely to be limited[116]. Elderly patients are most likely to experience side effects[66,69,117,118]. Cyclosporine can interact with antibiotics, such as gentamicin and vancomycin, leading to increased nephrotoxicity; and with NSAIDs, melphalan and histamine-2 receptor antagonists[14]. Cytochrome P450 inhibitors, such as verapamil and allopurinol, decrease the metabolism of cyclosporine and increase its serum levels[14]. Phenytoin, rifampin, carbamazepine and phenobarbital reduce cyclosporine blood levels *via* increased hepatic metabolism[14,119].

Biological therapies

The role of anti-TNF therapy in the induction and maintenance of clinical and histological remission of moderate to severely active IBD has been established through pivotal trials with evidence that it reduces hospitalisation rates and surgery and improves quality of life[64,84]. There is a lack of data demonstrating how they affect older patients. Nonetheless, indications are similar to those for younger patients[120-122].

Data on anti-TNF therapy in the elderly however are conflicting with some studies demonstrating similar results in older and younger patients[122-124] and others showing them to be less effective in elderly patients[125,126]. One study from the Massachusetts General Hospital found a lower response in older patients (61%) as compared to 83% in younger Anti-TNF treated patients[126]. A recent Belgian study however reported similar clinical response rates[123].

In the EPIMAD study between 2.5%-10% of patients with elderly onset IBD received immunosuppressive agents after 1 year. Only 26 patients with CD and 4 with UC received anti-TNF agents, which mirrors trends in other countries. This reflects relative underutilization of anti-TNF therapy perhaps driven by physician concern with adverse effects[15].

Data on biologics safety in elderly patients are predominantly from the rheumatological literature and conflicting, with some studies showing no increased risk of infection and others showing a higher rate of discontinuation owing to adverse effects[127-129]. Early experience from the Mayo Clinic demonstrated that 3 of 4 deaths due to infliximab treatment were in elderly patients. The independent contribution of age was unclear as these patients had a long disease course, more severe disease and co-morbidities[125]. Data from the Stockholm cohort study showed an increased risk of severe adverse effects and mortality in patients aged 60 years and above with severe infections occurring in 11% of elderly patients as compared to 2% in clinical trials and post marketing studies[120,130]. Notably, these patients were from a tertiary referral cohort and the control population in this study was retrospectively recruited which might lead one to speculate that patients treated with biologics may experience more serious disease and further complications[120]. Cottone *et al*[124] reported a 12% risk of serious infection when treated with biological therapy and 3% died from septic shock. Desai *et al*[126] reported that 70% discontinued biological therapy after a mean of two years.

Biological therapy has been associated with the risk of malignancy particularly lymphoma and melanoma skin cancer[131]. However, in most studies patients were prescribed concomitant immunosuppressant therapy and thus the risk of biological monotherapy is hard to extrapolate[115]. The TREAT registry, examining outcomes of CD treatment regimens in North America noted no increased risk of malignancy in patients treated with biologics as compared to other treatments. Furthermore, when compared to the background risk of other malignancies in the Surveillance, Epidemiology and End Results (SEER) database, no additional risk of malignancy was noted with biologics[62]. Long-term data are needed to define this risk.

Adverse effects relevant to clinical practice include exacerbation of congestive cardiac failure, psoriasis, infusion reactions and neurological sequelae such as demyelination[132,133]. The increase in mortality associated with exacerbation of congestive cardiac failure, a common comorbidity in the elderly population, contraindicates its use in the setting of NYHA class Ⅲ and Ⅳ heart failure[134]. Taken together these data emphasise the need for an astute clinical judgement, assessment of disease severity and careful counselling of therapy related risks.

SURGERY

Failure of medical therapy is the most likely cause for elderly IBD patients to have surgery[21,135]. Surgery associated complications and mortality have decreased significantly over the years from 50% between 1960-1984 to 20% between 1994-1999[136].

Recent studies show that there is not much difference in the risk of surgery for older and younger IBD patients[21,137,138]. Ananthakrishnan *et al*[135] reported that although elderly UC patients were less likely to undergo surgery there were similar surgical rates amongst younger and older CD patients. Factors attributable to adverse outcomes include advancing age, male gender, hypoalbuminemia and urgent surgery[22]. Clostridium difficile infection, colorectal cancer and dysplasia are more common indications for surgery in older patients.

Ileal pouch anal anastomosis (IPAA) is the surgical technique of choice in UC if the patient has good anal sphincter function and no history of faecal incontinence[83,139]. The American Society of Colon and Rectal Surgeons recommends that chronological age should not be an exclusion criterion for IPAA surgery[140]. Pouch function deteriorates with increasing age in all patients undergoing IPAA with faecal incontinence; this effect may be more pronounced in the elderly[141]. Despite this, patients report a high level of satisfaction with IPAA, with 89% of elderly UC patients stating that they would opt to undergo the procedure again and 96% willing to recommend it to others[142]. Careful patient selection, taking anal sphincter function, loco-motor and cognitive function into account could lead to favourable outcomes.

IBD RELATED COLORECTAL CANCER

Colitis-associated colorectal cancer (CAC) is one of the most feared complications of long-standing UC and Crohn's colitis. Several risk factors are associated with the development of colorectal neoplasia and include disease extent and duration, severity of histologic and endoscopic inflammation, colitis associated dysplasia, family history and primary sclerosing cholangitis[64,143,144]. Patients with subtotal colitis and pancolitis have the highest risk of developing CAC whereas those with proctitis or distal proctosigmoiditis are at no increased risk compared to the general population[64,144,145]. Patients with left-sided disease (to splenic flexure) carry an intermediate risk but their risk approaches that of patients with pancolitis as disease duration increases[145,146]. The relative risk (RR) increases after 8-10 years of disease and this is the rationale behind the initiation of surveillance colonoscopy[64,143,144,147,148].

Surveillance guidelines for the elderly are not different but need a considered approach. Elderly patients with IBD must be considered in two groups: Those diagnosed at a younger age, *i.e.,* before the age of 60 (long-standing IBD) and those with onset of IBD at a later age (late-onset IBD); with long-standing IBD conferring a higher risk of CAC[64,143,144,147,148]. Shaukat *et al*[149] using a SEER-Medicare linkage program database recently demonstrated that patients transitioning to older age with CD or UC had an OR of 1.93 ($P < 0.001$) and 1.45 ($P = 0.01$) respectively, of developing CAC thus reflecting disease duration, a risk factor for dysplasia in CAC. However, in a recent study comparing non-IBD patients over a six-year period, late-onset IBD was not associated with an increased risk of CAC[150]. This lower risk may be reflective of the shorter duration of the study although the immunobiology of CAC in older patients may be different.

The overriding principle governing colorectal cancer screening is that it should only be done in patients deemed fit to undergo colectomy should dysplasia be found and a life expectancy such that they would be expected to benefit[151]. Increasing age is an independent risk factor for complications at colonoscopy such as colonic perforation[152,153]. Careful patient selection and counselling is a key determinant to good outcomes[151].

VACCINATIONS

IBD patients treated with corticosteroids, immunomodulators and biological agents are at increased risk of developing infectious complications from immune suppression[154,155]. Elderly patients may have additional comorbidities and indeed immunosenescence makes them more susceptible to infection. Many of these diseases are vaccine preventable, yet there have been multiple case reports of infections including fulminant hepatitis and fatal varicella in IBD patients[156,157]. IBD patients are considered immunosuppressed with treatment if they are on 20 mg or more of prednisolone (or equivalent), on-going treatment with effective doses of thiopurines, methotrexate, biological therapies or indeed have had these agents discontinued within 3 mo[154]. There are no significant differences in vaccination guidelines for elderly and younger IBD patients but patients over 60 years old may have sub-optimal serological responses[154,158,159]. Recommended vaccinations include the inactivated influenza vaccine annually, pneumococcal vaccine given periodically (5-yearly), the initial dose followed by the vaccination in over 65 years after five years whether immunosuppressed or not, the hepatitis B series of vaccinations (after immunity is checked and if not immune), the meningococcal vaccine in certain instances (living in enclosed spaces such as dormitories) and those who have undergone splenectomy[154,158]. Live vaccines should be avoided in immunosuppressed patients and these typically include the intranasal influenza, BCG, typhoid oral, varicella, yellow fever, anthrax, measles mumps and rubella (Table 4). If required, live vaccines must be given at least 3 wk before commencing meaningful immunosuppressive therapy or 3 mo after stopping such therapy. Inactivated vaccines may be given at any time from the diagnosis of IBD but ideally at the earliest available opportunity after diagnosis. Recent data suggest that administration

Table 4 Live and attenuated vaccines

Live	Attenuated
Anthrax	Hepatitis B
Intranasal influenza	Human papilloma virus
Measles-mumps-rubella	Influenza
Polio oral vaccine	Pneumococcal
Small pox	
Tuberculosis BCG	
Typhoid	
Varicella	
Yellow fever	

BCG: Bacille Calmette-Guerin.

of the live herpes zoster vaccine may be safe even in patients prescribed Anti-TNF agents[159]. Clinician must carefully weigh the pros and cons of vaccinating *vs* not vaccinating, as consensus guidelines do not currently recommend live vaccinations in patients on immunosuppressive therapy[157].

FUNCTION, COGNITION AND QUALITY OF LIFE CONSIDERATIONS

An appreciation of the potential differences between chronological and biological age is vital for the holistic management of an elderly patient with IBD. Thus, the distinction between "fit and frail" will facilitate a more considered approach[28]. A frail patient with co-morbid illnesses and limited mobility would be at a higher risk of a medical or surgical intervention than a "fit" elderly patient. Health care utilisation by frail patients is notably higher and often related to multi-drug exposure, limited mobility, falls and cognitive impairment[160-166]. Data from the Nationwide Inpatient Sample showed worse outcomes from hospitalisations in elderly patients with 15.7% of patients aged 65-84 years and 35% of patients aged over 85 years requiring discharge to a nursing home or rehabilitation facility compared to less than 1% in individuals aged under 45 years. Furthermore, 12.6% of patients aged 65-84 years required home health care after discharge compared to 4.7% in those aged 18-45 years[135].

CONCLUSION

The rising global incidence of IBD and an ageing population implies that the prevalence of IBD in the elderly is set to increase. The clinical features and therapeutic options in elderly IBD patients are similar to those in younger patients but with important differences. The broad differential diagnosis and emerging patterns of phenotypic progression in the elderly, relative dearth of data specific to elderly IBD patients, their exclusion from pivotal clinical trials and the lack of consensus guidelines have made clinical decisions somewhat challenging. In addition, age specific concerns such as co-morbidity; loco-motor and cognitive function, poly-pharmacy and its consequences must all be taken into account. In applying modern treatment paradigms to the elderly, the clinician must pause to consider the potential for more pronounced adverse effects in this vulnerable group and set appropriate boundaries maximising benefit and minimising harm. There is an urgent need for more data on disease presentation and natural history, clinical trial data assessing treatment paradigms and medication safety, endoscopic complications and hospitalisation. Until then and as discussed, clinicians must make personalised decisions, as evidence based as possible in the holistic, considered and optimal management of elderly patients with IBD.

REFERENCES

1 **Abraham C**, Cho JH. Inflammatory bowel disease. *N Engl J Med* 2009; **361**: 2066-2078 [PMID: 19923578 DOI: 10.1056/NEJMra0804647]

2 **Cosnes J**, Gower-Rousseau C, Seksik P, Cortot A. Epidemiology and natural history of inflammatory bowel diseases. *Gastroenterology* 2011; **140**: 1785-1794 [PMID: 21530745 DOI: 10.1053/j.gastro.2011.01.055]

3 **Molinié F**, Gower-Rousseau C, Yzet T, Merle V, Grandbastien B, Marti R, Lerebours E, Dupas JL, Colombel JF, Salomez JL, Cortot A. Opposite evolution in incidence of Crohn's disease and ulcerative colitis in Northern France (1988-1999). *Gut* 2004; **53**: 843-848 [PMID: 15138211 DOI: 10.1136/gut.2003.025346]

4 **Kinsella H**. An Aging World: 2008, International Population Reports. 2009. Available from: URL: http://www.census.gov/prod/2009pubs/p95-09-1.pdf

5 **Jennifer M**, Ortman VAV, Hogan H. An Aging Nation: The Older Population in the United States Population Estimates and Projections. 2014. Available from: URL: https://www.census.gov/prod/2014pubs/p25-1140.pdf

6 **Charpentier C**, Salleron J, Savoye G, Fumery M, Merle V, Laberenne JE, Vasseur F, Dupas JL, Cortot A, Dauchet L, Peyrin-Biroulet L, Lerebours E, Colombel JF, Gower-Rousseau C. Natural history of elderly-onset inflammatory bowel disease: a population-based cohort study. *Gut* 2014; **63**: 423-432 [PMID: 23408350 DOI: 10.1136/gutjnl-2012-303864]

7 **Loftus EV**, Silverstein MD, Sandborn WJ, Tremaine WJ, Harmsen WS, Zinsmeister AR. Ulcerative colitis in Olmsted County, Minnesota, 1940-1993: incidence, prevalence, and survival. *Gut* 2000; **46**: 336-343 [PMID: 10673294]

8 **Kelsen J**, Baldassano RN. Inflammatory bowel disease: the difference between children and adults. *Inflamm Bowel Dis* 2008; **14** Suppl 2: S9-11 [PMID: 18816756 DOI: 10.1097/00054725-200810001-00005]

9 **Travis S**. Is IBD different in the elderly? *Inflamm Bowel Dis* 2008; **14** Suppl 2: S12-S13 [PMID: 18816682 DOI: 10.1002/ibd.20605]

10 **Bernstein CN**, Wajda A, Svenson LW, MacKenzie A, Koehoorn M, Jackson M, Fedorak R, Israel D, Blanchard JF. The epidemiology of inflammatory bowel disease in Canada: a population-based study. *Am J Gastroenterol* 2006; **101**: 1559-1568 [PMID: 16863561 DOI: 10.1111/j.1572-0241.2006.00603.x]

11 **Loftus EV**, Silverstein MD, Sandborn WJ, Tremaine WJ, Harmsen WS, Zinsmeister AR. Crohn's disease in Olmsted County, Minnesota, 1940-1993: incidence, prevalence, and survival. *Gastroenterology* 1998; **114**: 1161-1168 [PMID: 9609752 DOI: 10.1016/S0016-5085(98)70421-4]

12 **Molodecky NA**, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, Benchimol EI, Panaccione R, Ghosh S, Barkema HW, Kaplan GG. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology*

2012; **142**: 46-54.e42; quiz e30 [PMID: 22001864 DOI: 10.1053/j.gastro.2011.10.001]
13 **Russel MG**, Stockbrügger RW. Epidemiology of inflammatory bowel disease: an update. *Scand J Gastroenterol* 1996; **31**: 417-427 [PMID: 8734336 DOI: 10.3109/00365529609006759]
14 **Greenwald DA**, Brandt LJ. Inflammatory Bowel Disease After Age 60. *Curr Treat Options Gastroenterol* 2003; **6**: 213-225 [PMID: 12744821 DOI: 10.1007/s11938-003-0003-z]
15 **Gower-Rousseau C**, Vasseur F, Fumery M, Savoye G, Salleron J, Dauchet L, Turck D, Cortot A, Peyrin-Biroulet L, Colombel JF. Epidemiology of inflammatory bowel diseases: new insights from a French population-based registry (EPIMAD). *Dig Liver Dis* 2013; **45**: 89-94 [PMID: 23107487 DOI: 10.1016/j.dld.2012.09.005]
16 **Wild CP**. Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology. *Cancer Epidemiol Biomarkers Prev* 2005; **14**: 1847-1850 [PMID: 16103423 DOI: 10.1158/1055-9965.EPI-05-0456]
17 **Harper PC**, McAuliffe TL, Beeken WL. Crohn's disease in the elderly. A statistical comparison with younger patients matched for sex and duration of disease. *Arch Intern Med* 1986; **146**: 753-755 [PMID: 3963958 DOI: 10.1001/archinte.1986.00360160189025]
18 **Wagtmans MJ**, Verspaget HW, Lamers CB, van Hogezand RA. Crohn's disease in the elderly: a comparison with young adults. *J Clin Gastroenterol* 1998; **27**: 129-133 [PMID: 9754773 DOI: 10.1097/00004836-199809000-00005]
19 **Greth J**, Török HP, Koenig A, Folwaczny C. Comparison of inflammatory bowel disease at younger and older age. *Eur J Med Res* 2004; **9**: 552-554 [PMID: 15689301]
20 **Polito JM**, Childs B, Mellits ED, Tokayer AZ, Harris ML, Bayless TM. Crohn's disease: influence of age at diagnosis on site and clinical type of disease. *Gastroenterology* 1996; **111**: 580-586 [PMID: 8780560 DOI: 10.1053/gast.1996.v111.pm8780560]
21 **Lakatos PL**, David G, Pandur T, Erdelyi Z, Mester G, Balogh M, Szipocs I, Molnar C, Komaromi E, Kiss LS, Lakatos L. IBD in the elderly population: results from a population-based study in Western Hungary, 1977-2008. *J Crohns Colitis* 2011; **5**: 5-13 [PMID: 21272797 DOI: 10.1016/j.crohns.2010.08.004]
22 **Juneja M**, Baidoo L, Schwartz MB, Barrie A, Regueiro M, Dunn M, Binion DG. Geriatric inflammatory bowel disease: phenotypic presentation, treatment patterns, nutritional status, outcomes, and comorbidity. *Dig Dis Sci* 2012; **57**: 2408-2415 [PMID: 22359191 DOI: 10.1007/s10620-012-2083-x]
23 **Quezada SM**, Steinberger EK, Cross RK. Association of age at diagnosis and Crohn's disease phenotype. *Age Ageing* 2013; **42**: 102-106 [PMID: 22918090 DOI: 10.1093/ageing/afs107]
24 **Softley A**, Myren J, Clamp SE, Bouchier IA, Watkinson G, de Dombal FT. Inflammatory bowel disease in the elderly patient. *Scand J Gastroenterol Suppl* 1988; **144**: 27-30 [PMID: 3165553]
25 **Riegler G**, Tartaglione MT, Carratú R, D'Incá R, Valpiani D, Russo MI, Papi C, Fiorentini MT, Ingrosso M, Andreoli A, Vecchi M. Age-related clinical severity at diagnosis in 1705 patients with ulcerative colitis: a study by GISC (Italian Colon-Rectum Study Group). *Dig Dis Sci* 2000; **45**: 462-465 [PMID: 10749318 DOI: 10.1023/A:1005424603085]
26 **Zimmerman J**, Gavish D, Rachmilewitz D. Early and late onset ulcerative colitis: distinct clinical features. *J Clin Gastroenterol* 1985; **7**: 492-498 [PMID: 4086743 DOI: 10.1097/00004836-198512000-00010]
27 **del Val JH**. Old-age inflammatory bowel disease onset: a different problem? *World J Gastroenterol* 2011; **17**: 2734-2739 [PMID: 21734781 DOI: 10.3748/Wjg.V17.I22.2734]
28 **Katz S**, Pardi DS. Inflammatory bowel disease of the elderly: frequently asked questions (FAQs). *Am J Gastroenterol* 2011; **106**: 1889-1897 [PMID: 21862997 DOI: 10.1038/ajg.2011.271]
29 **Han SW**, McColl E, Barton JR, James P, Steen IN, Welfare MR. Predictors of quality of life in ulcerative colitis: the importance of symptoms and illness representations. *Inflamm Bowel Dis* 2005; **11**: 24-34 [PMID: 15674110 DOI: 10.1097/00054725-200501000-00004]
30 **Ananthakrishnan AN**, Binion DG. Treatment of ulcerative colitis in the elderly. *Dig Dis* 2009; **27**: 327-334 [PMID: 19786760 DOI: 10.1159/000228569]
31 **Bassi A**, Dodd S, Williamson P, Bodger K. Cost of illness of inflammatory bowel disease in the UK: a single centre retrospective study. *Gut* 2004; **53**: 1471-1478 [PMID: 15361497 DOI: 10.1136/gut.2004.041616]
32 **Odes S**, Vardi H, Friger M, Wolters F, Russel MG, Riis L, Munkholm P, Politi P, Tsianos E, Clofent J, Vermeire S, Monteiro E, Mouzas I, Fornaciari G, Sijbrandij J, Limonard C, Van Zeijl G, O'morain C, Moum B, Vatn M, Stockbrugger R. Cost analysis and cost determinants in a European inflammatory bowel disease inception cohort with 10 years of follow-up evaluation. *Gastroenterology* 2006; **131**: 719-728 [PMID: 16952541 DOI: 10.1053/j.gastro.2006.05.052]
33 **Loftus EV**. A matter of life or death: mortality in Crohn's disease. *Inflamm Bowel Dis* 2002; **8**: 428-429 [PMID: 12607512 DOI: 10.1097/00054725-200211000-00009]
34 **Wolters FL**, Russel MG, Sijbrandij J, Ambergen T, Odes S, Riis L, Langholz E, Politi P, Qasim A, Koutroubakis I, Tsianos E, Vermeire S, Freitas J, van Zeijl G, Hoie O, Bernklev T, Beltrami M, Rodriguez D, Stockbrügger RW, Moum B. Phenotype at diagnosis predicts recurrence rates in Crohn's disease. *Gut* 2006; **55**: 1124-1130 [PMID: 16361306 DOI: 10.1136/gut.2005.084061]
35 **Farrokhyar F**, Swarbrick ET, Irvine EJ. A critical review of epidemiological studies in inflammatory bowel disease. *Scand J Gastroenterol* 2001; **36**: 2-15 [PMID: 11218235 DOI: 10.1080/00365520150218002]
36 **Foxworthy DM**, Wilson JA. Crohn's disease in the elderly. Prolonged delay in diagnosis. *J Am Geriatr Soc* 1985; **33**: 492-495 [PMID: 4008848 DOI: 10.1111/j.1532-5415.1985.tb05462.x]
37 **Painter NS**, Burkitt DP. Diverticular disease of the colon: a deficiency disease of Western civilization. *Br Med J* 1971; **2**: 450-454 [PMID: 4930390 DOI: 10.1136/bmj.2.5759.450]
38 **Peppercorn MA**. Drug-responsive chronic segmental colitis associated with diverticula: a clinical syndrome in the elderly. *Am J Gastroenterol* 1992; **87**: 609-612 [PMID: 1595649]
39 **Hadithi M**, Cazemier M, Meijer GA, Bloemena E, Felt-Bersma RJ, Mulder CJ, Meuwissen SG, Pena AS, van Bodegraven AA. Retrospective analysis of old-age colitis in the Dutch inflammatory bowel disease population. *World J Gastroenterol* 2008; **14**: 3183-3187 [PMID: 18506923 DOI: 10.3748/wjg.14.3183]
40 **Faucheron JL**. Toxicity of non-steroidal anti-inflammatory drugs in the large bowel. *Eur J Gastroenterol Hepatol* 1999; **11**: 389-392 [PMID: 10321754 DOI: 10.1097/00042737-199904000-00005]
41 **Sandborn WJ**, Stenson WF, Brynskov J, Lorenz RG, Steidle GM, Robbins JL, Kent JD, Bloom BJ. Safety of celecoxib in patients with ulcerative colitis in remission: a randomized, placebo-controlled, pilot study. *Clin Gastroenterol Hepatol* 2006; **4**: 203-211 [PMID: 16469681 DOI: 10.1016/j.cgh.2005.12.002]
42 **Montoro MA**, Brandt LJ, Santolaria S, Gomollon F, Sánchez Puértolas B, Vera J, Bujanda L, Cosme A, Cabriada JL, Durán M, Mata L, Santamaría A, Ceña G, Blas JM, Ponce J, Ponce M, Rodrigo L, Ortiz J, Muñoz C, Arozena G, Ginard D, López-Serrano A, Castro M, Sans M, Campo R, Casalots A, Orive V, Loizate A, Titó L, Portabella E, Otazua P, Calvo M, Botella MT, Thomson C, Mundi JL, Quintero E, Nicolás D, Borda F, Martinez B, Gisbert JP, Chaparro M, Jimenez Bernadó A, Gómez-Camacho F, Cerezo A, Casal Nuñez E. Clinical patterns and outcomes of ischaemic colitis: results of the Working Group for the Study of Ischaemic Colitis in Spain (CIE study). *Scand J Gastroenterol* 2011; **46**: 236-246 [PMID: 20961178 DOI: 10.3109/00365521.2010.525794]
43 **Brandt L**, Boley S, Goldberg L, Mitsudo S, Berman A. Colitis in the elderly. A reappraisal. *Am J Gastroenterol* 1981; **76**: 239-245 [PMID: 7315820]
44 **Schumacher G**, Kollberg B, Sandstedt B. A prospective study of first attacks of inflammatory bowel disease and infectious colitis. Histologic course during the 1st year after presentation. *Scand J Gastroenterol* 1994; **29**: 318-332 [PMID: 8047806 DOI: 10.3109/00365529409094843]

45 **Pépin J**, Valiquette L, Cossette B. Mortality attributable to nosocomial Clostridium difficile-associated disease during an epidemic caused by a hypervirulent strain in Quebec. *CMAJ* 2005; **173**: 1037-1042 [PMID: 16179431 DOI: 10.1503/cmaj.050978]

46 **Cohen SH**, Gerding DN, Johnson S, Kelly CP, Loo VG, McDonald LC, Pepin J, Wilcox MH. Clinical practice guidelines for Clostridium difficile infection in adults: 2010 update by the society for healthcare epidemiology of America (SHEA) and the infectious diseases society of America (IDSA). *Infect Control Hosp Epidemiol* 2010; **31**: 431-455 [PMID: 20307191 DOI: 10.1086/651706]

47 **Colombel JF**, Sandborn WJ, Reinisch W, Mantzaris GJ, Kornbluth A, Rachmilewitz D, Lichtiger S, D'Haens G, Diamond RH, Broussard DL, Tang KL, van der Woude CJ, Rutgeerts P. Infliximab, azathioprine, or combination therapy for Crohn's disease. *N Engl J Med* 2010; **362**: 1383-1395 [PMID: 20393175 DOI: 10.1056/NEJMa0904492]

48 **D'Haens G**, Baert F, van Assche G, Caenepeel P, Vergauwe P, Tuynman H, De Vos M, van Deventer S, Stitt L, Donner A, Vermeire S, Van de Mierop FJ, Coche JC, van der Woude J, Ochsenkühn T, van Bodegraven AA, Van Hootegem PP, Lambrecht GL, Mana F, Rutgeerts P, Feagan BG, Hommes D. Early combined immunosuppression or conventional management in patients with newly diagnosed Crohn's disease: an open randomised trial. *Lancet* 2008; **371**: 660-667 [PMID: 18295023 DOI: 10.1016/S0140-6736(08)60304-9]

49 **van Duin D**, Mohanty S, Thomas V, Ginter S, Montgomery RR, Fikrig E, Allore HG, Medzhitov R, Shaw AC. Age-associated defect in human TLR-1/2 function. *J Immunol* 2007; **178**: 970-975 [PMID: 17202359 DOI: 10.4049/jimmunol.178.2.970]

50 **Ogawa T**, Kitagawa M, Hirokawa K. Age-related changes of human bone marrow: a histometric estimation of proliferative cells, apoptotic cells, T cells, B cells and macrophages. *Mech Ageing Dev* 2000; **117**: 57-68 [PMID: 10958923 DOI: 10.1016/S0047-6374(00)00137-8]

51 **Plowden J**, Renshaw-Hoelscher M, Engleman C, Katz J, Sambhara S. Innate immunity in aging: impact on macrophage function. *Aging Cell* 2004; **3**: 161-167 [PMID: 15268749 DOI: 10.1111/j.1474-9728.2004.00102.x]

52 **Borrego F**, Alonso MC, Galiani MD, Carracedo J, Ramirez R, Ostos B, Peña J, Solana R. NK phenotypic markers and IL2 response in NK cells from elderly people. *Exp Gerontol* 1999; **34**: 253-265 [PMID: 10363791 DOI: 10.1016/S0531-5565(98)00076-X]

53 **Hayhoe RP**, Henson SM, Akbar AN, Palmer DB. Variation of human natural killer cell phenotypes with age: identification of a unique KLRG1-negative subset. *Hum Immunol* 2010; **71**: 676-681 [PMID: 20394788 DOI: 10.1016/j.humimm.2010.03.014]

54 **Jing Y**, Shaheen E, Drake RR, Chen N, Gravenstein S, Deng Y. Aging is associated with a numerical and functional decline in plasmacytoid dendritic cells, whereas myeloid dendritic cells are relatively unaltered in human peripheral blood. *Hum Immunol* 2009; **70**: 777-784 [PMID: 19596035 DOI: 10.1016/j.humimm.2009.07.005]

55 **Lazuardi L**, Jenewein B, Wolf AM, Pfister G, Tzankov A, Grubeck-Loebenstein B. Age-related loss of naïve T cells and dysregulation of T-cell/B-cell interactions in human lymph nodes. *Immunology* 2005; **114**: 37-43 [PMID: 15606793 DOI: 10.1111/j.1365-2567.2004.02006.x]

56 **Drey M**, Kaiser MJ. [Malnutrition in the elderly]. *Dtsch Med Wochenschr* 2011; **136**: 176-178 [PMID: 21271475 DOI: 10.1055/s-0031-1272503]

57 **Gavazzi G**, Krause KH. Ageing and infection. *Lancet Infect Dis* 2002; **2**: 659-666 [PMID: 12409046 DOI: 10.1016/S1473-3099(02)00437-1]

58 **Stallmach A**, Hagel S, Gharbi A, Settmacher U, Hartmann M, Schmidt C, Bruns T. Medical and surgical therapy of inflammatory bowel disease in the elderly - prospects and complications. *J Crohns Colitis* 2011; **5**: 177-188 [PMID: 21575879 DOI: 10.1016/j.crohns.2011.02.001]

59 **Cross RK**, Wilson KT, Binion DG. Polypharmacy and Crohn's disease. *Aliment Pharmacol Ther* 2005; **21**: 1211-1216 [PMID: 15882241 DOI: 10.1111/j.1365-2036.2005.02429.x]

60 **MacLaughlin EJ**, Raehl CL, Treadway AK, Sterling TL, Zoller DP, Bond CA. Assessing medication adherence in the elderly: which tools to use in clinical practice? *Drugs Aging* 2005; **22**: 231-255 [PMID: 15813656 DOI: 10.2165/00002512-200522030-00005]

61 **Muller AF**, Stevens PE, McIntyre AS, Ellison H, Logan RF. Experience of 5-aminosalicylate nephrotoxicity in the United Kingdom. *Aliment Pharmacol Ther* 2005; **21**: 1217-1224 [PMID: 15882242 DOI: 10.1111/j.1365-2036.2005.02462.x]

62 **Lichtenstein GR**, Feagan BG, Cohen RD, Salzberg BA, Diamond RH, Langholff W, Londhe A, Sandborn WJ. Drug therapies and the risk of malignancy in Crohn's disease: results from the TREAT ™ Registry. *Am J Gastroenterol* 2014; **109**: 212-223 [PMID: 24394749 DOI: 10.1038/ajg.2013.441]

63 **Safdi M**, DeMicco M, Sninsky C, Banks P, Wruble L, Deren J, Koval G, Nichols T, Targan S, Fleishman C, Wiita B. A double-blind comparison of oral versus rectal mesalamine versus combination therapy in the treatment of distal ulcerative colitis. *Am J Gastroenterol* 1997; **92**: 1867-1871 [PMID: 9382054]

64 **Kornbluth A**, Sachar DB. Ulcerative colitis practice guidelines in adults: American College Of Gastroenterology, Practice Parameters Committee. *Am J Gastroenterol* 2010; **105**: 501-523; quiz 524 [PMID: 20068560 DOI: 10.1038/ajg.2009.727]

65 **Hussain SW**, Pardi DS. Inflammatory bowel disease in the elderly. *Drugs Aging* 2010; **27**: 617-624 [PMID: 20658790 DOI: 10.2165/11537340-000000000-00000]

66 **Kane SV**, Cohen RD, Aikens JE, Hanauer SB. Prevalence of nonadherence with maintenance mesalamine in quiescent ulcerative colitis. *Am J Gastroenterol* 2001; **96**: 2929-2933 [PMID: 11693328 DOI: 10.1111/j.1572-0241.2001.04683.x]

67 **Rao SS**. Diagnosis and management of fecal incontinence. American College of Gastroenterology Practice Parameters Committee. *Am J Gastroenterol* 2004; **99**: 1585-1604 [PMID: 15307881 DOI: 10.1111/j.1572-0241.2004.40105.x]

68 **Gisbert JP**, González-Lama Y, Maté J. 5-Aminosalicylates and renal function in inflammatory bowel disease: a systematic review. *Inflamm Bowel Dis* 2007; **13**: 629-638 [PMID: 17243140 DOI: 10.1002/ibd.20099]

69 **Stein RB**, Hanauer SB. Comparative tolerability of treatments for inflammatory bowel disease. *Drug Saf* 2000; **23**: 429-448 [PMID: 11085348 DOI: 10.2165/00002018-200023050-00006]

70 **Kapur KC**, Williams GT, Allison MC. Mesalazine induced exacerbation of ulcerative colitis. *Gut* 1995; **37**: 838-839 [PMID: 8537058 DOI: 10.1136/gut.37.6.838]

71 **Lewis LD**, Benin A, Szumlanski CL, Otterness DM, Lennard L, Weinshilboum RM, Nierenberg DW. Olsalazine and 6-mercaptopurine-related bone marrow suppression: a possible drug-drug interaction. *Clin Pharmacol Ther* 1997; **62**: 464-475 [PMID: 9357398 DOI: 10.1016/S0009-9236(97)90125-9]

72 **de Boer NK**, Wong DR, Jharap B, de Graaf P, Hooymans PM, Mulder CJ, Rijmen F, Engels LG, van Bodegraven AA. Dose-dependent influence of 5-aminosalicylates on thiopurine metabolism. *Am J Gastroenterol* 2007; **102**: 2747-2753 [PMID: 17764493 DOI: 10.1111/j.1572-0241.2007.01511.x]

73 **Gisbert JP**, Gomollón F. Thiopurine-induced myelotoxicity in patients with inflammatory bowel disease: a review. *Am J Gastroenterol* 2008; **103**: 1783-1800 [PMID: 18557712 DOI: 10.1111/j.1572-0241.2008.01848.x]

74 **Wells PS**, Holbrook AM, Crowther NR, Hirsh J. Interactions of warfarin with drugs and food. *Ann Intern Med* 1994; **121**: 676-683 [PMID: 7944078 DOI: 10.7326/0003-4819-121-9-199411010-00009]

75 **Khan KJ**, Ullman TA, Ford AC, Abreu MT, Abadir A, Marshall JK, Talley NJ, Moayyedi P. Antibiotic therapy in inflammatory bowel disease: a systematic review and meta-analysis. *Am J Gastroenterol* 2011; **106**: 661-673 [PMID: 21407187 DOI: 10.1038/ajg.2011.72]

76 **Mantzaris GJ**, Archavlis E, Christoforidis P, Kourtessas D,

Amberiadis P, Florakis N, Petraki K, Spiliadi C, Triantafyllou G. A prospective randomized controlled trial of oral ciprofloxacin in acute ulcerative colitis. *Am J Gastroenterol* 1997; **92**: 454-456 [PMID: 9068468]

77 **Mantzaris GJ**, Hatzis A, Kontogiannis P, Triadaphyllou G. Intravenous tobramycin and metronidazole as an adjunct to corticosteroids in acute, severe ulcerative colitis. *Am J Gastroenterol* 1994; **89**: 43-46 [PMID: 8273796]

78 **Duffy LF**, Daum F, Fisher SE, Selman J, Vishnubhakat SM, Aiges HW, Markowitz JF, Silverberg M. Peripheral neuropathy in Crohn's disease patients treated with metronidazole. *Gastroenterology* 1985; **88**: 681-684 [PMID: 2981752]

79 **Freeman CD**, Klutman NE, Lamp KC. Metronidazole. A therapeutic review and update. *Drugs* 1997; **54**: 679-708 [PMID: 9360057 DOI: 10.2165/00003495-199754050-00003]

80 **Shakeri-Nejad K**, Stahlmann R. Drug interactions during therapy with three major groups of antimicrobial agents. *Expert Opin Pharmacother* 2006; **7**: 639-651 [PMID: 16556082 DOI: 10.1517/14656566.7.6.639]

81 **Yu C**, Giuffre B. Achilles tendinopathy after treatment with fluoroquinolone. *Australas Radiol* 2005; **49**: 407-410 [PMID: 16174181 DOI: 10.1111/j.1440-1673.2005.01470.x]

82 **Louie TJ**, Miller MA, Crook DW, Lentnek A, Bernard L, High KP, Shue YK, Gorbach SL. Effect of age on treatment outcomes in Clostridium difficile infection. *J Am Geriatr Soc* 2013; **61**: 222-230 [PMID: 23379974 DOI: 10.1111/jgs.12090]

83 **Dignass A**, Lindsay JO, Sturm A, Windsor A, Colombel JF, Allez M, D'Haens G, D'Hoore A, Mantzaris G, Novacek G, Oresland T, Reinisch W, Sans M, Stange E, Vermeire S, Travis S, Van Assche G. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 2: current management. *J Crohns Colitis* 2012; **6**: 991-1030 [PMID: 23040451 DOI: 10.1016/j.crohns.2012.09.002]

84 **Lichtenstein GR**, Hanauer SB, Sandborn WJ. Management of Crohn's disease in adults. *Am J Gastroenterol* 2009; **104**: 465-483; quiz 464, 484 [PMID: 19174807 DOI: 10.1038/ajg.2008.168]

85 **Thomas TP**. The complications of systemic corticosteroid therapy in the elderly. A retrospective study. *Gerontology* 1984; **30**: 60-65 [PMID: 6698408]

86 **Akerkar GA**, Peppercorn MA, Hamel MB, Parker RA. Corticosteroid-associated complications in elderly Crohn's disease patients. *Am J Gastroenterol* 1997; **92**: 461-464 [PMID: 9068470]

87 **Tilg H**, Moschen AR, Kaser A, Pines A, Dotan I. Gut, inflammation and osteoporosis: basic and clinical concepts. *Gut* 2008; **57**: 684-694 [PMID: 18408105 DOI: 10.1136/gut.2006.117382]

88 **Lichtenstein GR**, Sands BE, Pazianas M. Prevention and treatment of osteoporosis in inflammatory bowel disease. *Inflamm Bowel Dis* 2006; **12**: 797-813 [PMID: 16917235 DOI: 10.1097/00054725-200608000-00016]

89 **Swaroop PP**. Inflammatory bowel diseases in the elderly. *Clin Geriatr Med* 2007; **23**: 809-821, vi [PMID: 17923339 DOI: 10.1016/j.cger.2007.06.007]

90 **Frey BM**, Frey FJ. Clinical pharmacokinetics of prednisone and prednisolone. *Clin Pharmacokinet* 1990; **19**: 126-146 [PMID: 2199128 DOI: 10.2165/00003088-199019020-00003]

91 **Travis SP**, Danese S, Kupcinskas L, Alexeeva O, D'Haens G, Gibson PR, Moro L, Jones R, Ballard ED, Masure J, Rossini M, Sandborn WJ. Once-daily budesonide MMX in active, mild-to-moderate ulcerative colitis: results from the randomised CORE II study. *Gut* 2014; **63**: 433-441 [PMID: 23436336 DOI: 10.1136/gutjnl-2012-304258]

92 **Feagan BG**, Rochon J, Fedorak RN, Irvine EJ, Wild G, Sutherland L, Steinhart AH, Greenberg GR, Gillies R, Hopkins M. Methotrexate for the treatment of Crohn's disease. The North American Crohn's Study Group Investigators. *N Engl J Med* 1995; **332**: 292-297 [PMID: 7816064 DOI: 10.1056/NEJM199502023320503]

93 **Matsumoto S**, Miyatani H, Yoshida Y. Ulcerative colitis: comparison between elderly and young adult patients and between elderly patients with late-onset and long-standing disease. *Dig Dis Sci* 2013; **58**: 1306-1312 [PMID: 23306844 DOI: 10.1007/s10620-012-2517-5]

94 **Rodríguez-D'Jesus A**, Casellas F, Malagelada JR. Epidemiology of inflammatory bowel disease in the elderly. *Gastroenterol Hepatol* 2008; **31**: 269-273 [PMID: 18448054 DOI: 10.1157/13119877]

95 **Present DH**, Meltzer SJ, Krumholz MP, Wolke A, Korelitz BI. 6-Mercaptopurine in the management of inflammatory bowel disease: short- and long-term toxicity. *Ann Intern Med* 1989; **111**: 641-649 [PMID: 2802419 DOI: 10.7326/0003-4819-111-8-641]

96 **Weinshilboum RM**, Sladek SL. Mercaptopurine pharmacogenetics: monogenic inheritance of erythrocyte thiopurine methyltransferase activity. *Am J Hum Genet* 1980; **32**: 651-662 [PMID: 7191632]

97 **Connell WR**, Kamm MA, Ritchie JK, Lennard-Jones JE. Bone marrow toxicity caused by azathioprine in inflammatory bowel disease: 27 years of experience. *Gut* 1993; **34**: 1081-1085 [PMID: 8174958 DOI: 10.1136/gut.34.8.1081]

98 **Bouhnik Y**, Lémann M, Mary JY, Scemama G, Taï R, Matuchansky C, Modigliani R, Rambaud JC. Long-term follow-up of patients with Crohn's disease treated with azathioprine or 6-mercaptopurine. *Lancet* 1996; **347**: 215-219 [PMID: 8551879 DOI: 10.1016/S0140-6736(96)90402-X]

99 **Black AJ**, McLeod HL, Capell HA, Powrie RH, Matowe LK, Pritchard SC, Collie-Duguid ES, Reid DM. Thiopurine methyltransferase genotype predicts therapy-limiting severe toxicity from azathioprine. *Ann Intern Med* 1998; **129**: 716-718 [PMID: 9841604 DOI: 10.7326/0003-4819-129-9-199811010-00007]

100 **Cuffari C**, Dassopoulos T, Turnbough L, Thompson RE, Bayless TM. Thiopurine methyltransferase activity influences clinical response to azathioprine in inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2004; **2**: 410-417 [PMID: 15118980 DOI: 10.1016/S1542-3565(04)00127-2]

101 **Chaparro M**, Ordás I, Cabré E, Garcia-Sanchez V, Bastida G, Peñalva M, Gomollón F, García-Planella E, Merino O, Gutiérrez A, Esteve M, Márquez L, Garcia-Sepulcre M, Hinojosa J, Vera I, Muñoz F, Mendoza JL, Cabriada JL, Montoro MA, Barreiro-de Acosta M, Ceña G, Saro C, Aldeguer X, Barrio J, Maté J, Gisbert JP. Safety of thiopurine therapy in inflammatory bowel disease: long-term follow-up study of 3931 patients. *Inflamm Bowel Dis* 2013; **19**: 1404-1410 [PMID: 23665964 DOI: 10.1097/MIB.0b013e318281f28f]

102 **Ariyaratnam J**, Subramanian V. Association between thiopurine use and nonmelanoma skin cancers in patients with inflammatory bowel disease: a meta-analysis. *Am J Gastroenterol* 2014; **109**: 163-169 [PMID: 24419479 DOI: 10.1038/ajg.2013.451]

103 **Govani SM**, Higgins PD. Combination of thiopurines and allopurinol: adverse events and clinical benefit in IBD. *J Crohns Colitis* 2010; **4**: 444-449 [PMID: 21122542 DOI: 10.1016/j.crohns.2010.02.009]

104 **Sparrow MP**, Hande SA, Friedman S, Cao D, Hanauer SB. Effect of allopurinol on clinical outcomes in inflammatory bowel disease nonresponders to azathioprine or 6-mercaptopurine. *Clin Gastroenterol Hepatol* 2007; **5**: 209-214 [PMID: 17296529 DOI: 10.1016/j.cgh.2006.11.020]

105 **Siegel CA**, Marden SM, Persing SM, Larson RJ, Sands BE. Risk of lymphoma associated with combination anti-tumor necrosis factor and immunomodulator therapy for the treatment of Crohn's disease: a meta-analysis. *Clin Gastroenterol Hepatol* 2009; **7**: 874-881 [PMID: 19558997 DOI: 10.1016/j.cgh.2009.01.004]

106 **Khan N**, Abbas AM, Lichtenstein GR, Loftus EV, Bazzano LA. Risk of lymphoma in patients with ulcerative colitis treated with thiopurines: a nationwide retrospective cohort study. *Gastroenterology* 2013; **145**: 1007-1015.e3 [PMID: 23891975 DOI: 10.1053/j.gastro.2013.07.035]

107 **Beaugerie L**, Brousse N, Bouvier AM, Colombel JF, Lémann M, Cosnes J, Hébuterne X, Cortot A, Bouhnik Y, Gendre JP, Simon T, Maynadié M, Hermine O, Faivre J, Carrat F. Lymphoproliferative disorders in patients receiving thiopurines for inflammatory bowel disease: a prospective observational cohort study. *Lancet*

2009; **374**: 1617-1625 [PMID: 19837455 DOI: 10.1016/S0140-6736(09)61302-7]
108 **Patel V**, Wang Y, MacDonald JK, McDonald JW, Chande N. Methotrexate for maintenance of remission in Crohn's disease. *Cochrane Database Syst Rev* 2014; **8**: CD006884 [PMID: 25157445 DOI: 10.1002/14651858.cd006884.pub3]
109 **Chande N**, Wang Y, MacDonald JK, McDonald JW. Methotrexate for induction of remission in ulcerative colitis. *Cochrane Database Syst Rev* 2014; **8**: CD006618 [PMID: 25162749 DOI: 10.1002/14651858.cd006618.pub3]
110 **Busard C**, Zweegers J, Limpens J, Langendam M, Spuls PI. Combined use of systemic agents for psoriasis: a systematic review. *JAMA Dermatol* 2014; **150**: 1213-1220 [PMID: 25188393 DOI: 10.1001/jamadermatol.2014.1111]
111 **Buttgereit F**, Smolen JS, Coogan AN, Cajochen C. Clocking in: chronobiology in rheumatoid arthritis. *Nat Rev Rheumatol* 2015; **11**: 349-356 [PMID: 25800214 DOI: 10.1038/nrrheum.2015.31]
112 **Morgacheva O**, Furst DE. Use of MTX in the elderly and in patients with compromised renal function. *Clin Exp Rheumatol* 2010; **28**: S85-S94 [PMID: 21044439]
113 **Shea B**, Swinden MV, Ghogomu ET, Ortiz Z, Katchamart W, Rader T, Bombardier C, Wells GA, Tugwell P. Folic acid and folinic acid for reducing side effects in patients receiving methotrexate for rheumatoid arthritis. *J Rheumatol* 2014; **41**: 1049-1060 [PMID: 24737913 DOI: 10.3899/jrheum.130738]
114 **Bourré-Tessier J**, Haraoui B. Methotrexate drug interactions in the treatment of rheumatoid arthritis: a systematic review. *J Rheumatol* 2010; **37**: 1416-1421 [PMID: 20436072 DOI: 10.3899/jrheum.090153]
115 **Subramaniam K**, D'Rozario J, Pavli P. Lymphoma and other lymphoproliferative disorders in inflammatory bowel disease: a review. *J Gastroenterol Hepatol* 2013; **28**: 24-30 [PMID: 23094824 DOI: 10.1111/jgh.12015]
116 **Laharie D**, Bourreille A, Branche J, Allez M, Bouhnik Y, Filippi J, Zerbib F, Savoye G, Nachury M, Moreau J, Delchier JC, Cosnes J, Ricart E, Dewit O, Lopez-Sanroman A, Dupas JL, Carbonnel F, Bommelaer G, Coffin B, Roblin X, Van Assche G, Esteve M, Färkkilä M, Gisbert JP, Marteau P, Nahon S, de Vos M, Franchimont D, Mary JY, Colombel JF, Lémann M. Ciclosporin versus infliximab in patients with severe ulcerative colitis refractory to intravenous steroids: a parallel, open-label randomised controlled trial. *Lancet* 2012; **380**: 1909-1915 [PMID: 23063316 DOI: 10.1016/S0140-6736(12)61084-8]
117 **Gisbert JP**, Chaparro M. Systematic review with meta-analysis: inflammatory bowel disease in the elderly. *Aliment Pharmacol Ther* 2014; **39**: 459-477 [PMID: 24405149 DOI: 10.1111/apt.12616]
118 **Kovarik JM**, Koelle EU. Cyclosporin pharmacokinetics in the elderly. *Drugs Aging* 1999; **15**: 197-205 [PMID: 10503812 DOI: 10.2165/00002512-199915030-00003]
119 **Dunn CJ**, Wagstaff AJ, Perry CM, Plosker GL, Goa KL. Cyclosporin: an updated review of the pharmacokinetic properties, clinical efficacy and tolerability of a microemulsion-based formulation (neoral)1 in organ transplantation. *Drugs* 2001; **61**: 1957-2016 [PMID: 11708766 DOI: 10.2165/00003495-200161130-00006]
120 **Ljung T**, Karlén P, Schmidt D, Hellström PM, Lapidus A, Janczewska I, Sjöqvist U, Löfberg R. Infliximab in inflammatory bowel disease: clinical outcome in a population based cohort from Stockholm County. *Gut* 2004; **53**: 849-853 [PMID: 15138212 DOI: 10.1136/gut.2003.018515]
121 **Seiderer J**, Göke B, Ochsenkühn T. Safety aspects of infliximab in inflammatory bowel disease patients. A retrospective cohort study in 100 patients of a German University Hospital. *Digestion* 2004; **70**: 3-9 [PMID: 15297773 DOI: 10.1159/000080075]
122 **Chevillotte-Maillard H**, Ornetti P, Mistrih R, Sidot C, Dupuis J, Dellas JA, Tavernier C, Maillefert JF. Survival and safety of treatment with infliximab in the elderly population. *Rheumatology* (Oxford) 2005; **44**: 695-696 [PMID: 15705631 DOI: 10.1093/rheumatology/keh562]
123 **Lobatón T**, Ferrante M, Rutgeerts P, Ballet V, Van Assche G, Vermeire S. Efficacy and safety of anti-TNF therapy in elderly patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2015; **42**: 441-451 [PMID: 26104047 DOI: 10.1111/apt.13294]
124 **Cottone M**, Kohn A, Daperno M, Armuzzi A, Guidi L, D'Inca R, Bossa F, Angelucci E, Biancone L, Gionchetti P, Ardizzone S, Papi C, Fries W, Danese S, Riegler G, Cappello M, Castiglione F, Annese V, Orlando A. Advanced age is an independent risk factor for severe infections and mortality in patients given anti-tumor necrosis factor therapy for inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2011; **9**: 30-35 [PMID: 20951835 DOI: 10.1016/j.cgh.2010.09.026]
125 **Colombel JF**, Loftus EV, Tremaine WJ, Egan LJ, Harmsen WS, Schleck CD, Zinsmeister AR, Sandborn WJ. The safety profile of infliximab in patients with Crohn's disease: the Mayo clinic experience in 500 patients. *Gastroenterology* 2004; **126**: 19-31 [PMID: 14699483 DOI: 10.1053/j.gastro.2003.10.047]
126 **Desai A**, Zator ZA, de Silva P, Nguyen DD, Korzenik J, Yajnik V, Ananthakrishnan AN. Older age is associated with higher rate of discontinuation of anti-TNF therapy in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2013; **19**: 309-315 [PMID: 22605668 DOI: 10.1002/ibd.23026]
127 **Schneeweiss S**, Setoguchi S, Weinblatt ME, Katz JN, Avorn J, Sax PE, Levin R, Solomon DH. Anti-tumor necrosis factor alpha therapy and the risk of serious bacterial infections in elderly patients with rheumatoid arthritis. *Arthritis Rheum* 2007; **56**: 1754-1764 [PMID: 17530704 DOI: 10.1002/art.22600]
128 **Marchesoni A**, Zaccara E, Gorla R, Bazzani C, Sarzi-Puttini P, Atzeni F, Caporali R, Bobbio-Pallavicini F, Favalli EG. TNF-alpha antagonist survival rate in a cohort of rheumatoid arthritis patients observed under conditions of standard clinical practice. *Ann N Y Acad Sci* 2009; **1173**: 837-846 [PMID: 19758236 DOI: 10.1111/j.1749-6632.2009.04621.x]
129 **Filippini M**, Bazzani C, Favalli EG, Marchesoni A, Atzeni F, Sarzi-Puttini P, Pallavicini FB, Caporali R, Gorla R. Efficacy and safety of anti-tumour necrosis factor in elderly patients with rheumatoid arthritis: an observational study. *Clin Rev Allergy Immunol* 2010; **38**: 90-96 [PMID: 19548124 DOI: 10.1007/s12016-009-8142-1]
130 **Lichtenstein GR**, Feagan BG, Cohen RD, Salzberg BA, Diamond RH, Chen DM, Pritchard ML, Sandborn WJ. Serious infections and mortality in association with therapies for Crohn's disease: TREAT registry. *Clin Gastroenterol Hepatol* 2006; **4**: 621-630 [PMID: 16678077 DOI: 10.1016/j.cgh.2006.03.002]
131 **Long MD**, Martin CF, Pipkin CA, Herfarth HH, Sandler RS, Kappelman MD. Risk of melanoma and nonmelanoma skin cancer among patients with inflammatory bowel disease. *Gastroenterology* 2012; **143**: 390-399.e1 [PMID: 22584081 DOI: 10.1053/j.gastro.2012.05.004]
132 **O'Meara S**, Nanda KS, Moss AC. Antibodies to infliximab and risk of infusion reactions in patients with inflammatory bowel disease: a systematic review and meta-analysis. *Inflamm Bowel Dis* 2014; **20**: 1-6 [PMID: 24280879 DOI: 10.1097/01.MIB.0000436951.80898.6d]
133 **Denadai R**, Teixeira FV, Saad-Hossne R. Management of psoriatic lesions associated with anti-TNF therapy in patients with IBD. *Nat Rev Gastroenterol Hepatol* 2012; **9**: 744 [PMID: 23147656 DOI: 10.1038/nrgastro.2012.125-c1]
134 **Singh JA**, Wells GA, Christensen R, Tanjong Ghogomu E, Maxwell L, Macdonald JK, Filippini G, Skoetz N, Francis D, Lopes LC, Guyatt GH, Schmitt J, La Mantia L, Weberschock T, Roos JF, Siebert H, Hershan S, Lunn MP, Tugwell P, Buchbinder R. Adverse effects of biologics: a network meta-analysis and Cochrane overview. *Cochrane Database Syst Rev* 2011; **(2)**: CD008794 [PMID: 21328309 DOI: 10.1002/14651858.cd008794.pub2]
135 **Ananthakrishnan AN**, McGinley EL, Binion DG. Inflammatory bowel disease in the elderly is associated with worse outcomes: a national study of hospitalizations. *Inflamm Bowel Dis* 2009; **15**: 182-189 [PMID: 18668678 DOI: 10.1002/ibd.20628]

136 **Almogy G**, Sachar DB, Bodian CA, Greenstein AJ. Surgery for ulcerative colitis in elderly persons: changes in indications for surgery and outcome over time. *Arch Surg* 2001; **136**: 1396-1400 [PMID: 11735867 DOI: 10.1001/archsurg.136.12.1396]

137 **Jeuring S**, Van den Heuvel T, Zeegers M, Hameeteman W, Romberg-Camps M, Oostenbrug L, Masclee A, Jonkers D, Pierik M. OP005 Is elderly-onset ulcerative colitis a different entity? - Natural disease course and treatment response compared to adult-onset disease in the population-based IBD-SL cohort. *J Crohn's Colitis* 2014; **8** Suppl 1: S3-S4 [DOI: 10.1016/S1873-9946(14)60006-4]

138 Fries W, Viola A, Mannetti N, Coppola M, Frankovic I, Monterubbianesi R, Cantoro L, Pugliese D, Aratri A, Cappello M, Saibeni S, Principi M, Naccarato P, Moncci G, Castiglione F, Callela F, Magarotto A, Caprioli F, Belvedere A, Casella G, Samperi L, Privitera AC, Inserra G, Danese S, Papi C, Armuzzi A, Kohn A, D'Incà R, Annese V, Manguso F. P303 Ulcerative colitis (UC) in the elderly - Moderate at onset but then a milder course? An IG-IBD study. *J Crohn's Colitis* 2014; **8** Suppl 1: S190-S191 [DOI: 10.1016/S1873-9946(14)60424-4]

139 **Delaney CP**, Fazio VW, Remzi FH, Hammel J, Church JM, Hull TL, Senagore AJ, Strong SA, Lavery IC. Prospective, age-related analysis of surgical results, functional outcome, and quality of life after ileal pouch-anal anastomosis. *Ann Surg* 2003; **238**: 221-228 [PMID: 12894015 DOI: 10.1097/01.sla.0000080825.95166.26]

140 **Cohen JL**, Strong SA, Hyman NH, Buie WD, Dunn GD, Ko CY, Fleshner PR, Stahl TJ, Kim DG, Bastawrous AL, Perry WB, Cataldo PA, Rafferty JF, Ellis CN, Rakinic J, Gregorcyk S, Shellito PC, Kilkenny JW, Ternent CA, Koltun W, Tjandra JJ, Orsay CP, Whiteford MH, Penzer JR. Practice parameters for the surgical treatment of ulcerative colitis. *Dis Colon Rectum* 2005; **48**: 1997-2009 [PMID: 16258712 DOI: 10.1007/s10350-005-0180-z]

141 **Church JM**. Functional outcome and quality of life in an elderly patient with an ileal pouch-anal anastomosis: a 10-year follow up. *Aust N Z J Surg* 2000; **70**: 906-907 [PMID: 11167585 DOI: 10.1046/j.1440-1622.2000.02006.x]

142 **Delaney CP**, Dadvand B, Remzi FH, Church JM, Fazio VW. Functional outcome, quality of life, and complications after ileal pouch-anal anastomosis in selected septuagenarians. *Dis Colon Rectum* 2002; **45**: 890-894; discussion 894 [PMID: 12130876 DOI: 10.1007/s10350-004-6323-9]

143 **Cairns SR**, Scholefield JH, Steele RJ, Dunlop MG, Thomas HJ, Evans GD, Eaden JA, Rutter MD, Atkin WP, Saunders BP, Lucassen A, Jenkins P, Fairclough PD, Woodhouse CR. Guidelines for colorectal cancer screening and surveillance in moderate and high risk groups (update from 2002). *Gut* 2010; **59**: 666-689 [PMID: 20427401 DOI: 10.1136/gut.2009.179804]

144 **Farraye FA**, Odze RD, Eaden J, Itzkowitz SH, McCabe RP, Dassopoulos T, Lewis JD, Ullman TA, James T, McLeod R, Burgart LJ, Allen J, Brill JV. AGA medical position statement on the diagnosis and management of colorectal neoplasia in inflammatory bowel disease. *Gastroenterology* 2010; **138**: 738-745 [PMID: 20141808 DOI: 10.1053/j.gastro.2009.12.037]

145 **Ekbom A**, Helmick C, Zack M, Adami HO. Ulcerative colitis and colorectal cancer. A population-based study. *N Engl J Med* 1990; **323**: 1228-1233 [PMID: 2215606 DOI: 10.1056/NEJM199011013231802]

146 **Sugita A**, Sachar DB, Bodian C, Ribeiro MB, Aufses AH, Greenstein AJ. Colorectal cancer in ulcerative colitis. Influence of anatomical extent and age at onset on colitis-cancer interval. *Gut* 1991; **32**: 167-169 [PMID: 1864536 DOI: 10.1136/gut.32.2.167]

147 **Eaden JA**, Abrams KR, Mayberry JF. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. *Gut* 2001; **48**: 526-535 [PMID: 11247898 DOI: 10.1136/gut.48.4.526]

148 **Rutter MD**, Saunders BP, Wilkinson KH, Rumbles S, Schofield G, Kamm MA, Williams CB, Price AB, Talbot IC, Forbes A. Thirty-year analysis of a colonoscopic surveillance program for neoplasia in ulcerative colitis. *Gastroenterology* 2006; **130**: 1030-1038 [PMID: 16618396 DOI: 10.1053/j.gastro.2005.12.035]

149 **Shaukat A**, Virnig DJ, Salfiti NI, Howard DH, Sitaraman SV, Liff JM. Is inflammatory bowel disease an important risk factor among older persons with colorectal cancer in the United States? A population-based case-control study. *Dig Dis Sci* 2011; **56**: 2378-2383 [PMID: 21409378 DOI: 10.1007/s10620-011-1632-z]

150 **Cheddani H**, Dauchet L, Charpentier C, Fumery M, Salleron J, Bouvier A-M, Dupas JL, Vasseur F, Lerebours E, Armengol-Debeir L, Laberenne E, Peyrin-Biroulet L, Colombel J-F, Savoye G, Gower-Rousseau C. DOP094 Cancer in elderly-onset inflammatory bowel disease: A population-based study. *J Crohn's Colitis* 2014; **8** Suppl 1: S60 [DOI: 10.1016/S1873-9946(14)60119-7]

151 **Rex DK**, Johnson DA, Anderson JC, Schoenfeld PS, Burke CA, Inadomi JM. American College of Gastroenterology guidelines for colorectal cancer screening 2009 [corrected]. *Am J Gastroenterol* 2009; **104**: 739-750 [PMID: 19240699 DOI: 10.1038/ajg.2009.104]

152 **Bielawska B**, Day AG, Lieberman DA, Hookey LC. Risk factors for early colonoscopic perforation include non-gastroenterologist endoscopists: a multivariable analysis. *Clin Gastroenterol Hepatol* 2014; **12**: 85-92 [PMID: 23891916 DOI: 10.1016/j.cgh.2013.06.030]

153 **Navaneethan U**, Parasa S, Venkatesh PG, Trikudanathan G, Shen B. Prevalence and risk factors for colonic perforation during colonoscopy in hospitalized inflammatory bowel disease patients. *J Crohns Colitis* 2011; **5**: 189-195 [PMID: 21575880 DOI: 10.1016/j.crohns.2010.12.005]

154 **Rahier JF**, Magro F, Abreu C, Armuzzi A, Ben-Horin S, Chowers Y, Cottone M, de Ridder L, Doherty G, Ehehalt R, Esteve M, Katsanos K, Lees CW, Macmahon E, Moreels T, Reinisch W, Tilg H, Tremblay L, Veereman-Wauters G, Viget N, Yazdanpanah Y, Eliakim R, Colombel JF. Second European evidence-based consensus on the prevention, diagnosis and management of opportunistic infections in inflammatory bowel disease. *J Crohns Colitis* 2014; **8**: 443-468 [PMID: 24613021 DOI: 10.1016/j.crohns.2013.12.013]

155 **Melmed GY**, Ippoliti AF, Papadakis KA, Tran TT, Birt JL, Lee SK, Frenck RW, Targan SR, Vasiliauskas EA. Patients with inflammatory bowel disease are at risk for vaccine-preventable illnesses. *Am J Gastroenterol* 2006; **101**: 1834-1840 [PMID: 16817843 DOI: 10.1111/j.1572-0241.2006.00646.x]

156 **Selby L**, Kane S, Wilson J, Balla P, Riff B, Bingcang C, Hoellein A, Pande S, de Villiers WJ. Receipt of preventive health services by IBD patients is significantly lower than by primary care patients. *Inflamm Bowel Dis* 2008; **14**: 253-258 [PMID: 17932966 DOI: 10.1002/ibd.20266]

157 **Keene JK**, Lowe DK, Grosfeld JL, Fitzgerald JF, Gonzales-Crussi F. Disseminated varicella complicating ulcerative colitis. *JAMA* 1978; **239**: 45-46 [PMID: 579232 DOI: 10.1001/jama.1978.03280280045025]

158 **Wasan SK**, Baker SE, Skolnik PR, Farraye FA. A practical guide to vaccinating the inflammatory bowel disease patient. *Am J Gastroenterol* 2010; **105**: 1231-1238 [PMID: 20104218 DOI: 10.1038/ajg.2009.733]

159 **Horton HA**, Kim H, Melmed GY. Vaccinations in older adults with gastrointestinal diseases. *Clin Geriatr Med* 2014; **30**: 17-28 [PMID: 24267599 DOI: 10.1016/j.cger.2013.10.002]

160 **Jyrkkä J**, Enlund H, Lavikainen P, Sulkava R, Hartikainen S. Association of polypharmacy with nutritional status, functional ability and cognitive capacity over a three-year period in an elderly population. *Pharmacoepidemiol Drug Saf* 2011; **20**: 514-522 [PMID: 21308855 DOI: 10.1002/pds.2116]

161 **Cahir C**, Fahey T, Teeling M, Teljeur C, Feely J, Bennett K. Potentially inappropriate prescribing and cost outcomes for older people: a national population study. *Br J Clin Pharmacol* 2010; **69**: 543-552 [PMID: 20573091 DOI: 10.1111/j.1365-2125.2010.03628.x]

162 **Siddiqi N**, House AO, Holmes JD. Occurrence and outcome of delirium in medical in-patients: a systematic literature review. *Age Ageing* 2006; **35**: 350-364 [PMID: 16648149 DOI: 10.1093/ageing/afl005]

163 **Alagiakrishnan K**, Wiens CA. An approach to drug induced delirium in the elderly. *Postgrad Med J* 2004; **80**: 388-393 [PMID: 15254302 DOI: 10.1136/pgmj.2003.017236]

164 **Kojima T**, Akishita M, Nakamura T, Nomura K, Ogawa S, Iijima K,

Eto M, Ouchi Y. Association of polypharmacy with fall risk among geriatric outpatients. *Geriatr Gerontol Int* 2011; **11**: 438-444 [PMID: 21545384 DOI: 10.1111/j.1447-0594.2011.00703.x]

165 **Boyle N**, Naganathan V, Cumming RG. Medication and falls: risk and optimization. *Clin Geriatr Med* 2010; **26**: 583-605 [PMID: 20934612 DOI: 10.1016/j.cger.2010.06.007]

166 **Sergi G**, De Rui M, Sarti S, Manzato E. Polypharmacy in the elderly: can comprehensive geriatric assessment reduce inappropriate medication use? *Drugs Aging* 2011; **28**: 509-518 [PMID: 21721596 DOI: 10.2165/11592010-000000000-00000]

P- Reviewer: Lee CL, Yu CG **S- Editor**: Kong JX **L- Editor**: A
E- Editor: Wang CH

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i1.66

World J Gastrointest Pharmacol Ther 2016 February 6; 7(1): 66-77
ISSN 2150-5349 (online)

REVIEW

Eosinophilic esophagitis: New insights in pathogenesis and therapy

Michele Pier Luca Guarino, Michele Cicala, Jose Behar

Michele Pier Luca Guarino, Michele Cicala, Unit of Digestive Disease, Campus Bio Medico University of Rome, 00128 Rome, Italy

Jose Behar, Brown University Medical School, Providence, RI 02912, United States

Author contributions: All the authors contributed equally to the manuscript, drafting the article and revising it critically for important intellectual content; all approve the final version for publication.

Conflict-of-interest statement: The authors have nothing to disclose.

Correspondence to: Michele Cicala, MD, PhD, Unit of Digestive Disease, Campus Bio Medico University of Rome, Via Alvaro del Portillo 200, 00128 Rome,
Italy. m.cicala@unicampus.it
Telephone: +39-06-22541560
Fax: +39-06-22541520

Received: June 12, 2015
Peer-review started: June 14, 2015
First decision: August 5, 2015
Revised: November 24, 2015
Accepted: December 1, 2015
Article in press: December 2, 2015
Published online: February 6, 2016

Abstract

Eosinophilic esophagitis (EoE) is a clinico-pathological entity with esophageal symptoms and dense esophageal eosinophilic infiltration throughout the esophagus that may persist despite treatment with proton pump inhibitors. This eosinophilic infiltration is usually absent in the stomach, small intestine and colon, although there are a number of reports of patients with a multi-organ involvement. EoE is associated with abnormalities involving TH2-dependent immunity, with multiple environmental factors strongly contributing to disease expression. The layer of the esophagus affected by the eosinophilic infiltration causes the specific symptoms. Esophageal involvement results mostly in dysphagia for solids that can be severe enough to cause recurrent esophageal obstruction with typical endoscopic features suggesting esophageal remodeling and pathological changes of eosinophilic infiltration of the mucosa, sub-epithelial fibrosis and muscle hypertrophy. This disease is frequently associated with other allergic conditions such as allergic asthma, allergic dermatitis and eosinophilia. The treatment of patients with EoE depends on the severity of the symptoms and of the inflammatory process as well as to their response to a gradual step-up treatment. The first line of treatment consists of steroid containing local inhalers. If unresponsive they are then treated with oral steroids. Intravenous interleukin blockers seem to have a consistent positive therapeutic effect.

Key words: Eosinophilic esophagitis; Gastro-esophageal reflux disease; Esophagus; Esophagitis; Eosinophilia; Cytokines

Core tip: Eosinophilic esophagitis is a clinico-pathological entity characterized by esophageal symptoms and dense esophageal eosinophilic infiltration throughout the esophagus. Our manuscript provides a deep description of the disease showing that many efforts have been made in the last decades in understanding

its pathogenesis paving the way to new therapeutic targets which are reviewed in the manuscript. For these reasons, the story would not appear to end herewith but deserves further attention and investigation.

Guarino MPL, Cicala M, Behar J. Eosinophilic esophagitis: New insights in pathogenesis and therapy. *World J Gastrointest Pharmacol Ther* 2016; 7(1): 66-77 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i1/66.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i1.66

INTRODUCTION

One of the first reports of eosinophilic involvement of the esophagus dates from 1977 when Dobbins *et al*[1] described a man with dysphagia who had dense esophageal eosinophilia. However, it has been classified as a distinct clinical disease limited to the esophagus later[2,3]. It is now recognized as a chronic inflammatory disorder of the esophagus. It is characterized by dysphagia and gastro-esophageal reflux disease (GERD) symptoms, including vomiting, regurgitation, nausea and epigastric pain. Esophageal mucosal biopsy contains more than 15 to 20 eosinophils per high-power field in the absence of GERD disease. Occasionally it is associated with various other conditions including celiac disease[4].

PREVALENCE

In a 16-year follow-up study, an average annual incidence of 1.4% was reported[5]. The marked increase in incidence does not appear to be explained by a greater recognition of this disease since it is higher than the increased number of endoscopic procedures that are being performed[6]. The prevalence of eosinophilic esophagitis (EoE) is higher in cold climate zones of the United States than in tropical or arid zones suggesting a possible relationship between climate and the disease[7]. There is also a higher incidence of this disease in westernized countries and in urban areas of the United States with a significant Caucasian predominance of almost 90%[8,9].

More recent studies have updated the estimates of the incidence (7/100000) and prevalence (43/100000) of EoE[10] similar to the estimates reported from Olmsted County, Minnesota (incidence 9/100000; prevalence 55/100000) in patients identified retrospectively[11]. A similar prevalence estimate (52/100000) was derived from physician surveys. It is far more common in males than in females with a 3:1 to 3:2 ratios[11]. In some reports the incidence in males is even as high as 86% of patients. This gender difference remains unexplained and it contrasts with the higher incidence of allergic asthma in females than in males[8]. There is also an increased rate of family history of atopy in patients with EoE, and the disease tends to occur with familial clustering[12-14].

CLINICAL PRESENTATION

Patients with EoE complain of dysphagia mostly with solid foods. Food impaction tends to occur in the mid-esophagus unless the inflammatory process affects the esophageal innervation resulting in motility disorders consistent with achalasia or diffuse esophageal spasm. Fifty to eighty per cent of these patients have a prior history of atopic symptoms such as allergic rhinitis, asthma or atopic dermatitis and some of these allergic conditions are frequently associated with EoE clinical presentation, particularly allergic asthma and rhinitis[14,15]. Children generally present with non-specific symptoms of GERD, abdominal pain or failure to thrive; other present with dysphagia and esophageal food impaction[4].

The entire esophagus is often infiltrated with increased number of eosinophils [> 15 to 20 eosinophils per high power field (HPF)] a differential feature from the increased number of eosinophils found occasionally in the distal esophagus in patients with GERD of less than 15 eosinophils per HPF[16,17]. A recent study demonstrated that a cutoff value of at least 15 eosinophils per high-power field has a sensitivity of 100% and specificity of 96% for establishing the histologic diagnosis of eosinophilic esophagitis, irrespective of location of the sample (proximal or distal esophagus). As patients with lower levels of eosinophilia and phenotypic features of eosinophilic esophagitis have been described and GERD may be difficult to rule out, in some cases the diagnosis could be better established by biopsy of the proximal esophagus[17]. In addition the biopsies showed increased number of mast cells. Anyway, the relationship between patient symptoms and endoscopic features as well as histological severity of the disease is not known. A modest correlation was found between symptoms and histology in newly diagnosed, untreated patients differently form patients with longstanding EoE[18].

The eosinophilic infiltrate in GERD frequently but not always tends to respond to omeprazole probably because it blocks stat-6 that appears to be involved in eosinophilic chemotaxis in the esophagus[19]. Occasionally, the differential diagnosis between EoE and GERD can be quite difficult particularly in the pediatric population with both conditions having similar symptoms and endoscopic appearance of the esophagus. It is important to treat these patients initially with high proton pump inhibitors (PPI) doses before GERD is ruled out[20]. In some cases this differential diagnosis is helped by biochemical and genetic studies. Measurements of eotaxin-3 levels may be useful in the diagnosis of EoE since its levels are higher than in GERD[21]. Another biochemical test that may be helpful is the level of AOL-15. High levels are more common in biopsies from patients with EoE than from GERD and controls[22].

However, there is a group of patients with biopsies showing 15 eosinophils or higher per HPF who respond to PPI therapy. These patients have been labeled PPI responsive esophageal eosinophilia (PPIREE). They have similar clinical, endoscopic and pathological features of those with EoE[23]. It has been suggested that PPI reduces the eosinophilic infiltration in this subgroup of patients by restoring the mucosal integrity (erosions and ulcerations) that had facilitated the transport of antigenic proteins[24].

More advanced cases of eosinophilic involvement the dysphagia for solid foods is caused by remodeling of the esophageal body due to extensive sub-epithelial fibrosis that tend to occur more frequently in adults[25]. The incidence of fibrostenotic disease increases with age with the incidence doubling every 10 years of age.

ENDOSCOPIC FINDINGS

The mucosa of esophagus can appear normal particularly in the pediatric population. It is estimated that in one-third of pediatric patients with EoE the esophagus has a normal endoscopic appearance[15]. However, in most adult patients the endoscopic examination of the esophagus reveal various types of abnormalities such as longitudinal furrows, white nodule- or plaque-like exudates, transient or fixed corrugated rings (esophageal "trachealization"), crepe-paper mucosa due to a loss of the mucosal elasticity and strictures of variable length. Whitish exudates and longitudinal furrowing occur secondary to local edema and acute inflammation[26-28]. Hirano *et al*[28] reported a good inter-observer agreement in these patients when these three esophageal features rings, furrows, exudates were present.

MOTILITY DISORDERS ASSOCIATED TO EOE

Patients with EoE exhibit a variety of esophageal functional disorders depending upon the esophageal layer involved by the chronic inflammatory process. Motility disorders occur when this process affect muscle or neuronal cells. Since the initial studies there have been at least 22 published series or case reports in which the results of esophageal manometry have been reported, 19 adult and 3 pediatric studies. These esophageal motility studies have included a total of 144 patients, 115 adults and 29 children[29]. One study examined the esophageal motility features in 48 EoE patients, 48 GERD patients and 50 controls. Motility disorders were more frequent in EoE patients than in controls; the observed abnormal motility features were similar in EoE and GERD patients[30]. It has been suggested that this motor pattern is probably due to an impaired response to neural stimulation probably due to impaired acetylcholine release since the muscle response to this neurotransmitter is normal and in more advanced cases to reduced esophageal compliance due to extensive sub-epithelial fibrosis[31]. Furthermore, previous conventional motility studies assessing esophageal motor function in patients with EoE have reported few cases of achalasia, diffuse esophageal spasm, nutcracker esophagus and high amplitude contractions[28]. One patient with dyphagia and manometric abnormalities consistent with achalasia responded to treatment with prednisone[32].

NATURAL HISTORY

EoE is a chronic condition that usually starts in childhood. In some patients it may become clinically apparent in adulthood when they begin to complain of dysphagia. Swallowing difficulties are due to remodeling of the esophagus, strictures and motility disorders. Strictures are uncommon in children suggesting that over time the chronic infiltration with eosinophils leads to this complication in adults. This is particularly evident when there is a delay in the diagnosis that may lead to the remodeling of the esophagus and stricture formation[33]. In some cases, esophageal food impaction can be the initial presentation of the disease. Endoscopic procedures to relieve food impaction may lead to complications such as esophageal perforation and, even if rare, spontaneous transmural esophageal rupture (Boerhaave's syndrome) has also been reported as a primary manifestation of EoE.

Most studies support a similar pathogenesis in both pediatric and adult populations suggesting that the clinical and pathological differences are the consequence of the evolution of the diseased process[34]. Occasionally heavy infiltration of eosinophils is found in the esophagus of patients complaining of symptoms originated in other segments of the upper gastrointestinal tract. However, a longitudinal study that followed 30 patients for up to 11 years and with a mean follow-up of 7.3 years revealed recurrent episodes of dysphagia but without nutritional abnormalities and without extension to other gastrointestinal segments or blood eosinophilia. Furthermore, in this study there was a partial spontaneous reduction of the eosinophilic count even in symptomatic patients[35]. However, the prognosis of this disease is still in its early stages, but there is no relation between esophageal cancer and EE described[35].

PATHOLOGICAL ABNORMALITIES

Pathological studies in patients with EoE reveal four abnormalities: Diffuse eosinophilic infiltration of the mucosa along the entire esophagus, increase basal cell hyperplasia, sub-epithelial fibrosis and muscle hypertrophy. Epithelial cells are highly hyperplasic in EoE patients[36]. In addition the epithelial layer shows a significant dilation of the intercellular spaces in patients with active disease compared to patients with inactive

disease suggesting that this epithelial abnormality is due to the inflammatory process[37].

The cardinal pathological abnormality is the presence of high levels of eosinophils in the epithelial layer. The pathogenic effects of eosinophils include the promotion of basal cell proliferation and mast cell recruitment. The finding that eosinophils are mostly present in the epithelial layer of this disease is probably because most of the diagnostic studies are based on endoscopic biopsies that are relatively superficial. If deeper esophageal specimens are obtained eosinophils are frequently present in the muscularis mucosa and circular muscle layer. These deeper specimens also revealed the presence of mast cells that may contribute to some of the esophageal functional abnormalities[38]. In atopic patients these mast cells contain IgE whereas they are IgE-negative in the non-atopic patients. These deeper specimens also show various degrees of sub-epithelial fibrosis and muscle hypertrophy that in some patients can be quite prominent resulting in short and long strictures[38].

Occasionally the differential diagnosis of EoE is with erosive esophagitis due to gastro-esophageal reflux disease. Although most patients with GERD complain of heartburn, in some patients dysphagia may be the most prominent symptom. Biopsies from the esophagus of these patients may reveal increased number of eosinophils but almost always they are present only in the distal segment and they are fewer than 15 cells per HPF[39]. This segmental eosinophilia resolves after treatment with PPI's probably because it heals the inflammatory process and blocks the function of stat-6 that contributes to the eosinophilic infiltration into the esophagus in both disorders[40]. In immortalized squamous epithelial cells omeprazole had no effect on eotaxin-3 mRNA stability or on STAT6 phosphorylation and its nuclear translocation. Rather, omeprazole appears to block the binding of interleukin stimulated STAT6 and RNA polymerase Ⅱ to the eotaxin-3 promoter lowering its expression. However, in few cases the differential diagnosis can be quite difficult. Therefore it is possible that in these patient's determinations of eotaxin-3 mRNA and protein levels that strongly correlate with tissue eosinophilia may be able to differentiate between these two entities[40].

GENETIC *VS* ENVIRONMENTAL ETIOLOGICAL FACTORS

Evidence has been presented that both genetic and environmental factors contribute to the development of EoE. This disease is associated with a variety of mendelian genetic based disorders[41]. The most frequent associations of EoE are with hereditary collagen disorders such as Marfan and Ehlers-Danlos syndromes with an incidence of about 1%[42]. Genetic studies have also identified a number of abnormalities. The one that appears more frequently is the presence of a common single nucleotide polyphormism in the 3' untranslated region of CCL-26 that encodes eotaxin-3 that is an over-expressed esophageal transcript in the EoE transcriptome[43]. It is well known that CCL-26 mRNA and protein are overexpressed in the majority of these patients. Although other genetic abnormalities have been reported perhaps two that have been persistently demonstrated in unbiased manner are present in chromosome 5q22. It contains a single locus in the spanning of the thymic stromal lymphoprotein (TSLP) and of the WD repeat domain 36 (WDR36) genes that are significantly associated with EoE[44]. TSLP mRNA is increased in esophageal tissues from patients with EoE, compared with controls. TSLP is a cytokine that is produced by keratinocytes and promotes the development of Th2 cells.

The genetic contribution of this disease, however, is limited despite of the higher incidence of EoE in members of the same families. Although EoE is observed in 1.8% to 2.4% of relatives depending on the relationship and sex with higher values for men, there is only a 40% concordance between monozygotic twins and 30% concordance between dizygotic twins. This latter low incidence suggests a strong environmental contribution to acquiring this disease. This conclusion is supported by the finding that although the nuclear-family heritability appeared to be high (72.0%), the twins cohort analysis revealed that this high levels are likely due to a powerful role of a common environment that occurs in 81.0% of the cases[45].

PATHOGENESIS

Epidemiologic studies and therapeutic trials have provided indirect evidence about the pathogenesis of this disease. Most patients with EoE (approximately 75% of cases) show signs of atopy, defined by reactivity to allergens by skin-prick testing (SPT) or by identifying specific serum IgE[46,47]. The majority of these patients have evidence of either aeroallergen and/or food sensitization. This was illustrated by one subject that developed acute eosinophilic esophagitis after exposure to sublingual immunotherapy that included typical airborne antigens such as hazelnut, birch and alder. This esophageal response resolved clinically and endoscopically after the immunotherapy was discontinued[48]. There is also experimental evidence in wild mice that repeated nasal inoculation with allergens such Aspergillus fumigatus triggers an eosinophilic infiltration of the esophagus. In contrast, these mice had no response when the allergen was inoculated in the oral cavity or in the stomach[49,50]. Occasionally the specific trigger of the eosinophilic infiltration of the esophagus is a drug as illustrated in two patients found to have had an anticonvulsant hypersensitivity syndrome[51]. The allergic component of EoE also is quite apparent from its strong association with other allergic diseases. About 70% of

EoE patients have current or past allergic diseases or positive skin pricks test especially to a variety of foods. However, despite these isolated reports the role of airborne antigens in the pathogenesis of human EoE is controversial and at best it may be a contributing factor in a small percentage of patients.

The immunoglobulin mediating this allergic reaction has not been conclusively determined. Fifty percent to seventy-five percent of patients with EoE are atopic, with a high prevalence of food-induced allergen-specific IgE[52]. This conclusion has been questioned after omalizumab an IgE antibody failed to improve the symptoms and reduce the eosinophilic infiltration in patients with EoE[53]. On the other hand a recent study shows that the most prominent immunoglobulin abnormality appears to be IgG4. There are abundant IgG4-containing plasma cells and serum levels of IgG4 appear to react to specific foods suggesting that EoE is more likely to be an IgG4 associated allergy[54].

The role of food in the pathogenesis of EoE is supported by the consistent observation that disease activity is responsive to elemental diets and resumption of unrestricted diets result in disease recurrence[55]. A prospective study in adult patients with EoE confirmed previous observations that a six-food elimination diet significantly improved their symptoms, endoscopic and histological abnormalities. Moreover, re-introduction of specific foods was frequently associated with recurrence of EoE with wheat in 60% of cases and milk in 50% of cases. In contrast, skin-prick testing of these foodstuffs predicted that only 13% of foods were associated with EoE. Even in patients without an associated allergic disease, EoE is likely caused by allergic reactions to certain foods since they still respond to an elemental diet. However, several studies have also suggested that aeroallergens may also play a role in the pathogenesis of this disease. This conclusion is supported by the fact that the severity of this disease may be affected by seasonal variations that correlated with pollen counts. Some of these studies have even suggested differences between younger from older patients with the former showing more IgE and patch sensitivity to certain foods whereas older children exhibiting greater IgE sensitization to inhalant aeroallergens[56].

This disease consists of highly reactive esophageal epithelial cells, high levels of cytokines, eotaxins, particularly eotaxin-3, eosinophils and mast cells that mediate this type-1 hypersensitivity involving Th2 cells[57]. However, only a minority of EoE patients present with food anaphylaxis suggesting that the mechanisms involved in this condition are different from those causing the classical IgE-mediated mast cell and basophile activation. These conclusions also are supported by studies performed in experimental EoE in wild mice[58].

There is evidence that epithelial cells are capable of mediating this food-induced allergic reaction. *In vitro* studies have shown that esophageal epithelial cell lines act as antigen presentation cells in the presence of interferon-γ (IFNγ) by inducing the major histocompatibility complex (MHC) class Ⅱ system. These findings suggest that the antigen presentation by esophageal epithelial cell may contribute to the pathophysiology of EoE[59].

Similarly to GERD patients with esophagitis[60,61], EoE is associated with high levels of cytokines particularly IL-3, IL-5, IL-13, increase production of eotaxin chemokines CCL11, CCL24 and CCL26 (or eotaxins 1, 2 and 3 respectively) that attract inflammatory cells, predominantly eosinophils[62,63]. Studies performed in esophageal rings showed that stimulation of the rings with IL-13 for 48 h resulted in a significant attraction of eosinophils into the lower chamber containing TH-2 lymphocytes while chambers containing only IL-13 did not[64,65]. These data demonstrate that IL-13 supports eosinophil migration and that CCL11 and CCL24 also are both important in promoting eosinophil infiltration[66,67]. Although patients with EoE have higher levels of several cytokines, the cytokines that are most consistently increased in this allergic disease are IL-5, IL-13 and eotaxin-3 (CCL26)[68,69]. Genome-wide microarray expression studies have shown that the gene-encoding eotaxin-3 is the most highly induced gene in EoE patients compared to healthy individuals[43]. Furthermore, a single-nucleotide polymorphism in the human eotaxin-3 gene seems to be associated with disease susceptibility and mice deficient in the eotaxin receptor were protected from experimental EoE[63,64]. Eosinophils are born from the bone marrow progenitor stem cells under the influence of interleukin-3 (IL-3), interleukin-5 (IL-5) and granulocyte-macrophage colony-stimulating factor (GM-CSF). Eosinophils contain cytokines, GM-CSF, transforming growth factor (TGF)-β, tumor necrosis factor (TNF)-α, RANTES (or CCL5)[67]. Eosinophils are also potent pro-inflammatory cells capable of causing severe host tissue damage[68,69].

There is also increasing evidence that suggests a strong role of mast cells in the pathogenesis of EoE. Although mast cells have been studied indirectly in EoE, the published data suggest that the number of mast cells present in the esophageal epithelium is higher with respect to controls and to patients with gastro-esophageal reflux disease[70,71]. However, history of anaphylactic reactions after exposure to allergens is uncommon in these patients. These findings suggest that the mast cells function in EoE may be dependent on T lymphocytes and that there may be a bi-directional crosstalk between mast cells and eosinophils. This relationship may contribute to EoE physiopathology. Furthermore, even though mast cells are present in healthy subjects, only the EoE patients have IgE-bearing mast cells. Their role in the pathogenesis of EoE was tested in experimentally induced EoE in wild-type mice, mast cell-deficient WWv mice, and mast cell-reconstituted WWv mice[38,72]. The results of these studies reveal that esophageal mast

Table 1 Possible mechanisms involved in eosinophilic esophagitis pathogenesis

Mechanism	Evidence	Ref.
Atopy	Reactivity to allergens by skin-prick testing Presence of specific serum IgE Experimental evidence in animals undergone nasal inoculation with allergens Strong association with other allergic diseases High association with food-induced allergen-specific IgE Abundant IgG4-containing plasma cells	[46,47,49,50,52,54]
Food	Disease activity is responsive to elemental diets	[55]
Aeroallergens	Severity of disease affected by seasonal variations which correlate with pollen counts	[56]
Chemo attractants	Increased IL-13 supports eosinophil migration by stimulating the chemo attractants production Increased levels of eotaxin-3 Gene-encoding eotaxin-3 the most highly induced gene in EoE patients Single-nucleotide polymorphism in the eotaxin-3 gene associated with disease susceptibility Mice deficient in the eotaxin receptor (CCR3) protected from experimental EoE	[43,63,64,66,67,68,69]
Mast cells	Increased number of mast cells in the esophageal epithelium Mast cells linked to IgE	[70,71]
TGF-β	Obstructive symptoms seem to occur secondary to epithelial cell proliferation and extracellular matrix remodeling, processes linked to eosinophil-derived TGF-β TGF-β is known to increase smooth muscle cell hyperplasia	[61]
Leukotriene C4	Leukotriene C4 is metabolized to LTD4 and LTE4 both of which stimulate smooth muscle contraction	[74]

EoE: Eosinophilic esophagitis; IL-3: Interleukin-3; TGF-β: Transforming growth factor-β.

cell numbers increase in parallel with eosinophils in a dose- and time-dependent manner. Incorporation 5'-bromodeoxyuridine analysis have indicated that mast cells contribute to the development of muscle cell hyperplasia and hypertrophy suggesting that these cells may have a significant role in promoting esophageal remodeling in EoE[73]. However, therapies that inhibit mast cell functions have been ineffective in treating EoE in contrast to their effectiveness in other respiratory tract diseases. It has been therefore suggested that mast cells may have a limited role in the pathogenesis of EoE.

While the natural history of EoE remains obscure, the fact that some patients develop esophageal narrowing and strictures is of concern, particularly as patients become adults. Obstructive symptoms seem to occur secondary to epithelial cell proliferation and extracellular matrix remodeling, processes linked to eosinophil-derived TGF-β[61]. Leukotriene C4 may be an additional contributing factor since it is metabolized to LTD4 and LTE4 both of which stimulate smooth muscle contraction. This action may be quite important because obstructive symptoms may be also related to active smooth muscle contraction[74].

It is also conceivable that EoE is an allergic reaction of the esophageal mucosa to a variety of stimuli that may be mediated by different immunologic pathways. This complexity may explain the discrepancies in the therapeutic responses to specific immune suppressing agents. Therefore, these studies also raised a number of questions regarding this disease: (1) whether the eosinophilic response through nasal or oral cavity depends to a greater extent on the type of the allergen applied; (2) why the esophagus is singularly affected by this allergic reaction since other segments of the upper gastrointestinal tract like the pharynx appears to be spared despite of its contact with foods and aero allergens that could also trigger this reaction; and (3) whether its pathogenesis in humans have similar molecular processes to those induced experimentally in wild mice.

Possible mechanisms involved in EoE pathogenesis are summarized in Table 1.

TREATMENT

The treatment of patients with EoE depends on the severity of symptoms and of the inflammatory process as well as on their response to a gradual step-up treatment. It is a possible that with appropriate and persistent treatment the symptoms, endoscopic and histological abnormalities can be completely controlled in most patients, including reversal of the sub-epithelial fibrosis and fibro-stenotic complications. However, treatment needs to be maintained for prolonged periods since EoE is a chronic disease that tends to relapse once the treatment is discontinued.

Initially patients should be treated with PPIs since some patients may respond to this treatment either by reducing the acid secretion in patients with co-existent GERD, or, by means of other still undefined anti-inflammatory mechanisms[23]. A small-randomized trial showed that EoE patients with coexisting GERD treated with esomeprazole, were significantly more likely to have resolution of esophageal eosinophilia as compared with fluticasone alone (100% *vs* 0%) and even in a few patients regardless of the presence of GERD. Treatment with esomeprazole, but not fluticasone, was associated with a significant improvement of dysphagia[75]. As mentioned before PPIs also may be effective in small subgroup of patients that have been labeled PPI responsive

EoE (PPI-REE) with symptomatic improvement and resolution of the esophageal eosinophilia[76]. However, a 24-ph-test performed prior to treatment to select patients that could be responsive to PPI therapy was unable to predict the response to PPI treatment. Neither positive nor negative tests were able to determine whether patients would respond to acid suppressing treatment[77]. The PPI responsive patients with EoE are genetically and phenoptypically indistinguishable from patients that are unresponsive. Some studies have suggested that up to 30% to 40% of patients with EoE may be responsive to PPI's. It is therefore recommended that PPI's should be the first line of treatment.

The PPI unresponsive patients are then treated with steroid containing local inhalers or with intravenous interleukin blockers. Patients complaining of dysphagia due to strictures or impaired esophageal poor distensibility due to subepithelial fibrosis are treated with esophageal dilation using Savary or Malone dilators. Most of the reports indicate that the risk of perforation in patients with EoE is not different from these complications observed when this procedure is performed in patients with other types of esophageal strictures[78,79]. A meta-analysis confirmed the safety of this procedure with reported complication rate of less than 1%[80]. However, irrespective of the type of esophageal disorder this therapeutic procedure should be performed carefully.

Topical treatment is as effective as systemic corticosteroids. Topical fluticasone was just as effective as oral prednisone in terms of histological and symptomatic remission[81]. Its local anti-inflammatory effects induce the development of esophageal steroid responsive genes such as FK-506 binding protein and miRs and can even partially reverse the eosinophil transcriptome. Furthermore, there is an occasional discrepancy between clinical and histological improvement, defined by the reduction of the eosinophilic infiltration to normal levels. It is unclear, however, why symptoms may persist in some patients despite of the resolution of the inflammatory process. In some of these patients the symptomatic unresponsiveness may be due to the sub-epithelial fibrosis and muscle hypertrophy.

Topical corticosteroids improve symptoms and reduce esophageal eosinophilia and have become the "gold standard" of the pharmacotherapy of this disease. Anyway, due to lack of approved drugs for EoE, ''off-label'' drugs, designed for other allergic diseases, such as asthma or rhinitis are used. One-year treatment with fluticasone propionate caused a slight reduction in the sub-epithelial fibrosis although the differences did not reach significance probably because the number of patients included in the study was relatively small[81]. Moreover viscous steroids are more effective than nebulized steroids[82]. However even high doses of fluticasone have a 30% failure. Other therapeutic studies have shown that some patients have remained symptomatic despite the histological improvement defined by the reduction in the number of eosinophils[83]. Pediatric patients are more responsive probably because adult patients have a significant remodeling of the esophagus that may be responsible for the persistent dysphagia[84]. In addition, treating patients with EoE with this topical steroid for three months significantly down regulated the levels of cytokines IL-5 and eotaxin-3 although control levels were not reached[85].

Viscous budesonide has also been successfully used in both children and adults. Remission is usually obtained after 12-wk treatment. A control trial showed that budesonide 1 mg twice daily administered as viscous slurry was more effective in reducing the eosinophilic infiltration of the esophagus than the nebulized form[85]. This is due to greater mucosal contact time with former than with the latter as measured by esophageal scintigraphy[82,86,87]. However, the disease commonly recurs after the drugs are withdrawn. Therefore maintenance treatment with either steroid drug should be considered to avoid symptomatic recurrences and development of sub-epithelial fibrosis[83].

Those conclusions are supported by a meta-analysis that included seven clinical studies that treated a total of 226 patients[88]. Despite of the substantial heterogeneity of the studies it showed topical steroids induced a significant reduction in the number of eosinophils compared to controls. Subgroup analysis also showed that the reduction in eosinophil count was only present in patients who where previously treated with PPI's used to rule out GERD. As mentioned in previous reports eleven out of 127 patients that received the oral steroid developed asymptomatic esophageal candidiasis[88]. Therefore, topical steroid therapy seems to be safe in general and, to date, there has been no evidence of adrenal suppression[88].

Specific treatments have also been developed in an attempt to inhibit immune mechanisms presumed to be involved in this disease. These therapies have been directed toward inhibition of IgE and IL-5 induced immunological abnormalities. While these approaches are often effective, no pharmaceutical agents have yet been approved by the Food and Drug Administration. The effect of omalizumab, an antibody against IgE, was evaluated in a double blind placebo controlled study in patients with EoE. This antibody, however, did not alter symptoms of eosinophilic esophagitis or eosinophil counts in biopsy samples compared with placebo[54,89].

The therapies against IL-5 were selected because this cytokine plays a pivotal role in innate and acquired immune responses and eosinophilia. In humans, the biologic effects of antibodies against IL-5 are best characterized by the reduction in the levels of eosinophils due to this cytokine involvement in the mechanisms of eosinophil development and activation.

These findings have led to therapeutic trials using humanized antibodies against IL-5 or the IL-5R[90,91]. However, probably due to the heterogeneity of this disease the symptomatic improvement induced by mepolizumab has not been consistent and not all patients respond to this form of treatment. In addition there have been discrepancies between the symptomatic improvement and the reduction of the esophageal eosinophils. It is more effective in reducing eosinophilic infiltration than in improving patients' symptoms.

Straumann *et al*[91] performed a placebo control study that enrolled few patients showed that mepolizumab reduced the number of eosinophils in the esophagus and reversed the esophageal remodeling. Moreover the safety profile was acceptable even at higher doses. It was partially supported by a double blind study performed in children using increasing doses of 3 infusions of mepolizumab of 0.55, 2.5, or 10 mg/kg every 4 wk over a 12-wk period followed by no treatment until week 24. After 3 mo, this antibody induced a mild to moderate symptomatic improvement associated with reduction in the number of eosinophils to normal levels (less than 20 per HPF) but only in 31.6% of the patients[90]. A sub analysis of the esophageal biopsies obtained from these children examined the effect of this antibody on eosinophils and mast cells. Forty per cent of patients responded with reductions in eosinophils and 77% in the number of mast cells[92]. Moreover prior to treatment eosinophils and mast cells were found in couplets that were significantly reduced after therapy. This treatment appears to be well tolerated.

This clinical trial was supported by a larger study showing that mepolizumab, assessed by immunofluorescence, induced a marked reduction in the mean esophageal eosinophilia (P = 0.03). Mepolizumab induced a 54% reduction in the eosinophil numbers of the esophageal mucosa compared to only 5% reduction in the placebo group 4 wk after initiation of treatment. Mepolizumab also reduced the number of mast cells in the esophageal mucosa[90].

Another IL-5 antibody reslizumab was studied in douple-blind placebo controlled study. Reslizumab or placebo was administered to 226 children and adolescent with EoE. Four infusions of 1, 2 and 3 mg were administered at weeks 0, 4, 8 and 12 wk. Peak eosinophil count was reduced by 59% to 64% from baseline in the antibody treated group and 24% in the placebo treated group. Symptomatic improvement was observed in the antibody and placebo treated groups but were not significantly different[92].

The limited percentage of patients that respond to these antibodies raise a number of questions regarding patient selection and the immune mechanisms involved in this disease. The selection of topical corticosteroid-refractory patients in these studies may have had a negative impact on the clinical results and possibly because targeting a single molecule IL-5 may prove insufficient to optimally control symptoms and disease progression, particularly in the adults who already have develop sub-epithelial fibrosis and strictures. These complications may also explain the discrepancy between symptomatic improvement and reduction of eosinophilic infiltration.

There is also increasing evidence that most patients with EoE respond to elemental diets or to specific dietary restrictions. However, even in pediatric patients these dietary restrictions are limited by the usefulness of predicting therapeutic response using skin-prick testing and atopy patch testing for food allergies. Moreover, it is still uncertain how to formulate the optimal dietary restrictions for the management of this disease[93].

Although these elemental/amino acid-based formula diets have shown to be effective in children they do not appear to be well tolerated by adults because of taste, volume or high expense. However, patients treated with specific and nutritionally acceptable diets have not been examined[94]. Moreover there are conflicting reports as to the efficacy of treating patients with elemental diets. It is possible as mentioned with previous treatments that the partial or lack of response to these relatively short dietary treatments may depend on the degree of the sub-epithelial fibrosis. In one clinical trial treatment an elemental diet reduced the number of eosinophils in the biopsies from adult patients with EoE. However, symptoms did not significantly improve and the eosinophilic infiltration recurred after the elemental diet was discontinued suggesting that persistent dietary treatment may be necessary[55]. Another study, however, showed that treatment with an elemental diet for 6 wk significantly improve the esophageal symptoms and markedly reduced the number of eosinophils in the esophageal biopsies. These studies showed that four foods were the most likely to trigger the eosinophilic reaction. Twenty-eight of 52 patients achieved clinical-pathological remission. Milk induced remission in 11 patients (50%), eggs in 8 patients (36%), wheat in 7 (31%) and legumes in 4 (18%)[95]. These conclusions were supported by a meta-analysis[96]. The reasons for the therapeutic responses to different foods are not known. An elimination diet also significantly improved symptoms and reduced endoscopic and pathologic features of EoE in adults. The systematic reintroduction food allergens in these patients lead to the recurrence of clinical and histological features of EoE supporting their role in its pathogenesis. It is conceivable that in some patients, as it occurs with other forms of therapy, the lack of correlation between reductions in the eosinophilic infiltration in the esophagus and symptomatic improvement after an elemental diet may be due to presence of esophageal remodeling and sub-epithelial fibrosis that may not respond to the treatment during the relatively short period of these clinical trials[97].

A more practical and acceptable treatment was

conducted in a prospective trial comparing swallowed fluticasone and elimination of cow's milk[98]. After 6-8 wk, esophageal eosinophil counts decreased in 64% of patients treated with cow's milk elimination and 80% of patients treated with fluticasone. Cow's milk elimination also significantly improved the Mean pediatric quality of life (PedsQL) EoE Module total scores and total symptoms scores. Cow's milk elimination may be more acceptable and desirable for EoE patients who do not want to take chronic, long-term steroid medications[98]. The benefits of dietary restrictions is further supported by the reduction in the number of mast cells and its proteases since these cells seem to play a role in the pathophysiology and symptoms of EoE[99].

In conclusion, from the data available to date it is reasonable to conclude that in most patients EoE is primarily caused by food hypersensitivity in subjects with a genetic predisposition induced by an early exposure to allergens that may abnormally stimulate T-helper type cytokines. However, the partial and selective response to specific treatments carried out in the above mentioned clinical trials do not provide conclusive evidence regarding the mechanisms involved in its pathogenesis. It is conceivable that more than one allergen or immune mechanism may contribute to this disease and therefore therapies may not only have to be individualized but also be acceptable to patients since long-term therapy may be necessary in order to avoid sub-epithelial fibrosis and remodeling of the esophagus.

REFERENCES

1 **Dobbins JW**, Sheahan D, Behar J. Eosinophilic gastroenteritis with esophageal involvement. *Gastroenterology* 1977; **72**: 1312-1316
2 **Attwood SE**, Smyrk TC, Demeester TR, Jones JB. Esophageal eosinophilia with dysphagia. A distinct clinicopathologic syndrome. *Dig Dis Sci* 1993; **38**: 109-116
3 **Straumann A,** Spichtin HP, Bernoulli R, Loosli J, Vögtlin J. Idiopathic eosinophilic esophagitis: a frequently overlooked disease with typical clinical aspects and discrete endoscopic findings. *Schweiz Med Wochenschr* 1994; **124:** 1419-1429
4 **Pellicano R**, De Angelis C, Ribaldone DG Fagoonee S, Astegiano M. 2013 update on celiac disease and eosinophilic esophagitis. *Nutrients* 2013; **5**: 3329-3336 [DOI: 10.3390/nu5093329]
5 **Straumann A**, Simon HU. Eosinophilic esophagitis: escalating epidemiology? *J Allergy Clin Immunol* 2005; **115**: 418-419 [PMID: 15696105]
6 **Hruz P**, Straumann A, Bussmann C, Heer P, Simon HU, Zwahlen M, Beglinger C, Schoepfer AM; Swiss EoE study group. Escalating incidence of eosinophilic esophagitis: a 20-year prospective, population-based study in Olten County, Switzerland. *J Allergy Clin Immunol* 2011; **128**: 1349-1350.e5 [PMID: 22019091 DOI: 10.1016/j.jaci.2011.09.013]
7 **Hurrell JM**, Genta RM, Dellon ES. Prevalence of esophageal eosinophilia varies by climate zone in the United States. *Am J Gastroenterol* 2012; **107**: 698-706 [PMID: 22310220 DOI: 10.1038/ajg.2012.6]
8 **Franciosi JP**, Tam V, Liacouras CA, Spergel JM. A case-control study of sociodemographic and geographic characteristics of 335 children with eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2009; **7**: 415-419 [PMID: 19118642 DOI: 10.1016/j.cgh.2008.10.006]
9 **Cherian S**, Smith NM, Forbes DA. Rapidly increasing prevalence of eosinophilic oesophagitis in Western Australia. *Arch Dis Child* 2006; **91**: 1000-1004 [PMID: 16877474]
10 **Spergel JM**, Book WM, Mays E, Song L, Shah SS, Talley NJ, Bonis PA. Variation in prevalence, diagnostic criteria, and initial management options for eosinophilic gastrointestinal diseases in the United States. *J Pediatr Gastroenterol Nutr* 2011; **52**: 300-306 [PMID: 21057327 DOI: 10.1097/MPG.0b013e3181eb5a9f]
11 **Prasad GA**, Alexander JA, Schleck CD, Zinsmeister AR, Smyrk TC, Elias RM, Locke GR, Talley NJ. Epidemiology of eosinophilic esophagitis over three decades in Olmsted County, Minnesota. *Clin Gastroenterol Hepatol* 2009; **7**: 1055-1061 [PMID: 19577011 DOI: 10.1016/j.cgh.2009.06.023]
12 **Spergel JM**, Brown-Whitehorn TF, Beausoleil JL, Franciosi J, Shuker M, Verma R, Liacouras CA. 14 years of eosinophilic esophagitis: clinical features and prognosis. *J Pediatr Gastroenterol Nutr* 2009; **48**: 30-36 [PMID: 19172120 DOI: 10.1097/MPG.0b013e3181788282]
13 **Zink DA**, Amin M, Gebara S, Desai TK. Familial dysphagia and eosinophilia. *Gastrointest Endosc* 2007; **65**: 330-334 [PMID: 17258999]
14 **Leynaert B**, Sunyer J, Garcia-Esteban R, Svanes C, Jarvis D, Cerveri I, Dratva J, Gislason T, Heinrich J, Janson C, Kuenzli N, de Marco R, Omenaas E, Raherison C, Gómez Real F, Wjst M, Zemp E, Zureik M, Burney PG, Anto JM, Neukirch F. Gender differences in prevalence, diagnosis and incidence of allergic and non-allergic asthma: a population-based cohort. *Thorax* 2012; **67**: 625-631 [PMID: 22334535 DOI: 10.1136/thoraxjnl-2011-201249]
15 **Blatman KH**, Ditto AM. Chapter 26: Eosinophilic esophagitis. *Allergy Asthma Proc* 2012; **33** Suppl 1: S88-S90 [PMID: 22794699 DOI: 10.2500/aap.2012.33.3559]
16 **Furuta GT**, Katzka DA. Eosinophilic Esophagitis. *N Engl J Med* 2015; **373**: 1640-1648 [PMID: 26488694 DOI: 10.1056/NEJMra1502863]
17 **Dellon ES**, Speck O, Woodward K, Covey S, Rusin S, Shaheen NJ, Woosley JT. Distribution and variability of esophageal eosinophilia in patients undergoing upper endoscopy. *Mod Pathol* 2015; **28**: 383-390 [PMID: 25216228 DOI: 10.1038/modpathol.2014.110]
18 **Pentiuk S**, Putnam PE, Collins MH, Rothenberg ME. Dissociation between symptoms and histological severity in pediatric eosinophilic esophagitis. *J Pediatr Gastroenterol Nutr* 2009; **48**: 152-160 [PMID: 19179876 DOI: 10.1097/MPG.0b013e31817f0197]
19 **Hirano I**. Editorial: Should patients with suspected eosinophilic esophagitis undergo a therapeutic trial of proton pump inhibition? *Am J Gastroenterol* 2013; **108**: 373-375 [PMID: 23459046 DOI: 10.1038/ajg.2012.459]
20 **Dellon ES**, Speck O, Woodward K, Gebhart JH, Madanick RD, Levinson S, Fritchie KJ, Woosley JT, Shaheen NJ. Clinical and endoscopic characteristics do not reliably differentiate PPI-responsive esophageal eosinophilia and eosinophilic esophagitis in patients undergoing upper endoscopy: a prospective cohort study. *Am J Gastroenterol* 2013; **108**: 1854-1860 [PMID: 24145677 DOI: 10.1038/ajg.2013.363]
21 **Bhattacharya B**, Carlsten J, Sabo E, Kethu S, Meitner P, Tavares R, Jakate S, Mangray S, Aswad B, Resnick MB. Increased expression of eotaxin-3 distinguishes between eosinophilic esophagitis and gastroesophageal reflux disease. *Hum Pathol* 2007; **38**: 1744-1753 [PMID: 17900656 DOI: 10.1016/j.humpath.2007.05.008]
22 **Matoso A**, Allen D, Herzlinger M, Ferreira J, Chen S, Lu S, Fabre V, Monahan R, Yang D, Noble L, Mangray S, Resnick MB. Correlation of ALOX15 expression with eosinophilic or reflux esophagitis in a cohort of pediatric patients with esophageal eosinophilia. *Hum Pathol* 2014; **45**: 1205-1212 [PMID: 24742828 DOI: 10.1016/j.humpath.2014.01.021]
23 **van Rhijn BD**, Weijenborg PW, Verheij J, van den Bergh Weerman MA, Verseijden C, van den Wijngaard RM, de Jonge WJ, Smout AJ, Bredenoord AJ. Proton pump inhibitors partially restore mucosal integrity in patients with proton pump inhibitor-responsive esophageal eosinophilia but not eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2014; **12**: 1815-1823.e2 [PMID: 24657840 DOI: 10.1016/j.cgh.2014.02.037]
24 **Croese J**, Fairley SK, Masson JW, Chong AK, Whitaker DA,

Kanowski PA, Walker NI. Clinical and endoscopic features of eosinophilic esophagitis in adults. *Gastrointest Endosc* 2003; **58**: 516-522 [PMID: 14520283 DOI: 10.1067/S0016-5107(03)01870-4]

25 **Dellon ES**, Kim HP, Sperry SL, Rybnicek DA, Woosley JT, Shaheen NJ. A phenotypic analysis shows that eosinophilic esophagitis is a progressive fibrostenotic disease. *Gastrointest Endosc* 2014; **79**: 577-85.e4 [PMID: 24275329 DOI: 10.1016/j.gie.2013.10.027]

26 **Straumann A**, Rossi L, Simon HU, Heer P, Spichtin HP, Beglinger C. Fragility of the esophageal mucosa: a pathognomonic endoscopic sign of primary eosinophilic esophagitis? *Gastrointest Endosc* 2003; **57**: 407-412 [PMID: 12612531 DOI: 10.1067/mge.2003.123]

27 **Sundaram S**, Sunku B, Nelson SP, Sentongo T, Melin-Aldana H, Kumar R, Li BU. Adherent white plaques: an endoscopic finding in eosinophilic esophagitis. *J Pediatr Gastroenterol Nutr* 2004; **38**: 208-212 [PMID: 14734886 DOI: 10.1097/00005176-200402000-00020]

28 **Hirano I**, Moy N, Heckman MG, Thomas CS, Gonsalves N, Achem SR. Endoscopic assessment of the oesophageal features of eosinophilic oesophagitis: validation of a novel classification and grading system. *Gut* 2013; **62**: 489-495 [PMID: 22619364 DOI: 10.1136/gutjnl-2011-301817]

29 **Nurko S**, Rosen R. Esophageal dysmotility in patients who have eosinophilic esophagitis. *Gastrointest Endosc Clin N Am* 2008; **18**: 73-89; ix [PMID: 18061103 DOI: 10.1016/j.giec.2007.09.006]

30 **Roman S**, Hirano I, Kwiatek MA, Gonsalves N, Chen J, Kahrilas PJ, Pandolfino JE. Manometric features of eosinophilic esophagitis in esophageal pressure topography. *Neurogastroenterol Motil* 2011; **23**: 208-214, e111 [PMID: 21091849 DOI: 10.1111/j.1365-2982.2010.01633.x]

31 **Lipka S**, Kumar A, Richter JE. Impact of Diagnostic Delay and Other Risk Factors on Eosinophilic Esophagitis Phenotype and Esophageal Diameter. *J Clin Gastroenterol* 2016; **50**: 134-140 [PMID: 25710524]

32 **Savarino E**, Gemignani L, Zentilin P, De Bortoli N, Malesci A, Mastracci L, Fiocca R, Savarino V. Achalasia with dense eosinophilic infiltrate responds to steroid therapy. *Clin Gastroenterol Hepatol* 2011; **9**: 1104-1106 [PMID: 21839711 DOI: 10.1016/j.cgh.2011.08.002]

33 **Rieder F**, Nonevski I, Ma J, Ouyang Z, West G, Protheroe C, DePetris G, Schirbel A, Lapinski J, Goldblum J, Bonfield T, Lopez R, Harnett K, Lee J, Hirano I, Falk G, Biancani P, Fiocchi C. T-helper 2 cytokines, transforming growth factor β1, and eosinophil products induce fibrogenesis and alter muscle motility in patients with eosinophilic esophagitis. *Gastroenterology* 2014; **146**: 1266-1277.e1-9 [PMID: 24486052 DOI: 10.1053/j.gastro.2014.01.051]

34 **Lucendo AJ**, Sánchez-Cazalilla M. Adult versus pediatric eosinophilic esophagitis: important differences and similarities for the clinician to understand. *Expert Rev Clin Immunol* 2012; **8**: 733-745 [PMID: 23167685 DOI: 10.1586/eci.12.68]

35 **Straumann A**, Spichtin HP, Grize L, Bucher KA, Beglinger C, Simon HU. Natural history of primary eosinophilic esophagitis: a follow-up of 30 adult patients for up to 11.5 years. *Gastroenterology* 2003; **125**: 1660-1669 [PMID: 14724818 DOI: 10.1053/j.gastro.2003.09.024]

36 **Steiner SJ**, Kernek KM, Fitzgerald JF. Severity of basal cell hyperplasia differs in reflux versus eosinophilic esophagitis. *J Pediatr Gastroenterol Nutr* 2006; **42**: 506-509 [PMID: 16707971 DOI: 10.1097/01.mpg.0000221906.06899.1b]

37 **Katzka DA**, Ravi K, Geno DM, Smyrk TC, Iyer PG, Alexander JA, Mabary JE, Camilleri M, Vaezi MF. Endoscopic Mucosal Impedance Measurements Correlate With Eosinophilia and Dilation of Intercellular Spaces in Patients With Eosinophilic Esophagitis. *Clin Gastroenterol Hepatol* 2015; **13**: 1242-1248.e1 [PMID: 25592662 DOI: 10.1016/j.cgh.2014.12.032]

38 **Niranjan R**, Mavi P, Rayapudi M, Dynda S, Mishra A. Pathogenic role of mast cells in experimental eosinophilic esophagitis. *Am J Physiol Gastrointest Liver Physiol* 2013; **304**: G1087-G1094 [PMID: 23599040 DOI: 10.1152/ajpgi.00070.2013]

39 **Lucendo AJ**, Pascual-Turrión JM, Navarro M, Comas C, Castillo P, Letrán A, Caballero MT, Larrauri J. Endoscopic, bioptic, and manometric findings in eosinophilic esophagitis before and after steroid therapy: a case series. *Endoscopy* 2007; **39**: 765-771 [PMID: 17703383]

40 **Cheng E**, Zhang X, Huo X, Yu C, Zhang Q, Wang DH, Spechler SJ, Souza RF. Omeprazole blocks eotaxin-3 expression by oesophageal squamous cells from patients with eosinophilic oesophagitis and GORD. *Gut* 2013; **62**: 824-832 [PMID: 22580413 DOI: 10.1136/gutjnl-2012-302250]

41 **Rothenberg ME**. Molecular, genetic, and cellular bases for treating eosinophilic esophagitis. *Gastroenterology* 2015; **148**: 1143-1157 [PMID: 25666870 DOI: 10.1053/j.gastro.2015.02.002]

42 **Abonia JP**, Wen T, Stucke EM, Grotjan T, Griffith MS, Kemme KA, Collins MH, Putnam PE, Franciosi JP, von Tiehl KF, Tinkle BT, Marsolo KA, Martin LJ, Ware SM, Rothenberg ME. High prevalence of eosinophilic esophagitis in patients with inherited connective tissue disorders. *J Allergy Clin Immunol* 2013; **132**: 378-386 [PMID: 23608731 DOI: 10.1016/j.jaci.2013.02.030]

43 **Blanchard C**, Wang N, Stringer KF, Mishra A, Fulkerson PC, Abonia JP, Jameson SC, Kirby C, Konikoff MR, Collins MH, Cohen MB, Akers R, Hogan SP, Assa'ad AH, Putnam PE, Aronow BJ, Rothenberg ME. Eotaxin-3 and a uniquely conserved gene-expression profile in eosinophilic esophagitis. *J Clin Invest* 2006; **116**: 536-547 [PMID: 16453027 DOI: 10.1172/JCI26679]

44 **Rothenberg ME**, Spergel JM, Sherrill JD, Annaiah K, Martin LJ, Cianferoni A, Gober L, Kim C, Glessner J, Frackelton E, Thomas K, Blanchard C, Liacouras C, Verma R, Aceves S, Collins MH, Brown-Whitehorn T, Putnam PE, Franciosi JP, Chiavacci RM, Grant SF, Abonia JP, Sleiman PM, Hakonarson H. Common variants at 5q22 associate with pediatric eosinophilic esophagitis. *Nat Genet* 2010; **42**: 289-291 [PMID: 20208534 DOI: 10.1038/ng.547]

45 **Alexander ES**, Martin LJ, Collins MH, Kottyan LC, Sucharew H, He H, Mukkada VA, Succop PA, Abonia JP, Foote H, Eby MD, Grotjan TM, Greenler AJ, Dellon ES, Demain JG, Furuta GT, Gurian LE, Harley JB, Hopp RJ, Kagalwalla A, Kaul A, Nadeau KC, Noel RJ, Putnam PE, von Tiehl KF, Rothenberg ME. Twin and family studies reveal strong environmental and weaker genetic cues explaining heritability of eosinophilic esophagitis. *J Allergy Clin Immunol* 2014; **134**: 1084-1092.e1 [PMID: 25258143 DOI: 10.1016/j.jaci.2014.07.021]

46 **Noel RJ**, Putnam PE, Rothenberg ME. Eosinophilic esophagitis. *N Engl J Med* 2004; **351**: 940-941 [PMID: 15329438 DOI: 10.1056/NEJM200408263510924]

47 **Vicario M**, Blanchard C, Stringer KF, Collins MH, Mingler MK, Ahrens A, Putnam PE, Abonia JP, Santos J, Rothenberg ME. Local B cells and IgE production in the oesophageal mucosa in eosinophilic oesophagitis. *Gut* 2010; **59**: 12-20 [PMID: 19528036 DOI: 10.1136/gut.2009.178020]

48 **Miehlke S**, Alpan O, Schröder S, Straumann A. Induction of eosinophilic esophagitis by sublingual pollen immunotherapy. *Case Rep Gastroenterol* 2013; **7**: 363-368 [PMID: 24163646 DOI: 10.1159/000355161]

49 **Mishra A**, Hogan SP, Brandt EB, Rothenberg ME. An etiological role for aeroallergens and eosinophils in experimental esophagitis. *J Clin Invest* 2001; **107**: 83-90 [PMID: 11134183 DOI: 10.1172/JCI10224]

50 **Bove M**, Tegtmeyer B, Persson S, Bergquist H. The pharyngeal mucosa is not involved in eosinophilic oesophagitis. *Aliment Pharmacol Ther* 2009; **30**: 495-500 [PMID: 19508404 DOI: 10.1111/j.1365-2036.2009.04059]

51 **Balatsinou C**, Milano A, Caldarella MP, Laterza F, Pierdomenico SD, Cuccurullo F, Neri M. Eosinophilic esophagitis is a component of the anticonvulsant hypersensitivity syndrome: description of two cases. *Dig Liver Dis* 2008; **40**: 145-148 [PMID: 17395557 DOI: 10.1016/j.dld.2007.01.017]

52 **Blanchard C**, Rothenberg ME. Basic pathogenesis of eosinophilic esophagitis. *Gastrointest Endosc Clin N Am* 2008; **18**: 133-143; x [PMID: 18061107 DOI: 10.1016/j.giec.2007.09.016]

53 **Foroughi S**, Foster B, Kim N, Bernardino LB, Scott LM, Hamilton RG, Metcalfe DD, Mannon PJ, Prussin C. Anti-IgE treatment of eosinophil-associated gastrointestinal disorders. *J Allergy Clin*

Immunol 2007; **120**: 594-601 [PMID: 17765756]
54 **Clayton F**, Fang JC, Gleich GJ, Lucendo AJ, Olalla JM, Vinson LA, Lowichik A, Chen X, Emerson L, Cox K, O'Gorman MA, Peterson KA. Eosinophilic esophagitis in adults is associated with IgG4 and not mediated by IgE. *Gastroenterology* 2014; **147**: 602-609 [PMID: 24907494 DOI: 10.1053/j.gastro.2014.05.036]
55 **Gonsalves N**, Yang GY, Doerfler B, Ritz S, Ditto AM, Hirano I. Elimination diet effectively treats eosinophilic esophagitis in adults; food reintroduction identifies causative factors. *Gastroenterology* 2012; **142**: 1451-1459.e1; quiz e14-15 [PMID: 22391333 DOI: 10.1053/j.gastro.2012.03.001]
56 **Sugnanam KK**, Collins JT, Smith PK, Connor F, Lewindon P, Cleghorn G, Withers G. Dichotomy of food and inhalant allergen sensitization in eosinophilic esophagitis. *Allergy* 2007; **62**: 1257-1260 [PMID: 17711545]
57 **Khan S**, Orenstein SR. Eosinophilic gastroenteritis. *Gastroenterol Clin North Am* 2008; **37**: 333-348, v [PMID: 18499023 DOI: 10.1016/j.gtc.2008.02.003]
58 **Mishra A**, Schlotman J, Wang M, Rothenberg ME. Critical role for adaptive T cell immunity in experimental eosinophilic esophagitis in mice. *J Leukoc Biol* 2007; **81**: 916-924 [PMID: 17194734 DOI: 10.1189/jlb.1106653]
59 **Mulder DJ**, Pooni A, Mak N, Hurlbut DJ, Basta S, Justinich CJ. Antigen presentation and MHC class II expression by human esophageal epithelial cells: role in eosinophilic esophagitis. *Am J Pathol* 2011; **178**: 744-753 [PMID: 21281807 DOI: 10.1016/j.ajpath.2010.10.027]
60 **Altomare A**, Ma J, Guarino MP, Cheng L, Rieder F, Ribolsi M, Fiocchi C, Biancani P, Harnett K, Cicala M. Platelet-activating factor and distinct chemokines are elevated in mucosal biopsies of erosive compared with non-erosive reflux disease patients and controls. *Neurogastroenterol Motil* 2012; **24**: 943-e463 [PMID: 22734465 DOI: 10.1111/j.1365-2982.2012.01963]
61 **Ma J**, Altomare A, Guarino M, Cicala M, Rieder F, Fiocchi C, Li D, Cao W, Behar J, Biancani P, Harnett KM. HCl-induced and ATP-dependent upregulation of TRPV1 receptor expression and cytokine production by human esophageal epithelial cells. *Am J Physiol Gastrointest Liver Physiol* 2012; **303**: G635-G645 [PMID: 22790593 DOI: 10.1152/ajpgi.00097]
62 **Blanchard C**, Stucke EM, Rodriguez-Jimenez B, Burwinkel K, Collins MH, Ahrens A, Alexander ES, Butz BK, Jameson SC, Kaul A, Franciosi JP, Kushner JP, Putnam PE, Abonia JP, Rothenberg ME. A striking local esophageal cytokine expression profile in eosinophilic esophagitis. *J Allergy Clin Immunol* 2011; **127**: 208-217, 217.e1-7 [PMID: 21211656 DOI: 10.1016/j.jaci.2010.10.039]
63 **Matthews AN**, Friend DS, Zimmermann N, Sarafi MN, Luster AD, Pearlman E, Wert SE, Rothenberg ME. Eotaxin is required for the baseline level of tissue eosinophils. *Proc Natl Acad Sci USA* 1998; **95**: 6273-6278 [PMID: 9600955 DOI: 10.1073/pnas.95.11.6273]
64 **Bullock JZ**, Villanueva JM, Blanchard C, Filipovich AH, Putnam PE, Collins MH, Risma KA, Akers RM, Kirby CL, Buckmeier BK, Assa'ad AH, Hogan SP, Rothenberg ME. Interplay of adaptive th2 immunity with eotaxin-3/c-C chemokine receptor 3 in eosinophilic esophagitis. *J Pediatr Gastroenterol Nutr* 2007; **45**: 22-31 [PMID: 17592361 DOI: 10.1097/MPG.0b013e318043c097]
65 **Ponath PD**, Qin S, Ringler DJ, Clark-Lewis I, Wang J, Kassam N, Smith H, Shi X, Gonzalo JA, Newman W, Gutierrez-Ramos JC, Mackay CR. Cloning of the human eosinophil chemoattractant, eotaxin. Expression, receptor binding, and functional properties suggest a mechanism for the selective recruitment of eosinophils. *J Clin Invest* 1996; **97**: 604-612 [PMID: 8609214]
66 **Mishra A**, Hogan SP, Brandt EB, Rothenberg ME. IL-5 promotes eosinophil trafficking to the esophagus. *J Immunol* 2002; **168**: 2464-2469 [PMID: 11859139 DOI: 10.4049/jimmunol.168.5.2464]
67 **Rothenberg ME**, Ownbey R, Mehlhop PD, Loiselle PM, van de Rijn M, Bonventre JV, Oettgen HC, Leder P, Luster AD. Eotaxin triggers eosinophil-selective chemotaxis and calcium flux via a distinct receptor and induces pulmonary eosinophilia in the presence of interleukin 5 in mice. *Mol Med* 1996; **2**: 334-348 [PMID: 8784786]
68 **Hogan SP**, Rothenberg ME. Eosinophil Function in Eosinophil-associated Gastrointestinal Disorders. *Curr Allergy Asthma Rep* 2006; **6**: 65-71 [PMID: 16476198 DOI: 10.1007/s11882-006-0013-8]
69 **Dellon ES**. Eosinophilic esophagitis. *Gastroenterol Clin North Am* 2013; **42**: 133-153 [PMID: 23452635 DOI: 10.1016/j.gtc.2012.11.008]
70 **Wershil BK**. Exploring the role of mast cells in eosinophilic esophagitis. *Immunol Allergy Clin North Am* 2009; **29**: 189-195, xiii [PMID: 19141354 DOI: 10.1016/j.iac.2008.09.006]
71 **Lucendo AJ**, Bellón T, Lucendo B. The role of mast cells in eosinophilic esophagitis. *Pediatr Allergy Immunol* 2009; **20**: 512-518 [PMID: 18681944 DOI: 10.1111/j.1399-3038.2008.00798]
72 **Niranjan R**, Rayapudi M, Mishra A, Dutt P, Dynda S, Mishra A. Pathogenesis of allergen-induced eosinophilic esophagitis is independent of interleukin (IL)-13. *Immunol Cell Biol* 2013; **91**: 408-15 [DOI: 10.1038/icb.2013.21]
73 **Aceves SS**, Newbury RO, Dohil R, Bastian JF, Broide DH. Esophageal remodeling in pediatric eosinophilic esophagitis. *J Allergy Clin Immunol* 2007; **119**: 206-212 [PMID: 17208603 DOI: 10.1016/j.jaci.2006.10.016]
74 **Kim N**, Sohn UD, Mangannan V, Rich H, Jain MK, Behar J, Biancani P. Leukotrienes in acetylcholine-induced contraction of esophageal circular smooth muscle in experimental esophagitis. *Gastroenterology* 1997; **112**: 1548-1558 [PMID: 9136833]
75 **Moawad FJ**, Veerappan GR, Dias JA, Baker TP, Maydonovitch CL, Wong RK. Randomized controlled trial comparing aerosolized swallowed fluticasone to esomeprazole for esophageal eosinophilia. *Am J Gastroenterol* 2013; **108**: 366-372 [PMID: 23399553 DOI: 10.1038/ajg.2012.443]
76 **Molina-Infante J**, Katzka DA, Dellon ES. Proton pump inhibitor-responsive esophageal eosinophilia: a historical perspective on a novel and evolving entity. *Rev Esp Enferm Dig* 2015; **107**: 29-36 [PMID: 25603329]
77 **Francis DL**, Foxx-Orenstein A, Arora AS, Smyrk TC, Jensen K, Nord SL, Alexander JA, Romero Y, Katzka DA. Results of ambulatory pH monitoring do not reliably predict response to therapy in patients with eosinophilic oesophagitis. *Aliment Pharmacol Ther* 2012; **35**: 300-307 [PMID: 22111863 DOI: 10.1111/j.1365-2036.2011.04922.x]
78 **Ally MR**, Dias J, Veerappan GR, Maydonovitch CL, Wong RK, Moawad FJ. Safety of dilation in adults with eosinophilic esophagitis. *Dis Esophagus* 2013; **26**: 241-245 [PMID: 22676406 DOI: 10.1111/j.1442-2050.2012.01363.x]
79 **Bohm M**, Richter JE, Kelsen S, Thomas R. Esophageal dilation: simple and effective treatment for adults with eosinophilic esophagitis and esophageal rings and narrowing. *Dis Esophagus* 2010; **23**: 377-385 [PMID: 20353444 DOI: 10.1111/j.1442-2050.2010.01051.x]
80 **Moawad FJ**, Cheatham JG, DeZee KJ. Meta-analysis: the safety and efficacy of dilation in eosinophilic oesophagitis. *Aliment Pharmacol Ther* 2013; **38**: 713-720 [PMID: 23915046 DOI: 10.1111/apt.12438]
81 **Konikoff MR**, Noel RJ, Blanchard C, Kirby C, Jameson SC, Buckmeier BK, Akers R, Cohen MB, Collins MH, Assa'ad AH, Aceves SS, Putnam PE, Rothenberg ME. A randomized, double-blind, placebo-controlled trial of fluticasone propionate for pediatric eosinophilic esophagitis. *Gastroenterology* 2006; **131**: 1381-1391 [PMID: 17101314 DOI: 10.1053/j.gastro.2006.08.033]
82 **Dellon ES**, Sheikh A, Speck O, Woodward K, Whitlow AB, Hores JM, Ivanovic M, Chau A, Woosley JT, Madanick RD, Orlando RC, Shaheen NJ. Viscous topical is more effective than nebulized steroid therapy for patients with eosinophilic esophagitis. *Gastroenterology* 2012; **143**: 321-324.e1 [PMID: 22561055 DOI: 10.1053/j.gastro.2012.04.049]
83 **Alexander JA**, Jung KW, Arora AS, Enders F, Katzka DA, Kephardt GM, Kita H, Kryzer LA, Romero Y, Smyrk TC, Talley NJ. Swallowed fluticasone improves histologic but not symptomatic response of adults with eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2012; **10**: 742-749.e1 [PMID: 22475741 DOI: 10.1016/j.cgh.2012.03.018]
84 **Lucendo AJ**, Arias A, De Rezende LC, Yagüe-Compadre JL, Mota-Huertas T, González-Castillo S, Cuesta RA, Tenias JM, Bellón T.

Subepithelial collagen deposition, profibrogenic cytokine gene expression, and changes after prolonged fluticasone propionate treatment in adult eosinophilic esophagitis: a prospective study. *J Allergy Clin Immunol* 2011; **128**: 1037-1046 [PMID: 21880354 DOI: 10.1016/j.jaci.2011.08.007]

85 **Lucendo AJ**, De Rezende L, Comas C, Caballero T, Bellón T. Treatment with topical steroids downregulates IL-5, eotaxin-1/CCL11, and eotaxin-3/CCL26 gene expression in eosinophilic esophagitis. *Am J Gastroenterol* 2008; **103**: 2184-2193 [PMID: 18844613 DOI: 10.1111/j.1572-0241.2008.01937.x]

86 **Dohil R**, Newbury R, Fox L, Bastian J, Aceves S. Oral viscous budesonide is effective in children with eosinophilic esophagitis in a randomized, placebo-controlled trial. *Gastroenterology* 2010; **139**: 418-429 [PMID: 20457157 DOI: 10.1053/j.gastro.2010.05.001]

87 **Straumann A**, Conus S, Degen L, Frei C, Bussmann C, Beglinger C, Schoepfer A, Simon HU. Long-term budesonide maintenance treatment is partially effective for patients with eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2011; **9**: 400-409.e1 [DOI: 10.1016/j.cgh.2011.01.017]

88 **Chuang MY**, Chinnaratha MA, Hancock DG, Woodman R, Wong GR, Cock C, Fraser RJ. Topical Steroid Therapy for the Treatment of Eosinophilic Esophagitis (EoE): A Systematic Review and Meta-Analysis. *Clin Transl Gastroenterol* 2015; **6**: e82 [PMID: 25809314 DOI: 10.1038/ctg.2015.9]

89 **Assa'ad AH**, Gupta SK, Collins MH, Thomson M, Heath AT, Smith DA, Perschy TL, Jurgensen CH, Ortega HG, Aceves SS. An antibody against IL-5 reduces numbers of esophageal intraepithelial eosinophils in children with eosinophilic esophagitis. *Gastroenterology* 2011; **141**: 1593-1604 [PMID: 21835135 DOI: 10.1053/j.gastro.2011.07.044]

90 **Otani IM**, Anilkumar AA, Newbury RO, Bhagat M, Beppu LY, Dohil R, Broide DH, Aceves SS. Anti-IL-5 therapy reduces mast cell and IL-9 cell numbers in pediatric patients with eosinophilic esophagitis. *J Allergy Clin Immunol* 2013; **131**: 1576-1582 [PMID: 23623266 DOI: 10.1016/j.jaci.2013.02.042]

91 **Straumann A**, Conus S, Grzonka P, Kita H, Kephart G, Bussmann C, Beglinger C, Smith DA, Patel J, Byrne M, Simon HU. Anti-interleukin-5 antibody treatment (mepolizumab) in active eosinophilic oesophagitis: a randomised, placebo-controlled, double-blind trial. *Gut* 2010; **59**: 21-30 [PMID: 19828470 DOI: 10.1136/gut.2009.178558]

92 **Spergel JM**, Rothenberg ME, Collins MH, Furuta GT, Markowitz JE, Fuchs G, O'Gorman MA, Abonia JP, Young J, Henkel T, Wilkins HJ, Liacouras CA. Reslizumab in children and adolescents with eosinophilic esophagitis: results of a double-blind, randomized, placebo-controlled trial. *J Allergy Clin Immunol* 2012; **129**: 456-463, 463.e1-3 [PMID: 22206777 DOI: 10.1016/j.jaci.2011.11.044]

93 **Lin SK**, Sabharwal G, Ghaffari G. A review of the evidence linking eosinophilic esophagitis and food allergy. *Allergy Asthma Proc* 2015; **36**: 26-33 [PMID: 25562553 DOI: 10.2500/aap.2015.36.3804]

94 **Peterson KA**, Byrne KR, Vinson LA, Ying J, Boynton KK, Fang JC, Gleich GJ, Adler DG, Clayton F. Elemental diet induces histologic response in adult eosinophilic esophagitis. *Am J Gastroenterol* 2013; **108**: 759-766 [PMID: 23381017 DOI: 10.1038/ajg.2012.468]

95 **Molina-Infante J**, Arias A, Barrio J, Rodríguez-Sánchez J, Sanchez-Cazalilla M, Lucendo AJ. Four-food group elimination diet for adult eosinophilic esophagitis: A prospective multicenter study. *J Allergy Clin Immunol* 2014; **134**: 1093-1099.e1 [PMID: 25174868 DOI: 10.1016/j.jaci.2014.07.023]

96 **Arias A**, González-Cervera J, Tenias JM, Lucendo AJ. Efficacy of dietary interventions for inducing histologic remission in patients with eosinophilic esophagitis: a systematic review and meta-analysis. *Gastroenterology* 2014; **146**: 1639-1648 [PMID: 24534634 DOI: 10.1053/j.gastro.2014.02.006]

97 **Lucendo AJ**, Molina-Infante J. Emerging therapeutic strategies for eosinophilic esophagitis. *Curr Treat Options Gastroenterol* 2014; **12**: 1-17 [PMID: 24370990 DOI: 10.1007/s11938-013-0001-8]

98 **Kruszewski PG**, Russo JM, Franciosi JP, Varni JW, Platts-Mills TA, Erwin EA. Prospective, comparative effectiveness trial of cow's milk elimination and swallowed fluticasone for pediatric eosinophilic esophagitis. *Dis Esophagus* 2015; Epub ahead of print [PMID: 25721813 DOI: 10.1111/dote.12339]

99 **Arias Á**, Lucendo AJ, Martínez-Fernández P, González-Castro AM, Fortea M, González-Cervera J, Yagüe-Compadre JL, Mota-Huertas T, Vicario M. Dietary treatment modulates mast cell phenotype, density, and activity in adult eosinophilic oesophagitis. *Clin Exp Allergy* 2016; **46**: 78-91 [PMID: 25640519 DOI: 10.1111/cea.12504]

P- Reviewer: Mir SAV, Nagahara A, Peixoto A **S- Editor**: Song XX
L- Editor: A **E- Editor**: Wang CH

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i1.78

World J Gastrointest Pharmacol Ther 2016 February 6; 7(1): 78-90
ISSN 2150-5349 (online)

REVIEW

Diagnosis and management of functional symptoms in inflammatory bowel disease in remission

Carlos Teruel, Elena Garrido, Francisco Mesonero

Carlos Teruel, Elena Garrido, Francisco Mesonero, Gastroenterology Department, Hospital Ramón y Cajal, 28034 Madrid, Spain

Author contributions: All authors contributed equally to this work.

Conflict-of-interest statement: The authors declare no conflicts of interest.

Correspondence to: Carlos Teruel, MD, Gastroenterology Department, Hospital Ramón y Cajal, Ctra. Colmenar Viejo; km. 9100, 28034 Madrid, Spain. cteruelvegazo@yahoo.es
Telephone: +34-91-3368354
Fax: +34-91-3368354

Received: April 28, 2015
Peer-review started: May 6, 2015
First decision: July 17, 2015
Revised: September 3, 2015
Accepted: October 20, 2015
Article in press: October 27, 2015
Published online: February 6, 2016

Abstract

Inflammatory bowel disease (IBD) patients in remission may suffer from gastrointestinal symptoms that resemble irritable bowel syndrome (IBS). Knowledge on this issue has increased considerably in the last decade, and it is our intention to review and summarize it in the present work. We describe a problematic that comprises physiopathological uncertainties, diagnostic difficulties, as IBS-like symptoms are very similar to those produced by an inflammatory flare, and the necessity of appropriate management of these patients, who, although in remission, have impaired quality of life. Ultimately, from almost a philosophical point of view, the presence of IBS-like symptoms in IBD patients in remission supposes a challenge to the traditional functional-organic dichotomy, suggesting the need for a change of paradigm.

Key words: Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Irritable bowel syndrome; Functional gastrointestinal disease

Core tip: Many inflammatory bowel disease patients in remission suffer from ongoing gastrointestinal symptoms that resemble those of irritable bowel syndrome and that hinder their quality of life. We review the pathogenesis of these symptoms, their prevalence and the best management strategies.

Teruel C, Garrido E, Mesonero F. Diagnosis and management of functional symptoms in inflammatory bowel disease in remission. *World J Gastrointest Pharmacol Ther* 2016; 7(1): 78-90 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i1/78.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i1.78

INTRODUCTION

More than thirty years ago, in 1983, it was first reported that a significant proportion of patients with inflammatory bowel disease (IBD) with normal

mucosa in endoscopic examination nevertheless suffered from gastrointestinal symptoms allegedly of functional origin[1]. Almost twenty years passed until the next significant investigation addressing this issue was published, in 2002 by Simrén *et al*[2]. From that year on, an important number of studies have been published providing a more profound knowledge about functional symptoms in IBD in remission. It is our objective in the present work to review this knowledge.

Within functional digestive disorders, we will focus on irritable bowel syndrome (IBS) because its symptoms (abdominal pain, diarrhea, constipation, fecal incontinence) resemble those of flare-up of IBD. Differential diagnosis between IBD flare and IBS-like symptoms is a diagnostic challenge with critical consequences. Immunomodulators could be prescribed unnecessarily or ongoing inflammation managed inadequately with, for example, antispasmodics, delaying initiation of proper treatment when not resulting in adverse events.

Some authors have explored the prevalence of IBS symptoms in active IBD[3-5]. We consider, as many others, that it is very difficult to discriminate reliably symptoms attributable to IBD from those secondary to a functional disorder in that setting, so we will not explore this aspect further.

Increasing knowledge about the occurrence of IBS symptoms in IBD patients with no macroscopic inflammation, has led to the definition of so-called "IBD-IBS". This concept, together with further awareness of links between the two entities, challenges the traditional functional-organic dichotomy and leads to the elaboration of a broader biopsychosocial model of disease that should allow a better understanding of patients' medical condition that the classical dogma fails to achieve[6-8].

We have structured the review following a question-and-answer scheme with intent to reproduce what comes into mind of the physician in the outpatient clinic. Key conclusions in each section are presented.

HOW DO I KNOW THAT IBD IS IN REMISSION?

To confidently define remission non-invasively we should use not only clinical indexes but also C-reactive protein (CRP) and fecal calprotectin. Radiological procedures could be of help in unclear cases. Endoscopy would still be necessary to confirm remission in many cases.

The definition of remission in IBD is not straightforward, as it is a broad concept that includes several aspects: (1) clinical remission (absence of symptoms); (2) endoscopic remission (mucosal healing); and (3) deep remission (no symptoms and mucosal healing).

To enhance the optimal diagnosis of remission several indices are available to monitor IBD inflammatory activity, such as the Crohn's Disease Activity Index (CDAI) or the Harvey-Bradshaw index for Crohn's disease (CD), or the Mayo score for ulcerative colitis (UC). They are a composite of objective items (such as analytical determinations), objective items self-reported by the patient (such as number of stools per day) and purely subjective items such as pain intensity or degree of wellbeing. Although valuable in monitoring disease progression and activity[9] and therefore widely used in clinical trials, these indices do not correlate perfectly with endoscopic/histological activity or analytical parameters, probably because they all include subjective items[10-12]. Sub-analyses of some recent clinical trials show that up to 18% of randomized patients, all with scores in the range of active disease as defined in the inclusion criteria, actually had no evidence of endoscopic inflammation[13]. Lahiff *et al*[10] measured CDAI in 44 CD patients and in 47 IBS patients, and noticed that 62% of IBS patients had a score higher than 150 (the usual threshold for inclusion in IBD clinical trials) and that mean scores were higher in IBS patients (183 points *vs* 157, $P < 0.05$). More intense pain and worse perception of general wellbeing were the main contributors to the final score in IBS. Authors conclude that remission cannot be determined solely on clinical indices, as their subjective components makes it impossible to separate functional from inflammatory symptoms[10,14].

According to all this, additional tools are needed to establish a reliable diagnosis of remission in IBD[15]: (1) Endoscopic examination of the intestinal mucosa is the gold standard to determine inflammatory activity and the most reliable test to define remission. However, endoscopy is invasive and uncomfortable, not devoid of side effects. Moreover, there are no clear or widely accepted criteria of endoscopic remission. On top of that, small bowel inflammatory activity can be very difficult to evaluate, even though the availability of the endoscopic capsule has increased in recent years; (2) Radiological imaging in IBD has lately experienced great advances. Ultrasound, computerized tomography (CT) and magnetic resonance imaging (MRI) are no longer used solely to rule out complications such as abscesses or perforation. They all play an increasing role in assessing the extent of disease, presence of active inflammation, transmural involvement and even response to treatment, all of which are especially true for MRI[16]; (3) CRP is the best serum marker of inflammatory activity in IBD, and reliably predicts treatment response[17,18]. It is therefore widely used in clinical practice. CRP is however an unspecific acute phase reactant, and up to 25% of patients with inflammatory activity will have normal levels[19,20]; and (4) Determination of fecal markers of neutrophilic activity in intestinal mucosa is a simple tool that reliably predicts the presence of significant mucosal inflammation[21,22]. Calprotectin is the most used

marker and has excellent diagnostic performance. In a meta-analysis the area under the receiver operating characteristic (ROC) curve was 0.97, with an optimal cut-off value of 50 mcg/g that yielded 93% sensitivity and 94% specificity in discriminating IBD from IBS[22]. Further on, calprotectin levels correlate very well with endoscopic activity[23-27], predict occurrence of a flare[28] and are capable of predicting response to treatment[27,29-31]. However, calprotectin also rises in other situations, such as with non-steroidal anti-inflammatory drugs (NSAIDs) use, and we lack a cut-off point that defines remission reliably[32]. As we describe in detail later, measurement of fecal calprotectin in IBD patients in remission could determine if the presence of symptoms is due to true functional syndromes or to ongoing inflammation[5,33-35].

ARE ALL PATIENTS IN REMISSION SUSCEPTIBLE TO SUFFERING IBS-LIKE SYMPTOMS?

Some IBD patients have risk factors for developing conditions such as bile salt diarrhea that can mimic IBS-like symptoms.

IBD patients in remission with previous intestinal surgery, stenosis or stomas are at risk of developing small intestine bacterial overgrowth (SIBO), sub-occlusive crisis or bile acid malabsorption. These three conditions cause symptoms that resemble IBS. Many authors exclude such patients when considering IBD-IBS prevalence, but others do not (Table 1). None have systematically carried out tests to rule out such conditions before definitive diagnosis, such as breath tests, enteroclysis, or a SeHCAT [tauroselcholic (75 selenium) acid] test respectively. Before establishing a purely functional nature of symptoms, it seems reasonable to consider such tests in IBD patients at risk of suffering these conditions or to carry out an empirical therapeutic trial for example with non-absorbable antibiotics or cholestyramine[36-38].

WHICH CRITERIA ARE VALID TO DIAGNOSE FUNCTIONAL SYMPTOMS IN IBD PATIENTS?

In absence of better alternatives, Rome Ⅲ criteria can be used to diagnose IBS in IBD patients in remission.

Irritable bowel syndrome is defined by the presence of abdominal pain or discomfort associated with changes in stool frequency or consistency. To date there is no biomarker available that can reliably confirm or exclude it. It is therefore a clinical diagnosis based on exclusion of organic disease in the presence of alarm symptoms (weight loss, anemia, gastrointestinal bleeding, *etc.*) and fulfillment of predefined criteria. Currently Rome Ⅲ criteria are without a doubt the most used to define IBS in epidemiological studies, clinical trials and everyday practice (Table 2)[39-41].

There are no consensus definitions for diagnosis of IBS-like symptoms in quiescent IBD. In general, investigators have used diagnostic criteria that were validated in non-IBD patients (Table 1). In 2005 Barratt *et al*[42] observed that several symptoms were more frequent when their etiology was functional and not inflammatory (exclusively daytime symptoms, bloating, excess gas, fatigue). They elaborated the St Mark's Diary Score, a tool to diagnose IBS-like symptoms in quiescent IBD. However, this score has not been validated externally to date.

HOW MANY IBD PATIENTS IN REMISSION HAVE IBS-LIKE SYMPTOMS?

IBD-IBS prevalence data are very variable due to heterogeneity in remission definition, diagnostic criteria and exclusion criteria. Pooling prevalences of the most homogeneous studies shows that about a third of quiescent IBD patients will suffer from IBS-like symptoms.

From 1983 until 2014 a total of 19 studies have measured the prevalence of IBS-like symptoms in IBD[1-5,33-35,42-52]. Two of these publications refer to the same IBD cohort[34,49], leaving 18 prevalence estimations. Two studies explore IBS symptoms in IBD patients regardless their inflammatory activity status[42,47]. The 16 remaining studies are summarized in Table 1. Three studies report IBS-like prevalences in both active and quiescent IBD[3-5]; from these studies we have extracted only data referring to IBD in remission. There are 10 cross-sectional studies, one prospective study (patients are followed systematically and in each visit they are assessed for presence of IBS-like symptoms) and 5 are case-control studies in which IBS-like symptoms prevalence in IBD patients is compared to IBS prevalence in non-IBD patients. Fourteen studies use the Rome criteria for IBS diagnosis (6 Rome Ⅱ, 6 Rome Ⅲ, one both and another Rome Ⅱ and Manning criteria), one uses Manning criteria only[1,53] and the last one uses a validated gastrointestinal symptom questionnaire[2].

The range of reported prevalences is quite wide (11%-64%), also in CD (12%-68%) and in UC (9%-60%) separately. Pooled prevalence is 30.9%, 38.1% in CD and 27.8% in UC (Table 1). These differences in prevalence were not attributable to type of diagnostic criteria used, as a meta-analysis by Halpin *et al*[54] that analyzed the studies published up to 2012 concludes. Possible alternative explanations are the small sample size of many of the studies, the variability in diagnostic criteria used to define IBD remission, the variability of exclusion criteria, and the absence of controls in most of them.

Table 1 Summary of studies that have determined prevalence of irritable bowel syndrome-like symptoms in quiescent inflammatory bowel disease

Ref.	Type	Sample size (CD/UC)	Criteria used to define IBD remission	Exclusions (IBD characteristics or previous surgery)	Criteria used to define IBS	IBD-IBS prevalence *n* (%)
Isgar *et al*[1]	Case-control	98 (0/98) 98 non-IBD controls	Endoscopic remission, steroid-free	Not further specified	Manning	UC: 33 (33.7)
Simrén *et al*[2]	Cross-sectional	83 (40/43)	CD: Physician global assessment, endoscopic/radiological remission and normal inflammatory markers (Hb, ESR, CRP, platelets, albumin) UC: Endoscopic remission, no blood nor mucus, normal CRP	Stenotic CD CD patients with > 2 surgeries Significant comorbidities	Gastrointestinal symptom questionnaire validated	37 (44.6) CD: 23 (57) UC: 14 (33)
Zaman *et al*[43]	Cross-sectional	55 (30/25)	Stable symptoms, no changes in medication for 3 mo	Not available	Rome Ⅱ	35 (63.6) CD: 20 (66.7) UC: 15 (60)
Minderhoud *et al*[44]	Case-control	107 (34/73) 66 non-IBD controls	CD: CDAI < 150 UC: CAIUC < 10	Significant comorbidities	Manning Rome Ⅱ	Manning: 33 (30.8) CD: 8 (23.5) UC: 25 (34.2) Rome Ⅱ: 37 (34.6) CD: 14 (41.7) UC: 23 (31.5)
Farrokhyar *et al*[45]	Cross-sectional	149 (105/44)	No changes/addition of medication nor change dosage in the last year	Not further specified	Rome Ⅱ	31 (20.8) CD: 27 (26) UC: 4 (9.1)
Ansari *et al*[46]	Case-control	50 (0/50) 100 non-IBD controls	Mayo score ≤ 2 (bleeding score = 0, endoscopic score 0-1)	Not further specified	Rome Ⅱ	UC: 23 (46)
Keohane *et al*[33]	Cross-sectional	106 (62/44)	CD: CDAI < 150 UC: UCAI ≤ 3 For both: physician's global assessment, CRP < 10 mg/L, no use of steroids or biological agents in previous 6 mo	Not further specified	Rome Ⅱ	54 (50.9) CD: 37 (59.7) UC: 17 (38.6)
Piche *et al*[48]	Cross-sectional	92 (92/0)	CDAI < 150 for > 6 mo, endoscopic/radiologic remission (CDEIS < 6), normal inflammatory markers (CRP, Hb, ESR, platelets, albumin)	Stenosis CD patients with previous surgery Recent corticoid use	Rome Ⅲ	CD: 42 (45.7)
Barratt *et al*[3]	Case-control	276 (110/166) 348 non-IBD controls	CD: HBI < 5 UC: SCCAI < 5	Not further specified	Rome Ⅱ	31 (11.2) CD: 14 (12) UC: 17 (9)
Bryant *et al*[4]	Cross-sectional	93 (47/43)[1]	Physician's global assessment using inflammatory markers, histological and endoscopic activity and clinical data	Not further specified	Rome Ⅲ	12 (12.9) (no CD/UC differentiation)
Jelsness-Jørgensen *et al*[49]	Cross-sectional	89 (28/61)	CD: SCDAI < 4 UC: SCCAI < 3 No current steroid treatment	Not further specified	Rome Ⅱ Rome Ⅲ	Rome Ⅱ: 21 (23.6) CD: 6 (21.4) UC: 15 (24.6) Rome Ⅲ: 30 (33.7) CD: 8 (28.6) UC: 22 (36.1)
Kim *et al*[50]	Cross-sectional	226 (107/119)	No changes on therapy in last year, normal limits of CRP, hemoglobin, no blood or mucus in stools for UC	CD with stenotic/penetrating phenotype Previous surgery	Rome Ⅲ	82 (36.3) CD: 50 (46.7) UC: 32 (26.9)
Berrill *et al*[5]	Cross-sectional	97 (40/57)	CD: HBI < 5, CRP < 10 mg/L UC: SCCAI < 3, CRP < 10 mg/L	Ileostomy, colostomy or total colectomy	Rome Ⅲ	31 (32) CD: 13 (32.5) UC: 18 (31.6)
Jonefjäll *et al*[35]	Pro-spective	94 (0/94)	Mayo ≤ 2 (endoscopic < 1) No relapse during 3 mo before inclusion	Significant comorbidities	Rome Ⅱ	UC: 25 (27)
Vivinus-Nébot *et al*[51]	Cross-sectional	49 (31/18)	CD: CDAI < 150,CDEIS ≤ 4 UC: UCAI ≤ 3, Mayo = 0 For both: physician's global assessment, CRP < 10 mg/L, no use of steroids over the last year	Stenotic or complicated CD Significant comorbidities	Rome Ⅲ	18 (36.7) CD: 11 (35.4) UC: 7 (38)
Fukuba *et al*[52]	Case control	172 (0/172) 330 non-IBD controls	CAI ≤ 4, CRP < 5 mg/L	Colectomy	Rome Ⅲ	UC: 46 (26.7)

Total	-	1836 (726/1107)[1]	-	-	-	Total: 567 (30.9) CD: 259 (38.1)[2] UC: 296 (27.8)[2]

[1]In the original article the numbers given by the authors do not add up to the total of patients and they do not specify if the rest correspond to undetermined colitis; [2]For the pooled prevalences in CD and UC, patients from study of Bryant et al have not been included as they do not state a differentiated IBS-IBD prevalence for CD and UC respectively. CD: Crohn's disease; UC: Ulcerative colitis; IBD: Inflammatory bowel disease; IBS: Irritable bowel disease; CRP: C reactive protein; Hb: Haemoglobin; ESR: Erythrocyte sedimentation rate; CDAI: Crohn's disease activity index; CAIUC: Clinical activity index for ulcerative colitis; UCAI: Ulcerative colitis activity index; CDEIS: Crohn's disease endoscopic index of severity; HBI: Harvey-Bradshaw index; SCCAI: Simple clinical colitis activity index; SCDAI: Simple Crohn's disease activity index; CAI: Colitis activity index.

Table 2 Irritable bowel syndrome Rome Ⅲ criteria

Recurrent abdominal pain or discomfort at least 3 d per month in the last 3 mo (with onset at least 6 mo prior to diagnosis) associated with two or more of the following
Relieved with defecation
Onset associated with a change in frequency of stools
Onset associated with a change in form (appearance) of stools

Adapted from Longstreth *et al*[40].

With respect to definitions of remission there are few coincidences between the different studies, ranging from simple clinical assessment[43,45] to a well-defined combination of IBD activity indexes and CRP quantification[5,33,48,51]. Strikingly only 7 studies include endoscopic evaluation to define remission[1,2,4,35,46,48,51]. Of those, some allow a low grade of inflammation[1,46] and only four use endoscopic indexes[35,46,48,51]. The prevalence variability seems greater in those studies without endoscopic criteria in remission definition (11.2%-63.6%) than in those with (12.9%-46%). In the five studies that included only patients with normal-appearing mucosa, variability persisted (range 12.9%-45.7%).

Regarding variability in exclusion criteria, it is interesting to focus on the six studies that exclude patients with previous abdominal surgery (mainly in CD cohorts) to reduce confusion and bias (see previous sections)[2,5,48,50-52]. In these studies, which include a total of 719 patients (310 CD, 409 UC), the prevalence range is narrower but still considerable and still greater in CD: range 32%-44.6% for all patients, 35.4%-57% in CD and 26.7%-38% in UC.

All five case-control studies reported a higher prevalence of IBS-like symptoms in IBD patients in remission than in non-IBD controls. The meta-analysis by Halpin *et al*[54] calculated for IBD as a whole an OR of 4.39 (95%CI: 2.24-8.61). In the study by Fukuba *et al*[52], not included in the meta-analysis, the numbers were similar for UC (OR = 7.17; 95%CI: 3.94-13.0).

Some studies have investigated if there are any variables associated with the occurrence of IBS-like symptoms. Although the conclusions are hampered by small sample size of many of them, these are the main results: (1) Gender: two studies report that women have a higher risk of suffering IBS-like symptoms while in remission, as happens in conventional IBS[4,5]; another study reports a higher risk in men[46]; (2) Age: leaving aside the study by Farrokhyar *et al*[45] that reported a higher risk for patients over 40 years of age (OR = 3.2) most studies have failed to show an association between age and risk of suffering IBS-like symptoms while in remission; (3) Extension and/or location of IBD: two studies analyzed this variable and did not find a significant association with the prevalence of functional symptoms[5,33]; (4) IBD duration: only analyzed in one study with a small sample size, no significant association was found[2]; and (5) Previous surgery: analyzed only in three studies, pooled prevalence of functional symptoms was higher in operated patients (60% *vs* 32.4%)[2,30,41].

IF THEY ARE IN REMISSION, WHY DO THEY HAVE SYMPTOMS?

Several pathogenic mechanisms can explain the occurrence of IBD-IBS beyond a merely random effect. Dysmotility, visceral hypersensitivity and increased mucosal permeability are the best studied ones.

Several studies have investigated why some IBD patients in remission remain asymptomatic while others experience ongoing symptoms of functional origin. We summarize the different explanations that have been considered.

It is just a random effect

IBS is one of the most frequent gastrointestinal diseases worldwide, with a prevalence that ranges from 10% to 15% of the general population[55-58]. A similar prevalence in quiescent IBD patients would be expected. However, as we have described in the previous section, prevalence of IBD-IBS is more than double[54]. It is therefore obvious that there must be further reasons other than merely chance that explain the occurrence of IBS symptoms in IBD.

Inflammation persists

Microscopic inflammation in normal-appearing mucosa could persist after resolution of an acute flare and justify persistence of symptoms in spite of mucosal healing[59,60].

In 2010 Keohane *et al*[33] analyzed 106 IBD patients in remission, all with normal CRP levels, and found significantly higher fecal calprotectin levels in patients

with functional symptoms than in asymptomatic patients (414.7 mg/kg *vs* 174.9 mg/kg in CD patients, 591.1 mg/kg *vs* 229.8 mg/kg in UC). They concluded against a real overlap between IBD and IBS, as persistence of symptoms would be provoked by inflammation persistence ("IBD is IBD unless proven otherwise"). It should be highlighted however, that they did not perform endoscopy systematically, so active inflammation could not be ruled out completely. Along the same lines, the work of Vivinus-Nébot *et al*[51] reported that symptomatic patients had higher levels of tumor necrotic factor alpha (TNF-α), as well as a trend towards a higher amount of intraepithelial lymphocytes and eosinophils. These studies imply that these patients could theoretically be candidates for IBD treatment escalation, a hypothesis neither proven nor tested to date.

Against this perspective several publications appeared in the following years reporting a considerable proportion of symptomatic patients with normal levels of fecal calprotectin[5,35,61], which means that there must be additional pathogenic factors and that treatment escalation in these patients should be considered very carefully, if at all.

IBD induces dysmotility

Several studies have detected similar colonic motility patterns in quiescent IBD than those described in IBS (higher number of low-amplitude propagated contractions)[62-64]. Subtle changes in antroduodenal motility in quiescent CD have also been described[65]. These alterations in intestinal motility could be related to autonomic nerve system dysfunction that has been described in IBD patients in remission[66-69].

IBD induces hypersensitivity

Several experiments with rectal balloon distension showed initially that IBD patients in remission had higher visceral pain thresholds (*i.e.*, tolerance to pain was higher) than IBS patients[70,71]. Cerebral activity induced by peripheral stimuli in quiescent CU patients resembled more that of healthy population than the one of IBS patients[72]. IBD patients would therefore have reduced visceral sensitivity, which could be interpreted as an adaptive response in the context of chronic-recurrent inflammation.

However, when visceral sensitivity in IBD patients in remission has been studied based on the presence or absence of functional symptoms, findings have been different. A study in pediatric IBD patients in remission with residual abdominal pain observed that they presented rectal pain thresholds lower than those of healthy volunteers and similar to those of patients with functional digestive diseases[73]. Further on, two studies measured in rectosigmoid junction biopsies the density of nerve fibers that presented the transient potential vanilloid receptor type 1 (TRPV1), implicated in nociception and in IBS visceral hypersensitivity[74,75]. They observed that in symptomatic IBD patients in remission the density was significantly higher than in healthy controls and in asymptomatic IBD patients. Additionally, the number of TRPV1 fibers was proportional to pain intensity referred by the patient. IBD-IBS patients would therefore have hypersensitivity induced by up-regulation of TRPV1, which could in turn be mediated by central generated stimuli. Mast cells, which participate in the generation of abdominal pain in IBS[76,77], have also been involved in pain generation in IBS-IBD[78]. At the molecular level, it has been described additionally an up-regulation of serotonin synthesis, a well-known mediator in visceral motility and sensitivity[79].

IBD induces mucosal permeability increment

In IBS, intestinal permeability is constantly increased and is proportional to symptom intensity[51,80]. This phenomenon would increase mucosal exposure to endoluminal antigens that could in turn induce inflammatory, sensitive or motor responses responsible for patients' symptoms. In IBD in remission similar findings have been reported: Permeability is higher than that of healthy volunteers and is even higher in symptomatic patients[51,81]. The permeability increment has been also related to the risk of suffering a relapse[82,83].

Microbiota disturbance plays a role

Composition of the gut flora is disturbed in both IBS and IBD, although it is not really established whether it is a cause or a consequence of the physiological alterations that characterize such entities or of the treatments patients receive[84-86]. No studies have explored flora composition in IBD-IBS patients or compared it with asymptomatic IBD patients in remission.

Similarities with post-infectious IBS

After acute gastroenteritis odds of developing IBS are increased 6-7 fold[87,88]. This post-infectious IBS (PI-IBS) is conceptually very similar to IBS in quiescent IBD, as both would be caused by post-inflammatory mechanisms. Although dysmotility and hypersensitivity do not seem to explain the development of symptoms after the infection[89], mast cells and increased intestinal permeability have been involved as in IBD-IBS[7,90].

Psychological stress: Pivotal role as well?

Specific role of psychological distress in pathogenesis of IBS symptoms in quiescent IBD remains to our knowledge uninvestigated. Evidence from its implication in IBS pathogenesis (both PI-IBS and conventional IBS)[89,91] and in IBD flare induction[92] makes it highly possible, in our opinion, that an important link exists with IBS-IBD.

Table 3 Studies that explore quality of life and anxiety in inflammatory bowel disease patients in remission with irritable bowel syndrome-like symptoms

Ref.	Sample size (CD/UC)	Questionnaires used	Results
Simrén *et al*[2]	83 (40/43) 37 IBD-IBS (23/14)	GSRS HADS STAI PGWB	Higher anxiety and depression scores in IBD-IBS (worst in CD)
Minderhoud *et al*[44]	107 (34/73) 37 IBD-IBS (14/23)	IBDQ	Lower QoL scores in IBD-IBS
Farrokhyar *et al*[45]	149 (105/44) 31 IBD-IBS (27/4)	sIBDQ EQ-5D	Occurence of any FGID taken in count (not only IBS-like) Lower QoL scores in CD patients with FGID Difference not significant for UC
Ansari *et al*[46]	50 (0/50) IBD-IBS: 23 (0/23)	SF-36	Lower QoL scores in IBD-IBS than in asymptomatic and similar to patients in flare
Keohane *et al*[33]	106 (62/44) 54 IBD-IBS (37/17)	IBSQ HADS	QoL scores only significantly lower in UC-IBS Levels of anxiety and depression only significantly higher in UC-IBS
Piche *et al*[48]	92 (92/0) 42 IBD-IBS (42/0)	French-validated IBS severity scoring system Likert scales FIS Short BDI HADS	Higher severity, impact, depression and fatigue scores in CD-IBS No significant differences in anxiety level
Barratt *et al*[3]	276 (110/166) 31 IBD-IBS (14/17)	SF-36 HADS	No differentiation between IBD patients in remission or in active phase Lower QoL scores and higher anxiety, depression scores in IBD-IBS
Bryant *et al*[4]	93 (47/43) 12 IBD-IBS (no CD/UC differentiation)	sIBDQ HADS BDQ-6	Occurrence of any FGID taken in count (not only IBS-like) Lower QoL scores and higher anxiety and depression scores in IBD patients with any FGID Lowest scores in IBS-like symptoms
Jelsness-Jørgensen *et al*[49]	89(28/61) 30 IBD-IBS (8/22)	FQ RFIPC	More fatigue scores in IBD-IBS (worst in UC) More concerns in UC-IBD (difference non significant for CD)
Kim *et al*[50]	226 (107/119) 82 IBD-IBS (50/32)	EQ-5D HADS	Occurrence of any FGID taken in count (not only IBS-like) Lower QoL scores and higher anxiety and depression scores in UC-IBS (difference non-significant for CD)
Berrill *et al*[5]	97 (40/57) 31 IBD-IBS (13/18)	HADS	No differentiation between IBD patients in remission or in active phase Higher anxiety and depression scores in IBD-IBS
Jonefjäll *et al*[35]	94 (0/94) 25 IBD-IBS (0/25)	HADS SF-36	Lower QoL scores and higher anxiety scores in UC-IBS (difference in depression score non-significant)
Vivinus-Nébot *et al*[51]	49 (31/18) 18 IBD-IBS (11/7)	French-validated IBS severity scoring system	Higher severity and impact scores in IBD-IBS

IBD: Inflammatory bowel disease; UC: Ulcerative colitis; CD: Crohn's disease; GSRS: Gastrointestinal symptom rating scale; HADS: Hospital anxiety and depression scale; STAI: Spielberger state trait anxiety inventory; PGWB: Psychological general well-being index; IBSQ: Inflammatory bowel disease questionnaire; QoL: Quality of life; sIBDQ: Inflammatory bowel disease questionnaire; EQ-5D: EuroQOL-5D; FGID: Functional gastrointestinal disorders; FIS: Fatigue impact scale; BDI: Becks depression inventory; BDQ-6: Bowel disease questionnaire-6; FQ: Fatigue questionnaire; RFIPC: Rating form of inflammatory bowel disease patients concerns.

I HAVE ACHIEVED REMISSION IN MY PATIENT. WHY SHOULD I CONTINUE TO BE CONCERNED?

In spite of being in remission, IBD-IBS patients have an impaired quality of life, and deserve attention and care.

IBD patients in general have a worse quality of life (QoL) than controls, mostly related to disease activity[93-95]. Even though quiescent IBD patients with IBS-like symptoms are not at risk of suffering direct complications of IBD such as perforations, abscesses or toxic megacolon, their symptoms can be very intense and significantly reduce their QoL, maybe as much as an actual flare does. In Table 3 we summarize all studies that have analyzed the effect of IBS-like symptom occurrence in different QoL scores and in anxiety and depression scales[2-5,33,35,44-46,48-51]. Although they are quite heterogeneous, all of them consistently show that IBD patients in remission who have IBS-like symptoms have a worse QoL and a greater probability of suffering from depression or anxiety than those who remain asymptomatic.

HOW DO WE TREAT FUNCTIONAL SYMPTOMS IN IBD PATIENTS IN REMISSION?

Evidence in IBD-IBS treatment is very poor to date. It is reasonable to apply usual IBS treatment strategies (diet modification, antispasmodics, antidepressants, probiotics, *etc.*) in the meantime.

The literature addressing this question is very scarce and of little quality, mostly based on experts'

recommendations[8,38,96,97]. It seems very reasonable to follow a step-up approach to avoid over-medication in patients who very probably are already on immunosuppressive maintenance drugs.

Diet interventions

Some authors recommend elaborating a diary to detect foods and beverages that provoke symptoms and eliminate them whenever feasible[97]. This is not easy as noxious effects can be accumulative (*i.e.,* symptoms may appear after eating them for several days and not immediately) and dose-dependent (*i.e.,* small amounts could be well tolerated)[96]. IBS literature is rich in food lists that have been involved in symptom triggering, and most include lactose, caffeine, fat-rich foods, deep fried foods, chewing gums, alcohol and sorbitol[98,99]. In recent years efforts are being made in order to establish a more systematic and more evidence-based approach to diet management in IBS. Diets with low quantities of fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs) reduce symptoms in well-designed clinical trials[100]. This strategy could be useful in IBD-IBS patients, but remains unproven[101].

Fiber supplementation

Hallert *et al*[102] carried out a small, placebo-controlled trial of ispaghula husk in ulcerative colitis patients in endoscopic remission who complained of several gastrointestinal symptoms. Although not defined by the authors as IBS, the symptoms they describe were those typically present in it (pain, diarrhea, urgency, constipation, bloating, *etc.*). Ispaghula was more effective than placebo in improving those symptoms so fiber supplementation could be beneficial. In contrast, other authors recommend fiber reduction based on the fact that fiber, especially the insoluble subtype, can exacerbate symptoms in IBS (mainly bloating and flatulence)[103-105].

Antispasmodics and antidiarrheals

Antispasmodics as a group are useful in IBS management and have a favorable safety profile[105,106]. They could be used in quiescent IBD patients with IBS-like symptoms, although no specific trials have been published to explore their efficacy. The theoretical risk exists of inducing toxic megacolon if IBD is active[103,107].

Loperamide has no clearly proven efficacy in treating IBS but can nevertheless be useful to manage diarrhea[105,106]. It has to be used with caution if at all during a flare, as it could induce a toxic megacolon[108,109]. A placebo-controlled trial in CD patients with chronic diarrhea showed benefits of loperamide in alleviating symptoms, so its judicious use could offer satisfactory relief in patients in remission with ongoing diarrhea[103,110].

Antidepressants

Antidepressants as a group, both tricyclic antidepressants (TCA) and selective serotonin reuptake inhibitors (SSRI), are effective in providing global symptom relief in IBS[105,106]. Besides their neuromodulatory action they could exert an anti-inflammatory effect, as shown in animal models[111]. Most publications[112] referring to use of antidepressants in IBD are case reports that globally suggest a beneficial effect of these drugs not only in psychological symptoms but also in somatic ones. A more recent retrospective study has shown that IBD patients with concurrent depression who were under high dose antidepressant regimens had fewer disease flares and had less need of steroids[113]. Iskandar *et al*[114] have published the only original work to date that addresses specifically the potential utility of antidepressants in IBD-IBS. In their retrospective cohort study they compared the efficacy of low dose TCAs in 81 IBD patients in remission or with mild disease who complained of ongoing gastrointestinal symptoms, with that registered in a cohort of IBS patients. At least moderate improvement was achieved in 59.3% of IBD patients (higher in UC patients), similar to the proportion obtained in IBS. Data on other antidepressants such as SSRI in IBD are lacking.

Any other options?

No other treatments have been explored in IBD-IBS, but there are several fields very interesting for future research. Involvement of gut flora in both IBS and IBD (see above), efficacy of non-absorbable antibiotic rifaximin and of probiotics in IBS[105,106], and promising results of probiotics in UC[115] suggest that intestinal microbiome modulation could be useful in IBD-IBS treatment. Hypersensitivity and central nervous system involvement make psychological therapies, also tested in IBS[105,106], an additional interesting option to be evaluated in the future.

CONCLUSION

IBD patients in remission suffer quite frequently from symptoms that resemble IBS. So-called IBD-IBS is probably secondary to several factors, including post-inflammatory dysmotility and hypersensitivity, mast cell activation and increased epithelial permeability. Even though these patients are in remission their quality of life can be as low as during an acute flare, so an effort should be undertaken to recognize and treat this condition as satisfactorily as possible. Evidence on best management options of these patients is almost nonexistent. It seems reasonable to use the same drugs that have proven efficacy in IBS, such as antispasmodics and antidepressants, while we wait for future prospective trials.

Concurrence of IBS and IBD, existence of post-

infectious IBS and the common pathogenic mechanisms between both entities, teach us that the classic functional-organic dichotomy is very probably obsolete. The old schema would be substituted by a unifying biopsychosocial model according to which we should not limit our therapeutic efforts to resolving inflammation but rather extend our scope to treatment of any symptoms patients report that impair their QoL and psychological well-being.

ACKNOWLEDGMENTS

We would like to thank Dr. Antonio López San Román for his valuable advice and recommendations in the drafting of the manuscript.

REFERENCES

1 **Isgar B**, Harman M, Kaye MD, Whorwell PJ. Symptoms of irritable bowel syndrome in ulcerative colitis in remission. *Gut* 1983; **24**: 190-192 [PMID: 6826101 DOI: 10.1136/gut.24.3.190]
2 **Simrén M**, Axelsson J, Gillberg R, Abrahamsson H, Svedlund J, Björnsson ES. Quality of life in inflammatory bowel disease in remission: the impact of IBS-like symptoms and associated psychological factors. *Am J Gastroenterol* 2002; **97**: 389-396 [PMID: 11866278 DOI: 10.1016/S0002-9270(01)04037-0]
3 **Barratt SM**, Leeds JS, Robinson K, Shah PJ, Lobo AJ, McAlindon ME, Sanders DS. Reflux and irritable bowel syndrome are negative predictors of quality of life in coeliac disease and inflammatory bowel disease. *Eur J Gastroenterol Hepatol* 2011; **23**: 159-165 [PMID: 21178777 DOI: 10.1097/MEG.0b013e328342a547]
4 **Bryant RV**, van Langenberg DR, Holtmann GJ, Andrews JM. Functional gastrointestinal disorders in inflammatory bowel disease: impact on quality of life and psychological status. *J Gastroenterol Hepatol* 2011; **26**: 916-923 [PMID: 21214889 DOI: 10.1111/j.1440-1746.2011.06624.x]
5 **Berrill JW**, Green JT, Hood K, Campbell AK. Symptoms of irritable bowel syndrome in patients with inflammatory bowel disease: examining the role of sub-clinical inflammation and the impact on clinical assessment of disease activity. *Aliment Pharmacol Ther* 2013; **38**: 44-51 [PMID: 23668698 DOI: 10.1111/apt.12335]
6 **Bercik P**, Verdu EF, Collins SM. Is irritable bowel syndrome a low-grade inflammatory bowel disease? *Gastroenterol Clin North Am* 2005; **34**: 235-245, vi-vii [PMID: 15862932 DOI: 10.1016/j.gtc.2005.02.007]
7 **Grover M**, Herfarth H, Drossman DA. The functional-organic dichotomy: postinfectious irritable bowel syndrome and inflammatory bowel disease-irritable bowel syndrome. *Clin Gastroenterol Hepatol* 2009; **7**: 48-53 [PMID: 18848909 DOI: 10.1016/j.cgh.2008.08.032]
8 **Long MD**, Drossman DA. Inflammatory bowel disease, irritable bowel syndrome, or what?: A challenge to the functional-organic dichotomy. *Am J Gastroenterol* 2010; **105**: 1796-1798 [PMID: 20686466 DOI: 10.1038/ajg.2010.162]
9 **Sandborn WJ**, Feagan BG, Hanauer SB, Lochs H, Löfberg R, Modigliani R, Present DH, Rutgeerts P, Schölmerich J, Stange EF, Sutherland LR. A review of activity indices and efficacy endpoints for clinical trials of medical therapy in adults with Crohn's disease. *Gastroenterology* 2002; **122**: 512-530 [PMID: 11832465 DOI: 10.1053/gast.2002.31072]
10 **Lahiff C**, Safaie P, Awais A, Akbari M, Gashin L, Sheth S, Lembo A, Leffler D, Moss AC, Cheifetz AS. The Crohn's disease activity index (CDAI) is similarly elevated in patients with Crohn's disease and in patients with irritable bowel syndrome. *Aliment Pharmacol Ther* 2013; **37**: 786-794 [PMID: 23432394 DOI: 10.1111/apt.12262]
11 **Papay P**, Ignjatovic A, Karmiris K, Amarante H, Milheller P, Feagan B, D'Haens G, Marteau P, Reinisch W, Sturm A, Steinwurz F, Egan L, Panés J, Louis E, Colombel JF, Panaccione R. Optimising monitoring in the management of Crohn's disease: a physician's perspective. *J Crohns Colitis* 2013; **7**: 653-669 [PMID: 23562672 DOI: 10.1016/j.crohns.2013.02.005]
12 **Bouguen G**, Levesque BG, Feagan BG, Kavanaugh A, Peyrin-Biroulet L, Colombel JF, Hanauer SB, Sandborn WJ. Treat to target: a proposed new paradigm for the management of Crohn's disease. *Clin Gastroenterol Hepatol* 2015; **13**: 1042-1050.e2 [PMID: 24036054 DOI: 10.1016/j.cgh.2013.09.006]
13 **Bruining DH**, Sandborn WJ. Do not assume symptoms indicate failure of anti-tumor necrosis factor therapy in Crohn's disease. *Clin Gastroenterol Hepatol* 2011; **9**: 395-399 [PMID: 21277392 DOI: 10.1016/j.cgh.2011.01.019]
14 **Chen WC**, Quigley EM. Commentary: irritable bowel syndrome and the CDAI--misleading activity by straw men. *Aliment Pharmacol Ther* 2013; **37**: 1020-1021 [PMID: 23590538 DOI: 10.1111/apt.12287]
15 **D'Incà R**, Caccaro R. Measuring disease activity in Crohn's disease: what is currently available to the clinician. *Clin Exp Gastroenterol* 2014; **7**: 151-161 [PMID: 24876789 DOI: 10.2147/CEG.S41413]
16 **Tielbeek JA**, Makanyanga JC, Bipat S, Pendsé DA, Nio CY, Vos FM, Taylor SA, Stoker J. Grading Crohn disease activity with MRI: interobserver variability of MRI features, MRI scoring of severity, and correlation with Crohn disease endoscopic index of severity. *AJR Am J Roentgenol* 2013; **201**: 1220-1228 [PMID: 24261360 DOI: 10.2214/AJR.12.10341]
17 **Kiss LS**, Szamosi T, Molnar T, Miheller P, Lakatos L, Vincze A, Palatka K, Barta Z, Gasztonyi B, Salamon A, Horvath G, Tóth GT, Farkas K, Banai J, Tulassay Z, Nagy F, Szenes M, Veres G, Lovasz BD, Vegh Z, Golovics PA, Szathmari M, Papp M, Lakatos PL. Early clinical remission and normalisation of CRP are the strongest predictors of efficacy, mucosal healing and dose escalation during the first year of adalimumab therapy in Crohn's disease. *Aliment Pharmacol Ther* 2011; **34**: 911-922 [PMID: 21883326 DOI: 10.1111/j.1365-2036.2011.04827]
18 **Jürgens M**, Mahachie John JM, Cleynen I, Schnitzler F, Fidder H, van Moerkercke W, Ballet V, Noman M, Hoffman I, van Assche G, Rutgeerts PJ, van Steen K, Vermeire S. Levels of C-reactive protein are associated with response to infliximab therapy in patients with Crohn's disease. *Clin Gastroenterol Hepatol* 2011; **9**: 421-427.e1 [PMID: 21334460 DOI: 10.1016/j.cgh.2011.02.008]
19 **Henriksen M**, Jahnsen J, Lygren I, Stray N, Sauar J, Vatn MH, Moum B. C-reactive protein: a predictive factor and marker of inflammation in inflammatory bowel disease. Results from a prospective population-based study. *Gut* 2008; **57**: 1518-1523 [PMID: 18566104 DOI: 10.1136/gut.2007]
20 **Denis MA**, Reenaers C, Fontaine F, Belaïche J, Louis E. Assessment of endoscopic activity index and biological inflammatory markers in clinically active Crohn's disease with normal C-reactive protein serum level. *Inflamm Bowel Dis* 2007; **13**: 1100-1105 [PMID: 17508418 DOI: 10.1002/ibd.20178]
21 **Däbritz J**, Musci J, Foell D. Diagnostic utility of faecal biomarkers in patients with irritable bowel syndrome. *World J Gastroenterol* 2014; **20**: 363-375 [PMID: 24574706 DOI: 10.3748/wjg.v20.i2.363]
22 **Waugh N**, Cummins E, Royle P, Kandala NB, Shyangdan D, Arasaradnam R, Clar C, Johnston R. Faecal calprotectin testing for differentiating amongst inflammatory and non-inflammatory bowel diseases: systematic review and economic evaluation. *Health Technol Assess* 2013; **17**: xv-xix, 1-211 [PMID: 24286461 DOI: 10.3310/hta17550]
23 **Sipponen T**, Savilahti E, Kolho KL, Nuutinen H, Turunen U, Färkkilä M. Crohn's disease activity assessed by fecal calprotectin and lactoferrin: correlation with Crohn's disease activity index and endoscopic findings. *Inflamm Bowel Dis* 2008; **14**: 40-46 [PMID: 18022866]

24 **Langhorst J**, Elsenbruch S, Koelzer J, Rueffer A, Michalsen A, Dobos GJ. Noninvasive markers in the assessment of intestinal inflammation in inflammatory bowel diseases: performance of fecal lactoferrin, calprotectin, and PMN-elastase, CRP, and clinical indices. *Am J Gastroenterol* 2008; **103**: 162-169 [PMID: 17916108 DOI: 10.1111/j.1572-0241.2007.01556.x]

25 **Schoepfer AM**, Beglinger C, Straumann A, Trummler M, Vavricka SR, Bruegger LE, Seibold F. Fecal calprotectin correlates more closely with the Simple Endoscopic Score for Crohn's disease (SES-CD) than CRP, blood leukocytes, and the CDAI. *Am J Gastroenterol* 2010; **105**: 162-169 [PMID: 19755969 DOI: 10.1038/ajg.2009.545]

26 **Røseth AG**, Aadland E, Jahnsen J, Raknerud N. Assessment of disease activity in ulcerative colitis by faecal calprotectin, a novel granulocyte marker protein. *Digestion* 1997; **58**: 176-180 [PMID: 9144308 DOI: 10.1159/000201441]

27 **D'Haens G**, Ferrante M, Vermeire S, Baert F, Noman M, Moortgat L, Geens P, Iwens D, Aerden I, Van Assche G, Van Olmen G, Rutgeerts P. Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. *Inflamm Bowel Dis* 2012; **18**: 2218-2224 [PMID: 22344983 DOI: 10.1002/ibd.22917]

28 **Mao R**, Xiao YL, Gao X, Chen BL, He Y, Yang L, Hu PJ, Chen MH. Fecal calprotectin in predicting relapse of inflammatory bowel diseases: a meta-analysis of prospective studies. *Inflamm Bowel Dis* 2012; **18**: 1894-1899 [PMID: 22238138 DOI: 10.1002/ibd.22861]

29 **Sands BE**, Ooi CJ. A survey of methodological variation in the Crohn's disease activity index. *Inflamm Bowel Dis* 2005; **11**: 133-138 [PMID: 15677906 DOI: 10.1097/00054725-200502000-00006]

30 **Sipponen T**, Savilahti E, Kärkkäinen P, Kolho KL, Nuutinen H, Turunen U, Färkkilä M. Fecal calprotectin, lactoferrin, and endoscopic disease activity in monitoring anti-TNF-alpha therapy for Crohn's disease. *Inflamm Bowel Dis* 2008; **14**: 1392-1398 [PMID: 18484671 DOI: 10.1002/ibd.20490]

31 **Sipponen T**, Björkesten CG, Färkkilä M, Nuutinen H, Savilahti E, Kolho KL. Faecal calprotectin and lactoferrin are reliable surrogate markers of endoscopic response during Crohn's disease treatment. *Scand J Gastroenterol* 2010; **45**: 325-331 [PMID: 20034360 DOI: 10.3109/00365520903483650]

32 **Lin JF**, Chen JM, Zuo JH, Yu A, Xiao ZJ, Deng FH, Nie B, Jiang B. Meta-analysis: fecal calprotectin for assessment of inflammatory bowel disease activity. *Inflamm Bowel Dis* 2014; **20**: 1407-1415 [PMID: 24983982 DOI: 10.1097/MIB.0000000000000057]

33 **Keohane J**, O'Mahony C, O'Mahony L, O'Mahony S, Quigley EM, Shanahan F. Irritable bowel syndrome-type symptoms in patients with inflammatory bowel disease: a real association or reflection of occult inflammation? *Am J Gastroenterol* 2010; **105**: 1788, 1789-1794; quiz 1795 [PMID: 20389294 DOI: 10.1038/ajg.2010.156]

34 **Jelsness-Jørgensen LP**, Bernklev T, Moum B. Calprotectin Is a Useful Tool in Distinguishing Coexisting Irritable Bowel-Like Symptoms from That of Occult Inflammation among Inflammatory Bowel Disease Patients in Remission. *Gastroenterol Res Pract* 2013; **2013**: 620707 [PMID: 23476638 DOI: 10.1155/2013/620707]

35 **Jonefjäll B**, Strid H, Ohman L, Svedlund J, Bergstedt A, Simren M. Characterization of IBS-like symptoms in patients with ulcerative colitis in clinical remission. *Neurogastroenterol Motil* 2013; **25**: 756-e578 [PMID: 23731196 DOI: 10.1111/nmo.12163]

36 **Castiglione F**, Rispo A, Di Girolamo E, Cozzolino A, Manguso F, Grassia R, Mazzacca G. Antibiotic treatment of small bowel bacterial overgrowth in patients with Crohn's disease. *Aliment Pharmacol Ther* 2003; **18**: 1107-1112 [PMID: 14653830 DOI: 10.1046/j.1365-2036.2003.01800.x]

37 **Lenicek M**, Duricova D, Komarek V, Gabrysova B, Lukas M, Smerhovsky Z, Vitek L. Bile acid malabsorption in inflammatory bowel disease: assessment by serum markers. *Inflamm Bowel Dis* 2011; **17**: 1322-1327 [PMID: 21058331 DOI: 10.1002/ibd.21502]

38 **Camilleri M**. Managing symptoms of irritable bowel syndrome in patients with inflammatory bowel disease. *Gut* 2011; **60**: 425-428 [PMID: 21292684 DOI: 10.1136/gut.2010.234583]

39 **Thompson WG**, Longstreth GF, Drossman DA, Heaton KW, Irvine EJ, Müller-Lissner SA. Functional bowel disorders and functional abdominal pain. *Gut* 1999; **45** Suppl 2: II43-II47 [PMID: 10457044 DOI: 10.1136/gut.45.2008.ii43]

40 **Longstreth GF**, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC. Functional bowel disorders. *Gastroenterology* 2006; **130**: 1480-1491 [PMID: 16678561 DOI: 10.1053/j.gastro.2005.11.061]

41 **Mearin F**. [Irritable bowel syndrome: new Rome III criteria]. *Med Clin* (Barc) 2007; **128**: 335-343 [PMID: 17376361 DOI: 10.1157/13099805]

42 **Barratt HS**, Kalantzis C, Polymeros D, Forbes A. Functional symptoms in inflammatory bowel disease and their potential influence in misclassification of clinical status. *Aliment Pharmacol Ther* 2005; **21**: 141-147 [PMID: 15679763 DOI: 10.1111/j.1365-2036.2005.02314.x]

43 **Zaman MS**, Robson KM, Lembo AJ. Overlap of irritable bowel syndrome (IBS) symptoms in patients with inflammatory bowel disease (IBD). *Am J Gastroenterol* 2002; **97** Suppl: S284 [DOI: 10.1016/S0002-9270(02)05348-0]

44 **Minderhoud IM**, Oldenburg B, Wismeijer JA, van Berge Henegouwen GP, Smout AJ. IBS-like symptoms in patients with inflammatory bowel disease in remission; relationships with quality of life and coping behavior. *Dig Dis Sci* 2004; **49**: 469-474 [PMID: 15139501 DOI: 10.1023/B:DDAS.0000020506.84248.f9]

45 **Farrokhyar F**, Marshall JK, Easterbrook B, Irvine EJ. Functional gastrointestinal disorders and mood disorders in patients with inactive inflammatory bowel disease: prevalence and impact on health. *Inflamm Bowel Dis* 2006; **12**: 38-46 [PMID: 16374257 DOI: 10.1097/01.MIB.0000195391.49762.89]

46 **Ansari R**, Attari F, Razjouyan H, Etemadi A, Amjadi H, Merat S, Malekzadeh R. Ulcerative colitis and irritable bowel syndrome: relationships with quality of life. *Eur J Gastroenterol Hepatol* 2008; **20**: 46-50 [PMID: 18090990 DOI: 10.1097/MEG.0b013e3282f16a62]

47 **Mikocka-Walus AA**, Turnbull DA, Andrews JM, Moulding NT, Holtmann GJ. The effect of functional gastrointestinal disorders on psychological comorbidity and quality of life in patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2008; **28**: 475-483 [PMID: 18532989 DOI: 10.1111/j.1365-2036.2008.03754.x]

48 **Piche T**, Ducrotté P, Sabate JM, Coffin B, Zerbib F, Dapoigny M, Hua M, Marine-Barjoan E, Dainese R, Hébuterne X. Impact of functional bowel symptoms on quality of life and fatigue in quiescent Crohn disease and irritable bowel syndrome. *Neurogastroenterol Motil* 2010; **22**: 626-e174 [PMID: 20403099 DOI: 10.1111/j.1365-2982.2010.01502.x]

49 **Jelsness-Jørgensen LP**, Bernklev T, Moum B. Fatigue and disease-related worries among inflammatory bowel disease patients in remission; is it a reflection of coexisting IBS-like symptoms? A short report. *J Psychosom Res* 2012; **73**: 469-472 [PMID: 23148817 DOI: 10.1016/j.jpsychores.2012.08.009]

50 **Kim ES**, Cho KB, Park KS, Jang BI, Kim KO, Jeon SW, Jung MK, Kim EY, Yang CH. Predictive factors of impaired quality of life in Korean patients with inactive inflammatory bowel disease: association with functional gastrointestinal disorders and mood disorders. *J Clin Gastroenterol* 2013; **47**: e38-e44 [PMID: 23090047 DOI: 10.1097/MCG.0b013e318266fff5]

51 **Vivinus-Nébot M**, Frin-Mathy G, Bzioueche H, Dainese R, Bernard G, Anty R, Filippi J, Saint-Paul MC, Tulic MK, Verhasselt V, Hébuterne X, Piche T. Functional bowel symptoms in quiescent inflammatory bowel diseases: role of epithelial barrier disruption and low-grade inflammation. *Gut* 2014; **63**: 744-752 [PMID: 23878165 DOI: 10.1136/gutjnl-2012-304066]

52 **Fukuba N**, Ishihara S, Tada Y, Oshima N, Moriyama I, Yuki T, Kawashima K, Kushiyama Y, Fujishiro H, Kinoshita Y. Prevalence

of irritable bowel syndrome-like symptoms in ulcerative colitis patients with clinical and endoscopic evidence of remission: prospective multicenter study. *Scand J Gastroenterol* 2014; **49**: 674-680 [PMID: 24646420 DOI: 10.3109/00365521.2014.898084]

53 **Manning AP**, Thompson WG, Heaton KW, Morris AF. Towards positive diagnosis of the irritable bowel. *Br Med J* 1978; **2**: 653-654 [PMID: 698649 DOI: 10.1136/bmj.2.6138.653]

54 **Halpin SJ**, Ford AC. Prevalence of symptoms meeting criteria for irritable bowel syndrome in inflammatory bowel disease: systematic review and meta-analysis. *Am J Gastroenterol* 2012; **107**: 1474-1482 [PMID: 22929759 DOI: 10.1038/ajg.2012.260]

55 **Drossman DA**, Camilleri M, Mayer EA, Whitehead WE. AGA technical review on irritable bowel syndrome. *Gastroenterology* 2002; **123**: 2108-2131 [PMID: 12454866 DOI: 10.1053/gast.2002.37095]

56 **Hungin AP**, Whorwell PJ, Tack J, Mearin F. The prevalence, patterns and impact of irritable bowel syndrome: an international survey of 40,000 subjects. *Aliment Pharmacol Ther* 2003; **17**: 643-650 [PMID: 12641512 DOI: 10.1046/j.1365-2036.2003.01456.x]

57 **Hungin AP**, Chang L, Locke GR, Dennis EH, Barghout V. Irritable bowel syndrome in the United States: prevalence, symptom patterns and impact. *Aliment Pharmacol Ther* 2005; **21**: 1365-1375 [PMID: 15932367 DOI: 10.1111/j.1365-2036.2005.02463.x]

58 **Lovell RM**, Ford AC. Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. *Clin Gastroenterol Hepatol* 2012; **10**: 712-721.e4 [PMID: 22426087 DOI: 10.1016/j.cgh.2012.02.029]

59 **Kristjánsson G**, Venge P, Wanders A, Lööf L, Hällgren R. Clinical and subclinical intestinal inflammation assessed by the mucosal patch technique: studies of mucosal neutrophil and eosinophil activation in inflammatory bowel diseases and irritable bowel syndrome. *Gut* 2004; **53**: 1806-1812 [PMID: 15542519 DOI: 10.1136/gut.2003.036418]

60 **Ahn JY**, Lee KH, Choi CH, Kim JW, Lee HW, Kim JW, Kim MK, Kwon GY, Han S, Kim SE, Kim SM, Chang SK. Colonic mucosal immune activity in irritable bowel syndrome: comparison with healthy controls and patients with ulcerative colitis. *Dig Dis Sci* 2014; **59**: 1001-1011 [PMID: 24282051 DOI: 10.1007/s10620-013-2930-4]

61 **Keszthelyi D**, Jonkers DM, Hamer HM, Masclee AA. Letter: the role of sub-clinical inflammation and TRPV1 in the development of IBS-like symptoms in ulcerative colitis in remission. *Aliment Pharmacol Ther* 2013; **38**: 560-561 [PMID: 23937469 DOI: 10.1111/apt.12409]

62 **Annese V**, Bassotti G, Napolitano G, Usai P, Andriulli A, Vantrappen G. Gastrointestinal motility disorders in patients with inactive Crohn's disease. *Scand J Gastroenterol* 1997; **32**: 1107-1117 [PMID: 9399391 DOI: 10.3109/00365529709002989]

63 **Bassotti G**, de Roberto G, Chistolini F, Sietchiping-Nzepa F, Morelli O, Morelli A. Twenty-four-hour manometric study of colonic propulsive activity in patients with diarrhea due to inflammatory (ulcerative colitis) and non-inflammatory (irritable bowel syndrome) conditions. *Int J Colorectal Dis* 2004; **19**: 493-497 [PMID: 15083326 DOI: 10.1007/s00384-004-0604-6]

64 **Bassotti G**, Villanacci V, Mazzocchi A, Castellani D, Giuliano V, Corsi S, Morelli A. Colonic propulsive and postprandial motor activity in patients with ulcerative colitis in remission. *Eur J Gastroenterol Hepatol* 2006; **18**: 507-510 [PMID: 16607145 DOI: 10.1097/00042737-200605000-00008]

65 **Minderhoud IM**, Smout AJ, Oldenburg B, Samsom M. A pilot study on chemospecific duodenal visceral sensitivity in inflammatory bowel disease in remission. *Digestion* 2006; **73**: 151-159 [PMID: 16837799 DOI: 10.1159/000094522]

66 **Lindgren S**, Stewenius J, Sjölund K, Lilja B, Sundkvist G. Autonomic vagal nerve dysfunction in patients with ulcerative colitis. *Scand J Gastroenterol* 1993; **28**: 638-642 [PMID: 8362220 DOI: 10.3109/00365529309096103]

67 **Coruzzi P**, Castiglioni P, Parati G, Brambilla V, Brambilla L, Gualerzi M, Cademartiri F, Franzè A, De Angelis G, Di Rienzo M, Di Mario F. Autonomic cardiovascular regulation in quiescent ulcerative colitis and Crohn's disease. *Eur J Clin Invest* 2007; **37**: 964-970 [PMID: 18036030 DOI: 10.1111/j.1365-2362.2007.01887.x]

68 **Sharma P**, Makharia GK, Ahuja V, Dwivedi SN, Deepak KK. Autonomic dysfunctions in patients with inflammatory bowel disease in clinical remission. *Dig Dis Sci* 2009; **54**: 853-861 [PMID: 18712478 DOI: 10.1007/s10620-008-0424-6]

69 **Ananthakrishnan AN**, Issa M, Barboi A, Jaradeh S, Zadvornova Y, Skaros S, Johnson K, Otterson MF, Binion DG. Impact of autonomic dysfunction on inflammatory bowel disease. *J Clin Gastroenterol* 2010; **44**: 272-279 [PMID: 19727003 DOI: 10.1097/MCG.0b013e3181b2682a]

70 **Bernstein CN**, Niazi N, Robert M, Mertz H, Kodner A, Munakata J, Naliboff B, Mayer EA. Rectal afferent function in patients with inflammatory and functional intestinal disorders. *Pain* 1996; **66**: 151-161 [PMID: 8880836 DOI: 10.1016/0304-3959(96)03062-X]

71 **Chang L**, Munakata J, Mayer EA, Schmulson MJ, Johnson TD, Bernstein CN, Saba L, Naliboff B, Anton PA, Matin K. Perceptual responses in patients with inflammatory and functional bowel disease. *Gut* 2000; **47**: 497-505 [PMID: 10986209 DOI: 10.1136/gut.47.4.497]

72 **Mayer EA**, Berman S, Suyenobu B, Labus J, Mandelkern MA, Naliboff BD, Chang L. Differences in brain responses to visceral pain between patients with irritable bowel syndrome and ulcerative colitis. *Pain* 2005; **115**: 398-409 [PMID: 15911167 DOI: 10.1016/j.pain.2005.03.023]

73 **Faure C**, Giguère L. Functional gastrointestinal disorders and visceral hypersensitivity in children and adolescents suffering from Crohn's disease. *Inflamm Bowel Dis* 2008; **14**: 1569-1574 [PMID: 18521915 DOI: 10.1002/ibd.20506]

74 **Akbar A**, Yiangou Y, Facer P, Brydon WG, Walters JR, Anand P, Ghosh S. Expression of the TRPV1 receptor differs in quiescent inflammatory bowel disease with or without abdominal pain. *Gut* 2010; **59**: 767-774 [PMID: 20551462 DOI: 10.1136/gut.2009.194449]

75 **Keszthelyi D**, Troost FJ, Jonkers DM, Helyes Z, Hamer HM, Ludidi S, Vanhoutvin S, Venema K, Dekker J, Szolcsányi J, Masclee AA. Alterations in mucosal neuropeptides in patients with irritable bowel syndrome and ulcerative colitis in remission: a role in pain symptom generation? *Eur J Pain* 2013; **17**: 1299-1306 [PMID: 23529955 DOI: 10.1002/j.1532-2149.2013.00309.x]

76 **Akbar A**, Yiangou Y, Facer P, Walters JR, Anand P, Ghosh S. Increased capsaicin receptor TRPV1-expressing sensory fibres in irritable bowel syndrome and their correlation with abdominal pain. *Gut* 2008; **57**: 923-929 [PMID: 18252749 DOI: 10.1136/gut.2007.138982]

77 **Barbara G**, Stanghellini V, De Giorgio R, Cremon C, Cottrell GS, Santini D, Pasquinelli G, Morselli-Labate AM, Grady EF, Bunnett NW, Collins SM, Corinaldesi R. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. *Gastroenterology* 2004; **126**: 693-702 [PMID: 14988823 DOI: 10.1053/j.gastro.2003.11.055]

78 **van Hoboken EA**, Thijssen AY, Verhaaren R, van der Veek PP, Prins FA, Verspaget HW, Masclee AA. Symptoms in patients with ulcerative colitis in remission are associated with visceral hypersensitivity and mast cell activity. *Scand J Gastroenterol* 2011; **46**: 981-987 [PMID: 21623672 DOI: 10.3109/00365521.2011.579156]

79 **Minderhoud IM**, Oldenburg B, Schipper ME, ter Linde JJ, Samsom M. Serotonin synthesis and uptake in symptomatic patients with Crohn's disease in remission. *Clin Gastroenterol Hepatol* 2007; **5**: 714-720 [PMID: 17481962 DOI: 10.1016/j.cgh.2007.02.013]

80 **Piche T**, Barbara G, Aubert P, Bruley des Varannes S, Dainese R, Nano JL, Cremon C, Stanghellini V, De Giorgio R, Galmiche JP, Neunlist M. Impaired intestinal barrier integrity in the colon of patients with irritable bowel syndrome: involvement of soluble mediators. *Gut* 2009; **58**: 196-201 [PMID: 18824556 DOI:

10.1136/gut.2007.140806]
81 **Gecse K**, Róka R, Séra T, Rosztóczy A, Annaházi A, Izbéki F, Nagy F, Molnár T, Szepes Z, Pávics L, Bueno L, Wittmann T. Leaky gut in patients with diarrhea-predominant irritable bowel syndrome and inactive ulcerative colitis. *Digestion* 2012; **85**: 40-46 [PMID: 22179430 DOI: 10.1159/000333083]
82 **D'Incà R**, Di Leo V, Corrao G, Martines D, D'Odorico A, Mestriner C, Venturi C, Longo G, Sturniolo GC. Intestinal permeability test as a predictor of clinical course in Crohn's disease. *Am J Gastroenterol* 1999; **94**: 2956-2960 [PMID: 10520851 DOI: 10.1111/j.1572-0241.1999.01444.x]
83 **Arnott ID**, Kingstone K, Ghosh S. Abnormal intestinal permeability predicts relapse in inactive Crohn disease. *Scand J Gastroenterol* 2000; **35**: 1163-1169 [PMID: 11145287 DOI: 10.1080/003655200750056637]
84 **Salonen A**, de Vos WM, Palva A. Gastrointestinal microbiota in irritable bowel syndrome: present state and perspectives. *Microbiology* 2010; **156**: 3205-3215 [PMID: 20705664 DOI: 10.1099/mic.0.043257-0]
85 **Carroll IM**, Ringel-Kulka T, Siddle JP, Ringel Y. Alterations in composition and diversity of the intestinal microbiota in patients with diarrhea-predominant irritable bowel syndrome. *Neurogastroenterol Motil* 2012; **24**: 521-530, e248 [PMID: 22339879 DOI: 10.1111/j.1365-2982.2012.01891.x]
86 **Nagalingam NA**, Lynch SV. Role of the microbiota in inflammatory bowel diseases. *Inflamm Bowel Dis* 2012; **18**: 968-984 [PMID: 21936031 DOI: 10.1002/ibd.21866]
87 **Halvorson HA**, Schlett CD, Riddle MS. Postinfectious irritable bowel syndrome--a meta-analysis. *Am J Gastroenterol* 2006; **101**: 1894-1899; quiz 1942 [PMID: 16928253 DOI: 10.1111/j.1572-0241.2006.00654.x]
88 **Thabane M**, Kottachchi DT, Marshall JK. Systematic review and meta-analysis: The incidence and prognosis of post-infectious irritable bowel syndrome. *Aliment Pharmacol Ther* 2007; **26**: 535-544 [PMID: 17661757 DOI: 10.1111/j.1365-2036.2007.03399.x]
89 **Gwee KA**, Leong YL, Graham C, McKendrick MW, Collins SM, Walters SJ, Underwood JE, Read NW. The role of psychological and biological factors in postinfective gut dysfunction. *Gut* 1999; **44**: 400-406 [PMID: 10026328 DOI: 10.1136/gut.44.3.400]
90 **Gwee KA**. Post-Infectious Irritable Bowel Syndrome, an Inflammation-Immunological Model with Relevance for Other IBS and Functional Dyspepsia. *J Neurogastroenterol Motil* 2010; **16**: 30-34 [PMID: 20535323 DOI: 10.5056/jnm.2010.16.1.30]
91 **Qin HY**, Cheng CW, Tang XD, Bian ZX. Impact of psychological stress on irritable bowel syndrome. *World J Gastroenterol* 2014; **20**: 14126-14131 [PMID: 25339801 DOI: 10.3748/wjg.v20.i39.14126]
92 **Triantafillidis JK**, Merikas E, Gikas A. Psychological factors and stress in inflammatory bowel disease. *Expert Rev Gastroenterol Hepatol* 2013; **7**: 225-238 [PMID: 23445232 DOI: 10.1586/egh.13.4]
93 **Mitchell A**, Guyatt G, Singer J, Irvine EJ, Goodacre R, Tompkins C, Williams N, Wagner F. Quality of life in patients with inflammatory bowel disease. *J Clin Gastroenterol* 1988; **10**: 306-310 [PMID: 2980766 DOI: 10.1097/00004836-198806000-00014]
94 **Irvine EJ**, Feagan B, Rochon J, Archambault A, Fedorak RN, Groll A, Kinnear D, Saibil F, McDonald JW. Quality of life: a valid and reliable measure of therapeutic efficacy in the treatment of inflammatory bowel disease. Canadian Crohn's Relapse Prevention Trial Study Group. *Gastroenterology* 1994; **106**: 287-296 [PMID: 8299896]
95 **Hjortswang H**, Ström M, Almer S. Health-related quality of life in Swedish patients with ulcerative colitis. *Am J Gastroenterol* 1998; **93**: 2203-2211 [PMID: 9820397 DOI: 10.1111/j.1572-0241.1998.00537.x]
96 **Ginsburg PM**, Bayless TM. Managing Functional Disturbances in Patients with Inflammatory Bowel Disease. *Curr Treat Options Gastroenterol* 2005; **8**: 211-221 [PMID: 15913510 DOI: 10.1007/s11938-005-0013-0]
97 **MacDermott RP**. Treatment of irritable bowel syndrome in outpatients with inflammatory bowel disease using a food and beverage intolerance, food and beverage avoidance diet. *Inflamm Bowel Dis* 2007; **13**: 91-96 [PMID: 17206644 DOI: 10.1002/ibd.20048]
98 **Dapoigny M**, Stockbrügger RW, Azpiroz F, Collins S, Coremans G, Müller-Lissner S, Oberndorff A, Pace F, Smout A, Vatn M, Whorwell P. Role of alimentation in irritable bowel syndrome. *Digestion* 2003; **67**: 225-233 [PMID: 12966230 DOI: 10.1159/000072061]
99 **Lea R**, Whorwell PJ. The role of food intolerance in irritable bowel syndrome. *Gastroenterol Clin North Am* 2005; **34**: 247-255 [PMID: 15862933 DOI: 10.1016/j.gtc.2005.02.005]
100 **Halmos EP**, Power VA, Shepherd SJ, Gibson PR, Muir JG. A diet low in FODMAPs reduces symptoms of irritable bowel syndrome. *Gastroenterology* 2014; **146**: 67-75.e5 [PMID: 24076059 DOI: 10.1053/j.gastro.2013.09.046]
101 **Schwender B**, Floch MH. Should FODMAP withdrawal be tried in inflammatory bowel disease patients with irritable bowel syndrome? *J Clin Gastroenterol* 2014; **48**: 393-394 [PMID: 24705090 DOI: 10.1097/MCG.0000000000000127]
102 **Hallert C**, Kaldma M, Petersson BG. Ispaghula husk may relieve gastrointestinal symptoms in ulcerative colitis in remission. *Scand J Gastroenterol* 1991; **26**: 747-750 [PMID: 1654592 DOI: 10.3109/00365529108998594]
103 **Meng J**, Agrawal A, Whorwell PJ. Refractory inflammatory bowel disease-could it be an irritable bowel? *Nat Rev Gastroenterol Hepatol* 2013; **10**: 58-61 [PMID: 22965430 DOI: 10.1038/nrgastro.2012.173]
104 **Bijkerk CJ**, Muris JW, Knottnerus JA, Hoes AW, de Wit NJ. Systematic review: the role of different types of fibre in the treatment of irritable bowel syndrome. *Aliment Pharmacol Ther* 2004; **19**: 245-251 [PMID: 14984370 DOI: 10.1111/j.0269-2813.2004.01862.x]
105 **Moayyedi P**, Quigley EM, Lacy BE, Lembo AJ, Saito YA, Schiller LR, Soffer EE, Spiegel BM, Ford AC. The effect of fiber supplementation on irritable bowel syndrome: a systematic review and meta-analysis. *Am J Gastroenterol* 2014; **109**: 1367-1374 [PMID: 25070054 DOI: 10.1038/ajg.2014.187]
106 **Weinberg DS**, Smalley W, Heidelbaugh JJ, Sultan S. American Gastroenterological Association Institute Guideline on the pharmacological management of irritable bowel syndrome. *Gastroenterology* 2014; **147**: 1146-1148 [PMID: 25224526 DOI: 10.1053/j.gastro.2014.09.001]
107 **Pezzone MA**, Wald A. Functional bowel disorders in inflammatory bowel disease. *Gastroenterol Clin North Am* 2002; **31**: 347-357 [PMID: 12122742 DOI: 10.1016/S0889-8553(01)00021-8]
108 **Whorwell PJ**, Isaacson P. Toxic dilatation of colon in Crohn's disease. *Lancet* 1981; **2**: 1334-1337 [PMID: 6118726 DOI: 10.1016/S0140-6736(81)91350-7]
109 **Cuadrado-Gómez LM**, Arranz-Caso A, Albarrán-Hernández F, Alvarez de Mon M. [Toxic megacolon caused by loperamide as initial form of Crohn disease]. *Rev Clin Esp* 1994; **194**: 201-202 [PMID: 8008961]
110 **van Outryve M**, Toussaint J. Loperamide oxide for the treatment of chronic diarrhoea in Crohn's disease. *J Int Med Res* 1995; **23**: 335-341 [PMID: 8529776]
111 **Ghia JE**, Blennerhassett P, Collins SM. Impaired parasympathetic function increases susceptibility to inflammatory bowel disease in a mouse model of depression. *J Clin Invest* 2008; **118**: 2209-2218 [PMID: 18451995 DOI: 10.1172/JCI32849]
112 **Mikocka-Walus AA**, Turnbull DA, Moulding NT, Wilson IG, Andrews JM, Holtmann GJ. Antidepressants and inflammatory bowel disease: a systematic review. *Clin Pract Epidemiol Ment Health* 2006; **2**: 24 [PMID: 16984660 DOI: 10.1186/1745-0179-2-24]
113 **Goodhand JR**, Greig FI, Koodun Y, McDermott A, Wahed M, Langmead L, Rampton DS. Do antidepressants influence the disease course in inflammatory bowel disease? A retrospective case-matched observational study. *Inflamm Bowel Dis* 2012; **18**:

1232-1239 [PMID: 22234954 DOI: 10.1002/ibd.21846]
114 **Iskandar HN**, Cassell B, Kanuri N, Gyawali CP, Gutierrez A, Dassopoulos T, Ciorba MA, Sayuk GS. Tricyclic antidepressants for management of residual symptoms in inflammatory bowel disease. *J Clin Gastroenterol* 2014; **48**: 423-429 [PMID: 24406434 DOI: 10.1097/MCG.0000000000000049]
115 **Whelan K**, Quigley EM. Probiotics in the management of irritable bowel syndrome and inflammatory bowel disease. *Curr Opin Gastroenterol* 2013; **29**: 184-189 [PMID: 23286925 DOI: 10.1097/MOG.0b013e32835d7bba]

P- Reviewer: Gaertner W, Raman M, Sipahi AM **S- Editor**: Tian YL
L- Editor: A **E- Editor**: Wang CH

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i1.91

World J Gastrointest Pharmacol Ther 2016 February 6; 7(1): 91-106
ISSN 2150-5349 (online)

REVIEW

Clinical applications, limitations and future role of transient elastography in the management of liver disease

Pik Eu Chang, George Boon-Bee Goh, Jing Hieng Ngu, Hiang Keat Tan, Chee Kiat Tan

Pik Eu Chang, George Boon-Bee Goh, Jing Hieng Ngu, Hiang Keat Tan, Chee Kiat Tan, Department of Gastroenterology and Hepatology, Singapore General Hospital, Singapore 169586, Singapore

Pik Eu Chang, Chee Kiat Tan, George Boon-Bee Goh, Duke-NUS Graduate Medical School, Singapore 169587, Singapore

Author contributions: Chang PE designed the framework of the review, performed the final edits and revision of the manuscript; Chang PE, Goh GBB, Ngu JH, Tan HK and Tan CK contributed towards drafting the article and final approval of the manuscript.

Conflict-of-interest statement: None of the authors have any conflict of interests related to this study.

Correspondence to: Dr. Pik Eu Chang, Jason, Department of Gastroenterology and Hepatology, Singapore General Hospital, 20 College Road, Level 3, Academia Building, Singapore 169856, Singapore. jason.chang@sgh.com.sg
Telephone: +65-63214684
Fax: +65-62273623

Received: July 2, 2015
Peer-review started: July 9, 2015
First decision: August 25, 2015
Revised: September 22, 2015
Accepted: October 23, 2015
Article in press: October 27, 2015
Published online: February 6, 2016

Abstract

Transient elastography (TE) is a reliable tool for the non-invasive assessment of liver fibrosis in routine clinical practice. TE is currently approved for use in Europe, Asia and the United States. The widespread adoption of this technology is certain to increase the use of TE worldwide. Although TE has been well validated in chronic viral hepatitis, its clinical role in other liver diseases remains less clear. The advent of new treatment for chronic hepatitis C and emerging prevalence of non-alcoholic steatohepatitis raises new questions on the role of TE in current clinical practice. This review aims to examine the clinical applications, limitations and future role of TE in current clinical practice in light of the changing epidemiology of liver diseases and new clinical management paradigms. In current clinical practice, TE is the most accurate non-invasive method for diagnosis of liver cirrhosis. TE is useful to rule out fibrosis and cirrhosis but does not have sufficient accuracy to discern between various stages of fibrosis. The clinical role of TE has evolved from cross-sectional point-in-time assessment of fibrosis and cirrhosis to the more relevant role of prediction of vital clinical end-points. This provides clinicians with the ability to modify treatment strategies based on the information provided by TE. TE has evolved over the past decade to become an essential tool to assist the clinician in the management of chronic liver disease.

Key words: Liver stiffness; Transient elastography; Non-invasive; Fibrosis; Chronic

Core tip: Transient elastography (TE) is a reliable tool for the non-invasive assessment of liver fibrosis in routine clinical practice. Although TE has been well validated in chronic viral hepatitis, its clinical role in other liver diseases remains less clear. The advent of new treatment for chronic hepatitis C and emerging prevalence of non-alcoholic steatohepatitis raises new questions on the role of TE in current clinical practice. This review aims to examine the clinical applications,

limitations and future role of TE in current clinical practice in light of the changing epidemiology of liver diseases and new clinical management paradigms.

Chang PE, Goh GBB, Ngu JH, Tan HK, Tan CK. Clinical applications, limitations and future role of transient elastography in the management of liver disease. *World J Gastrointest Pharmacol Ther* 2016; 7(1): 91-106 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i1/91.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i1.91

INTRODUCTION

Liver fibrosis is the common end-point of a variety of chronic liver diseases. The progression of liver fibrosis leads to cirrhosis, decompensation, liver failure, hepatocellular carcinoma (HCC) and death[1]. Accurate diagnosis of liver fibrosis and cirrhosis is essential for prognostication of liver disease and for timely intervention to prevent negative outcome. Liver biopsy was the traditional gold standard for diagnosis of fibrosis, but significant progress has been made in the field of non-invasive assessment of liver fibrosis over the past decade such that the role of liver biopsy has been diminishing in clinical practice. Non-invasive markers of fibrosis include serum markers which assess the biochemical properties of fibrosis and elastography devices which assess the physical stiffness of the fibrotic liver. Transient elastography (TE) measured by Fibroscan® (Echosens, France) was the first of such elastography devices, followed by magnetic resonance elastography (MRE), acoustic radiation force impulse (ARFI) and shear wave elastography (SWE). In current clinical practice, TE is the most widely used elastography device for non-invasive assessment of liver fibrosis and is popular in Europe, Asia and recently North America as well.

TE works by measuring shear wave speed through the liver[2]. A handheld probe is placed in the intercostal space of the patient over the right lobe of the liver[3]. A vibration pulse of mild amplitude and low frequency is transmitted by the transducer. This induces a shear wave that propagates through the liver. Pulse-echo ultrasonic acquisitions are simultaneously performed by the machine to follow the shear wave and to measure its velocity. The velocity of the returning shear waves is measured at a depth of 25-65 mm when using the standard M probe and 35-75 mm with the XL probe. This provides an indication of the stiffness of the liver, which is expressed in kPa. The stiffer the liver, the faster the shear wave and hence the higher the liver stiffness measurement (LSM) value. At least 10 successful measurements are required for a valid assessment. The TE result is reported as the median value of at least 10 successful LSMs.

LIMITATIONS OF TE

Before we review the use of TE in clinical practice, it is important to be familiar with the limitations of this new technology. Although TE has been proposed as a non-invasive tool to measure liver fibrosis, TE actually measures the the shear wave speed through the liver which reflects liver stiffness and not actual amount of fibrosis in the liver. Hence, conditions which increase the stiffness of the liver independent of fibrosis will result in an increased LSM and will result in a falsely high estimate of liver fibrosis.

Acute hepatitis

TE has been demonstrated to be unreliable in acute hepatitis, with LSM values increasing 1.3 to 3 fold during alanine transaminase (ALT) flares[4,5]. This can lead to inaccurate diagnosis of cirrhosis in patients with acute transaminitis. A clear correlation between aminotransferases and LSM has been described, with LSM values falling to normal range after resolution of the acute liver injury[6]. It is thus advised that TE be avoided in situations where there is acute hepatitis as the LSM result is likely to overestimate the degree of fibrosis. The LSM should be repeated when or delayed till recovery from the acute liver injury when the ALT levels return to the baseline. It has been suggested that caution should be applied in the interpretation of LSM values when the ALT level is above 100 IU/L. This poses a clinical dilemma in conditions where there is constant fluctuation of transaminitis, for example in chronic hepatitis B (CHB). Initial validation studies of LSM were largely performed in patients with chronic hepatitis C (CHC) and did not report any association between LSM and ALT[7]. However studies in patients with CHB reported a significant correlation between ALT and LSM[8]. From a clinical perspective, it is not feasible to discount the LSM in every CHB patient who has an ALT level > 100 IU/L since many CHB patients would be expected to have fluctuations in ALT. This has led to proposals to use different LSM cut-off values and algorithms for fibrosis estimation for patients with normal and elevated ALT[9]. A large multicentre study recently demonstrated that ALT and LSM maintain a weak linear relationship for each fibrosis stage up to an ALT of 300 IU/L and proposed using probability-based interpretation of LSM using the LiFA-HBV score[10]. This new score helps the clinician to assess the probability of severity of fibrosis based on the LSM and ALT. For example, a patient with an LSM of 18.4 kPa and a normal ALT of 35 IU/L would have a 0.97 probability of F2 fibrosis, a 0.89 probability of F3 fibrosis and a 0.73 probability of cirrhosis. Another patient with the same LSM of 18.4 kPa but an elevated ALT of 350 IU/L would have a 0.97 probability of F2 fibrosis, 0.77 probability of F3 fibrosis but only 0.35 probability of cirrhosis. This provides the clinician with a practical and useful way

of interpreting LSM in patients with elevated ALT in order to make appropriate clinical decisions. However, the LiFA-HBV score was developed based on untreated CHB patients and requires further validation in other liver diseases.

Hepatic congestion

LSM values has been shown to increase significantly after a liquid meal, suggesting that TE should be performed after at least a 3 h fast in order to ensure accuracy of fibrosis assessment[11,12]. Liver stiffness is affected by the central venous pressure[13,14] and has been used as a potential non-invasive measure of decompensated chronic heart failure[15] and in congenital heart disease[16]. It is thus important for clinicians to be aware that TE is not suitable for assessment of liver fibrosis in patients in cardiac failure and those with tricuspid regurgitation as it will lead to an overestimation in the severity of liver fibrosis. This poses a clinical challenge in the assessment of patients with cardiac causes of fibrosis, *e.g.*, those with chronic congestive hepatopathy as a result of Fontan procedure for complex congenital heart disease[17]. TE has been reported to be useful for identifying Fontan patients with significant liver fibrosis and cirrhosis[18,19]. However in the absence of biopsy confirmation, LSM cannot be considered to be a reliable predictor for liver cirrhosis as an elevated liver stiffness value cannot differentiate between hepatic congestion and hepatic fibrosis.

Cholestasis

Extrahepatic cholestasis leads to increased liver stiffness values and results in false estimation of severity of fibrosis. Studies in patients with extrahepatic biliary obstruction either due to neoplasm or choledocholithiasis report elevated LSM readings which declined significantly on repeat TE after biliary drainage[20-23]. It has been suggested that TE should be avoided in patients with significant hyperbilirubinemia (bilirubin > 100 $\geqslant$ μmol/L) and should be repeated after biliary drainage when the bilirubin levels return to baseline[20,23].

Operator experience

TE has been described to be an operator-independent procedure with a high inter-observer agreement of up to 98%[24]. However a large review of 13369 TE examinations over 5 years demonstrated LSM failure in 3.1% and unreliable LSM in 15.8%. Both were associated with two main factors: Elevated body mass index (BMI) > 30 kg/m^2 and operator experience of less than 500 examinations[25]. In a separate French study of TE in 935 patients, the odds ratio (OR) for successful LSM were significantly higher for operators with prior experience of 50-99 measurements and even higher with > 100 previous measurements[26]. Poor operator technique may result in a higher variability of LSMs, which is reflected by a higher interquartile range (IQR). LSM measurements with an IQR greater than 30% of the median value (IQR/M > 0.3) are considered to be invalid and should be either repeated or discarded. In a study examining factors affecting accuracy of TE in patients with CHC fibrosis, an IQR/M $\geqslant$ 0.21 was associated with an increased likelihood of inaccurate TE assessment, with an OR of 2.23[27]. The authors suggest that TE measurements with IQR/M $\geqslant$ 0.21 should be repeated, and if the repeat LSM has a consistent IQR/M $\geqslant$ 0.21, the assessment should be discarded and alternative methods to assess liver fibrosis should be explored. One of the most important factors related to operator technique is the maintenance of perpendicularity of the probe to the liver surface. Correct positioning of the probe is also important to achieve reliable LSM readings[28]. The available data suggests that while a minimal experience of 50 prior measurements may be sufficient for an operator to perform TE, the reliability of LSM measurements is increased in experienced operators with > 500 previous examinations[29].

Obesity

Early studies in TE using the standard M probe encountered a high rate of TE failure between 5%-22% in obese patients with high BMI (> 30 kg/m^2) and increased waist circumference[24,25]. This has been attributed to the interference with the transmission of shear waves and ultrasound waves through the liver parenchyma by thick subcutaneous adipose tissue[30]. However, further studies established that the thoracic fatty belt and not BMI per se was the main determinant of TE failures in obese individuals[31]. Subsequent studies established that the primary factor that was responsible for the failure to obtain a LSM result in obese patients was the distance between the skin and the liver capsule. Patients with a skin-capsule distance (SCD) > 2.6 cm due to increased subcutaneous thoracic fat were more likely to have unsuccessful TE examinations using the M probe[32]. This has led to the development of the XL probe, which differs from the M probe in the following features: a lower ultrasound frequency of 3.5 MHz compared to 5 MHz, a greater transducer focal length of 50 mm *vs* 35 mm, a larger probe tip diameter of 12 mm *vs* 9 mm, higher vibration amplitude of 3 mm *vs* 2 mm and measurement depths of 35-75 mm *vs* 25-65 mm. The XL probe is able to provide a valid TE result in approximately 60% of M probe failures[33]. XL probe failures occur when the SCD is > 3.4 cm, which exceeds the measurement depth of the XL probe. Such patients should undergo alternative assessments for liver fibrosis such as MRE which is not affected by subcutaneous thoracic fat.

Optimal cut-off levels for diagnosis of fibrosis and cirrhosis in different etiologies of liver disease

One of the difficulties in using TE in routine clinical

Table 1 Optimal cut-off values for liver stiffness measurement in different etiologies of chronic liver disease

	Optimal cut-off LSM for F2	Optimal cut-off LSM for F3	Optimal cut-off for LSM F4	Ref.
Chronic hepatitis C	7.6 (5.1-10.1)	10.9 (8.0-15.4)	15.3 (11.9-26.5)	[33]
Chronic hepatitis B	7.0 (6.9-7.2)	8.2 (7.3-9.0)	11.3 (9.0-13.4)	[33]
Alcoholic liver disease	8.9 (2.8-46.4)	10.3 (7.7-20.8)	18.4 (12.2-75.0)	[66]
Non-alcoholic fatty liver disease	7.0 (6.7-7.8)	8.7 (7.1-10.4)	10.3 (10.3-22.3)	[35-37]
Cholestatic liver disease	7.3	9.8	17.3	[54]

LSM: Liver stiffness measurement.

practice is the variability of optimal cut-off levels for the diagnosis of fibrosis and cirrhosis in different etiologies of liver disease. In a meta-analysis of 40 studies evaluating the diagnostic accuracy of TE in various chronic liver disease[34], the optimal cut-off LSMs for CHC are 7.6 kPa for significant fibrosis and 15.3 kPa for cirrhosis (Table 1). Cut-off levels in CHB are similar although some studies demonstrate a slightly lower LSM cut-off for cirrhosis in CHB compared to CHC[35,36]. TE has been shown to be useful for detection of fibrosis and cirrhosis in non-alcoholic fatty liver disease (NAFLD), but the reported optimal cut-off levels for diagnosis of cirrhosis vary from 10.3 kPa to 17.5 kPa[37-39]. In alcoholic liver disease and cholestatic liver disease, the optimal cut-off levels for diagnosis of cirrhosis are significantly higher than viral hepatitis or NASH. Given the variability of cut-off LSMs, LSM results should be interpreted by based on the underlying etiology of liver disease. However, this poses challenges when patients have concomitant liver disease, *e.g.*, CHC and alcoholic liver disease or CHB and NASH. In such situations, most clinicians intuitively use the lower cut-off value to determine the fibrosis stage. However, there have been no studies to date that specifically address this clinical predicament.

One of the underlying reasons for the variability of cut-off levels is that although TE measures amount of fibrosis tissue in the liver, it does not grade the severity of the fibrosis. The METAVIR classification, which is the fibrosis staging system used in most biopsy-paired TE studies, grades severity of fibrosis based on the pattern of fibrosis distribution (*i.e.*, portal fibrosis *vs* portal-central bridging). In contrast, TE simply measures the stiffness of the liver which reflects overall amount of fibrosis tissue in the liver. TE cannot assess the distribution or pattern of fibrosis. This may in part explain the variability of cut-off levels in different diseases. Another contributing factor is that a majority of biopsy-paired validation studies for TE were performed using the METAVIR scoring system as the comparator. While this is relevant for chronic viral hepatitis since the METAVIR system is accurate for staging severity of portal-based fibrosis, it is less relevant for NASH and alcoholic liver disease where the distribution of fibrosis is not predominantly portal-based but pericellular or perivenular, respectively. In a study of accuracy of LSM for the diagnosis of cirrhosis in 1257 patients with various chronic liver disease, Ganne-Carrié *et al*[40] observed that false-positive LSM results were mainly observed in patients with extensive fibrosis. This could reflect a situation where either the liver biopsy has under-staged cirrhosis due to sampling error or there is extensive fibrosis (reflecting a large amount fibrous tissue) but without the nodular architecture required for a pathological diagnosis of cirrhosis.

Differences in the optimal cut-off values reported in different studies can also result from statistical bias. The identification of a specific cut-off value to diagnose a particular fibrosis grade is dependent on the choice of sensitivity and specificity parameters, which in turn depend on the indication for the test and the prevalence of the condition in the study population. For purposes of screening (*e.g.*, diagnosis of fibrosis in NAFLD patients), a lower LSM cut-off level would be more clinically applicable so as not to miss subjects who may require treatment. However, this would reduce the specificity of the test and result in more false-positive tests. In contrast, in clinical situations where accurate identification is important, a LSM cut-off level which provides a high specificity is more relevant than sensitivity. For example, accurate identification of patients with cirrhosis in viral hepatitis is important as these subjects would require antiviral treatment, endoscopic variceal screening and routine surveillance for liver cancer. Some authors have proposed the use of dual cut-off LSMs to rule in or rule out fibrosis and cirrhosis in clinical practice[41].

Cut-off values identified for one population may not be applicable to another which has a different prevalence of disease. For this reason, the performance of TE is more accurate for the identification of more advanced degrees of fibrosis compared to mild fibrosis in biopsy-paired studies because there is an inherent bias to biopsy patients in whom severe fibrosis is clinically more likely. In clinical practice, the use of a specific LSM cut-off value to determine fibrosis stage is less reliable, especially when the LSM value is close to the cut-off value or when there are confounding factors present like necroinflammation, congestion or steatosis. The LSM result should be interpreted in a range or continuum as this provides more reliable clinical interpretation of this non-invasive marker. For example, patients with LSM values ranging from 2.5 to 7 kPa are unlikely to have significant fibrosis, whereas patients with LSM > 13 kPa are likely to have

cirrhosis[42]. A patient with LSM of 25 kPa is more likely to have definite cirrhosis as compared to a patient with an LSM of 13.5 kPa. Hence, the use of probability-based interpretation of LSM results promise to be the most useful way to interpret LSM in routine clinical practice[10].

Reliability criteria

Initial studies in TE defined reliable results as those with at least 10 validated measurements, a success rate of at least 60% and an IQR/M ratio less than 0.3[7]. These criteria were based on the manufacturer's recommendations. However, the impact of these un-reliable TE measurements on accuracy for diagnosis for fibrosis and cirrhosis was not known. Boursier *et al*[43] evaluated the relevance of the recommended reliability criteria in a large multicentre cohort with the aim of improving reliability by using diagnostic accuracy as the primary outcome. They demonstrated that TE success rate and $\geqslant$ 10 valid measurements had no significant influence on reliability for accurate fibrosis staging. The reliability of LSM was shown to be due to the IQR/M according to the liver stiffness median level, which defined three reliability categories: Very reliable (IQR/M $\leqslant$ 0.10), reliable (IQR/M between 0.10 and 0.30 or IQR/M > 0.30 with median LSM < 7.1 kPa) and poorly reliable (IQR/M > 0.30 with median LSM $\geqslant$ 7.1 kPa).

CLINICAL APPLICATIONS OF TE IN CURRENT PRACTICE

Non-invasive diagnosis of fibrosis and cirrhosis in chronic liver disease

CHC: The primary role of TE is for the non-invasive diagnosis of liver fibrosis with the aim of reducing the need for liver biopsy in the clinical management of chronic liver disease. TE was first developed for and extensively validated in patients with CHC[7,44]. Numerous meta-analyses have demonstrated that TE has a high diagnostic accuracy for the diagnosis of CHC cirrhosis with a mean AUROC of 0.94[45,46]. Castéra *et al*[47] established TE as the most accurate non-invasive method for detection of early cirrhosis when compared with other available tests and algorithms. In this study involving 298 CHC patients, the AUROC of TE for detection of cirrhosis was 0.96 compared to 0.82 for Fibrotest®, 0.80 for Lok index and APRI, 0.79 for platelet count, 0.73 for prothrombin index and 0.61 for AST/ALT ratio (P < 0.0001). A subsequent larger study of 1839 French patients with CHC confirmed a similar significant superiority of TE over serum markers in excluding cirrhosis[48]. The performance of TE has also been shown to be equally accurate in special populations of CHC patients. These include patients with HCV/HIV co-infection[49,50] and post-transplant HCV[51,52]. The introduction of TE has resulted in a significant reduction in the numbers of liver biopsy in Europe[53].

The recent introduction of highly effective direct antiviral treatment (DAA) for CHC has provided cure rates exceeding 95% with minimal side-effects. With the availability of DAA, all CHC patients should be considered for treatment irrespective of severity of fibrosis since cure is possible. With this paradigm shift in CHC management, the role of non-invasive markers for fibrosis becomes diminished. However, the high cost of such treatment has necessitated prioritization for CHC treatment based on severity of fibrosis. Hence for present day clinicians, TE plays a role to assist in stratifying patients for CHC treatment (Table 2). Based on the latest EASL guidelines, DAA should be prioritized for CHC patients with cirrhosis and advanced fibrosis (F3 and F4), justified in those with significant fibrosis (F2) and individualized in those with no or mild fibrosis (F1 and F0) in whom risk of decompensated cirrhosis and HCC remains low[54].

CHB: In the management of patients with CHB, it is most important to distinguish those with inactive disease from those with active hepatitis, as the latter group of patients is more likely to progress to advanced fibrosis and cirrhosis. Even among patients with persistently normal transaminases, a subgroup will present with higher degree of fibrosis and are more likely to have adverse long-term outcomes, particularly those with greater viraemia[55,56]. The main role of TE in CHB is to differentiate patients with significant fibrosis from those with inactive disease without fibrosis. Maimone *et al*[57] demonstrated that the LSM in patients with inactive CHB was significantly lower than those with e-antigen negative CHB. In another study by Fung *et al*[58], TE demonstrated excellent diagnostic accuracy across the entire spectrum of liver fibrosis with good negative predictive value, although caution needs to be exercised when encountering patients with elevated transaminases. Interpretation of LSM is sometimes challenging due to the confounding effect of ALT, but several strategies can be used to circumvent this problem. One is to use different LSM cut-off levels for those with normal and elevated ALT[9] and the other is to use probability-based scores that correct for the ALT level[10]. In routine clinical practice, TE can be used to select patients with higher risk of disease progression and targeted for closer surveillance and consideration of early antiviral therapy.

NAFLD: NAFLD is one of the most common chronic liver diseases worldwide, with increasing disease prevalence in parallel with the burgeoning obesity and metabolic syndrome epidemic[59]. NAFLD is a spectrum of disease, ranging in severity from simple steatosis, which is considered relatively benign, to non-alcoholic steatohepatitis (NASH), the more aggressive, severe end of the spectrum. NASH can potentially progress to cirrhosis and accompanying complications such as HCC[60]. Accurate staging of liver fibrosis is important

Table 2 What the clinician needs to know about transient elastography (Fibroscan®)

1 Clinical indications for TE		
Liver disease	Indications for TE	Potential clinical applications
Chronic liver disease	To assess for severity of fibrosis	Assist in treatment decisions in CHC and CHB Selection of patients for treatment trials Decision to continue or stop MTX
	To diagnose early cirrhosis	Commence variceal screening and HCC surveillance, monitor for decompensation
	Longitudinal assessment of fibrosis	Assess for progression of fibrosis in untreated patients and for regression of fibrosis/cirrhosis in treated patients
Patients with NAFLD	Assess severity of fibrosis and steatosis (with Fibroscan-CAP)	Aggressive control of risk factors Selection of patients for treatment trials Selection of patients for liver biopsy
Post-liver transplant	Assess for fibrosis in recurrent CHC post liver transplant	Avoid protocol liver biopsies for diagnosis of fibrosis
Non-cirrhotic portal hypertension	Exclude cirrhosis	Assists in differentiating cirrhotic *vs* non-cirrhotic portal hypertension
Patients with cirrhosis	Predict significant portal hypertension and risk of liver-related events	Stratify frequency of follow-up in low-risk *vs* high-risk cirrhotics
	Predict absence of varices	Avoid/delay endoscopy screening in cirrhotics at low risk for varices
2 Conditions that affect accuracy of TE		
Condition	How it affects the TE result	What the clinician should do
Post-meal	LSMs are elevated after meals due to increased hepatic venous flow	Patients should fast for at least 3 h before TE measurement
Elevated ALT	LSMs are elevated due to hepatic inflammation	Repeat or delay TE till after ALT has returned to baseline/normal levels Use ALT-based LSM cut-off values to interpret LSM result Use probability-based LSM interpretation scores which account for ALT
Cardiac failure	LSMs are elevated due to hepatic congestion in right heart failure	Repeat or delay TE until after patient's heart failure is treated
Cholestasis	LSMs are elevated due to increased stiffness from biliary dilatation	Repeat or delay TE until after biliary obstruction is resolved
Operator experience	Operator inexperience may lead to higher rate of unsuccessful or invalid LSM results	TE should be performed by operators with prior experience of at least 50-100 examinations
Obesity	Higher rate of unsuccessful LSMs due to increased SCD because of increased subcutaneous fat	Use XL probe if SCD > 3.4 cm (with the current Fibroscan 502 Touch®, the machine will automatically advise when the XL probe should be used) If LSM is unsuccessful with XL probe, use alternative non-invasive test
Ascites	High rate of unsuccessful LSM due to interruption of shear waves by ascites	Use alternative non-invasive test
Pregnancy, cardiac pacemaker, AICD	Safety of TE in these conditions have not been assessed	TE contraindicated

TE: Transient elastography; CHC: Chronic hepatitis C; CHB: Chronic hepatitis B; MTX: Methotrexate; HCC: Hepatocellular carcinoma; CAP: Controlled attenuation parameter; NAFLD: Non-alcoholic fatty liver disease; LSM: Liver stiffness measurement; ALT: Alanine transaminase; SCD: Skin-capsule distance; AICD: Activation-induced cell death.

in the management algorithm of NAFLD for aiding treatment decisions and prognostication to monitoring disease progression or treatment response. As such, there have been a myriad of studies exploring the use of TE in patients with NAFLD, with data derived from both Asian and Western series in addition to adult and paediatric cohorts[61-66]. Based on these studies, variable LSM cut-off values for each stage of fibrosis have been reported, with readings of 6.6-7.8, 7.1-10.4 and 10.3-22.3 kPa corresponding to stage F2, F3 and F4, respectively[67]. A recent meta-analysis on the utility of TE in the context of NAFLD included 9 studies consisting of 1047 NAFLD patients[39]. The analysis suggested excellent accuracy in diagnosing F3 or higher (85% sensitivity, 82% specificity) and F4 (92% sensitivity, 92% specificity) while performance was moderate for stage F2 or higher (79% sensitivity, 75% specificity).

Alcoholic liver disease: A recent Cochrane Database review examined the diagnostic accuracy of TE for diagnosis and staging of liver fibrosis in patients with alcoholic liver disease[68]. Five retrospective and nine prospective cohort studies with a total of 834 subjects were reviewed. The authors concluded that TE may be used to rule out liver cirrhosis in patients with alcoholic liver disease when the pre-test probability is about 51% (range 15%-79%) using a cut-off value of 12.5 kPa. However the authors cautioned that the optimal cut-off values for assessing fibrosis cannot be established due to the wide range of cut-off values used in individual studies. In a recent study comparing different non-invasive modalities for the diagnosis of advanced fibrosis and cirrhosis in alcoholic liver disease, TE performed better than FibroTest, APRI, Forns and FIB-4 with an optimal LSM of 10.3 kPa for F3 and 18.0 kPa for F4 disease[69,70].

Cholestatic liver diseases: TE is a reliable non-invasive means for assessing fibrosis stages in cho-

lestatic liver diseases such as primary biliary cirrhosis (PBC) and primary sclerosing cholangitis[71-74]. Optimal stiffness cutoff values of 7.3, 9.8, and 17.3 kPa for F ⩾ 2, F ⩾ 3 and F ⩾ 4 respectively have been proposed[71]. TE has been shown to be significantly superior to biochemical markers such as aspartate aminotransferase (AST)/platelet ratio, FIB-4, hyaluronic acid, AST/alanine aminotransferase ratio, and Mayo score in assessing fibrosis stages in PBC. Furthermore, it has also been shown that serial TE can provide prognostic information, as a 2.1 kPa-per-year increase is associated with an 8.4 fold increased risk of liver decompensations, liver transplantations, or deaths in patients with PBC[72].

Autoimmune hepatitis: The utility of TE in autoimmune hepatitis (AIH) is less well validated. There are case reports that described markedly increased liver stiffness in acute AIH. However, liver stiffness normalised after 4 mo of therapy suggesting that liver stiffness measurement can be greatly influenced by florid inflammatory liver process in AIH[75]. Another small case series supported the use of TE in assessing fibrosis in non-viral chronic liver diseases including AIH[73]. Optimal TE cut-off value for AIH has not been established. Therefore, care needs to be taken when performing TE on AIH patients, bearing in mind that uncontrolled inflammation from AIH will increase liver stiffness.

Post liver transplant: Several studies have evaluated the role of TE as a non-invasive tool for the detection of hepatic fibrosis due to recurrent hepatitis C following living donor and deceased donor liver transplantation[76-79]. All studies confirmed the excellent correlation of LSM to fibrosis on histology. In addition, Carrión *et al*[76] also showed that TE is an excellent tool to diagnose portal hypertension among patients with advanced fibrosis, cirrhosis and fibrosing cholestatic hepatitis. TE was also shown to be superior to serum markers[77] and other more complex scoring systems for the diagnosis of advanced fibrosis and cirrhosis in this group of liver transplant recipients[78]. These studies suggest that in patients with very low liver stiffness values, liver biopsy may safely be avoided. However, the main drawback of TE is the interpretation of results which correspond to the intermediate stages of fibrosis, where liver biopsy is still mandatory for accurate staging of liver fibrosis[79].

Other liver diseases: The availability of reliable non-invasive tools to diagnose liver fibrosis is of tremendous clinical relevance for patients on long-term treatment with methotrexate (MTX) as it helps to avoid routine liver biopsy for assessment of MTX toxicity. Despite a lack of high-quality, prospective studies providing biopsy-paired correlation of the accuracy of TE in this population, the existing literature suggests that TE is an effective non-invasive tool for monitoring MTX toxicity in patients with inflammatory bowel disease, rheumatoid arthritis and psoriasis[80-84]. The prevalence of abnormal LSM values > 7.1 kPa was generally low in this patient population and LSM values are not correlated with the cumulative MTX dose[85]. A recent review from the International Society of Dermatology states that both TE and MRE have outstanding efficacy in detection of liver fibrosis and can help the physician in the decision to use a therapeutic alternative to MTX[86].

In patients with hemochromatosis, an algorithm using serum ferritin levels together with TE was shown to accurately classify the presence of severe fibrosis in 61% of patients, thus avoiding liver biopsy in this group[87]. Together with other studies on patients with hemochromatosis, the evidence suggests a role for TE although more longitudinal prospective studies are required to clearly establish the clinical role of TE in hemochromatosis[88,89].

TE plays a role in the clinical evaluation of individuals with non-cirrhotic portal hypertension[90]. The primary role of TE in this setting is to exclude cirrhosis in patients who present with clinical features suggesting cirrhosis such as splenomegaly, esophageal varices and thrombocytopenia. Compared to cirrhotics, patients with non-cirrhotic portal hypertension have much lower liver stiffness values in the range of 8-9 kPa, which is clearly not compatible with the diagnosis of cirrhosis[91,92].

In summary, TE is the most accurate non-invasive test for the diagnosis of cirrhosis with a high negative predictive value to exclude liver cirrhosis[93]. One important use of TE in clinical practice is to exclude cirrhosis in patients with chronic liver disease, thus avoiding the need for patients to undergo invasive and expensive investigations such as screening gastroscopy for varices and routine HCC surveillance. This translates to a greater cost-effective management for this group of patients. The performance of TE is only moderate for the non-invasive diagnosis of fibrosis and it cannot reliably replace liver biopsy to diagnose milder stages of fibrosis.

Longitudinal assessment of fibrosis regression

All the preceding applications of TE were based on a single, point-in-time TE assessment. Intuitively, serial TE measurements should allow one to assess the progression of fibrosis over time or the regression of fibrosis after successful treatment of the underlying liver disease. This has been shown to be possible in chronic viral hepatitis. Vergniol *et al*[94] showed that there was a significant reduction of TE readings in CHC patients successfully treated with pegylated interferon and ribavirin. Regression of fibrosis in CHB patients on antiviral treatment is associated with good outcomes[95-97]. Several short and long-term studies have shown that LSM values consistently decrease over

time during continuous antiviral treatment[98-100]. This decrease in liver stiffness is not restricted to patients with milder degree of fibrosis. Kim *et al*[101] reported that a higher liver stiffness value was the only significant factor associated with a decline in liver stiffness value during prolonged antiviral therapy. However, it is known that liver stiffness measurement by TE is increased by elevation of aminotransferases[102]. In a study by Lim *et al*[103], the decrease in liver stiffness value during antiviral therapy was correlated to decrease in liver inflammation on histology but not fibrosis, while contradicting findings were reported by Wong *et al*[104]. Therefore, it remains unclear based on available evidence if a decrease in LSM in treated CHB reflects a regression of liver fibrosis or a decrease in hepatic necroinflammation as a result of viral suppression. In current clinical practice, an emerging role for TE is for the longitudinal monitoring for regression of cirrhosis and fibrosis in patients on antiviral therapy. This role is likely to expand with recent advances in antifibrotic treatment.

Non-invasive prediction of significant portal hypertension

In patients with compensated liver cirrhosis, the presence of clinically significant portal hypertension predicts clinical decompensation and poor outcomes[105]. One area of interest in TE is the correlation between LSM and hepatic venous pressure gradient (HVPG). Five studies have evaluated the diagnostic accuracy of TE for diagnosis of clinically significant portal hypertension (defined as HVPG $\geqslant$ 10 mmHg)[106-110]. A recent meta-analysis[111] evaluating 18 studies involving 3644 patients with chronic liver disease showed that TE was a good screening tool for detecting significant portal hypertension with 81% probability of correctly detecting significant portal hypertension when the pre-test probability was 50%. Cut-off LSM values ranged from 13.6 to 34.9 kPa with summary sensitivity of 0.90 and 0.79, with PPV of 0.88 and NPV of 0.88. Bureau *et al*[108] reported that a LSM of 21 kPa accurately predicted significant portal hypertension in 92% of patients undergoing paired HVPG and TE with an OR of 120 for HVPG $\geqslant$ 10 mmHg. However, Vizzutti *et al*[106] demonstrated that while a strong correlation existed between LSM and HVPG up to a HVPG of 12 mmHg, the correlation was poor at HVPG values beyond 12 mmHg. As such, although there may be a potential role of LSM for screening for presence of significant portal hypertension, it cannot replace HVPG for the quantitative assessment of portal pressures.

Prediction of liver-related clinical outcomes

While earlier studies exploring the role of TE in clinical practice focused on cross-sectional studies, there is a wealth of convincing literature which demonstrates that TE has a prognostic role for prediction of important clinical end-points related to progression of fibrosis and cirrhosis[112-114]. In our opinion, this has greater clinical significance compared to point-in-time assessment of cirrhosis. Foucher *et al*[115] were the first to demonstrate that progressively higher LSM values were correlated with clinical decompensation events such as ascites, HCC and variceal bleeding. In this study of 711 CHC patients, various LSM cut-offs had NPV exceeding 90% for different associations, *e.g*., 27.5 kPa for large esophageal varices, 37.5 kPa for Child-Pugh B or C, 49.1 kPa for past history of ascites, 53.7 kPa for HCC and 62.7 kPa for esophageal variceal bleeding.

A significant correlation was shown between TE and presence of esophageal varices[116,117]. In a meta-analysis of 12 studies examining the accuracy of TE for detection of esophageal varices[111], there was a wide range of cut-off LSM values from 15.1 to 28.0 kPa, with a summary sensitivity of 0.87 but poor specificity of 0.53. In a setting of a low pre-test index of suspicion, the probability of a correct diagnosis following a "correct" LSM measurement was less than 70%. Recently Kim *et al*[118] developed a liver stiffness measurement based prediction model which included spleen diameter to platelet ratio, to enable identification of patients with very low likelihood of high risk esophageal varices with a negative predictive value of 94.0%. However, this was a single-centre study where external validation is necessary before the prediction model may be widely used. At present, TE is not sufficiently reliable to replace endoscopy for assessment of esophageal varices in routine clinical practice[119].

Importantly, TE has the potential to predict clinical liver-related events. A prospective study by Robic *et al*[120], demonstrated that a LSM > 21.1 kPa proved as effective as HVPG to predict clinical decompensation and liver-related events (ascites, variceal bleeding, HCC, HE and death). A Japanese study demonstrated in a large 3-year study of 866 CHC patients that a TE value of > 10 kPa carried a significantly higher risk of developing HCC[121]. This finding is not surprising as a TE value of 10 kPa really denotes that a patient has significant fibrosis which is a known association with HCC. Kim *et al*[122] correlated liver stiffness values according to histological sub-classifications of cirrhosis according to Laennec system, and showed that the proportion of liver-related events increased according to the baseline histological sub-classification and LSM prior to starting antiviral therapy. In another study by Lee *et al*[123], TE was shown to be a useful tool to predict liver-related events among CHB patients with complete viral suppression, where patients with LSM > 13.0 kPa had a hazard ratio of 12.0 for any cirrhosis-related decompensation, HCC and liver-related mortality as compared to patients with liver stiffness < 8.0 kPa. These two studies suggest that baseline as well as dynamic change in the liver stiffness value among patients on antiviral therapy can risk stratify

patients into those at higher risk of decompensation and mortality, even among those with complete viral suppression. Serial TE in cirrhotic patients may be clinically relevant as increases in serial LSM has been shown to predict clinical outcomes including decompensation, need for liver transplant and death[72].

Assessment of hepatic steatosis

The rising prevalence of NAFLD worldwide is becoming an increasing problem in tandem with rising rates of obesity and metabolic syndrome. This raises the need to screen, diagnose and quantify hepatic steatosis in the large population at risk. TE has been shown to be useful for the non-invasive prediction of fibrosis in NAFLD patients and helps to select patients at high risk for progression to cirrhosis and HCC. The introduction and widespread adoption of the XL probe has resolved issues with TE failure in obese NAFLD patients. Apart from fibrosis assessment, the recent introduction of the novel controlled attenuation parameter (CAP) function allows for the non-invasive measurement of hepatic steatosis[124]. CAP measures ultrasound attenuation to quantify hepatic steatosis using the M probe and is expressed in dB/m. Studies have shown that CAP is able to detect more than 5% hepatic steatosis which intuitively is more sensitive than conventional ultrasound which can only detect more than 30% steatosis. In addition, CAP provides comparable accuracy in detection and quantification of hepatic steatosis across a range of liver disease etiologies[125,126]. Further studies are required to explore the robustness and validity of CAP in the study of liver disease. Interestingly, the combination of TE and CAP can simultaneously evaluate hepatic fibrosis and steatosis in a single examination. However, clinicians need to be mindful that this combination of TE and CAP can only predict for fibrosis and steatosis but cannot assess lobular inflammation and balloon degeneration. Hence the reliability of TE to predict clinical progression in NAFLD is limited considering that balloon degeneration is the most important histological feature that predicts disease progression. As such, in contrast to viral hepatitis, TE is unlikely to replace liver biopsy for NAFLD. Currently, the main clinical role for TE in NAFLD is for population screening to detect those with significant steatosis and fibrosis who would benefit from specialty care or treatment. Confirmation of NASH and assessment of severity will still require liver biopsy.

COMPARISON BETWEEN TE AND OTHER NON-INVASIVE MARKERS OF FIBROSIS

TE vs serum markers

There have been numerous studies comparing the performance of TE against serum markers for the non-invasive diagnosis of liver fibrosis. Overall, the diagnostic accuracy of TE and serum markers are comparable for the diagnosis of significant fibrosis but TE has improved accuracy for the diagnosis of cirrhosis[127]. A large multi-center prospective study comparing TE to serum markers (FIBROSTIC study) of 1307 patients with chronic viral hepatitis concluded that the accuracy of TE was significantly higher than serum markers for predicting cirrhosis. However, all non-invasive markers including TE had only moderate accuracy for predicting significant fibrosis[48]. In another multicentre study, TE was compared against nine serum markers for the diagnosis of fibrosis and cirrhosis in untreated CHC patients. FibroTest, FibroMeter, Hepascore and TE had similar superior performance compared to the other tests[128]. Overall performance of TE was reduced because 22% had uninterpretable results using the M probe. The advantage of serum markers is that it is easily available, inexpensive and does not require specialized equipment and training. However, serum markers can be confounded by biochemical abnormalities (*e.g.*, transaminitis, hemolysis, *etc*.) and do not provide a reflection of the physical degree of fibrosis in the liver. TE provides a more reliable assessment of liver fibrosis but is limited by invalid measurements in obese individuals or those with ascites (Table 3).

Combining TE and serum markers

Combination of serum markers with TE can improve the accuracy of fibrosis staging. TE may falsely record high fibrosis scores due to increased stiffness of an inflamed liver. To overcome this weakness, a simple serum marker such as ALT can be used to improve its accuracy. ALT based algorithms for TE measurement of liver fibrosis has been proposed for CHB[9,10]. In addition, it has been demonstrated that spleen diameter and platelet ratio can also be used in combination with TE to improve accuracy[129]. Other markers such as haptoglobin, apolipoprotein A1, and α2-macroglobulin levels have been used in combination with TE to establish a prediction model, called the HALF index, for better estimation of fibrosis staging[130]. Combination of serum markers with TE has been shown to improve the accuracy of detecting fibrosis and cirrhosis[7,128]. The latest clinical practice guidelines from the EASL and AASLD both recommend combination of TE and serum markers as the most efficient method of assessing liver fibrosis in making treatment decisions for patients with CHC[54,131]. Liver biopsy is reserved only in situations where there is discordance between the two non-invasive modalities.

TE vs MRE

MRE uses a modified phase-contrast technique to visualise the propagation characteristics of acoustic shear waves generated by an acoustic driver placed over the liver[132]. Early studies have demonstrated that MRE indeed is a feasible alternative method to

Table 3 Comparison of non-invasive modalities for assessment of fibrosis

Non-invasive test	Advantages	Disadvantages
Transient elastography	Easy to perform	Requires costly equipment
	Painless and comfortable	Unreliable in patients with severe obesity and ascites
	Can be done in clinic or office	Requires technical expertise
	Provides immediate results for clinician	Requires fasting
	Well-validated	Interpretation of LSM result dependent on etiology, ALT, *etc.*
	Can be performed reliably in obese patients with the use of XL probe	Only assesses part of the liver
	Readily available in most centres	
Serum markers	Easy to perform	Results can be confounded by biochemical abnormalities
	Inexpensive	Indirect reflection of liver fibrosis
	Does not require training or equipment	Does not assess liver stiffness directly
	Well-validated	Some tests are proprietary and are relatively costly
	Easily repeatable	
MRE	Multi-dimensional assessment	High cost
	Able to assess whole liver	Limited availability
	Operator independence	Cannot be performed in subjects with claustrophobia
	Can be performed in obese patients and those with ascites	Long examination time
	Can be integrated as part of a comprehensive MRI examination	Cannot be performed in livers with iron overload
ARFI/SWE	Higher success rate compared to TE (using M probe)	Requires special equipment and technical expertise
	Similar accuracy to TE	Operator-dependent
	Can be performed in obese patients and those with ascites	Not widely available
	Can assess whole liver	
	Can assess specific part of the liver (*i.e.*, region of interest)	

TE: Transient elastography; MRE: Magnetic resonance elastography; ARFI: Acoustic radiation force impulse; SWE: Shear wave elastography; LSM: Liver stiffness measurement; ALT: Alanine transaminase; MRI: Magnetic resonance imaging.

assess liver elasticity[133-135]. Like TE, MRE has been shown to be repeatable and reproducible[136,137], has been validated against histological fibrosis in various chronic liver diseases including CHB, CHC and NAFLD[138-140] and has been shown to predict esophageal varices[141,142]. MRE is also falsely elevated by necroinflammation[143] but is not affected by steatosis[135].

In a study by Huwart *et al*[144] comparing the performance of TE and MRE in 141 patients with various liver diseases, MRE was shown to be superior to TE in predicting liver fibrosis stage. The better performance of MRE over TE was attributed to several reasons. In MRE, a multi-dimensional displacement vector is assessed as opposed to the 1-dimensional model of TE which improves the shear elastic parameter measured. Also, in MRE, a volume that includes several liver sections is analysed, in contrast to TE which analyses a single cylindrical liver sample of 20-40 mm. Hence, the volume analysed by MRE is far more representative of the liver parenchyma. However, in another study by Bohte *et al*[145], the diagnostic accuracies of TE and MRE for detecting METAVIR F > 2 and F > 3 in patients with CHB and CHC did not differ significantly.

Although there is no conclusive data on superiority of MRE over TE, there are several advantages of MRE over TE. Unlike TE, MRE has a freely oriented field of view without the need for an acoustic window and the latter is one of the important reasons for TE failure. MRE is operator independent and can be used in obese patients and patients with ascites. Perhaps most importantly, MRE can be integrated as part of a comprehensive liver MR imaging examination that can include a conventional diagnostic liver MRI in addition to MRE as well as protocols for assessment of steatosis. The disadvantages of MRE include the high cost, longer examination time, facility constraints and the inability to perform MRE in livers with iron overload due to signal-to-noise limitation. Importantly TE offers the convenience of a rapid bedside procedure which can be done in the clinic and can provide immediate results to the physician.

TE vs ARFI

In the last few years, several non-invasive methods have been developed to evaluate liver fibrosis, including TE and ARFI elastography.

ARFI is performed with a Siemens AcusonS2000TM (Siemens AG, Erlangen, Germany) ultrasound system. The ultrasound probe automatically generates shearwaves which propagate into the tissue. The propagation speed increases with fibrosis severity, providing an estimation of the elasticity which is expressed in m/s[146]. Both TE and ARFI have been validated and advocated for assessment of liver fibrosis across a range of liver diseases. In a meta-analysis comparing diagnostic performance of ARFI and TE involving 13 studies and 1163 patients, failure rates were higher in TE compared to ARFI (6.6% *vs* 2.1%); caveat being that the TE evaluations were performed using M probe[146]. In terms of diagnostic accuracy, there were no significant differences between either modality to detect significant fibrosis or cirrhosis. For

detection of F2, sensitivity of 0.74 and specificity of 0.83 while sensitivity of 0.78 and specificity of 0.84 was reported for ARFI and TE, respectively. For detection of F4, sensitivity of 0.87 and specificity of 0.87 while sensitivity of 0.89 and specificity of 0.87 was reported for ARFI and TE, respectively.

CONCLUSION

The role of TE in clinical practice has evolved over the past decade in tandem with changing trends in clinical management of chronic liver disease. The diagnostic accuracy of TE has been clearly defined for the diagnosis of cirrhosis. In current clinical practice, TE has replaced ultrasound and CT as the most accurate non-invasive method for diagnosis of liver cirrhosis. TE is useful to rule out fibrosis and cirrhosis but does not have sufficient accuracy to discern between various stages of fibrosis. This has led to the recommendation to use TE in combination with serum markers for clinical assessment of fibrosis in CHC. Importantly, the clinical role of TE has evolved from cross-sectional point-in-time assessment of fibrosis and cirrhosis to the more relevant role of prediction of vital clinical end-points. This provides clinicians with the ability to modify treatment strategies based on the information provided by TE. In addition, recent advances in development of antifibrotic therapy will increase the role of serial TE for longitudinal assessment of progression and regression of fibrosis. The availability of the combination of TE and CAP will provide the opportunity to screen at-risk populations with NAFLD for fibrosis and steatosis in a single convenient examination. TE has evolved over the past decade to become an essential tool to assist the clinician in management of chronic liver disease.

REFERENCES

1 **D'Amico G**, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. *J Hepatol* 2006; **44**: 217-231 [PMID: 16298014 DOI: 10.1016/j.hep.2005.10.013]

2 **Ziol M**, Handra-Luca A, Kettaneh A, Christidis C, Mal F, Kazemi F, de Lédinghen V, Marcellin P, Dhumeaux D, Trinchet JC, Beaugrand M. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. *Hepatology* 2005; **41**: 48-54 [PMID: 15690481 DOI: 10.1002/hep20506]

3 **Shiina T**, Nightingale KR, Palmeri ML, Hall TJ, Bamber JC, Barr RG, Castera L, Choi BI, Chou YH, Cosgrove D, Dietrich CF, Ding H, Amy D, Farrokh A, Ferraioli G, Filice C, Friedrich-Rust M, Nakashima K, Schafer F, Sporea I, Suzuki S, Wilson S, Kudo M. WFUMB guidelines and recommendations for clinical use of ultrasound elastography: Part 1: basic principles and terminology. *Ultrasound Med Biol* 2015; **41**: 1126-1147 [PMID: 25805059 DOI: 10.1016/j.ultrasmedbio.2015.03.009]

4 **Sagir A**, Erhardt A, Schmitt M, Häussinger D. Transient elastography is unreliable for detection of cirrhosis in patients with acute liver damage. *Hepatology* 2008; **47**: 592-595 [PMID: 18098325 DOI: 10.1002/hep.22056]

5 **Coco B**, Oliveri F, Maina AM, Ciccorossi P, Sacco R, Colombatto P, Bonino F, Brunetto MR. Transient elastography: a new surrogate marker of liver fibrosis influenced by major changes of transaminases. *J Viral Hepat* 2007; **14**: 360-369 [PMID: 17439526 DOI: 10.1111/j.1365-2893.2006.00811.x]

6 **Arena U**, Vizzutti F, Corti G, Ambu S, Stasi C, Bresci S, Moscarella S, Boddi V, Petrarca A, Laffi G, Marra F, Pinzani M. Acute viral hepatitis increases liver stiffness values measured by transient elastography. *Hepatology* 2008; **47**: 380-384 [PMID: 18095306 DOI: 10.1002/hep.22007]

7 **Castéra L**, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, Darriet M, Couzigou P, De Lédinghen V. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005; **128**: 343-350 [PMID: 15685546 DOI: 10.1053/j.gastro.2004.11.018]

8 **Marcellin P**, Ziol M, Bedossa P, Douvin C, Poupon R, de Lédinghen V, Beaugrand M. Non-invasive assessment of liver fibrosis by stiffness measurement in patients with chronic hepatitis B. *Liver Int* 2009; **29**: 242-247 [PMID: 18637064 DOI: 10.1111/j.1478-3231.2008.01802.x]

9 **Chan HL**, Wong GL, Choi PC, Chan AW, Chim AM, Yiu KK, Chan FK, Sung JJ, Wong VW. Alanine aminotransferase-based algorithms of liver stiffness measurement by transient elastography (Fibroscan) for liver fibrosis in chronic hepatitis B. *J Viral Hepat* 2009; **16**: 36-44 [PMID: 18673426]

10 **Wong VW**, Lampertico P, de Lédinghen V, Chang PE, Kim SU, Chen Y, Chan HL, Mangia G, Foucher J, Chow WC, Ahn SH, Hou J. Probability-based interpretation of liver stiffness measurement in untreated chronic hepatitis B patients. *Dig Dis Sci* 2015; **60**: 1448-1456 [PMID: 25563720 DOI: 10.1007/s10620-014-3488-5]

11 **Berzigotti A**, De Gottardi A, Vukotic R, Siramolpiwat S, Abraldes JG, García-Pagan JC, Bosch J. Effect of meal ingestion on liver stiffness in patients with cirrhosis and portal hypertension. *PLoS One* 2013; **8**: e58742 [PMID: 23520531 DOI: 10.1371/journal.pone.0058742]

12 **Mederacke I**, Wursthorn K, Kirschner J, Rifai K, Manns MP, Wedemeyer H, Bahr MJ. Food intake increases liver stiffness in patients with chronic or resolved hepatitis C virus infection. *Liver Int* 2009; **29**: 1500-1506 [PMID: 19732330 DOI: 10.1111/j.1478-3231.2009.02100.x]

13 **Hopper I**, Kemp W, Porapakkham P, Sata Y, Condon E, Skiba M, Farber L, Porapakkham P, Williams TJ, Menahem S, Roberts S, Krum H. Impact of heart failure and changes to volume status on liver stiffness: non-invasive assessment using transient elastography. *Eur J Heart Fail* 2012; **14**: 621-627 [PMID: 22523374 DOI: 10.1093/eurjhf/hfs044]

14 **Millonig G**, Friedrich S, Adolf S, Fonouni H, Golriz M, Mehrabi A, Stiefel P, Pöschl G, Büchler MW, Seitz HK, Mueller S. Liver stiffness is directly influenced by central venous pressure. *J Hepatol* 2010; **52**: 206-210 [PMID: 20022130 DOI: 10.1016/j.jhep.2009.11.018]

15 **Colli A**, Pozzoni P, Berzuini A, Gerosa A, Canovi C, Molteni EE, Barbarini M, Bonino F, Prati D. Decompensated chronic heart failure: increased liver stiffness measured by means of transient elastography. *Radiology* 2010; **257**: 872-878 [PMID: 20935077 DOI: 10.1148/radiol.10100013]

16 **Jalal Z**, Iriart X, De Lédinghen V, Barnetche T, Hiriart JB, Vergniol J, Foucher J, Thambo JB. Liver stiffness measurements for evaluation of central venous pressure in congenital heart diseases. *Heart* 2015; **101**: 1499-1504 [PMID: 26085526 DOI: 10.1136/heartjnl-2014-307385]

17 **Yoo BW**, Choi JY, Eun LY, Park HK, Park YH, Kim SU. Congestive hepatopathy after Fontan operation and related factors assessed by transient elastography. *J Thorac Cardiovasc Surg* 2014; **148**: 1498-1505 [PMID: 24823284 DOI: 10.1016/j.jtcvs.2014.04.010]

18 **Friedrich-Rust M**, Koch C, Rentzsch A, Sarrazin C, Schwarz P, Herrmann E, Lindinger A, Sarrazin U, Poynard T, Schäfers HJ, Zeuzem S, Abdul-Khaliq H. Noninvasive assessment of liver fibrosis in patients with Fontan circulation using transient elastography and biochemical fibrosis markers. *J Thorac Cardiovasc Surg* 2008; **135**: 560-567 [PMID: 18329470 DOI: 10.1016/j.jtcvs.2007.09.039]

19 **Wu FM**, Opotowsky AR, Raza R, Harney S, Ukomadu C, Landzberg MJ, Valente AM, Breitbart RE, Singh MN, Gauvreau K, Jonas MM. Transient elastography may identify Fontan patients with

unfavorable hemodynamics and advanced hepatic fibrosis. *Congenit Heart Dis* 2014; **9**: 438-447 [PMID: 24418160 DOI: 10.1111/chd.12159]

20 **Trifan A**, Sfarti C, Cojocariu C, Dimache M, Cretu M, Hutanasu C, Stanciu C. Increased liver stiffness in extrahepatic cholestasis caused by choledocholithiasis. *Hepat Mon* 2011; **11**: 372-375 [PMID: 22087164]

21 **Millonig G**, Reimann FM, Friedrich S, Fonouni H, Mehrabi A, Büchler MW, Seitz HK, Mueller S. Extrahepatic cholestasis increases liver stiffness (FibroScan) irrespective of fibrosis. *Hepatology* 2008; **48**: 1718-1723 [PMID: 18836992 DOI: 10.1002/hep.22577]

22 **Yashima Y**, Tsujino T, Masuzaki R, Nakai Y, Hirano K, Tateishi R, Sasahira N, Isayama H, Tada M, Yoshida H, Kawabe T, Omata M. Increased liver elasticity in patients with biliary obstruction. *J Gastroenterol* 2011; **46**: 86-91 [PMID: 20814804 DOI: 10.1007/s00535-010-0290-9]

23 **Chang PE**, Lui HF, Chau YP, Lim KH, Yap WM, Tan CK, Chow WC. Prospective evaluation of transient elastography for the diagnosis of hepatic fibrosis in Asians: comparison with liver biopsy and aspartate transaminase platelet ratio index. *Aliment Pharmacol Ther* 2008; **28**: 51-61 [PMID: 18410556 DOI: 10.1111/j.1365-2036.2008.03711.x]

24 **Fraquelli M**, Rigamonti C, Casazza G, Conte D, Donato MF, Ronchi G, Colombo M. Reproducibility of transient elastography in the evaluation of liver fibrosis in patients with chronic liver disease. *Gut* 2007; **56**: 968-973 [PMID: 17255218]

25 **Castéra L**, Foucher J, Bernard PH, Carvalho F, Allaix D, Merrouche W, Couzigou P, de Lédinghen V. Pitfalls of liver stiffness measurement: a 5-year prospective study of 13,369 examinations. *Hepatology* 2010; **51**: 828-835 [PMID: 20063276 DOI: 10.1002/hep.23425]

26 **Kettaneh A**, Marcellin P, Douvin C, Poupon R, Ziol M, Beaugrand M, de Lédinghen V. Features associated with success rate and performance of FibroScan measurements for the diagnosis of cirrhosis in HCV patients: a prospective study of 935 patients. *J Hepatol* 2007; **46**: 628-634 [PMID: 17258346]

27 **Lucidarme D**, Foucher J, Le Bail B, Vergniol J, Castera L, Duburque C, Forzy G, Filoche B, Couzigou P, de Lédinghen V. Factors of accuracy of transient elastography (fibroscan) for the diagnosis of liver fibrosis in chronic hepatitis C. *Hepatology* 2009; **49**: 1083-1089 [PMID: 19140221 DOI: 10.1002/hep.22748]

28 **Ingiliz P**, Chhay KP, Munteanu M, Lebray P, Ngo Y, Roulot D, Benhamou Y, Thabut D, Ratziu V, Poynard T. Applicability and variability of liver stiffness measurements according to probe position. *World J Gastroenterol* 2009; **15**: 3398-3404 [PMID: 19610141]

29 **Pang JX**, Pradhan F, Zimmer S, Niu S, Crotty P, Tracey J, Schneider C, Heitman SJ, Kaplan GG, Swain MG, Myers RP. The feasibility and reliability of transient elastography using Fibroscan®: a practice audit of 2335 examinations. *Can J Gastroenterol Hepatol* 2014; **28**: 143-149 [PMID: 24619636]

30 **Wong GL**, Wong VW, Chim AM, Yiu KK, Chu SH, Li MK, Chan HL. Factors associated with unreliable liver stiffness measurement and its failure with transient elastography in the Chinese population. *J Gastroenterol Hepatol* 2011; **26**: 300-305 [PMID: 21261720]

31 **de Lédinghen V**, Vergniol J. Transient elastography (FibroScan). *Gastroenterol Clin Biol* 2008; **32**: 58-67 [PMID: 18973847 DOI: 10.1016/S0399-8320(08)73994-0]

32 **de Lédinghen V**, Vergniol J, Foucher J, El-Hajbi F, Merrouche W, Rigalleau V. Feasibility of liver transient elastography with FibroScan using a new probe for obese patients. *Liver Int* 2010; **30**: 1043-1048 [PMID: 20492500 DOI: 10.1111/j.1478-3231.2010.02258.x]

33 **Şirli R**, Sporea I, Deleanu A, Culcea L, Szilaski M, Popescu A, Dănilă M. Comparison between the M and XL probes for liver fibrosis assessment by transient elastography. *Med Ultrason* 2014; **16**: 119-122 [PMID: 24791843]

34 **Tsochatzis EA**, Gurusamy KS, Ntaoula S, Cholongitas E, Davidson BR, Burroughs AK. Elastography for the diagnosis of severity of fibrosis in chronic liver disease: a meta-analysis of diagnostic accuracy. *J Hepatol* 2011; **54**: 650-659 [PMID: 21146892]

35 **Cardoso AC**, Carvalho-Filho RJ, Stern C, Dipumpo A, Giuily N, Ripault MP, Asselah T, Boyer N, Lada O, Castelnau C, Martinot-Peignoux M, Valla DC, Bedossa P, Marcellin P. Direct comparison of diagnostic performance of transient elastography in patients with chronic hepatitis B and chronic hepatitis C. *Liver Int* 2012; **32**: 612-621 [PMID: 22103765 DOI: 10.1111/j.1478-3231.2011.02660.x]

36 **Sporea I**, Sirli R, Deleanu A, Tudora A, Popescu A, Curescu M, Bota S. Liver stiffness measurements in patients with HBV vs HCV chronic hepatitis: a comparative study. *World J Gastroenterol* 2010; **16**: 4832-4837 [PMID: 20939112]

37 **Yoneda M**, Yoneda M, Mawatari H, Fujita K, Endo H, Iida H, Nozaki Y, Yonemitsu K, Higurashi T, Takahashi H, Kobayashi N, Kirikoshi H, Abe Y, Inamori M, Kubota K, Saito S, Tamano M, Hiraishi H, Maeyama S, Yamaguchi N, Togo S, Nakajima A. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with nonalcoholic fatty liver disease (NAFLD). *Dig Liver Dis* 2008; **40**: 371-378 [PMID: 18083083]

38 **Wong VW**, Vergniol J, Wong GL, Foucher J, Chan HL, Le Bail B, Choi PC, Kowo M, Chan AW, Merrouche W, Sung JJ, de Lédinghen V. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. *Hepatology* 2010; **51**: 454-462 [PMID: 20101745]

39 **Kwok R**, Tse YK, Wong GL, Ha Y, Lee AU, Ngu MC, Chan HL, Wong VW. Systematic review with meta-analysis: non-invasive assessment of non-alcoholic fatty liver disease--the role of transient elastography and plasma cytokeratin-18 fragments. *Aliment Pharmacol Ther* 2014; **39**: 254-269 [PMID: 24308774 DOI: 10.1111/apt.12569]

40 **Ganne-Carrié N**, Ziol M, de Ledinghen V, Douvin C, Marcellin P, Castera L, Dhumeaux D, Trinchet JC, Beaugrand M. Accuracy of liver stiffness measurement for the diagnosis of cirrhosis in patients with chronic liver diseases. *Hepatology* 2006; **44**: 1511-1517 [PMID: 17133503]

41 **Viganò M**, Paggi S, Lampertico P, Fraquelli M, Massironi S, Ronchi G, Rigamonti C, Conte D, Colombo M. Dual cut-off transient elastography to assess liver fibrosis in chronic hepatitis B: a cohort study with internal validation. *Aliment Pharmacol Ther* 2011; **34**: 353-362 [PMID: 21631559 DOI: 10.1111/j.1365-2036.2011.04722.x]

42 **Castera L**. Noninvasive methods to assess liver disease in patients with hepatitis B or C. *Gastroenterology* 2012; **142**: 1293-1302.e4 [PMID: 22537436 DOI: 10.1053/j.gastro.2012.02.017]

43 **Boursier J**, Zarski JP, de Ledinghen V, Rousselet MC, Sturm N, Lebail B, Fouchard-Hubert I, Gallois Y, Oberti F, Bertrais S, Calès P. Determination of reliability criteria for liver stiffness evaluation by transient elastography. *Hepatology* 2013; **57**: 1182-1191 [PMID: 22899556 DOI: 10.1002/hep.25993]

44 **Sandrin L**, Fourquet B, Hasquenoph JM, Yon S, Fournier C, Mal F, Christidis C, Ziol M, Poulet B, Kazemi F, Beaugrand M, Palau R. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. *Ultrasound Med Biol* 2003; **29**: 1705-1713 [PMID: 14698338]

45 **Friedrich-Rust M**, Ong MF, Martens S, Sarrazin C, Bojunga J, Zeuzem S, Herrmann E. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. *Gastroenterology* 2008; **134**: 960-974 [PMID: 18395077]

46 **Talwalkar JA**, Kurtz DM, Schoenleber SJ, West CP, Montori VM. Ultrasound-based transient elastography for the detection of hepatic fibrosis: systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2007; **5**: 1214-1220 [PMID: 17916549]

47 **Castéra L**, Le Bail B, Roudot-Thoraval F, Bernard PH, Foucher J, Merrouche W, Couzigou P, de Lédinghen V. Early detection in routine clinical practice of cirrhosis and oesophageal varices in chronic hepatitis C: comparison of transient elastography (FibroScan) with standard laboratory tests and non-invasive scores. *J Hepatol* 2009; **50**: 59-68 [PMID: 19013661 DOI: 10.1016/j.jhep.2008.08.018]

48 **Degos F**, Perez P, Roche B, Mahmoudi A, Asselineau J, Voitot H, Bedossa P. Diagnostic accuracy of FibroScan and comparison to liver fibrosis biomarkers in chronic viral hepatitis: a multicenter prospective study (the FIBROSTIC study). *J Hepatol* 2010; **53**:

1013-1021 [PMID: 20850886 DOI: 10.1016/j.jhep.2010.05.035]
49 **de Lédinghen V**, Douvin C, Kettaneh A, Ziol M, Roulot D, Marcellin P, Dhumeaux D, Beaugrand M. Diagnosis of hepatic fibrosis and cirrhosis by transient elastography in HIV/hepatitis C virus-coinfected patients. *J Acquir Immune Defic Syndr* 2006; **41**: 175-179 [PMID: 16394849]
50 **Vergara S**, Macías J, Rivero A, Gutiérrez-Valencia A, González-Serrano M, Merino D, Ríos MJ, García-García JA, Camacho A, López-Cortés L, Ruiz J, de la Torre J, Viciana P, Pineda JA. The use of transient elastometry for assessing liver fibrosis in patients with HIV and hepatitis C virus coinfection. *Clin Infect Dis* 2007; **45**: 969-974 [PMID: 17879910]
51 **Rigamonti C**, Donato MF, Fraquelli M, Agnelli F, Ronchi G, Casazza G, Rossi G, Colombo M. Transient elastography predicts fibrosis progression in patients with recurrent hepatitis C after liver transplantation. *Gut* 2008; **57**: 821-827 [PMID: 18218676 DOI: 10.1136/gut.2007.135046]
52 **Harada N**, Soejima Y, Taketomi A, Yoshizumi T, Ikegami T, Yamashita Y, Itoh S, Kuroda Y, Maehara Y. Assessment of graft fibrosis by transient elastography in patients with recurrent hepatitis C after living donor liver transplantation. *Transplantation* 2008; **85**: 69-74 [PMID: 18192914 DOI: 10.1097/01.tp.0000297248.18483.16]
53 **Castera L**, Denis J, Babany G, Roudot-Thoraval F. Evolving practices of non-invasive markers of liver fibrosis in patients with chronic hepatitis C in France: time for new guidelines? *J Hepatol* 2007; **46**: 528-529; author reply 529-530 [PMID: 17239479]
54 EASL Recommendations on Treatment of Hepatitis C 2015. *J Hepatol* 2015; **63**: 199-236 [PMID: 25911336 DOI: 10.1016/j.jhep.2015.03.025]
55 **Lin CL**, Liao LY, Liu CJ, Yu MW, Chen PJ, Lai MY, Chen DS, Kao JH. Hepatitis B viral factors in HBeAg-negative carriers with persistently normal serum alanine aminotransferase levels. *Hepatology* 2007; **45**: 1193-1198 [PMID: 17464993]
56 **Nakazawa T**, Shibuya A, Takeuchi A, Shibata Y, Hidaka H, Okuwaki Y, Takada J, Tanaka Y, Watanabe M, Minamino T, Sakurai K, Koizumi W. Viral level is an indicator of long-term outcome of hepatitis B virus e antigen-negative carriers with persistently normal serum alanine aminotransferase levels. *J Viral Hepat* 2011; **18**: e191-e199 [PMID: 21692932 DOI: 10.1111/j.1365-2893.2010.01427.x]
57 **Maimone S**, Calvaruso V, Pleguezuelo M, Squadrito G, Amaddeo G, Jacobs M, Khanna P, Raimondo G, Dusheiko G. An evaluation of transient elastography in the discrimination of HBeAg-negative disease from inactive hepatitis B carriers. *J Viral Hepat* 2009; **16**: 769-774 [PMID: 19709363 DOI: 10.1111/j.1365-2893.2009.01120.x]
58 **Fung J**, Lai CL, Chan SC, But D, Seto WK, Cheng C, Wong DK, Lo CM, Fan ST, Yuen MF. Correlation of liver stiffness and histological features in healthy persons and in patients with occult hepatitis B, chronic active hepatitis B, or hepatitis B cirrhosis. *Am J Gastroenterol* 2010; **105**: 1116-1122 [PMID: 19920809 DOI: 10.1038/ajg.2009.665]
59 **Loomba R**, Sanyal AJ. The global NAFLD epidemic. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 686-690 [PMID: 24042449 DOI: 10.1038/nrgastro.2013.171]
60 **Vernon G**, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther* 2011; **34**: 274-285 [PMID: 21623852]
61 **Nobili V**, Vizzutti F, Arena U, Abraldes JG, Marra F, Pietrobattista A, Fruhwirth R, Marcellini M, Pinzani M. Accuracy and reproducibility of transient elastography for the diagnosis of fibrosis in pediatric nonalcoholic steatohepatitis. *Hepatology* 2008; **48**: 442-448 [PMID: 18563842 DOI: 10.1002/hep.22376]
62 **Lupsor M**, Badea R, Stefanescu H, Grigorescu M, Serban A, Radu C, Crişan D, Sparchez Z, Iancu S, Maniu A. Performance of unidimensional transient elastography in staging non-alcoholic steatohepatitis. *J Gastrointestin Liver Dis* 2010; **19**: 53-60 [PMID: 20361076]
63 **Petta S**, Di Marco V, Cammà C, Butera G, Cabibi D, Craxì A. Reliability of liver stiffness measurement in non-alcoholic fatty liver disease: the effects of body mass index. *Aliment Pharmacol Ther* 2011; **33**: 1350-1360 [PMID: 21517924]
64 **Wong VW**, Vergniol J, Wong GL, Foucher J, Chan AW, Chermak F, Choi PC, Merrouche W, Chu SH, Pesque S, Chan HL, de Lédinghen V. Liver stiffness measurement using XL probe in patients with nonalcoholic fatty liver disease. *Am J Gastroenterol* 2012; **107**: 1862-1871 [PMID: 23032979 DOI: 10.1038/ajg.2012.331]
65 **Myers RP**, Pomier-Layrargues G, Kirsch R, Pollett A, Duarte-Rojo A, Wong D, Beaton M, Levstik M, Crotty P, Elkashab M. Feasibility and diagnostic performance of the FibroScan XL probe for liver stiffness measurement in overweight and obese patients. *Hepatology* 2012; **55**: 199-208 [PMID: 21898479 DOI: 10.1002/hep.24624]
66 **Mahadeva S**, Mahfudz AS, Vijayanathan A, Goh KL, Kulenthran A, Cheah PL. Performance of transient elastography (TE) and factors associated with discordance in non-alcoholic fatty liver disease. *J Dig Dis* 2013; **14**: 604-610 [PMID: 23859493 DOI: 10.1111/1751-2980.12088]
67 **Yoshioka K**, Hashimoto S, Kawabe N. Measurement of liver stiffness as a non-invasive method for diagnosis of non-alcoholic fatty liver disease. *Hepatol Res* 2015; **45**: 142-151 [PMID: 25040931 DOI: 10.1111/hepr.12388]
68 **Pavlov CS**, Casazza G, Nikolova D, Tsochatzis E, Burroughs AK, Ivashkin VT, Gluud C. Transient elastography for diagnosis of stages of hepatic fibrosis and cirrhosis in people with alcoholic liver disease. *Cochrane Database Syst Rev* 2015; **1**: CD010542 [PMID: 25612182]
69 **Fernandez M**, Trépo E, Degré D, Gustot T, Verset L, Demetter P, Devière J, Adler M, Moreno C. Transient elastography using Fibroscan is the most reliable noninvasive method for the diagnosis of advanced fibrosis and cirrhosis in alcoholic liver disease. *Eur J Gastroenterol Hepatol* 2015; **27**: 1074-1079 [PMID: 26011235 DOI: 10.1097/MEG.0000000000000392]
70 **Mueller S**, Seitz HK, Rausch V. Non-invasive diagnosis of alcoholic liver disease. *World J Gastroenterol* 2014; **20**: 14626-14641 [PMID: 25356026 DOI: 10.3748/wjg.v20.i40.14626]
71 **Corpechot C**, El Naggar A, Poujol-Robert A, Ziol M, Wendum D, Chazouillères O, de Lédinghen V, Dhumeaux D, Marcellin P, Beaugrand M, Poupon R. Assessment of biliary fibrosis by transient elastography in patients with PBC and PSC. *Hepatology* 2006; **43**: 1118-1124 [PMID: 16628644]
72 **Corpechot C**, Carrat F, Poujol-Robert A, Gaouar F, Wendum D, Chazouillères O, Poupon R. Noninvasive elastography-based assessment of liver fibrosis progression and prognosis in primary biliary cirrhosis. *Hepatology* 2012; **56**: 198-208 [PMID: 22271046 DOI: 10.1002/hep.25599]
73 **Obara N**, Ueno Y, Fukushima K, Nakagome Y, Kakazu E, Kimura O, Wakui Y, Kido O, Ninomiya M, Kogure T, Inoue J, Kondo Y, Shiina M, Iwasaki T, Yamamoto T, Shimosegawa T. Transient elastography for measurement of liver stiffness measurement can detect early significant hepatic fibrosis in Japanese patients with viral and nonviral liver diseases. *J Gastroenterol* 2008; **43**: 720-728 [PMID: 18807134 DOI: 10.1007/s00535-008-2225-2]
74 **Friedrich-Rust M**, Müller C, Winckler A, Kriener S, Herrmann E, Holtmeier J, Poynard T, Vogl TJ, Zeuzem S, Hammerstingl R, Sarrazin C. Assessment of liver fibrosis and steatosis in PBC with FibroScan, MRI, MR-spectroscopy, and serum markers. *J Clin Gastroenterol* 2010; **44**: 58-65 [PMID: 19581812 DOI: 10.1097/MCG.0b013e3181a84b8d]
75 **Romanque P**, Stickel F, Dufour JF. Disproportionally high results of transient elastography in patients with autoimmune hepatitis. *Liver Int* 2008; **28**: 1177-1178 [PMID: 18783552 DOI: 10.1111/j.1478-3231.2008.01743.x]
76 **Carrión JA**, Navasa M, Bosch J, Bruguera M, Gilabert R, Forns X. Transient elastography for diagnosis of advanced fibrosis and portal hypertension in patients with hepatitis C recurrence after liver transplantation. *Liver Transpl* 2006; **12**: 1791-1798 [PMID: 16823833]
77 **Kamphues C**, Lotz K, Röcken C, Berg T, Eurich D, Pratschke J, Neuhaus P, Neumann UP. Chances and limitations of non-invasive tests in the assessment of liver fibrosis in liver transplant patients.

Clin Transplant 2010; **24**: 652-659 [PMID: 19925459 DOI: 10.1111/j.1399-0012.2009.01152.x]
78 **Beckebaum S**, Iacob S, Klein CG, Dechêne A, Varghese J, Baba HA, Sotiropoulos GC, Paul A, Gerken G, Cicinnati VR. Assessment of allograft fibrosis by transient elastography and noninvasive biomarker scoring systems in liver transplant patients. *Transplantation* 2010; **89**: 983-993 [PMID: 20335832 DOI: 10.1097/TP.0b013e3181cc66ca]
79 **Adebajo CO**, Talwalkar JA, Poterucha JJ, Kim WR, Charlton MR. Ultrasound-based transient elastography for the detection of hepatic fibrosis in patients with recurrent hepatitis C virus after liver transplantation: a systematic review and meta-analysis. *Liver Transpl* 2012; **18**: 323-331 [PMID: 22140010 DOI: 10.1002/lt.22460]
80 **Laharie D**, Zerbib F, Adhoute X, Boué-Lahorgue X, Foucher J, Castéra L, Rullier A, Bertet J, Couzigou P, Amouretti M, de Lédinghen V. Diagnosis of liver fibrosis by transient elastography (FibroScan) and non-invasive methods in Crohn's disease patients treated with methotrexate. *Aliment Pharmacol Ther* 2006; **23**: 1621-1628 [PMID: 16696812]
81 **Park SH**, Choe JY, Kim SK. Assessment of liver fibrosis by transient elastography in rheumatoid arthritis patients treated with methotrexate. *Joint Bone Spine* 2010; **77**: 588-592 [PMID: 20471892 DOI: 10.1016/j.jbspin.2010.02.024]
82 **Bray AP**, Barnova I, Przemioslo R, Kennedy CT. Liver fibrosis screening for patients with psoriasis taking methotrexate: a cross-sectional study comparing transient elastography and liver biopsy. *Br J Dermatol* 2012; **166**: 1125-1127 [PMID: 21967341 DOI: 10.1111/j.1365-2133.2011.10657.x]
83 **Barbero-Villares A**, Mendoza J, Trapero-Marugan M, Gonzalez-Alvaro I, Daudén E, Gisbert JP, Moreno-Otero R. Evaluation of liver fibrosis by transient elastography in methotrexate treated patients. *Med Clin* (Barc) 2011; **137**: 637-639 [PMID: 21719043 DOI: 10.1016/j.medcli.2010.12.024]
84 **Laharie D**, Seneschal J, Schaeverbeke T, Doutre MS, Longy-Boursier M, Pellegrin JL, Chabrun E, Villars S, Zerbib F, de Lédinghen V. Assessment of liver fibrosis with transient elastography and FibroTest in patients treated with methotrexate for chronic inflammatory diseases: a case-control study. *J Hepatol* 2010; **53**: 1035-1040 [PMID: 20801541 DOI: 10.1016/j.jhep.2010.04.043]
85 **Lynch M**, Higgins E, McCormick PA, Kirby B, Nolan N, Rogers S, Lally A, Vellinga A, Omar H, Collins P. The use of transient elastography and FibroTest for monitoring hepatotoxicity in patients receiving methotrexate for psoriasis. *JAMA Dermatol* 2014; **150**: 856-862 [PMID: 24964792 DOI: 10.1001/jamadermatol.2013.9336]
86 **Kaffenberger BH**, Kaffenberger JA, Wong H, Jarjour W, Levin D, Bechtel MA. Magnetic resonance elastography and transient elastography as non-invasive analyses for liver fibrosis: can they obviate the need for liver biopsy in psoriasis patients treated with methotrexate? *Int J Dermatol* 2015; **54**: 752-756 [PMID: 26108262 DOI: 10.1111/ijd.12923]
87 **Legros L**, Bardou-Jacquet E, Latournerie M, Guillygomarc'h A, Turlin B, Le Lan C, Désille Y, Lainé F, Moirand R, Brissot P, Deugnier Y, Guyader D. Non-invasive assessment of liver fibrosis in C282Y homozygous HFE hemochromatosis. *Liver Int* 2015; **35**: 1731-1738 [PMID: 25495562 DOI: 10.1111/liv.12762]
88 **Adhoute X**, Foucher J, Laharie D, Terrebonne E, Vergniol J, Castéra L, Lovato B, Chanteloup E, Merrouche W, Couzigou P, de Lédinghen V. Diagnosis of liver fibrosis using FibroScan and other noninvasive methods in patients with hemochromatosis: a prospective study. *Gastroenterol Clin Biol* 2008; **32**: 180-187 [PMID: 18496894]
89 **Castiella A**, Zapata E, Alústiza JM. Non-invasive methods for liver fibrosis prediction in hemochromatosis: One step beyond. *World J Hepatol* 2010; **2**: 251-255 [PMID: 21161006 DOI: 10.4254/wjh.v2.i7.251]
90 **Seijo S**, Reverter E, Miquel R, Berzigotti A, Abraldes JG, Bosch J, García-Pagán JC. Role of hepatic vein catheterisation and transient elastography in the diagnosis of idiopathic portal hypertension. *Dig Liver Dis* 2012; **44**: 855-860 [PMID: 22721839 DOI: 10.1016/j.dld.2012.05.005]
91 **Chang PE**, Miquel R, Blanco JL, Laguno M, Bruguera M, Abraldes JG, Bosch J, Garcia-Pagan JC. Idiopathic portal hypertension in patients with HIV infection treated with highly active antiretroviral therapy. *Am J Gastroenterol* 2009; **104**: 1707-1714 [PMID: 19471257 DOI: 10.1038/ajg.2009.165]
92 **Cesari M**, Schiavini M, Marchetti G, Caramma I, Ortu M, Franzetti F, Galli M, Antinori S, Milazzo L. Noncirrhotic portal hypertension in HIV-infected patients: a case control evaluation and review of the literature. *AIDS Patient Care STDS* 2010; **24**: 697-703 [PMID: 20969464 DOI: 10.1089/apc.2010.0160]
93 **Barr RG**, Ferraioli G, Palmeri ML, Goodman ZD, Garcia-Tsao G, Rubin J, Garra B, Myers RP, Wilson SR, Rubens D, Levine D. Elastography Assessment of Liver Fibrosis: Society of Radiologists in Ultrasound Consensus Conference Statement. *Radiology* 2015; **276**: 845-861 [PMID: 26079489 DOI: 10.1148/radiol.2015150619]
94 **Vergniol J**, Foucher J, Castéra L, Bernard PH, Tournan R, Terrebonne E, Chanteloup E, Merrouche W, Couzigou P, de Lédinghen V. Changes of non-invasive markers and FibroScan values during HCV treatment. *J Viral Hepat* 2009; **16**: 132-140 [PMID: 19175875 DOI: 10.1111/j.1365-2893.2008.01055.x]
95 **Chang TT**, Liaw YF, Wu SS, Schiff E, Han KH, Lai CL, Safadi R, Lee SS, Halota W, Goodman Z, Chi YC, Zhang H, Hindes R, Iloeje U, Beebe S, Kreter B. Long-term entecavir therapy results in the reversal of fibrosis/cirrhosis and continued histological improvement in patients with chronic hepatitis B. *Hepatology* 2010; **52**: 886-893 [PMID: 20683932 DOI: 10.1002/hep.23785]
96 **Dienstag JL**, Goldin RD, Heathcote EJ, Hann HW, Woessner M, Stephenson SL, Gardner S, Gray DF, Schiff ER. Histological outcome during long-term lamivudine therapy. *Gastroenterology* 2003; **124**: 105-117 [PMID: 12512035]
97 **Marcellin P**, Gane E, Buti M, Afdhal N, Sievert W, Jacobson IM, Washington MK, Germanidis G, Flaherty JF, Aguilar Schall R, Bornstein JD, Kitrinos KM, Subramanian GM, McHutchison JG, Heathcote EJ. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. *Lancet* 2013; **381**: 468-475 [PMID: 23234725 DOI: 10.1016/S0140-6736(12)61425-1]
98 **Kim SU**, Park JY, Kim do Y, Ahn SH, Choi EH, Seok JY, Lee JM, Park YN, Chon CY, Han KH. Non-invasive assessment of changes in liver fibrosis via liver stiffness measurement in patients with chronic hepatitis B: impact of antiviral treatment on fibrosis regression. *Hepatol Int* 2010; **4**: 673-680 [PMID: 21286337 DOI: 10.1007/s12072-010-9201-7]
99 **Enomoto M**, Mori M, Ogawa T, Fujii H, Kobayashi S, Iwai S, Morikawa H, Tamori A, Sakaguchi H, Sawada A, Takeda S, Habu D, Shiomi S, Kawada N. Usefulness of transient elastography for assessment of liver fibrosis in chronic hepatitis B: Regression of liver stiffness during entecavir therapy. *Hepatol Res* 2010; **40**: 853-861 [PMID: 20887589 DOI: 10.1111/j.1872-034X.2010.00687.x]
100 **Fung J**, Lai CL, Wong DK, Seto WK, Hung I, Yuen MF. Significant changes in liver stiffness measurements in patients with chronic hepatitis B: 3-year follow-up study. *J Viral Hepat* 2011; **18**: e200-e205 [PMID: 21692933 DOI: 10.1111/j.1365-2893.2010.01428.x]
101 **Kim MN**, Kim SU, Kim BK, Park JY, Kim do Y, Ahn SH, Han KH. Long-term changes of liver stiffness values assessed using transient elastography in patients with chronic hepatitis B receiving entecavir. *Liver Int* 2014; **34**: 1216-1223 [PMID: 24267737 DOI: 10.1111/liv.12377]
102 **Fung J**, Lai CL, Cheng C, Wu R, Wong DK, Yuen MF. Mild-to-moderate elevation of alanine aminotransferase increases liver stiffness measurement by transient elastography in patients with chronic hepatitis B. *Am J Gastroenterol* 2011; **106**: 492-496 [PMID: 21157442 DOI: 10.1038/ajg.2010.463]
103 **Lim SG**, Cho SW, Lee YC, Jeon SJ, Lee MH, Cho YJ, Kim SS, Kim YB, Seok JY, Cheong JY, Kim JH. Changes in liver stiffness measurement during antiviral therapy in patients with chronic hepatitis B. *Hepatogastroenterology* 2011; **58**: 539-545 [PMID: 21661428]
104 **Wong GL**, Wong VW, Choi PC, Chan AW, Chim AM, Yiu KK, Chu SH, Chan FK, Sung JJ, Chan HL. On-treatment monitoring of liver

fibrosis with transient elastography in chronic hepatitis B patients. *Antivir Ther* 2011; **16**: 165-172 [PMID: 21447865 DOI: 10.3851/IMP1726]

105 **Ripoll C**, Groszmann R, Garcia-Tsao G, Grace N, Burroughs A, Planas R, Escorsell A, Garcia-Pagan JC, Makuch R, Patch D, Matloff DS, Bosch J. Hepatic venous pressure gradient predicts clinical decompensation in patients with compensated cirrhosis. *Gastroenterology* 2007; **133**: 481-488 [PMID: 17681169]

106 **Vizzutti F**, Arena U, Romanelli RG, Rega L, Foschi M, Colagrande S, Petrarca A, Moscarella S, Belli G, Zignego AL, Marra F, Laffi G, Pinzani M. Liver stiffness measurement predicts severe portal hypertension in patients with HCV-related cirrhosis. *Hepatology* 2007; **45**: 1290-1297 [PMID: 17464971]

107 **Lemoine M**, Katsahian S, Ziol M, Nahon P, Ganne-Carrie N, Kazemi F, Grando-Lemaire V, Trinchet JC, Beaugrand M. Liver stiffness measurement as a predictive tool of clinically significant portal hypertension in patients with compensated hepatitis C virus or alcohol-related cirrhosis. *Aliment Pharmacol Ther* 2008; **28**: 1102-1110 [PMID: 18691352 DOI: 10.1111/j.1365-2036.2008.03825.x]

108 **Bureau C**, Metivier S, Peron JM, Selves J, Robic MA, Gourraud PA, Rouquet O, Dupuis E, Alric L, Vinel JP. Transient elastography accurately predicts presence of significant portal hypertension in patients with chronic liver disease. *Aliment Pharmacol Ther* 2008; **27**: 1261-1268 [PMID: 18397389 DOI: 10.1111/j.1365-2036.2008.03701.x]

109 **Llop E**, Berzigotti A, Reig M, Erice E, Reverter E, Seijo S, Abraldes JG, Bruix J, Bosch J, García-Pagan JC. Assessment of portal hypertension by transient elastography in patients with compensated cirrhosis and potentially resectable liver tumors. *J Hepatol* 2012; **56**: 103-108 [PMID: 21827733 DOI: 10.1016/j.jhep.2011.06.027]

110 **Sánchez-Conde M**, Miralles P, Bellón JM, Rincón D, Ramírez M, Gutiérrez I, Ripoll C, López JC, Cosín J, Clemente G, Lo Iacono O, Bañares R, Berenguer J. Use of transient elastography (FibroScan ®) for the noninvasive assessment of portal hypertension in HIV/HCV-coinfected patients. *J Viral Hepat* 2011; **18**: 685-691 [PMID: 21914085 DOI: 10.1111/j.1365-2893.2010.01371.x]

111 **Shi KQ**, Fan YC, Pan ZZ, Lin XF, Liu WY, Chen YP, Zheng MH. Transient elastography: a meta-analysis of diagnostic accuracy in evaluation of portal hypertension in chronic liver disease. *Liver Int* 2013; **33**: 62-71 [PMID: 22973991 DOI: 10.1111/liv.12003]

112 **Tapper EB**, Castera L, Afdhal NH. FibroScan (vibration-controlled transient elastography): where does it stand in the United States practice. *Clin Gastroenterol Hepatol* 2015; **13**: 27-36 [PMID: 24909907 DOI: 10.1016/j.cgh.2014.04.039]

113 **Singh S**, Fujii LL, Murad MH, Wang Z, Asrani SK, Ehman RL, Kamath PS, Talwalkar JA. Liver stiffness is associated with risk of decompensation, liver cancer, and death in patients with chronic liver diseases: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2013; **11**: 1573-1584.e1-2; quiz e88-89 [PMID: 23954643 DOI: 10.1016/j.cgh.2013.07.034]

114 **Kim BK**, Fung J, Yuen MF, Kim SU. Clinical application of liver stiffness measurement using transient elastography in chronic liver disease from longitudinal perspectives. *World J Gastroenterol* 2013; **19**: 1890-1900 [PMID: 23569334 DOI: 10.3748/wjg.v19.i12.1890]

115 **Foucher J**, Chanteloup E, Vergniol J, Castéra L, Le Bail B, Adhoute X, Bertet J, Couzigou P, de Lédinghen V. Diagnosis of cirrhosis by transient elastography (FibroScan): a prospective study. *Gut* 2006; **55**: 403-408 [PMID: 16020491]

116 **Kazemi F**, Kettaneh A, N'kontchou G, Pinto E, Ganne-Carrie N, Trinchet JC, Beaugrand M. Liver stiffness measurement selects patients with cirrhosis at risk of bearing large oesophageal varices. *J Hepatol* 2006; **45**: 230-235 [PMID: 16797100]

117 **Pritchett S**, Cardenas A, Manning D, Curry M, Afdhal NH. The optimal cut-off for predicting large oesophageal varices using transient elastography is disease specific. *J Viral Hepat* 2011; **18**: e75-e80 [PMID: 21040236 DOI: 10.1111/j.1365-2893.2010.01375.x]

118 **Kim BK**, Oh HJ, Park JY, Kim do Y, Ahn SH, Han KH, Park Y, Yoo EJ, Park YN, Kim SU. Early on-treatment change in liver stiffness predicts development of liver-related events in chronic hepatitis B patients receiving antiviral therapy. *Liver Int* 2013; **33**: 180-189 [PMID: 23295050 DOI: 10.1111/liv.12020]

119 **Castera L**, Pinzani M, Bosch J. Non invasive evaluation of portal hypertension using transient elastography. *J Hepatol* 2012; **56**: 696-703 [PMID: 21767510 DOI: 10.1016/j.jhep.2011.07.005]

120 **Robic MA**, Procopet B, Métivier S, Péron JM, Selves J, Vinel JP, Bureau C. Liver stiffness accurately predicts portal hypertension related complications in patients with chronic liver disease: a prospective study. *J Hepatol* 2011; **55**: 1017-1024 [PMID: 21354450 DOI: 10.1016/j.jhep.2011.01.051]

121 **Masuzaki R**, Tateishi R, Yoshida H, Goto E, Sato T, Ohki T, Imamura J, Goto T, Kanai F, Kato N, Ikeda H, Shiina S, Kawabe T, Omata M. Prospective risk assessment for hepatocellular carcinoma development in patients with chronic hepatitis C by transient elastography. *Hepatology* 2009; **49**: 1954-1961 [PMID: 19434742 DOI: 10.1002/hep.22870]

122 **Kim BK**, Han KH, Park JY, Ahn SH, Kim JK, Paik YH, Lee KS, Chon CY, Kim do Y. A liver stiffness measurement-based, noninvasive prediction model for high-risk esophageal varices in B-viral liver cirrhosis. *Am J Gastroenterol* 2010; **105**: 1382-1390 [PMID: 20087336 DOI: 10.1038/ajg.2009.750]

123 **Lee HW**, Yoo EJ, Kim BK, Kim SU, Park JY, Kim do Y, Ahn SH, Han KH. Prediction of development of liver-related events by transient elastography in hepatitis B patients with complete virological response on antiviral therapy. *Am J Gastroenterol* 2014; **109**: 1241-1249 [PMID: 24957159 DOI: 10.1038/ajg.2014.157]

124 **Sasso M**, Tengher-Barna I, Ziol M, Miette V, Fournier C, Sandrin L, Poupon R, Cardoso AC, Marcellin P, Douvin C, de Ledinghen V, Trinchet JC, Beaugrand M. Novel controlled attenuation parameter for noninvasive assessment of steatosis using Fibroscan(®): validation in chronic hepatitis C. *J Viral Hepat* 2012; **19**: 244-253 [PMID: 22404722 DOI: 10.1111/j.1365-2893.2011.01534.x]

125 **Kumar M**, Rastogi A, Singh T, Behari C, Gupta E, Garg H, Kumar R, Bhatia V, Sarin SK. Controlled attenuation parameter for non-invasive assessment of hepatic steatosis: does etiology affect performance? *J Gastroenterol Hepatol* 2013; **28**: 1194-1201 [PMID: 23425053 DOI: 10.1111/jgh.12134]

126 **Chon YE**, Jung KS, Kim SU, Park JY, Park YN, Kim do Y, Ahn SH, Chon CY, Lee HW, Park Y, Han KH. Controlled attenuation parameter (CAP) for detection of hepatic steatosis in patients with chronic liver diseases: a prospective study of a native Korean population. *Liver Int* 2014; **34**: 102-109 [PMID: 24028214 DOI: 10.1111/liv.12282]

127 **Castera L**, Winnock M, Pambrun E, Paradis V, Perez P, Loko MA, Asselineau J, Dabis F, Degos F, Salmon D. Comparison of transient elastography (FibroScan), FibroTest, APRI and two algorithms combining these non-invasive tests for liver fibrosis staging in HIV/HCV coinfected patients: ANRS CO13 HEPAVIH and FIBROSTIC collaboration. *HIV Med* 2014; **15**: 30-39 [PMID: 24007567 DOI: 10.1111/hiv.12082]

128 **Zarski JP**, Sturm N, Guechot J, Paris A, Zafrani ES, Asselah T, Boisson RC, Bosson JL, Guyader D, Renversez JC, Bronowicki JP, Gelineau MC, Tran A, Trocme C, De Ledinghen V, Lasnier E, Poujol-Robert A, Ziegler F, Bourliere M, Voitot H, Larrey D, Rosenthal-Allieri MA, Fouchard Hubert I, Bailly F, Vaubourdolle M. Comparison of nine blood tests and transient elastography for liver fibrosis in chronic hepatitis C: the ANRS HCEP-23 study. *J Hepatol* 2012; **56**: 55-62 [PMID: 21781944 DOI: 10.1016/j.jhep.2011.05.024]

129 **Kim BK**, Han KH, Park JY, Ahn SH, Chon CY, Kim JK, Paik YH, Lee KS, Park YN, Kim do Y. A novel liver stiffness measurement-based prediction model for cirrhosis in hepatitis B patients. *Liver Int* 2010; **30**: 1073-1081 [PMID: 20492510 DOI: 10.1111/j.1478-3231.2010.02269.x]

130 **Lee HJ**, Seo YS, Kim DJ, Kang HS, An H, Kim JH, Cheong JY, Yim HJ, Yeon JE, Lee HS, Byun KS, Cho SW, Kim DJ, Um SH, Kim CD, Ryu HS. Application of the HALF index obviates the need for liver biopsy in half of all patients with chronic hepatitis B. *J Gastroenterol Hepatol* 2011; **26**: 987-995 [PMID: 21198828 DOI: 10.1111/j.1440-1746.2010.06609.x]

131 **AASLD/IDSA HCV Guidance Panel**. Hepatitis C guidance: AASLD-IDSA recommendations for testing, managing, and treating

adults infected with hepatitis C virus. *Hepatology* 2015; **62**: 932-954 [PMID: 26111063 DOI: 10.1002/hep.27950]

132 **Muthupillai R**, Lomas DJ, Rossman PJ, Greenleaf JF, Manduca A, Ehman RL. Magnetic resonance elastography by direct visualization of propagating acoustic strain waves. *Science* 1995; **269**: 1854-1857 [PMID: 7569924]

133 **Huwart L**, Peeters F, Sinkus R, Annet L, Salameh N, ter Beek LC, Horsmans Y, Van Beers BE. Liver fibrosis: non-invasive assessment with MR elastography. *NMR Biomed* 2006; **19**: 173-179 [PMID: 16521091]

134 **Huwart L**, Sempoux C, Salameh N, Jamart J, Annet L, Sinkus R, Peeters F, ter Beek LC, Horsmans Y, Van Beers BE. Liver fibrosis: noninvasive assessment with MR elastography versus aspartate aminotransferase-to-platelet ratio index. *Radiology* 2007; **245**: 458-466 [PMID: 17940304]

135 **Yin M**, Talwalkar JA, Glaser KJ, Manduca A, Grimm RC, Rossman PJ, Fidler JL, Ehman RL. Assessment of hepatic fibrosis with magnetic resonance elastography. *Clin Gastroenterol Hepatol* 2007; **5**: 1207-1213.e2 [PMID: 17916548]

136 **Lee Yj**, Lee JM, Lee JE, Lee KB, Lee ES, Yoon JH, Yu MH, Baek JH, Shin CI, Han JK, Choi BI. MR elastography for noninvasive assessment of hepatic fibrosis: reproducibility of the examination and reproducibility and repeatability of the liver stiffness value measurement. *J Magn Reson Imaging* 2014; **39**: 326-331 [PMID: 23589232 DOI: 10.1002/jmri.24147]

137 **Runge JH**, Bohte AE, Verheij J, Terpstra V, Nederveen AJ, van Nieuwkerk KM, de Knegt RJ, Baak BC, Jansen PL, Sinkus R, Stoker J. Comparison of interobserver agreement of magnetic resonance elastography with histopathological staging of liver fibrosis. *Abdom Imaging* 2014; **39**: 283-290 [PMID: 24366108 DOI: 10.1007/s00261-013-0063-z]

138 **Venkatesh SK**, Wang G, Lim SG, Wee A. Magnetic resonance elastography for the detection and staging of liver fibrosis in chronic hepatitis B. *Eur Radiol* 2014; **24**: 70-78 [PMID: 23928932 DOI: 10.1007/s00330-013-2978-8]

139 **Shire NJ**, Yin M, Chen J, Railkar RA, Fox-Bosetti S, Johnson SM, Beals CR, Dardzinski BJ, Sanderson SO, Talwalkar JA, Ehman RL. Test-retest repeatability of MR elastography for noninvasive liver fibrosis assessment in hepatitis C. *J Magn Reson Imaging* 2011; **34**: 947-955 [PMID: 21751289 DOI: 10.1002/jmri.22716]

140 **Loomba R**, Wolfson T, Ang B, Hooker J, Behling C, Peterson M, Valasek M, Lin G, Brenner D, Gamst A, Ehman R, Sirlin C. Magnetic resonance elastography predicts advanced fibrosis in patients with nonalcoholic fatty liver disease: a prospective study. *Hepatology* 2014; **60**: 1920-1928 [PMID: 25103310 DOI: 10.1002/hep.27362]

141 **Shin SU**, Lee JM, Yu MH, Yoon JH, Han JK, Choi BI, Glaser KJ, Ehman RL. Prediction of esophageal varices in patients with cirrhosis: usefulness of three-dimensional MR elastography with echo-planar imaging technique. *Radiology* 2014; **272**: 143-153 [PMID: 24620910 DOI: 10.1148/radiol.14130916]

142 **Sun HY**, Lee JM, Han JK, Choi BI. Usefulness of MR elastography for predicting esophageal varices in cirrhotic patients. *J Magn Reson Imaging* 2014; **39**: 559-566 [PMID: 24115368 DOI: 10.1002/jmri.24186]

143 **Shi Y**, Guo Q, Xia F, Dzyubak B, Glaser KJ, Li Q, Li J, Ehman RL. MR elastography for the assessment of hepatic fibrosis in patients with chronic hepatitis B infection: does histologic necroinflammation influence the measurement of hepatic stiffness? *Radiology* 2014; **273**: 88-98 [PMID: 24893048 DOI: 10.1148/radiol.14132592]

144 **Huwart L**, Sempoux C, Vicaut E, Salameh N, Annet L, Danse E, Peeters F, ter Beek LC, Rahier J, Sinkus R, Horsmans Y, Van Beers BE. Magnetic resonance elastography for the noninvasive staging of liver fibrosis. *Gastroenterology* 2008; **135**: 32-40 [PMID: 18471441 DOI: 10.1053/j.gastro.2008.03.076]

145 **Bohte AE**, de Niet A, Jansen L, Bipat S, Nederveen AJ, Verheij J, Terpstra V, Sinkus R, van Nieuwkerk KM, de Knegt RJ, Baak BC, Jansen PL, Reesink HW, Stoker J. Non-invasive evaluation of liver fibrosis: a comparison of ultrasound-based transient elastography and MR elastography in patients with viral hepatitis B and C. *Eur Radiol* 2014; **24**: 638-648 [PMID: 24158528 DOI: 10.1007/s00330-013-3046-0]

146 **Bota S**, Herkner H, Sporea I, Salzl P, Sirli R, Neghina AM, Peck-Radosavljevic M. Meta-analysis: ARFI elastography versus transient elastography for the evaluation of liver fibrosis. *Liver Int* 2013; **33**: 1138-1147 [PMID: 23859217 DOI: 10.1111/liv.12240]

P- Reviewer: Cosgrove DO, Sirli R, Sporea I **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Wang CH

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i1.107

World J Gastrointest Pharmacol Ther 2016 February 6; 7(1): 107-111
ISSN 2150-5349 (online)

MINIREVIEWS

Review of vedolizumab for the treatment of ulcerative colitis

Michelle SY Lau, Her Hsin Tsai

Michelle SY Lau, Her Hsin Tsai, IBD Unit, Gastroenterology Department, Castle Hill Hospital, HEY NHS Trust, Cottingham HU16 5JQ, United Kingdom

Author contributions: Lau MSY carried out all the literature searches and prepared the manuscript; Tsai HH made several revisions and prepared the manuscript for publication.

Conflict-of-interest statement: The authors declare no conflicts of interest.

Correspondence to: Her Hsin Tsai, MD, FRCP, IBD Unit, Gastroenterology Department, Castle Hill Hospital, HEY NHS Trust, Castle Road, Cottingham HU16 5JQ, United Kingdom. hhtsai@doctors.org.uk
Telephone: +44-1482-875875
Fax: +44-1482-622049

Received: June 10, 2015
Peer-review started: June 11, 2015
First decision: August 25, 2015
Revised: December 12, 2015
Accepted: December 29, 2015
Article in press: January 4, 2016
Published online: February 6, 2016

Abstract

The review summarises the key data on the efficacy and the safety of vedolizumab in the management of ulcerative colitis.

Key words: Ulcerative colitis; Vedolizumab; Biological therapy; Pharmacology; Safety and efficacy

Core tip: Vedolizumab appears to be effective in the management of moderate to severe ulcerative colitis with a good safety profile.

Lau MSY, Tsai HH. Review of vedolizumab for the treatment of ulcerative colitis. *World J Gastrointest Pharmacol Ther* 2016; 7(1): 107-111 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i1/107.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i1.107

INTRODUCTION

The prevalence and incidence of inflammatory bowel disease (IBD) have been increasing globally, with the highest incidence in Europe and North America. Ulcerative colitis (UC) remains the commonest type of IBD, the annual incidence varying from 0 to 24 per 100000 person years, and prevalence between 4.9 and 505 cases per 100000 worldwide[1]. UC typically causes bloody diarrhoea, urgency and abdominal pain, and runs a relapsing and remitting course. It is associated with significant morbidity, with an estimated 30%-60% of patients experiencing at least one relapse per year, and approximately 20% of patients suffer from severe UC[2].

Conventional treatments for maintenance therapy for UC include 5-aminosalicylates (5-ASA), and additional immunosuppressants such as thiopurine analogues, *e.g.*, azathioprine and 6-mercaptopurine, are used in cases of frequent relapses. Thiopurine analogues have shown to prevent relapse in quiescent UC[3]. Corticosteroids remain the predominant therapy for induction of remission of moderate to severe acute

exacerbation of UC but limited by serious side effects. Over the last two decades, much research has been focussed on understanding the immune processes in the pathogenesis of IBD. More targeted therapies have been developed that specifically inhibit the mediators of gut inflammation, such as monoclonal antibodies, which have revolutionised the treatment for IBD. Infliximab is the first monoclonal antibody inhibitor to be developed which targets the tumour necrosis factor, the key pro-inflammatory cytokine in gut inflammation. The ACT 1 trial showed that patients with moderately to severe UC had a clinical response rate to infliximab of 65.5% at week 8, and almost 50% maintained response at week 30[4]. Adalimumab was subsequently developed, with the ULTRA 2 trial demonstrating a clinical response rate of nearly 50% at week 8, although only 17% patients maintained remission at week 52[5].

ROLE OF INTEGRINS

Recent advances in research have led to the development of drugs targeting alternative pathways of inflammation in IBD. One important pathway which propagates gut inflammation in IBD involves recruitment of circulating T lymphocytes into the intestinal vascular endothelial cells[6]. The trafficking of lymphocytes involve a complex adhesion cascade resulting in tethering, rolling, firm adhesion, and finally migration of lymphocytes from the vascular space into inflamed tissue[7]. Integrins play a critical role in the adhesion cascade. They are heterodimeric receptors composed of an α and β subunit, that is expressed on the surface of circulating lymphocytes where they are activated, and bind to their major ligand, the mucosal addressin-cell adhesion molecule (usually abbreviated, MadCAM-1), selectively expressed on the intestinal endothelium. This aids the binding of circulating lymphocytes onto the endothelium and migration into the lamina propria and tissue, contributing to the inflammatory process in IBD[8].

INTEGRIN ANTIBODY ANTAGONISTS

Integrin antagonists are monoclonal antibodies that block the trafficking of lymphocytes to the intestinal endothelium. The first integrin antagonist to emerge is Natalizumab, a humanised IgG4 monoclonal antibody eventually leading to inhibition of the α4 integrin. It was approved for use in Crohn's disease in 2008. The ENCORE trial reported a clinical response rate of nearly 48% for Natalizumab at 8 to 12 wk for Crohn's disease, compared to 32% in the placebo group[9]. However, the widespread use of Natalizumab was limited by associated increased incidence of progressive multifocal leukoencephalopathy (or PML), a fatal demyelinating disease of the CNS caused by the opportunistic human polyoma John Cunningham (JC) virus[10,11]. Natalizumab inhibits not only α4β7, which is expressed on T lymphocytes bound on the inflamed gut, but also α4β1, which mediates lymphocyte homing exclusively in the central nervous system (CNS).

VEDOLIZUMAB

Vedolizumab (previously known as LDP-02 or MLN02, MLN002), a humanised monoclonal IgG1 antibody, was subsequently developed as a gut selective anti-integrin specifically targeting α4β7 integrins in the gut. This paper reviews the safety and efficacy of vedolizumab as a novel therapy in the management of UC.

EFFICACY

Early clinical trial

Inhibition of monoclonal antibody to α4β7 integrin was initially reported to be effective at inducing remission of colitis in cotton-top tamarin in a double blinded RCT done by Hesterberg *et al*[12] in 1996. Cotton top tamarin monkeys, when kept in captivity, can develop chronic colitis, clinically and histologically resembling UC in humans. Eight cotton top tamarin moneys were diagnosed with chronic colitis endoscopically and histologically, before being administered either a cross-reactive antibody to human α4β7or a non-therapeutic control monoclonal antibody intramuscularly. The intervention group benefited from a rapid improvement of endoscopic and histological inflammatory activity and stool consistency[12]. These encouraging results propelled the study of vedolizumab in phase 1 clinical trials.

Phase Ⅰ trial

In 2000, Feagan *et al*[13] conducted a double-blinded, placebo-controlled, ascending dose trial of humanised α4β7 antibody (LDP-02) in 29 patients with moderate to severe UC. Their inclusion criteria included endoscopically verified UC for at least 25 cm from the anal verge, a minimum of 3 bowel motions a day, and a Mayo score of 5 or more. The median Mayo score being 10. Eligible patients (86%) received and continued on a same dose of concomitant 5-ASA for 3 wk or more, and 34% received some oral prednisolone during the study. The patients were administered either a single dose of humanised antibody (LDP-02) in an increasing dose (0.15 mg/kg subcutaneously, 0.15 mg/kg intravenously (*IV*), 0.5 mg/kg *IV*, and 3 mg/kg *IV*) or placebo in a 5:2 ratio in each group.

A dose of 0.5 mg/kg *IV* was found to be sufficient to completely saturate the antibody receptors for up to 30 d, and to give an endoscopic mucosal response at day 30 with at least a two grades improvement in the Baron score[13].

Phase Ⅱ trials

In 2005, Feagan *et al*[14] conducted a multicentre, double blinded, placebo controlled trial of α4β7

antibody (MLN02) on 181 patients with active UC. Active disease was defined as a UC clinical score of between 5 to 9 points, with a score of at least 1 on either stool frequency or rectal bleeding, with a modified Baron score of at least 2 on sigmoidoscopy, with the disease extending a minimum of 25 cm from the anal verge. Patients with active UC and either on no therapy or stable doses of mesalazine were eligible for inclusion. Excluded were patients on therapy with oral steroids within 4 wk or *IV* steroids within 6 wk prior to screening, topical therapy with mesalazine or steroids in the preceding 1 wk, immunosuppressive therapy the preceding 3 mo and severe active disease. The patients were randomly assigned to receive either 0.5 mg/kg of MLN02 (n = 58), 2 mg/kg of MLN02 (n = 60), or placebo (n = 63) *IV* on day 1 and day 29.

At week 6, the primary outcome of clinical remission (which was defined as UC clinical score of 0-1 plus a modified Baron score of 0-1 without rectal bleeding) was significantly higher in the MLN02 group as compared to placebo (33% in the 0.5 mg/kg, 32% in the 2 mg/kg and 14% in the placebo only group; P = 0.003).

Secondary outcome of clinical response (which was defined as improvement of UC clinical score by at least 3 points) were significantly higher in the MLN02 group compared to placebo (66% in the 0.5 mg/kg, 53% in the 2 mg/kg and 33% in the placebo only group; P = 0.002).

Endoscopically assessed remission rates at week 6 were observed in 28% in the 0.5 mg/kg MLN02 group, 12% in the 2 mg/kg of MLN02 group, and 8% in the placebo group (P = 0.007). A lower median modified Baron grade was also observed in the MLN02 group compared to placebo (1 *vs* 1.5; P = 0.02). The mean IBD questionnaire scores were significantly higher in the MLN02 group compared to placebo (171.5 *vs* 162.5; P = 0.03).

A further Phase Ⅱ dose ranging, randomised controlled trial was undertaken by Parikh *et al*[15] in 2012, using an improved formulation of vedolizumab made with a substance produced in hamster ovary. The study randomised 47 patients from 11 centres in Canada and Russia. Criteria for inclusion were patients aged 18-70 years who were diagnosed with UC, confirmed histopathologically or endoscopically (partial Mayo score of > 1), with minimum disease length of 10 years. These patients were randomised to receive either vedolizumab (n = 38) [2 mg/kg (n = 13), 6 mg/kg (n = 14), or 10 mg/kg (n = 11)] or just placebo (n = 9) on days 1, 15, 29 and 85, and were followed up for 253 d. This study used higher doses of vedolizumab administered with shorter frequency in between doses compared to previous trials. The aim of the study was to assess the safety, pharmacokinetics, pharmacodynamics and immunogenicity of the new formulation of vedolizumab. Clinical remission and response were assessed by partial Mayo score and faecal calprotectin. The study demonstrated pharmacokinetics which were dose-proportional and vedolizumab maximally saturated $\alpha4\beta7$ receptors over the tested dosing. Multiple doses up to 10 mg/kg were very well-tolerated with no adverse events leading to drug discontinuation. The clinical response rate of those receiving vedolizumab was over 50% compared to 22%-33% in the placebo group. Vedolizumab treatment was also shown to reduce faecal calprotectin levels compared to placebo.

Phase Ⅲ trial

The GEMINI 1 trial was published in 2013 by Feagan *et al*[16]. This phase Ⅲ trial was a randomised, double-blinded, placebo-controlled study on the efficacy, safety and tolerability of vedolizumab (MLN002) in patients with moderate to severe UC. The GEMINI 1 trial consisted of 2 separate trials on vedolizumab as both induction and maintenance therapy for UC involving 895 patients in 34 countries. Patients were eligible if they were 18 to 80 years of age with active UC (defined by a Mayo score of 6 to 12, with a sigmoidoscopy sub-score of at least 2, and disease extending 15 cm or more from the anal margin). An additional criteria was previously failed treatment with steroids, immunosuppressives or anti-TNF therapy.

In a trial of induction therapy, 374 patients received either vedolizumab 300 mg (n = 224) or only placebo (n = 149) *IV* at weeks zero and 2. Results showed a significantly greater percentage of patients receiving vedolizumab achieving clinical response (47% *vs* 26%; P < 0.001), with clinical remission (17% *vs* 5%; P = 0.0009) and with mucosal healing (41% *vs* 25%; P = 0.0012) compared to placebo. The study also included a second group of 521 patients receiving open-labelled vedolizumab in parallel with the first cohort, with similar results. Remission rates and clinical response were higher in the vedolizumab group amongst patients who had been anti-TNF naïve and also those who had prior anti-TNF failure, when compared to placebo (Table 1).

In a trial of maintenance therapy, patients in either of above cohorts who had responded to vedolizumab at week 6 were then randomly assigned to continue receiving vedolizumab 300 mg *IV* at 4 wk intervals (n = 125), vedolizumab 300 mg *IV* at 8 wk intervals (n = 122), or placebo (n = 126) for 52 wk. The authors assessed outcome measures of clinical remission rate at 52 wk, durable clinical response (defined as a response at weeks 6 and 52), durable clinical remission (which was defined as remission at weeks 6 and 52), mucosal healing at 52 wk and steroid free remission at 52 wk. Results showed that a significantly greater percentage of patients receiving vedolizumab reached clinical remission (45% for the vedolizumab 4 weekly group, 52% for the vedolizumab 8 weekly group, and 16% for the placebo group; P < 0.001) and mucosal healing at 52 wk (56% for the vedolizumab 4 weekly

Table 1 Principal trial results

Ref.	Sample size (*n*)	Phase of trial	Treatment arms (*n*)	Clinical remission (%)	Clinical response (%)
Feagan *et al*[14], 2005	181	II	Placebo	14	33
			0.5 mg/kg *IV*	33	66
			2 mg/kg *IV*	32	53
Parikh *et al*[15], 2012	47	II	Placebo	33	22-33
			2 mg/kg *IV*	68-89[1]	> 50[1]
			6 mg/kg *IV*		
			10 mg/kg *IV*		
Feagan *et al*[16], 2012	374	III			
		Induction phase	Placebo	5.4	25.5
			300 mg *IV*	16.9	47.1
		Maintenance phase	Placebo	15.9	23.8
			300 mg *IV* 4 weekly	44.8	56.6
			300 mg *IV* 8 weekly	41.8	52

[1]Collective results for all vedolizumab groups combined. *IV*: Intravenously.

Table 2 Adverse events

Ref.	Group	UC aggravated	Nausea/ vomiting	Headache	Frequent bowel movement	Fatigue	Upper respiratory tract infection	Abdominal pain	Arthralgia	Dizziness	Rash
Feagan *et al*[14],	Placebo	24	15	13	10	7	5	16	5	1	4
2005	0.5 mg/kg *IV*	29	21	12	10	8	8	10	4	6	6
	2 mg/kg *IV*	22	13	11	5	5	8	6	7	4	4
Parikh *et al*[15],	Placebo	4		1			4			1	
2012	2 mg/kg *IV*	2		2			4			1	
	6 mg/kg *IV*	1		3			3			0	
	10 mg/kg *IV*	0		2			1			0	
Feagan *et al*[16],	Placebo	58	19	28		10	21	10	25		
2012	300 mg *IV*	97	38	80		33	52	35	56		

IV: Intravenously; UC: Ulcerative colitis.

group, 52% for the vedolizumab 8 weekly group and 20% for the placebo group; $P < 0.001$) compared to placebo. The proportion of patients who were steroid-free at week 52 was also significantly more in the vedolizumab group (45% for the vedolizumab 4 weekly group, 31% for the vedolizumab 8 weekly group, and 14% for the placebo group; $P = 0.012$/$P < 0.0001$). The authors did not find a clear difference in efficacy between the 4 and 8 weekly vedolizumab regimes (Table 1).

ADVERSE EFFECTS

Vedolizumab displays a relatively benign adverse effect profile. The large GEMINI 1 trial[16] reported the most common adverse effects included an exacerbation of colitis, headache and nasopharyngitis. A similar set of adverse effects were reported by Parikh *et al*[15] However, overall the frequency for which these events occur are uncommon. Certainly Parikh *et al*[15] reported no withdrawal from their clinical trial as a consequence of adverse effects. The concern regarding PML has also not proven to be of significance[15,16]. There is yet to be an index case accountable to vedolizumab. Overall, it is reassuring that meta-analyses of several RCTs have found no significant difference in the number of serious adverse events of vedolizumab when compared to placebo (Table 2).

CONCLUSION

Vedolizumab is a novel, humanised, monoclonal IgG1-type antibody, developed as a gut selective anti-integrin specifically targeting $\alpha4\beta7$ integrins in the gut. Clinical studies have demonstrated efficacy in the induction and the maintenance of response and remission in UC. This places it amongst the biologicals that are currently available for treatment of UC. This includes the anti-TNF antibodies of infliximab, adalimumab and golimumab. It has a different target and thus represents a new front for the suppression of the inflammatory process that fuels colitis. It does not appear to have the same safety issues as natalizumab with no reports of PML.

Vedolizumab's role in the management algorithm of moderately to severe UC remains unclear. Further trials would be needed to answer several questions. Firstly, should vedolizumab be used as the primary biologic after failure of conventional treatment? Secondly, is vedolizumab effective in patients who are primary anti-TNF failures? Finally does it have a role in patients who

have had secondary loss of response to anti-TNFs? These answers would greatly help the clinician treat UC more effectively.

REFERENCES

1 **Molodecky NA**, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, Benchimol EI, Panaccione R, Ghosh S, Barkema HW, Kaplan GG. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology* 2012; **142**: 46-54.e42; quiz e30 [PMID: 22001864 DOI: 10.1053/j.gastro.2011.10.001]

2 **National Institute for Health and Clinical Excellence**. Management of ulcerative colitis. Clinical guideline 166, June 2013. Available from: URL: https://www.nice.org.uk/guidance/cg166

3 **Khan KJ**, Dubinsky MC, Ford AC, Ullman TA, Talley NJ, Moayyedi P. Efficacy of immunosuppressive therapy for inflammatory bowel disease: a systematic review and meta-analysis. *Am J Gastroenterol* 2011; **106**: 630-642 [PMID: 21407186 DOI: 10.1038/ajg.2011.64]

4 **Rutgeerts P**, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J, Travers S, Rachmilewitz D, Hanauer SB, Lichtenstein GR, de Villiers WJ, Present D, Sands BE, Colombel JF. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2005; **353**: 2462-2476 [PMID: 16339095 DOI: 10.1056/NEJMoa050516]

5 **Sandborn WJ**, van Assche G, Reinisch W, Colombel JF, D'Haens G, Wolf DC, Kron M, Tighe MB, Lazar A, Thakkar RB. Adalimumab induces and maintains clinical remission in patients with moderate-to-severe ulcerative colitis. *Gastroenterology* 2012; **142**: 257-265.e1-3 [PMID: 22062358]

6 **Cominelli F**. Inhibition of leukocyte trafficking in inflammatory bowel disease. *N Engl J Med* 2013; **369**: 775-776 [PMID: 23964940 DOI: 10.1056/NEJMe1307415]

7 **Yonekawa K**, Harlan JM. Targeting leukocyte integrins in human diseases. *J Leukoc Biol* 2005; **77**: 129-140 [PMID: 15548573]

8 **Tilg H**, Kaser A. Vedolizumab, a humanized mAb against the α4β7 integrin for the potential treatment of ulcerative colitis and Crohn's disease. *Curr Opin Investig Drugs* 2010; **11**: 1295-1304 [PMID: 21157649]

9 **Targan SR**, Feagan BG, Fedorak RN, Lashner BA, Panaccione R, Present DH, Spehlmann ME, Rutgeerts PJ, Tulassay Z, Volfova M, Wolf DC, Hernandez C, Bornstein J, Sandborn WJ. Natalizumab for the treatment of active Crohn's disease: results of the ENCORE Trial. *Gastroenterology* 2007; **132**: 1672-1683 [PMID: 17484865 DOI: 10.1053/j.gastro.2007.03.024]

10 **Chen Y**, Bord E, Tompkins T, Miller J, Tan CS, Kinkel RP, Stein MC, Viscidi RP, Ngo LH, Koralnik IJ. Asymptomatic reactivation of JC virus in patients treated with natalizumab. *N Engl J Med* 2009; **361**: 1067-1074 [PMID: 19741227 DOI: 10.1056/NEJMoa0904267]

11 **Bloomgren G**, Richman S, Hotermans C, Subramanyam M, Goelz S, Natarajan A, Lee S, Plavina T, Scanlon JV, Sandrock A, Bozic C. Risk of natalizumab-associated progressive multifocal leukoencephalopathy. *N Engl J Med* 2012; **366**: 1870-1880 [PMID: 22591293 DOI: 10.1056/NEJMoa1107829]

12 **Hesterberg PE**, Winsor-Hines D, Briskin MJ, Soler-Ferran D, Merrill C, Mackay CR, Newman W, Ringler DJ. Rapid resolution of chronic colitis in the cotton-top tamarin with an antibody to a gut-homing integrin alpha 4 beta 7. *Gastroenterology* 1996; **111**: 1373-1380 [PMID: 8898653 DOI: 10.1053/gast.1996.v111.pm8898653]

13 **Feagan B**, Macdonald J, Greenberg G, Wild G, Pare P, Fedorak RN, Landau SB, Brettrnan LR. An ascending dose of a humanized alpha 4 beta 7 antibody in ulcerative colitis (UC). *Gastroenterology* 2000; **118**: A874 [DOI: 10.1016/S0016-5085(00)85637-1]

14 **Feagan BG**, Greenberg GR, Wild G, Fedorak RN, Paré P, McDonald JW, Dubé R, Cohen A, Steinhart AH, Landau S, Aguzzi RA, Fox IH, Vandervoort MK. Treatment of ulcerative colitis with a humanized antibody to the alpha4beta7 integrin. *N Engl J Med* 2005; **352**: 2499-2507 [PMID: 15958805 DOI: 10.1056/NEJMoa042982]

15 **Parikh A**, Leach T, Wyant T, Scholz C, Sankoh S, Mould DR, Ponich T, Fox I, Feagan BG. Vedolizumab for the treatment of active ulcerative colitis: a randomized controlled phase 2 dose-ranging study. *Inflamm Bowel Dis* 2012; **18**: 1470-1479 [PMID: 22147460 DOI: 10.1002/ibd.21896]

16 **Feagan BG**, Rutgeerts PJ, Sands BE, Colombel J, Hanauer SB, Van Assche GA, Axler J, Kim H, Danese S, Fox I, Milch C, Sankoh S, Wyant T, Xu J, Parikh A. 943b Induction therapy for ulcerative colitis: results of GEMINI I, a randomised placebo-controlled, double-blind, multicentre phase 3 trial. *Gastroenterology* 2012; **142**: S160-S161 [DOI: 10.1016/S0016-5085(12)60607-6]

P- Reviewer: Ansari AA, Odes S **S- Editor**: Ji FF **L- Editor**: A **E- Editor**: Wang CH

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i1.112

World J Gastrointest Pharmacol Ther 2016 February 6; 7(1): 112-125
ISSN 2150-5349 (online)

MINIREVIEWS

Influence of environmental factors in the development of inflammatory bowel diseases

Evangelia Legaki, Maria Gazouli

Evangelia Legaki, Maria Gazouli, Department of Basic Medical Sciences, Laboratory of Biology, School of Medicine, University of Athens, 11527 Athens, Greece

Author contributions: Legaki E performed all the literature searching and wrote the manuscript; Gazouli M designed, wrote and edited the manuscript.

Supported by The Hellenic State Scholarships Foundation to Legaki E.

Conflict-of-interest statement: No conflict of interest to state.

Correspondence to: Maria Gazouli, PhD, Assistant Professor, Department of Basic Medical Sciences, Laboratory of Biology, School of Medicine, University of Athens, Michalakopoulou 176, 11527 Athens, Greece. mgazouli@med.uoa.gr
Telephone: +30-210-7462231
Fax: +30-210-7462231

Received: May 28, 2015
Peer-review started: June 1, 2015
First decision: October 14, 2015
Revised: October 20, 2015
Accepted: December 3, 2015
Article in press: December 4, 2015
Published online: February 6, 2016

Abstract

Idiopathic inflammatory bowel diseases (IBD), Crohn's disease (CD) and ulcerative colitis (UC), are multifactorial diseases that are manifested after disruption of a genetic predisposed individual and its intestinal microflora through an environmental stimulus. Urbanization and industrialization are associated with IBD. Epidemiological data, clinical observations and family/immigrants studies indicate the significance of environmental influence in the development of IBD. Some environmental factors have a different effect on the subtypes of IBD. Smoking and appendectomy is negatively associated with UC, but they are aggravating factors for CD. A westernized high fat diet, full of refined carbohydrates is strongly associated with the development of IBD, contrary to a high in fruit, vegetables and polyunsaturated fatty acid-3 diet that is protective against these diseases. High intake of nonsteroidal antiinflammatory drug and oral contraceptive pills as well as the inadequacy of vitamin D leads to an increased risk for IBD and a more malignant course of disease. Moreover, other factors such as air pollution, psychological factors, sleep disturbances and exercise influence the development and the course of IBD. Epigenetic mechanism like DNA methylation, histone modification and altered expression of miRNAS could explain the connection between genes and environmental factors in triggering the development of IBD.

Key words: Crohn's disease; Ulcerative colitis; Epigenetics; Environment

Core tip: Epidemiological data, clinical observations and family/ immigrants studies indicate the significance of environmental influence in the development of inflammatory bowel diseases (IBD). A westernized high fat diet, full of refined carbohydrates is strongly associated with the development of IBD, contrary to a high in fruit, vegetables and polyunsaturated fatty acid-3 diet that is protective against these diseases. Additional factors such as air pollution, psychological factors, sleep disturbances and exercise influence

the development and the course of IBD. Epigenetic mechanism like DNA methylation, histone modification and altered expression of miRNAS could explain the connection between genes and environmental factors in triggering the development of IBD.

Legaki E, Gazouli M. Influence of environmental factors in the development of inflammatory bowel diseases. *World J Gastrointest Pharmacol Ther* 2016; 7(1): 112-125 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i1/112.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i1.112

INTRODUCTION

Idiopathic inflammatory bowel diseases (IBD), Crohn's disease (CD) and ulcerative colitis (UC) are characterized by chronic relapsing inflammation without a particular infectious or environmental cause. IBD are heterogeneous, multifactorial diseases that are manifested after disruption of a genetic predisposed individual and its intestinal microflora through an environmental stimulus, as this leads to faulty response of both the innate (macrophages, neutrophils) and the acquired (T and B cells) immune system. This results in an intense recruitment of immune cells with prolonged survival due to the reduced cell apoptosis. These cells infiltrate the intestinal membrane, enhancing an ongoing inflammatory process[1-4]. IBD are called a disease of developed countries or a disease of the West as they occur more frequently in America and Europe compared with Asia. The incidence of IBD used to be rare in developing countries, but it is rising as these countries are industrialized[5-12]. Furthermore the incidence of IBD varies in different age groups and is primarily a disease of young ages. The pediatric IBD show an increasing trend worldwide, with more references to CD[13-18]. The maximum prevalence of CD is observed in the age group of 16-25 years while UC appears more at ages 30-40 years. The incidence gradually decreases with age for both diseases and present a new peak at the age of 76-85 years. The IBD pediatric cases are estimated at 7%-20% of all cases according to demographic studies[19,20]. Various incidences are observed between different sex and different nationalities. Generally, there is a higher incidence of 20%-30% in women for Crohn's disease, while there is a slight predominance of the male gender in UC appearance.

Urbanization and industrialization are associated with lifestyle changes. Epidemiological data, clinical and laboratory observations indicate the significance of environmental influence in the development of IBD. Family studies, mostly twin studies, provide an important tool for the identification of hereditary and environmental contribution in IBD pathogenesis. Family studies records increased prevalence in first degree relatives[21-23]. In large European studies conducted in Sweden, Denmark and the United Kingdom, the rate of CD in monozygotic twins was estimated to range between 20% and 50%, while the rate in dizygotic twins, who were brought up in the same environment, was less than 10%. The corresponding difference in monozygotic and dizygotic twins shows the relative effect of genes, however, the low rates highlight the most significant environmental effect on the pathogenesis of IBD[24-27]. Studies of immigrant populations suggest that ethnic and racial differences in the incidence of IBD may be more related to lifestyle and environmental influence rather than actual genetic differences[28]. Groups of immigrants who moved from areas with low incidence of IBD to areas with high incidence provide information on the environmental effects on the development of the disease. Migration from a low-incidence to a higher incidence region increases the risk of disease, particularly in the first generation children. The arrival in high risk areas at a younger age increases the risk of developing IBD in immigrants. For example, until recently, IBD thought to be rare in the Indian subcontinent. However, South Asians who moved to the United Kingdom, and their descendants, are at increased risk for UC compared to whites[29-38]. The changing epidemiology of IBD chronically and geographically suggests that environmental factors play an important role in modifying the development and the activity of disease. The rising incidence in developing countries, that have traditionally presented low incidence, shows that IBD is associated with both westernization of lifestyle and industrialization (Figure 1)[6,39].

Smoking

Smoking is one of the most important and well-characterized environmental risk factors for IBD, but its pathogenic mechanism is not clear. Much evidence from studies suggests smoking is a causative agent in CD while it supports the protective role against UC. Smoking cessation dramatically changes the composition and increases the variety of the intestinal microbiome[40-43]. There is a dose-dependent relationship between smoking and IBD. Ex-smokers have a higher risk for UC development, while quitting smoking in UC patients aggravates the clinical outcome of the disease. Similarly, a reduced risk is observed in smokers, where patients tend to a more benign course as flares, hospitalization, need for steroids and colectomy are experienced rarely[44,45], and there is an improvement of disease activity in former smokers who started to smoke again[46,47]. Cigarette smoking appears to have a different impact on men and women with UC, with the beneficial effects appear mostly in men[48,49]. It is remarkable that 52% of patients developed UC in the first three years after quitting smoking[50], while UC patients experienced flares during the first years after smoking cessation[46].

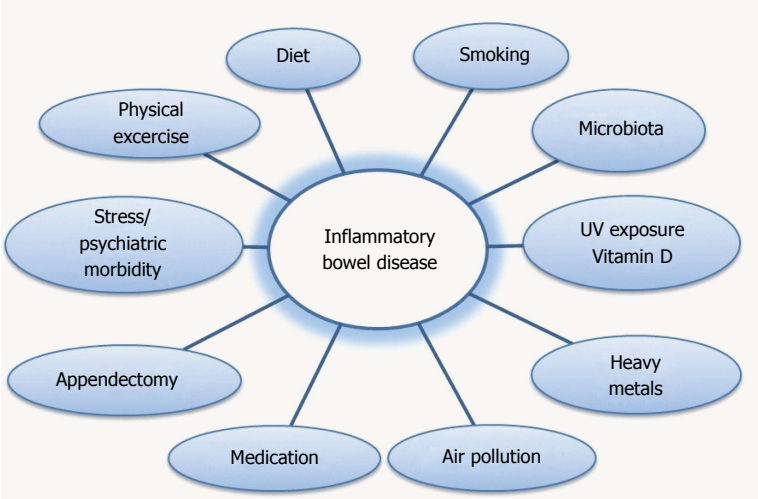

Figure 1 Schematic presentation of the main environmental risks for the development of inflammatory bowel diseases.

A population study confirms the protective role of smoking in UC, concluding that the prevalence of UC was raised 5 times in the Mormon Church population in England and Ireland than in the rest of the population, where smoking is strongly discouraged[51]. Additional pilot studies indicate that nicotine could effectively induce remission in active UC, although its use provokes various mild side-effects such as nausea, headache and sleep disturbance[52,53].

Conversely, smoking doubles the risk of CD compared to that of non-smokers[47,54] and leads to a worse clinical outcome and to a more aggressive disease[43,55,56]. Smoking has been associated with a higher risk of severe relapse, a more complicated disease with development of strictures or fistulae and a higher need for steroids and surgery[45,57-59]. Smoking cessation is a therapeutic strategy for the CD[60]. A study has shown that patients who stop smoking for at least six months have a lower risk of relapse for the next 12-18 mo. Smoking has a greater effect on women[61]. A meta-analysis showed that CD patients who smoke have 2.5 times increased risk of postsurgical recurrence and a double risk of recurrence than nonsmokers[62]. There is not much data for passive smokers, however a study showed that CD patients who are passive smokers needed immunosuppressants and infliximab more often than non-passive smokers. Therefore, secondhand smoke appears to show a similar effect as active smoking, but with weaker results[63]. In addition CD patients are more likely to have been prenatally exposed to tobacco smoke[59].

Appendectomy

Appendectomy also appears to have a different effect in UC and CD. Most studies show a strong negative association between appendectomy and UC suggesting that it can improve the course of disease and the need for colectomy[64-67], whereas a recent work in China found no significant association[68]. Children and adolescents experiencing appendicitis have a reduced risk for UC, as opposed to those who experience appendicitis during adulthood[69]. A population based cohort study of Sweden and Denmark concluded that the incidence of UC was 26% and 13% lower, respectively, in patients who had undergone appendectomy[70]. Also, a study from Spain showed that appendectomy was less common not only in patients with UC but also in their relatives[71]. In three different experimental mice models of colitis, removing the appendix prevented the development of colitis[72]. However, it is believed that appendicitis provides a protective role against UC, not its resection[73,74]. A meta-analysis of studies showed an increased risk for CD development in the first year after appendectomy, whereas five years later the risk for CD is no longer important[75].

Drugs

Many studies propose that high frequency use of non-steroid anti-inflammatory medicines, in a large dose and for a long time period increases the risk for UC or CD and leads to disease relapse[76-81]. A study based on the European population suggested that the risk for CD is 6 times increased in those who take aspirin, with a higher incidence in women and young people[82]. Since 1980, many studies have indicated an association between consumption of contraceptive pills and developing of IBD[83-87]. A major recent study confirmed that, recording a greater association with the risk of CD. Women with a history of smoking present a significant association between oral contraceptive pills and UC[88]. Furthermore, early exposure to antibiotics is associated with development of pediatric IBD in a dose dependent relationship. Specifically, antianaerobic antibiotic use during childhood could alter gut flora and promote inflammation[89,90]. Virta *el al*[91] showed that there is higher risk using antibiotics in childhood for CD development than UC. A meta-analysis study confirmed that antibiotic exposure increases the risk of

new- onset CD with a greater risk for children[92]. Two nested case-control analysis of the population-based University of Manitoba Inflammatory Bowel Disease Epidemiologic Database by Shaw *et al*[93] concluded that pediatric IBD patients are more likely to have been exposed to antibiotic use in their first year of life and that IBD patients may have been prescribed with antibiotics 2-5 years before their diagnosis[93,94].

Diet

A Western diet, a diet with high amount of fat and carbohydrates and low amount of fiber, is implicated in the increasing incidence of IBD[95]. Change in human nutritional standards has a great result in shaping the microbiome[96]. Children in Africa, whose diet is rich in fiber have a really different gut-microbial community to European children whose diet contains a high amount of sugar, fat and proteins[97].

Meat consumption has been associated with increased risk of developing IBD, and induce relapse[98,99]. A review of case-control studies and epidemiological data by Asakura *et al*[100] presented significant correlation between animal meat and CD. Likewise, meta-analyses of case studies show a positive correlation between consumption of animal protein or whole protein intake and CD[101]. A recent study population in middle-aged French women showed that high total protein intake, especially animal protein was associated with a significantly increased risk for IBD, while the consumption of eggs and dairy products were respectively associated with IBD[102]. Fish/tone consumption is negatively associated with both colonic and ileal CD[103]. In a study of pediatric patients whose CD was diagnosed before the age of 20 years, children who consumed a greater amount of fruit and vegetables had a lower risk for developing CD, with a significant dose-dependent manner[104].

A larger prospective study of adults also indicates a strong inverse association between fiber intake and risk for IBD, with a weaker effect on UC[105]. Many studies concluded in similar results with a negative association between both fruits and vegetables and development of IBD[103]. Russel *et al*[106] reported that consuming more than five citrus per week was significantly associated with decreased risk of UC. Low intake of raw fruits and vegetables is common in IBD patients. The meta-analysis of Hou *et al*[101] has shown that intake of high- fiber diet and fruits is associated with reduced risk for CD. The protective effect of fiber, however, appears to be related to the source of fiber. Dietary fiber from fruits and vegetables were associated with a reduced risk for CD in the population of Nurses' Health Study, but insoluble fiber from whole grains and bran have not the same significant effect[105]. A study in Japanese population indicated the role of fiber in suppressing patients' inflammation and recommends patients to consume more fiber, such as fruits, vegetables, seaweed, dried mushrooms and dried Japanese radish[107].

In 1976, both groups, Martini and Brandes[108] and Mayberry *et al*[109], were the first to report that CD patients consume excess amount of sugar and products containing refined carbohydrates. The increased consumption of refined sugar and processed carbohydrates can be a risk factor for CD and has also been demonstrated in some UC patients. Intake of refined carbohydrates, fizzy drinks, soft drinks cola, commercial desserts with added sugar, chocolate and/or pastry has been shown in several studies to affect the appearance of IBD. Intake of refined carbohydrates, fizzy drinks, soft drinks cola, commercial desserts with added sugar, chocolate and/or pastry has been implicated in the development of IBD[110-112].

High consumption of rice and pasta has been reported to increase but not significantly the risk for UC, while potato consumption reduces the risk for IBD[103]. High fat diet (HFD) prolongs and exacerbates inflammatory manifestations of chronic UC. In an experimental DSS-colitis model, colon analyses showed mild inflammation in DSS colitis group, which became more serious when HFD was administered[113]. Devkota *et al*[114] demonstrated that consumption of dietary fat can dramatically reshape the gut microflora, and trigger the initiation of colitis. The intake of long chain omega-6 polyunsaturated fatty acids, especially linoleic and arachidonic acid, may contribute to IBD development with UC incidence increased by two- and four-fold, respectively[115-117], in contrary n-3PUFA presents a protective role against IBD[118]. A prospective United Kingdom study showed that the total dietary intake of omega-3 PUFAs, eicosapentaenoic and docosahexaenoic acid, was associated with reduced risk for UC[119]. Similar results were presented in a North American study where it was demonstrated that higher intake of omega-3 long-chain PUFAs is associated with a lower risk for UC and a high long-term intake of trans unsaturated fatty acids is associated with an increased frequency of IBD development[120].

Meta-analysis studies in the role of breastfeeding in the development of IBD during childhood and adulthood presents a statistically significant protective effect for CD[121] and the early onset IBD[122]. Improved sanitary conditions are associated with increased risk of IBD. There is a negative association between IBD risk and family size, showing that many siblings are a protective factor against IBD with a graded manner, supporting the "'old friends' hypothesis", means the exposure to pathogenic microorganisms during childhood[123-125]. Another hygienic protective factor is the presence of a pet at home[126]. Children living in rural crowded homes, consuming unpasteurized milk are at lower IBD risk, mainly CD[127].

Supporting the case of hygiene, negative association exists between some microorganisms such as Helicobacter pylori[128-130] and colonization of parasitic

worms (*i.e.,* helminths)[131-137] and development of IBD. The *Mycobacterium Avium* Paraturbeculosis spp (MAP) is a pathogen that may be a causative agent for IBD. A study indicate that a high percentage of both CD and UC patients have been contaminated with MAP[138-140] and a meta-analysis of 28 case-control studies showed a positive correlation between MAP and CD[141]. Furthermore, other pathogens such as *Salmonella*, *Escherichia coli*, *Clostridium difficile* and *Campylobacter* appear to be involved in the pathogenesis of IBD[142-144]. Moreover the case of cold chain, the correlation of refrigerating food and IBD, mainly CD[145] implicates psychotrofic bacteria with pathogenic properties such as Listeria monocytogenes, *Yersinia enterocolitica*, *Clostridium botulinum* and *Bacillus cereus* identified in CD patients[134,146,147].

Microbiota

The human gastrointestinal tract contains approximately 10-100 trillion microorganisms, the majority of which are anaerobic bacteria. It is estimated that there are more than 500 different species of bacteria in the intestine whose number and composition varies along the gastrointestinal tract. The most commonly found bacteria in normal intestinal flora are *Firmicutes* (49%-76%), *Bacteroidetes* (16%-23%), followed to a less extent by *Proteobacteria*, *Actinobacteria*, *Fusobacteria* and *Verrucomicrobia*[148]. The intestinal microbial community plays an important role for the host, as it carries out many useful functions including the digestion of substrates that host enzymes are unable to digest; the production of vitamins and short chain fatty acid; the formation of enteric immune system; and the protection of enteric homoeostasis repressing the growth of harmful microorganisms[149,150]. Although the diversity of microbes is huge, it appears from recent post-genomic studies that there is a common core of microbial genes which are common for at least 50% of people[151]. A westernized diet and overexposure to drugs such as antibiotics, mainly during childhood, could alter the intestinal microbial composition and affect the number ratios between protective and pathogen microorganisms[152,153]. Patients with IBD present a different composition in their intestine characterized by a reduction in their microbial diversity, specifically reduction of the dominant members of the gut microbiota. This altered balance in the gut microbiota constituents, called dysbiosis, causes functional changes that seem to be involved in the pathophysiology of many diseases, including IBD[154-156]. The reduced abundance of the *Firmicutes phyla*, and the decrease in their diversity, are the most well studied changes in IBD patients. *Faecalibacterium prausnitzii*, *Butyricoccus pullicaecorum* and *Roseburia hominis* are members of the *Firmicutes* where a reduction has been found in IBD patients in comparison to controls[157-160]. The other important anaerobic phylum also found depleted in patients with IBD are Bacteriodetes[158]. The bacteria in these phyla are known for their anti-inflammatory role in the gut by producing short-chain fatty acid metabolites, such as butyrate and acetate, and inducing the expansion of Treg cells that suppress intestinal inflammation[161-163]. Although gut microbiota in healthy populations shows temporal change, IBD patients present an unstable gut microbiota even during remission. Ott *et al*[164] noticed that, normal anaerobic bacteria such as *Bacteroides*, *Escherichia*, *Eubacterium*, *Lactobacillus*, and *Ruminococcus* are decreased and the diversity of the gut microbiota is also reduced before a relapse of UC. On the other hand, as a result of this dysbiosis, pathogenic microorganisms are increased in IBD patients showing a preference for inflammatory environments. High levels of *Enterobacteriacae*, including adherent invasive *Escherichia coli*, *Klebsiella pneumonia* and *Proteus mirabilis* have been detected in IBD patients, indicating their provocative role in enteric inflammation[165-168]. Moreover, an increase in Fusobacteria has been reported in patients with UC compared to healthy individuals. Of note, when a rectal enema of Fusobacterium isolates from humans was administered in mice, colonic mucosa erosions were induced. Thus, a positive correlation between Fusobacterium and the IBD status of the host indicates that invasive Fusobacterium may have an influence on IBD pathology[169].

Vitamin D

Many references support the important role of vitamin D in both the pathogenesis and therapy of IBD[170,171]. Vitamin D appears to play an important role in innate and adaptive immunity and influences autophagy participating in IBD pathogenesis[172-177]. Several studies indicate a high rate of vitamin D deficiency in IBD patients[178,179]. Several groups have examined the geographic variability of IBD even within a given country and suggests a greater frequency in regions associated with reduced exposure to ultraviolet radiation[180,181]. In contrary, a high intake of vitamin D was associated with a reduced risk for IBD suggesting its pathophysiological role in IBD development, with a significant association to CD (increase 1 ng/mL of 25(OH)D plasma leads to a relative risk reduction of 6% for CD and 100 IU/d increase in total vitamin D intake was associated with a 10% relative risk reduction for UC[182]. A large study with 3217 IBD patients proved that lower 25(OH)D plasma levels are associated with an increased risk of surgery and hospitalization for both CD and UC, compared to those with adequate levels of vitamin[183]. Its role is also supported by animal experiments where administration of $1,25(OH)_2D3$ improves colitis through suppression of genes associated with TNF-a in the colon of mice[184,185]. Increased hospitalization rates and higher disease severity are recorded in regions with limited exposure to UV radiation. The precise mechanism of the effect of UV remains unknown but it is likely to

be related to vitamin D[186]. Additionally, studies have associated the month of birth with the emergence of various inflammatory diseases including IBD. Shaw *et al*[187] recorded a small but significant increase in spring births among IBD patients, specifically CD patients. Respectively, Disanto *et al*[188] indicated that people born in spring are 1.06 more likely to develop UC. The effect of the birth month on inflammatory diseases incidence is possibly related to the UV intake and the adequacy of vitamin D during pregnancy.

Air pollution

Young people living in areas with high concentrations of SO_2 show a greater tendency to develop UC and young people living in areas with high levels of NO_2 are more likely to develop CD. This association appeared to be dose and age-dependent and was strengthened when the study was restricted to urban areas[189]. Another study showed association of IBD patients hospitalizations with overall concentration of pollutants, registered in the US Wisconsin. Total emission was associated with a 40% increase in hospitalization per each registered increase of contaminants[190,191].

Other factors

Heavy metals are also environmental compounds that could contribute to inflammatory diseases like IBD. Ingested mercury causes various disturbances in the intestinal track such as abdominal pain, IBD, ulcers and bloody diarrhea[192]. Several studies have proved the association between major life stressors, anxiety, depression or psychiatric morbidity and onset IBD risk[193-200]. Stress reduces mucus secretion and increases the permeability of mice colon, both characteristics of IBD[201]. Levenstein *et al*[202] firstly, and Bitton *et al*[203] showed higher recruited stress associated with relapse of UC and CD, respectively. Bernstein *et al*[204] in their 704-patients study displayed stress as the only independent predictor of increased risk for disease flare. Also, the presence of anxiety or depression has been associated with increased disease activity and an increased risk of surgery in CD patients[205-207]. There is only a little data on whether anxiety and depression management leads to a more benign disease course. Results of these studies are controversial, however, it could improve the quality of life, particularly in UC patients[208].

Regular low intensity exercise seems constructive to the patients' health reducing both anxiety and depression, and generally improves the quality of life[209,210]. Employment requiring outdoor physical activity has been associated with a lower IBD incidence. Active women seem to have a 44% reduced risk of CD compared to sedentary women[211]. An interesting environmental influence with emerging data is sleep. Mainly reduced, but also increased sleep has been associated with health problems. IBD patients in clinical remission who have sleep disorders are twice more likely to experience flare at 6 mo and are more likely to subclinical disease activity compared to those without sleep disturbances[207,212].

Epigenetics

Epigenetics provides a connection between environmental exposure and the onset and continuation of the disease. Epigenetic modifications, including DNA methylation, are considered as the basis for Th cells differentiation and cytokines regulation. Consequently, methylation has emerged as a research priority for IBD pathogenesis. Nimmo *et al*[213] defined a global methylation profile for ileal CD and identifies altered epigenetic regulation of key host defense mechanisms including the Th17 pathway. DNA methylation changes in the colonic epithelial cells, normally occurred with aging, are accelerated in IBD because of higher cells recycling in inflammation. Increased DNA methylation is shown in dysplastic and surrounding non-dysplastic colonic tissue in UC patients. Four of the 15 loci related to cancer development (*Cdh1, GDNF, HPP1* and *MYOD1*) were differently methylated in surgical resection specimens from patients with active UC compared to those with normal mucosa[214]. Genes showing strongest evidence for hypermethylation in CD compared to healthy controls were *ATF2, CXCL5* and *IL12B* whereas *CCL25, CXCL14, CXCL3, CXCL6, IL12A, INHA, IL15, IL17RA, IL4R, IL6R, IL6ST, FADD, GATA3, IL7, TYK2* were found to be hypomethylated. Regarding UC, methylation status of *CXCL6* and *IL13RA1* in peripheral blood samples did not differ significantly from the methylation status of healthy individuals, whereas most of the genes (*ATF2, CXCL14, CXCL5, GATA3, IL12B, IL17C, IL4R, IL6R and IL6ST*) were found to be significantly hypermethylated in UC patients compared to healthy individuals. *CCL25, CXCL3, FADD, IL10RA, IL12A, IL13, IL15, IL17RA, INHA, TYK2* and *IL7* were hypomethylated in UC. Additionally, the genes *IL13, IL17C, CXCL6, IL10RA, CXCL14, GATA3, IL6ST, IL4R* and *IL6R* show different methylation profiles between UC and CD. Methylation profile in intestinal tissue and peripheral blood are in concordance[215].

Increased acetylation of H4 (the lysine residues 8 and 12) has been found in inflamed tissues and Peyer patches from patients and rats with colitis. Several mechanisms have been proposed to link histone modification with inflammation, involving the innate immune response to microbiota[216,217]. Deregulation of intestinal inflammatory response can occur through disruption in the balance between miRNA activity and threshold levels of specific target mRNAS[218]. Several studies have investigated the different expression of miRNAs in IBD patients. Altered expression patterns of miRNAs in IBD patients were first described in 2008. In biopsy samples of patients with sigmoid active UC, 8 miRNAs levels were significantly increased and 3 were decreased compared with normal. MiR-192, which

is expressed in normal colonic epithelial cells, was significantly reduced in tissues of patients with active UC[219]. Increased expression of miR-21 and -155, which promotes inflammation, has been reported in patients with active UC and colonic CD. The miR-196 is upregulated in inflamed epithelium of CD patients and can reduce the IRGM-mediated autophagy. Otherwise, different miR expression patterns have been identified in peripheral blood samples from IBD patients compared to controls and from CD patients compared to those with UC. Several miRs have been indicated to have negative or positive regulation, including miRs -16, -21, -28-5p, -149, -151-5p, -199-A, and -532-3p. Eleven miRs have also been found to be differently expressed in serum samples from pediatric CD patients and healthy children[220].

CONCLUSION

Environment plays a major role in the development and activity of IBD. The clarification of the pathophysiological mechanisms in relation with the environmental effect on the incidence of IBD can lead to more effective prevention and/or treatment of disease. More clinical studies could indicate if avoiding some drugs and a westernized diet followed by an intake of vitamin D, would lead to a remission even to colonic healing in IBD patients. Connection between environmental and genetic factors, through epigenetic alterations, may lead to a better understanding of IBD. The recent advances in our understanding of IBD-associated epigenetic mechanisms underlie many promising clinical applications such as molecular biomarkers for diagnosis and prognosis of the disease as well as prediction of treatment outcomes.

REFERENCES

1 **Duchmann R**, Kaiser I, Hermann E, Mayet W, Ewe K, Meyer zum Büschenfelde KH. Tolerance exists towards resident intestinal flora but is broken in active inflammatory bowel disease (IBD) *Clin Exp Immunol* 1995; **102**: 448-455 [PMID: 8536356 DOI: 10.1111/j.1365-2249.1995.tb03836.x]

2 **Abraham C**, Cho JH. Inflammatory bowel disease. *N Engl J Med* 2009; **361**: 2066-2078 [PMID: 19923578 DOI: 10.1056/NEJMra0804647]

3 **Baumgart DC**, Sandborn WJ. Crohn's disease. *Lancet* 2012; **380**: 1590-1605 [PMID: 22914295 DOI: 10.1016/S0140-6736(12)60026-9]

4 **Ordás I**, Eckmann L, Talamini M, Baumgart DC, Sandborn WJ. Ulcerative colitis. *Lancet* 2012; **380**: 1606-1619 [PMID: 22914296 DOI: 10.1016/S0140-6736(12)60150-0]

5 **Loftus EV**. Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. *Gastroenterology* 2004; **126**: 1504-1517 [PMID: 15168363 DOI: 10.1053/j.gastro.2004.01.063]

6 **Molodecky NA**, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, Benchimol EI, Panaccione R, Ghosh S, Barkema HW, Kaplan GG. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology* 2012; **142**: 46-54.e42; quiz e30 [PMID: 22001864 DOI: 10.1053/j.gastro.2011.10.001]

7 **Zhao J**, Ng SC, Lei Y, Yi F, Li J, Yu L, Zou K, Dan Z, Dai M, Ding Y, Song M, Mei Q, Fang X, Liu H, Shi Z, Zhou R, Xia M, Wu Q, Xiong Z, Zhu W, Deng L, Kamm MA, Xia B. First prospective, population-based inflammatory bowel disease incidence study in mainland of China: the emergence of "western" disease. *Inflamm Bowel Dis* 2013; **19**: 1839-1845 [PMID: 23669403 DOI: 10.1097/MIB.0b013e31828a6551]

8 **Park SJ**, Kim WH, Cheon JH. Clinical characteristics and treatment of inflammatory bowel disease: a comparison of Eastern and Western perspectives. *World J Gastroenterol* 2014; **20**: 11525-11537 [PMID: 25206259 DOI: 10.3748/wjg.v20.i33.11525]

9 **Ng SC**. Emerging leadership lecture: Inflammatory bowel disease in Asia: emergence of a "Western" disease. *J Gastroenterol Hepatol* 2015; **30**: 440-445 [PMID: 25469874 DOI: 10.1111/jgh.12859]

10 **Rehman A**, Rausch P, Wang J, Skieceviciene J, Kiudelis G, Bhagalia K, Amarapurkar D, Kupcinskas L, Schreiber S, Rosenstiel P, Baines JF, Ott S. Geographical patterns of the standing and active human gut microbiome in health and IBD. *Gut* 2016; **65**: 238-248 [PMID: 25567118 DOI: 10.1136/gutjnl-2014-308341]

11 **Ng SC**, Tang W, Ching JY, Wong M, Chow CM, Hui AJ, Wong TC, Leung VK, Tsang SW, Yu HH, Li MF, Ng KK, Kamm MA, Studd C, Bell S, Leong R, de Silva HJ, Kasturiratne A, Mufeena MN, Ling KL, Ooi CJ, Tan PS, Ong D, Goh KL, Hilmi I, Pisespongsa P, Manatsathit S, Rerknimitr R, Aniwan S, Wang YF, Ouyang Q, Zeng Z, Zhu Z, Chen MH, Hu PJ, Wu K, Wang X, Simadibrata M, Abdullah M, Wu JC, Sung JJ, Chan FK. Incidence and phenotype of inflammatory bowel disease based on results from the Asia-pacific Crohn's and colitis epidemiology study. *Gastroenterology* 2013; **145**: 158-165.e2 [PMID: 23583432 DOI: 10.1053/j.gastro.2013.04.007]

12 **Vogel H**, Halpert D, Horoupian DS. Hypoplasia of posterior spinal roots and dorsal spinal tracts with arthrogryposis multiplex congenita. *Acta Neuropathol* 1990; **79**: 692-696 [PMID: 2360413 DOI: 10.1136/gutjnl-2013-304636]

13 **Benchimol EI**, Fortinsky KJ, Gozdyra P, Van den Heuvel M, Van Limbergen J, Griffiths AM. Epidemiology of pediatric inflammatory bowel disease: a systematic review of international trends. *Inflamm Bowel Dis* 2011; **17**: 423-439 [PMID: 20564651 DOI: 10.1002/ibd.21349]

14 **El Mouzan MI**, Saadah O, Al-Saleem K, Al Edreesi M, Hasosah M, Alanazi A, Al Mofarreh M, Asery A, Al Qourain A, Nouli K, Al Hussaini A, Telmesani A, AlReheili K, Alghamdi S, Alrobiaa N, Alzaben A, Mehmadi A, Al Hebbi H, Al Sarkhy A, Al Mehaidib A, Al Saleem B, Assiri A, Wali S. Incidence of pediatric inflammatory bowel disease in Saudi Arabia: a multicenter national study. *Inflamm Bowel Dis* 2014; **20**: 1085-1090 [PMID: 24788219 DOI: 10.1097/MIB.0000000000000048]

15 **Benchimol EI**, Manuel DG, Guttmann A, Nguyen GC, Mojaverian N, Quach P, Mack DR. Changing age demographics of inflammatory bowel disease in Ontario, Canada: a population-based cohort study of epidemiology trends. *Inflamm Bowel Dis* 2014; **20**: 1761-1769 [PMID: 25159453 DOI: 10.1097/MIB.0000000000000103]

16 **Benchimol EI**, Mack DR, Nguyen GC, Snapper SB, Li W, Mojaverian N, Quach P, Muise AM. Incidence, outcomes, and health services burden of very early onset inflammatory bowel disease. *Gastroenterology* 2014; **147**: 803-813.e7; quiz e14-15 [PMID: 24951840 DOI: 10.1053/j.gastro.2014.06.023]

17 **El-Matary W**, Moroz SP, Bernstein CN. Inflammatory bowel disease in children of Manitoba: 30 years' experience of a tertiary center. *J Pediatr Gastroenterol Nutr* 2014; **59**: 763-766 [PMID: 25111222 DOI: 10.1097/MPG.0000000000000525]

18 **Jakobsen C**, Paerregaard A, Munkholm P, Faerk J, Lange A, Andersen J, Jakobsen M, Kramer I, Czernia-Mazurkiewicz J, Wewer V. Pediatric inflammatory bowel disease: increasing incidence, decreasing surgery rate, and compromised nutritional status: A prospective population-based cohort study 2007-2009. *Inflamm Bowel Dis* 2011; **17**: 2541-2550 [PMID: 21381152 DOI: 10.1002/ibd.21654]

19 **Vind I**, Riis L, Jess T, Knudsen E, Pedersen N, Elkjaer M, Bak Andersen I, Wewer V, Nørregaard P, Moesgaard F, Bendtsen F, Munkholm P. Increasing incidences of inflammatory bowel disease and decreasing surgery rates in Copenhagen City and County, 2003-2005: a population-based study from the Danish Crohn colitis database. *Am J Gastroenterol* 2006; **101**: 1274-1282 [PMID:

16771949]
20 **Kelsen J**, Baldassano RN. Inflammatory bowel disease: the difference between children and adults. *Inflamm Bowel Dis* 2008; **14** Suppl 2: S9-11 [PMID: 18816756 DOI: 10.1002/ibd.20560]
21 **Binder V**. Genetic epidemiology in inflammatory bowel disease. *Dig Dis* 1998; **16**: 351-355 [PMID: 10207221 DOI: 10.1159/000016891]
22 **Laharie D**, Debeugny S, Peeters M, Van Gossum A, Gower-Rousseau C, Bélaïche J, Fiasse R, Dupas JL, Lerebours E, Piotte S, Cortot A, Vermeire S, Grandbastien B, Colombel JF. Inflammatory bowel disease in spouses and their offspring. *Gastroenterology* 2001; **120**: 816-819 [PMID: 11231934 DOI: 10.1053/gast.2001.22574]
23 **Freeman HJ**. Familial Crohn's disease in single or multiple first-degree relatives. *J Clin Gastroenterol* 2002; **35**: 9-13 [PMID: 12080219 DOI: 10.1097/00004836-200207000-00004]
24 **Tysk C**, Lindberg E, Järnerot G, Flodérus-Myrhed B. Ulcerative colitis and Crohn's disease in an unselected population of monozygotic and dizygotic twins. A study of heritability and the influence of smoking. *Gut* 1988; **29**: 990-996 [PMID: 3396969 DOI: 10.1136/gut.29.7.990]
25 **Thompson NP**, Driscoll R, Pounder RE, Wakefield AJ. Genetics versus environment in inflammatory bowel disease: results of a British twin study. *BMJ* 1996; **312**: 95-96 [PMID: 8555939 DOI: 10.1136/bmj.312.7023.95]
26 **Orholm M**, Binder V, Sørensen TI, Rasmussen LP, Kyvik KO. Concordance of inflammatory bowel disease among Danish twins. Results of a nationwide study. *Scand J Gastroenterol* 2000; **35**: 1075-1081 [PMID: 11099061 DOI: 10.1080/003655200451207]
27 **Halfvarson J**, Bodin L, Tysk C, Lindberg E, Järnerot G. Inflammatory bowel disease in a Swedish twin cohort: a long-term follow-up of concordance and clinical characteristics. *Gastroenterology* 2003; **124**: 1767-1773 [PMID: 12806610 DOI: 10.1016/S0016-5085(03)00385-8]
28 **Bernstein CN**, Shanahan F. Disorders of a modern lifestyle: reconciling the epidemiology of inflammatory bowel diseases. *Gut* 2008; **57**: 1185-1191 [PMID: 18515412 DOI: 10.1136/gut.2007.122143]
29 **Pinsk V**, Lemberg DA, Grewal K, Barker CC, Schreiber RA, Jacobson K. Inflammatory bowel disease in the South Asian pediatric population of British Columbia. *Am J Gastroenterol* 2007; **102**: 1077-1083 [PMID: 17378907 DOI: 10.1111/j.1572-0241.2007.01124.x]
30 **Odes HS**, Fraser D, Krawiec J. Inflammatory bowel disease in migrant and native Jewish populations of southern Israel. *Scand J Gastroenterol Suppl* 1989; **170**: 36-38; discussion 50-55 [PMID: 2617190 DOI: 10.3109/00365528909091348]
31 **Probert CS**, Jayanthi V, Pinder D, Wicks AC, Mayberry JF. Epidemiological study of ulcerative proctocolitis in Indian migrants and the indigenous population of Leicestershire. *Gut* 1992; **33**: 687-693 [PMID: 1307684 DOI: 10.1136/gut.33.5.687]
32 **Carr I**, Mayberry JF. The effects of migration on ulcerative colitis: a three-year prospective study among Europeans and first- and second- generation South Asians in Leicester (1991-1994). *Am J Gastroenterol* 1999; **94**: 2918-2922 [PMID: 10520845]
33 **Goh K**, Xiao SD. Inflammatory bowel disease: a survey of the epidemiology in Asia. *J Dig Dis* 2009; **10**: 1-6 [PMID: 19236540 DOI: 10.1111/j.1751-2980.2008.00355.x]
34 **Li X**, Sundquist J, Hemminki K, Sundquist K. Risk of inflammatory bowel disease in first- and second-generation immigrants in Sweden: a nationwide follow-up study. *Inflamm Bowel Dis* 2011; **17**: 1784-1791 [PMID: 21744434 DOI: 10.1002/ibd.21535]
35 **Ko Y**, Kariyawasam V, Karnib M, Butcher R, Samuel D, Alrubaie A, Rahme N, McDonald C, Cowlishaw J, Katelaris P, Barr G, Jones B, Connor S, Paven G, Chapman G, Park G, Gearry R, Leong RW. Inflammatory Bowel Disease Environmental Risk Factors: A Population-Based Case-Control Study of Middle Eastern Migration to Australia. *Clin Gastroenterol Hepatol* 2015; **13**: 1453-63.e1 [PMID: 25771246 DOI: 10.1016/j.cgh.2015.02.045]
36 **Benchimol EI**, Mack DR, Guttmann A, Nguyen GC, To T, Mojaverian N, Quach P, Manuel DG. Inflammatory bowel disease in immigrants to Canada and their children: a population-based cohort study. *Am J Gastroenterol* 2015; **110**: 553-563 [PMID: 25756238 DOI: 10.1038/ajg.2015.52]
37 **Barreiro-de Acosta M**, Alvarez Castro A, Souto R, Iglesias M, Lorenzo A, Dominguez-Muñoz JE. Emigration to western industrialized countries: A risk factor for developing inflammatory bowel disease. *J Crohns Colitis* 2011; **5**: 566-569 [PMID: 22115376 DOI: 10.1016/j.crohns.2011.05.009]
38 **Benchimol EI**, Manuel DG, To T, Mack DR, Nguyen GC, Gommerman JL, Croitoru K, Mojaverian N, Wang X, Quach P, Guttmann A. Asthma, type 1 and type 2 diabetes mellitus, and inflammatory bowel disease amongst South Asian immigrants to Canada and their children: a population-based cohort study. *PLoS One* 2015; **10**: e0123599 [PMID: 25849480 DOI: 10.1371/journal.pone.0123599]
39 **Ng SC**, Bernstein CN, Vatn MH, Lakatos PL, Loftus EV, Tysk C, O'Morain C, Moum B, Colombel JF. Geographical variability and environmental risk factors in inflammatory bowel disease. *Gut* 2013; **62**: 630-649 [PMID: 23335431 DOI: 10.1136/gutjnl-2012-303661]
40 **Tuvlin JA**, Raza SS, Bracamonte S, Julian C, Hanauer SB, Nicolae DL, King AC, Cho JH. Smoking and inflammatory bowel disease: trends in familial and sporadic cohorts. *Inflamm Bowel Dis* 2007; **13**: 573-579 [PMID: 17345609]
41 **Biedermann L**, Zeitz J, Mwinyi J, Sutter-Minder E, Rehman A, Ott SJ, Steurer-Stey C, Frei A, Frei P, Scharl M, Loessner MJ, Vavricka SR, Fried M, Schreiber S, Schuppler M, Rogler G. Smoking cessation induces profound changes in the composition of the intestinal microbiota in humans. *PLoS One* 2013; **8**: e59260 [PMID: 23516617 DOI: 10.1371/journal.pone.0059260]
42 **Parkes GC**, Whelan K, Lindsay JO. Smoking in inflammatory bowel disease: impact on disease course and insights into the aetiology of its effect. *J Crohns Colitis* 2014; **8**: 717-725 [PMID: 24636140 DOI: 10.1016/j.crohns.2014.02.002]
43 **Naito T**, Kida H, Yokoyama H, Koshino Y, Tomosugi N, Hattori N, Kobayashi K. A case of diffuse panbronchiolitis (DPB) with benign monoclonal IgA gammopathy and IgA nephropathy with monoclonal IgA deposition. *Jpn J Med* 1989; **28**: 503-505 [PMID: 2509771 DOI: 10.5114/pg.2014.43577]
44 **Cosnes J**. Tobacco and IBD: relevance in the understanding of disease mechanisms and clinical practice. *Best Pract Res Clin Gastroenterol* 2004; **18**: 481-496 [PMID: 15157822 DOI: 10.1016/j.bpg.2003.12.003]
45 **Lakatos PL**, Szamosi T, Lakatos L. Smoking in inflammatory bowel diseases: good, bad or ugly? *World J Gastroenterol* 2007; **13**: 6134-6139 [PMID: 18069751 DOI: 10.3748/wjg.13.6134]
46 **Beaugerie L**, Massot N, Carbonnel F, Cattan S, Gendre JP, Cosnes J. Impact of cessation of smoking on the course of ulcerative colitis. *Am J Gastroenterol* 2001; **96**: 2113-2116 [PMID: 11467641 DOI: 10.1111/j.1572-0241.2001.03944.x]
47 **Mahid SS**, Minor KS, Soto RE, Hornung CA, Galandiuk S. Smoking and inflammatory bowel disease: a meta-analysis. *Mayo Clin Proc* 2006; **81**: 1462-1471 [PMID: 17120402 DOI: 10.4065/81.11.1462]
48 **Cosnes J**, Nion-Larmurier I, Afchain P, Beaugerie L, Gendre JP. Gender differences in the response of colitis to smoking. *Clin Gastroenterol Hepatol* 2004; **2**: 41-48 [PMID: 15017631 DOI: 10.1016/S1542-3565(03)00290-8]
49 **Jha P**, Ranson MK, Nguyen SN, Yach D. Estimates of global and regional smoking prevalence in 1995, by age and sex. *Am J Public Health* 2002; **92**: 1002-1006 [PMID: 12036796 DOI: 10.2105/AJPH.92.6.1002]
50 **Motley RJ**, Rhodes J, Ford GA, Wilkinson SP, Chesner IM, Asquith P, Hellier MD, Mayberry JF. Time relationships between cessation of smoking and onset of ulcerative colitis. *Digestion* 1987; **37**: 125-127 [PMID: 3622939 DOI: 10.1159/000199478]
51 **Penny WJ**, Penny E, Mayberry JF, Rhodes J. Prevalence of inflammatory bowel disease amongst Mormons in Britain and Ireland. *Soc Sci Med* 1985; **21**: 287-290 [PMID: 3875900 DOI: 10.1016/0277-9536(85)90103-0]
52 **Thomas GA**, Rhodes J, Ragunath K, Mani V, Williams GT, Newcombe RG, Russell MA, Feyerabend C. Transdermal nicotine

compared with oral prednisolone therapy for active ulcerative colitis. *Eur J Gastroenterol Hepatol* 1996; **8**: 769-776 [PMID: 8864674]
53 **Bastida G**, Beltrán B. Ulcerative colitis in smokers, non-smokers and ex-smokers. *World J Gastroenterol* 2011; **17**: 2740-2747 [PMID: 21734782 DOI: 10.3748/wjg.v17.i22.2740]
54 **Calkins BM**. A meta-analysis of the role of smoking in inflammatory bowel disease. *Dig Dis Sci* 1989; **34**: 1841-1854 [PMID: 2598752 DOI: 10.1007/BF01536701]
55 **Nos P**, Domènech E. Management of Crohn's disease in smokers: is an alternative approach necessary? *World J Gastroenterol* 2011; **17**: 3567-3574 [PMID: 21987601 DOI: 10.3748/wjg.v17.i31.3567]
56 **Higuchi LM**, Khalili H, Chan AT, Richter JM, Bousvaros A, Fuchs CS. A prospective study of cigarette smoking and the risk of inflammatory bowel disease in women. *Am J Gastroenterol* 2012; **107**: 1399-1406 [PMID: 22777340 DOI: 10.1038/ajg.2012.196]
57 **Lunney PC**, Kariyawasam VC, Wang RR, Middleton KL, Huang T, Selinger CP, Andrews JM, Katelaris PH, Leong RW. Smoking prevalence and its influence on disease course and surgery in Crohn's disease and ulcerative colitis. *Aliment Pharmacol Ther* 2015; **42**: 61-70 [PMID: 25968332 DOI: 10.1111/apt.13239]
58 **Picco MF**, Bayless TM. Tobacco consumption and disease duration are associated with fistulizing and stricturing behaviors in the first 8 years of Crohn's disease. *Am J Gastroenterol* 2003; **98**: 363-368 [PMID: 12591056 DOI: 10.1111/j.1572-0241.2003.07240.x]
59 **Mahid SS**, Minor KS, Stevens PL, Galandiuk S. The role of smoking in Crohn's disease as defined by clinical variables. *Dig Dis Sci* 2007; **52**: 2897-2903 [PMID: 17401688 DOI: 10.1007/s10620-006-9624-0]
60 **Johnson GJ**, Cosnes J, Mansfield JC. Review article: smoking cessation as primary therapy to modify the course of Crohn's disease. *Aliment Pharmacol Ther* 2005; **21**: 921-931 [PMID: 15813828 DOI: 10.1111/j.1365-2036.2005.02424.x]
61 **Cosnes J**, Beaugerie L, Carbonnel F, Gendre JP. Smoking cessation and the course of Crohn's disease: an intervention study. *Gastroenterology* 2001; **120**: 1093-1099 [PMID: 11266373 DOI: 10.1053/gast.2001.23231]
62 **Reese GE**, Nanidis T, Borysiewicz C, Yamamoto T, Orchard T, Tekkis PP. The effect of smoking after surgery for Crohn's disease: a meta-analysis of observational studies. *Int J Colorectal Dis* 2008; **23**: 1213-1221 [PMID: 18762954 DOI: 10.1007/s00384-008-0542-9]
63 **van der Heide F**, Dijkstra A, Weersma RK, Albersnagel FA, van der Logt EM, Faber KN, Sluiter WJ, Kleibeuker JH, Dijkstra G. Effects of active and passive smoking on disease course of Crohn's disease and ulcerative colitis. *Inflamm Bowel Dis* 2009; **15**: 1199-1207 [PMID: 19170191 DOI: 10.1002/ibd.20884]
64 **Koutroubakis IE**, Vlachonikolis IG, Kouroumalis EA. Role of appendicitis and appendectomy in the pathogenesis of ulcerative colitis: a critical review. *Inflamm Bowel Dis* 2002; **8**: 277-286 [PMID: 12131612 DOI: 10.1097/00054725-200207000-00007]
65 **Kurina LM**, Goldacre MJ, Yeates D, Seagroatt V. Appendicectomy, tonsillectomy, and inflammatory bowel disease: a case-control record linkage study. *J Epidemiol Community Health* 2002; **56**: 551-554 [PMID: 12080166 DOI: 10.1136/jech.56.7.551]
66 **Radford-Smith GL**, Edwards JE, Purdie DM, Pandeya N, Watson M, Martin NG, Green A, Newman B, Florin TH. Protective role of appendicectomy on onset and severity of ulcerative colitis and Crohn's disease. *Gut* 2002; **51**: 808-813 [PMID: 12427781 DOI: 10.1136/gut.51.6.808]
67 **de Saussure P**, Clerson P, Prost PL, Truong Tan N, Bouhnik Y. Appendectomy, smoking habits and the risk of developing ulcerative colitis: a case control study in private practice setting. *Gastroenterol Clin Biol* 2007; **31**: 493-497 [PMID: 17541339 DOI: 10.1016/S0399-8320(07)89417-6]
68 **Wang YF**, Ou-Yang Q, Xia B, Liu LN, Gu F, Zhou KF, Mei Q, Shi RH, Ran ZH, Wang XD, Hu PJ, Wu KC, Liu XG, Miao YL, Han Y, Wu XP, He GB, Zhong J, Liu GJ. Multicenter case-control study of the risk factors for ulcerative colitis in China. *World J Gastroenterol* 2013; **19**: 1827-1833 [PMID: 23555172 DOI: 10.3748/wjg.v19.i11.1827]
69 **Frisch M**, Gridley G. Appendectomy in adulthood and the risk of inflammatory bowel diseases. *Scand J Gastroenterol* 2002; **37**: 1175-1177 [PMID: 12408522 DOI: 10.1080/003655202760373380]
70 **Kaplan GG**, Pedersen BV, Andersson RE, Sands BE, Korzenik J, Frisch M. The risk of developing Crohn's disease after an appendectomy: a population-based cohort study in Sweden and Denmark. *Gut* 2007; **56**: 1387-1392 [PMID: 17494106 DOI: 10.1136/gut.2007.121467]
71 **López Ramos D**, Gabriel R, Cantero Perona J, Moreno Otero R, Fernández Bermejo M, Maté Jiménez J. Association of MALTectomy (appendectomy and tonsillectomy) and inflammatory bowel disease: a familial case-control study. *Rev Esp Enferm Dig* 2001; **93**: 303-314 [PMID: 11488108]
72 **Cheluvappa R**, Luo AS, Palmer C, Grimm MC. Protective pathways against colitis mediated by appendicitis and appendectomy. *Clin Exp Immunol* 2011; **165**: 393-400 [PMID: 21707591 DOI: 10.1111/j.1365-2249.2011.04434.x]
73 **Andersson RE**, Olaison G, Tysk C, Ekbom A. Appendectomy and protection against ulcerative colitis. *N Engl J Med* 2001; **344**: 808-814 [PMID: 11248156]
74 **Beaugerie L**, Sokol H. Appendicitis, not appendectomy, is protective against ulcerative colitis, both in the general population and first-degree relatives of patients with IBD. *Inflamm Bowel Dis* 2010; **16**: 356-357 [PMID: 19685454 DOI: 10.1002/ibd.21064]
75 **Kaplan GG**, Jackson T, Sands BE, Frisch M, Andersson RE, Korzenik J. The risk of developing Crohn's disease after an appendectomy: a meta-analysis. *Am J Gastroenterol* 2008; **103**: 2925-2931 [PMID: 18775018 DOI: 10.1111/j.1572-0241.2008.02118.x]
76 **Evans JM**, McMahon AD, Murray FE, McDevitt DG, MacDonald TM. Non-steroidal anti-inflammatory drugs are associated with emergency admission to hospital for colitis due to inflammatory bowel disease. *Gut* 1997; **40**: 619-622 [PMID: 9203940 DOI: 10.1136/gut.40.5.619]
77 **Felder JB**, Korelitz BI, Rajapakse R, Schwarz S, Horatagis AP, Gleim G. Effects of nonsteroidal antiinflammatory drugs on inflammatory bowel disease: a case-control study. *Am J Gastroenterol* 2000; **95**: 1949-1954 [PMID: 10950041 DOI: 10.1111/j.1572-0241.2000.02262.x]
78 **Gleeson MH**, Davis AJ. Non-steroidal anti-inflammatory drugs, aspirin and newly diagnosed colitis: a case-control study. *Aliment Pharmacol Ther* 2003; **17**: 817-825 [PMID: 12641504 DOI: 10.1046/j.1365-2036.2003.01519.x]
79 **Ananthakrishnan AN**, Higuchi LM, Huang ES, Khalili H, Richter JM, Fuchs CS, Chan AT. Aspirin, nonsteroidal anti-inflammatory drug use, and risk for Crohn disease and ulcerative colitis: a cohort study. *Ann Intern Med* 2012; **156**: 350-359 [PMID: 22393130 DOI: 10.7326/0003-4819-156-5-201203060-00007]
80 **Meyer AM**, Ramzan NN, Heigh RI, Leighton JA. Relapse of inflammatory bowel disease associated with use of nonsteroidal anti-inflammatory drugs. *Dig Dis Sci* 2006; **51**: 168-172 [PMID: 16416231 DOI: 10.1007/s10620-006-3103-5]
81 **Philpott HL**, Nandurkar S, Lubel J, Gibson PR. Drug-induced gastrointestinal disorders. *Postgrad Med J* 2014; **90**: 411-419 [PMID: 24942356 DOI: 10.1136/postgradmedj-2013-100316rep]
82 **Chan SS**, Luben R, Bergmann MM, Boeing H, Olsen A, Tjonneland A, Overvad K, Kaaks R, Kennedy H, Khaw KT, Riboli E, Hart AR. Aspirin in the aetiology of Crohn's disease and ulcerative colitis: a European prospective cohort study. *Aliment Pharmacol Ther* 2011; **34**: 649-655 [PMID: 21790683 DOI: 10.1111/j.1365-2036.2011.04784.x]
83 **Rhodes JM**, Cockel R, Allan RN, Hawker PC, Dawson J, Elias E. Colonic Crohn's disease and use of oral contraception. *Br Med J* (Clin Res Ed) 1984; **288**: 595-596 [PMID: 6421392 DOI: 10.1136/bmj.288.6417.595]
84 **Lesko SM**, Kaufman DW, Rosenberg L, Helmrich SP, Miller DR, Stolley PD, Shapiro S. Evidence for an increased risk of Crohn's disease in oral contraceptive users. *Gastroenterology* 1985; **89**: 1046-1049 [PMID: 4043662]
85 **Sandler RS**, Wurzelmann JI, Lyles CM. Oral contraceptive use and the risk of inflammatory bowel disease. *Epidemiology* 1992; **3**: 374-378 [PMID: 1637902 DOI: 10.1097/00001648-199207000-00

014]
86 **Cornish JA**, Tan E, Simillis C, Clark SK, Teare J, Tekkis PP. The risk of oral contraceptives in the etiology of inflammatory bowel disease: a meta-analysis. *Am J Gastroenterol* 2008; **103**: 2394-2400 [PMID: 18684177 DOI: 10.1111/j.1572-0241.2008.02064.x]
87 **Dubeau MF**, Iacucci M, Beck PL, Moran GW, Kaplan GG, Ghosh S, Panaccione R. Drug-induced inflammatory bowel disease and IBD-like conditions. *Inflamm Bowel Dis* 2013; **19**: 445-456 [PMID: 22573536 DOI: 10.1002/ibd.22990]
88 **Khalili H**, Higuchi LM, Ananthakrishnan AN, Richter JM, Feskanich D, Fuchs CS, Chan AT. Oral contraceptives, reproductive factors and risk of inflammatory bowel disease. *Gut* 2013; **62**: 1153-1159 [PMID: 22619368 DOI: 10.1136/gutjnl-2012-302362]
89 **Hviid A**, Svanström H, Frisch M. Antibiotic use and inflammatory bowel diseases in childhood. *Gut* 2011; **60**: 49-54 [PMID: 20966024 DOI: 10.1136/gut.2010.219683]
90 **Kronman MP**, Zaoutis TE, Haynes K, Feng R, Coffin SE. Antibiotic exposure and IBD development among children: a population-based cohort study. *Pediatrics* 2012; **130**: e794-e803 [PMID: 23008454 DOI: 10.1542/peds.2011-3886]
91 **Virta L**, Auvinen A, Helenius H, Huovinen P, Kolho KL. Association of repeated exposure to antibiotics with the development of pediatric Crohn's disease--a nationwide, register-based finnish case-control study. *Am J Epidemiol* 2012; **175**: 775-784 [PMID: 22366379 DOI: 10.1093/aje/kwr400]
92 **Ungaro R**, Bernstein CN, Gearry R, Hviid A, Kolho KL, Kronman MP, Shaw S, Van Kruiningen H, Colombel JF, Atreja A. Antibiotics associated with increased risk of new-onset Crohn's disease but not ulcerative colitis: a meta-analysis. *Am J Gastroenterol* 2014; **109**: 1728-1738 [PMID: 25223575 DOI: 10.1038/ajg.2014.246]
93 **Shaw SY**, Blanchard JF, Bernstein CN. Association between the use of antibiotics in the first year of life and pediatric inflammatory bowel disease. *Am J Gastroenterol* 2010; **105**: 2687-2692 [PMID: 20940708 DOI: 10.1038/ajg.2010.398]
94 **Shaw SY**, Blanchard JF, Bernstein CN. Association between the use of antibiotics and new diagnoses of Crohn's disease and ulcerative colitis. *Am J Gastroenterol* 2011; **106**: 2133-2142 [PMID: 21912437 DOI: 10.1038/ajg.2011.304]
95 **Chapman-Kiddell CA**, Davies PS, Gillen L, Radford-Smith GL. Role of diet in the development of inflammatory bowel disease. *Inflamm Bowel Dis* 2010; **16**: 137-151 [PMID: 19462428 DOI: 10.1002/ibd.20968]
96 **Muegge BD**, Kuczynski J, Knights D, Clemente JC, González A, Fontana L, Henrissat B, Knight R, Gordon JI. Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. *Science* 2011; **332**: 970-974 [PMID: 21596990 DOI: 10.1126/science.1198719]
97 **De Filippo C**, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G, Lionetti P. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci USA* 2010; **107**: 14691-14696 [PMID: 20679230 DOI: 10.1073/pnas.1005963107]
98 **Jowett SL**, Seal CJ, Pearce MS, Phillips E, Gregory W, Barton JR, Welfare MR. Influence of dietary factors on the clinical course of ulcerative colitis: a prospective cohort study. *Gut* 2004; **53**: 1479-1484 [PMID: 15361498 DOI: 10.1136/gut.2003.024828]
99 **D'Souza S**, Levy E, Mack D, Israel D, Lambrette P, Ghadirian P, Deslandres C, Morgan K, Seidman EG, Amre DK. Dietary patterns and risk for Crohn's disease in children. *Inflamm Bowel Dis* 2008; **14**: 367-373 [PMID: 18092347 DOI: 10.1002/ibd.20333]
100 **Asakura H**, Suzuki K, Kitahora T, Morizane T. Is there a link between food and intestinal microbes and the occurrence of Crohn's disease and ulcerative colitis? *J Gastroenterol Hepatol* 2008; **23**: 1794-1801 [PMID: 19120872 DOI: 10.1111/j.1440-1746.2008.05681.x]
101 **Hou JK**, Abraham B, El-Serag H. Dietary intake and risk of developing inflammatory bowel disease: a systematic review of the literature. *Am J Gastroenterol* 2011; **106**: 563-573 [PMID: 21468064 DOI: 10.1038/ajg.2011.44]
102 **Jantchou P**, Morois S, Clavel-Chapelon F, Boutron-Ruault MC, Carbonnel F. Animal protein intake and risk of inflammatory bowel disease: The E3N prospective study. *Am J Gastroenterol* 2010; **105**: 2195-2201 [PMID: 20461067 DOI: 10.1038/ajg.2010.192]
103 **Maconi G**, Ardizzone S, Cucino C, Bezzio C, Russo AG, Bianchi Porro G. Pre-illness changes in dietary habits and diet as a risk factor for inflammatory bowel disease: a case-control study. *World J Gastroenterol* 2010; **16**: 4297-4304 [PMID: 20818813]
104 **Amre DK**, D'Souza S, Morgan K, Seidman G, Lambrette P, Grimard G, Israel D, Mack D, Ghadirian P, Deslandres C, Chotard V, Budai B, Law L, Levy E, Seidman EG. Imbalances in dietary consumption of fatty acids, vegetables, and fruits are associated with risk for Crohn's disease in children. *Am J Gastroenterol* 2007; **102**: 2016-2025 [PMID: 17617201]
105 **Ananthakrishnan AN**, Khalili H, Konijeti GG, Higuchi LM, de Silva P, Korzenik JR, Fuchs CS, Willett WC, Richter JM, Chan AT. A prospective study of long-term intake of dietary fiber and risk of Crohn's disease and ulcerative colitis. *Gastroenterology* 2013; **145**: 970-977 [PMID: 23912083 DOI: 10.1053/j.gastro.2013.07.050]
106 **Russel MG**, Engels LG, Muris JW, Limonard CB, Volovics A, Brummer RJ, Stockbrügger RW. Modern life' in the epidemiology of inflammatory bowel disease: a case-control study with special emphasis on nutritional factors. *Eur J Gastroenterol Hepatol* 1998; **10**: 243-249 [PMID: 9585029]
107 **Kowal-Vern A**, McFadden J. Pseudomonas aeruginosa pneumonia as a presenting entity in an AIDS patient. *Clin Pediatr* (Phila) 1989; **28**: 403 [PMID: 2504528 DOI: 10.3904/kjim.2014.29.4.409]
108 **Martini GA**, Brandes JW. Increased consumption of refined carbohydrates in patients with Crohn's disease. *Klin Wochenschr* 1976; **54**: 367-371 [PMID: 1271690]
109 **Mayberry JF**, Rhodes J, Newcombe RG. Increased sugar consumption in Crohn's disease. *Digestion* 1980; **20**: 323-326 [PMID: 7390057]
110 **Bianchi Porro G**, Panza E. Smoking, sugar, and inflammatory bowel disease. *Br Med J* (Clin Res Ed) 1985; **291**: 971-972 [PMID: 3929989]
111 **Hansen TS**, Jess T, Vind I, Elkjaer M, Nielsen MF, Gamborg M, Munkholm P. Environmental factors in inflammatory bowel disease: a case-control study based on a Danish inception cohort. *J Crohns Colitis* 2011; **5**: 577-584 [PMID: 22115378 DOI: 10.1016/j.crohns.2011.05.010]
112 **Jakobsen C**, Paerregaard A, Munkholm P, Wewer V. Environmental factors and risk of developing paediatric inflammatory bowel disease -- a population based study 2007-2009. *J Crohns Colitis* 2013; **7**: 79-88 [PMID: 22748696 DOI: 10.1016/j.crohns.2012.05.024]
113 **Teixeira LG**, Leonel AJ, Aguilar EC, Batista NV, Alves AC, Coimbra CC, Ferreira AV, de Faria AM, Cara DC, Alvarez Leite JI. The combination of high-fat diet-induced obesity and chronic ulcerative colitis reciprocally exacerbates adipose tissue and colon inflammation. *Lipids Health Dis* 2011; **10**: 204 [PMID: 22073943 DOI: 10.1186/1476-511X-10-204]
114 **Devkota S**, Wang Y, Musch MW, Leone V, Fehlner-Peach H, Nadimpalli A, Antonopoulos DA, Jabri B, Chang EB. Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10-/- mice. *Nature* 2012; **487**: 104-108 [PMID: 22722865 DOI: 10.1038/nature11225]
115 **Shoda R**, Matsueda K, Yamato S, Umeda N. Epidemiologic analysis of Crohn disease in Japan: increased dietary intake of n-6 polyunsaturated fatty acids and animal protein relates to the increased incidence of Crohn disease in Japan. *Am J Clin Nutr* 1996; **63**: 741-745 [PMID: 8615358]
116 **Hart AR**, Luben R, Olsen A, Tjonneland A, Linseisen J, Nagel G, Berglund G, Lindgren S, Grip O, Key T, Appleby P, Bergmann MM, Boeing H, Hallmans G, Danielsson A, Palmqvist R, Sjodin H, Hagglund G, Overvad K, Palli D, Masala G, Riboli E, Kennedy H, Welch A, Khaw KT, Day N, Bingham S. Diet in the aetiology of ulcerative colitis: a European prospective cohort study. *Digestion* 2008; **77**: 57-64 [PMID: 18349539 DOI: 10.1159/000121412]
117 **de Silva PS**, Olsen A, Christensen J, Schmidt EB, Overvaad K, Tjonneland A, Hart AR. An association between dietary arachidonic acid, measured in adipose tissue, and ulcerative colitis.

Gastroenterology 2010; **139**: 1912-1917 [PMID: 20950616 DOI: 10.1053/j.gastro.2010.07.065]
118 **Kono H**, Fujii H, Ogiku M, Tsuchiya M, Ishii K, Hara M. Enteral diets enriched with medium-chain triglycerides and N-3 fatty acids prevent chemically induced experimental colitis in rats. *Transl Res* 2010; **156**: 282-291 [PMID: 20970751 DOI: 10.1016/j.trsl.2010.07.012]
119 **John S**, Luben R, Shrestha SS, Welch A, Khaw KT, Hart AR. Dietary n-3 polyunsaturated fatty acids and the aetiology of ulcerative colitis: a UK prospective cohort study. *Eur J Gastroenterol Hepatol* 2010; **22**: 602-606 [PMID: 20216220]
120 **Ananthakrishnan AN**, Khalili H, Konijeti GG, Higuchi LM, de Silva P, Fuchs CS, Willett WC, Richter JM, Chan AT. Long-term intake of dietary fat and risk of ulcerative colitis and Crohn's disease. *Gut* 2014; **63**: 776-784 [PMID: 23828881 DOI: 10.1136/gutjnl-2013-305304]
121 **Klement E**, Cohen RV, Boxman J, Joseph A, Reif S. Breastfeeding and risk of inflammatory bowel disease: a systematic review with meta-analysis. *Am J Clin Nutr* 2004; **80**: 1342-1352 [PMID: 15531685]
122 **Barclay AR**, Russell RK, Wilson ML, Gilmour WH, Satsangi J, Wilson DC. Systematic review: the role of breastfeeding in the development of pediatric inflammatory bowel disease. *J Pediatr* 2009; **155**: 421-426 [PMID: 19464699 DOI: 10.1016/j.jpeds.2009.03.017]
123 **Blanchard JF**, Bernstein CN, Wajda A, Rawsthorne P. Small-area variations and sociodemographic correlates for the incidence of Crohn's disease and ulcerative colitis. *Am J Epidemiol* 2001; **154**: 328-335 [PMID: 11495856]
124 **Klement E**, Lysy J, Hoshen M, Avitan M, Goldin E, Israeli E. Childhood hygiene is associated with the risk for inflammatory bowel disease: a population-based study. *Am J Gastroenterol* 2008; **103**: 1775-1782 [PMID: 18557710 DOI: 10.1111/j.1572-0241.2008.01905.x]
125 **Castiglione F**, Diaferia M, Morace F, Labianca O, Meucci C, Cuomo A, Panarese A, Romano M, Sorrentini I, D'Onofrio C, Caporaso N, Rispo A. Risk factors for inflammatory bowel diseases according to the "hygiene hypothesis": a case-control, multi-centre, prospective study in Southern Italy. *J Crohns Colitis* 2012; **6**: 324-329 [PMID: 22405169 DOI: 10.1016/j.crohns.2011.09.003]
126 **Bernstein CN**, Rawsthorne P, Cheang M, Blanchard JF. A population-based case control study of potential risk factors for IBD. *Am J Gastroenterol* 2006; **101**: 993-1002 [PMID: 16696783 DOI: 10.1111/j.1572-0241.2006.00381.x]
127 **Hampe J**, Heymann K, Krawczak M, Schreiber S. Association of inflammatory bowel disease with indicators for childhood antigen and infection exposure. *Int J Colorectal Dis* 2003; **18**: 413-417 [PMID: 12687394 DOI: 10.1007/s00384-003-0484-1]
128 **Wu XW**, Ji HZ, Yang MF, Wu L, Wang FY. Helicobacter pylori infection and inflammatory bowel disease in Asians: a meta-analysis. *World J Gastroenterol* 2015; **21**: 4750-4756 [PMID: 25914487 DOI: 10.3748/wjg.v21.i15.4750]
129 **Luther J**, Dave M, Higgins PD, Kao JY. Association between Helicobacter pylori infection and inflammatory bowel disease: a meta-analysis and systematic review of the literature. *Inflamm Bowel Dis* 2010; **16**: 1077-1084 [PMID: 19760778 DOI: 10.1002/ibd.21116]
130 **Papamichael K**, Konstantopoulos P, Mantzaris GJ. Helicobacter pylori infection and inflammatory bowel disease: is there a link? *World J Gastroenterol* 2014; **20**: 6374-6385 [PMID: 24914359 DOI: 10.3748/wjg.v20.i21.6374]
131 **Weinstock JV**, Elliott DE. Helminths and the IBD hygiene hypothesis. *Inflamm Bowel Dis* 2009; **15**: 128-133 [PMID: 18680198 DOI: 10.1002/ibd.20633]
132 **Szkudlapski D**, Labuzek K, Pokora Z, Smyla N, Gonciarz M, Mularczyk A, Maluch P, Okopien B. The emering role of helminths in treatment of the inflammatory bowel disorders. *J Physiol Pharmacol* 2014; **65**: 741-751 [PMID: 25554978]
133 **Mohammadi R**, Hosseini-Safa A, Ehsani Ardakani MJ, Rostami-Nejad M. The relationship between intestinal parasites and some immune-mediated intestinal conditions. *Gastroenterol Hepatol Bed Bench* 2015; **8**: 123-131 [PMID: 25926937]
134 **Koloski NA**, Bret L, Radford-Smith G. Hygiene hypothesis in inflammatory bowel disease: a critical review of the literature. *World J Gastroenterol* 2008; **14**: 165-173 [PMID: 18186549 DOI: 10.3748/wjg.14.165]
135 **Hunter MM**, McKay DM. Review article: helminths as therapeutic agents for inflammatory bowel disease. *Aliment Pharmacol Ther* 2004; **19**: 167-177 [PMID: 14723608 DOI: 10.1111/j.0269-2813.2004.01803.x]
136 **Ottow MK**, Klaver EJ, van der Pouw Kraan TC, Heijnen PD, Laan LC, Kringel H, Vogel DY, Dijkstra CD, Kooij G, van Die I. The helminth Trichuris suis suppresses TLR4-induced inflammatory responses in human macrophages. *Genes Immun* 2014; **15**: 477-486 [PMID: 25008860 DOI: 10.1038/gene.2014.38]
137 **Sun S**, Wang X, Wu X, Zhao Y, Wang F, Liu X, Song Y, Wu Z, Liu M. Toll-like receptor activation by helminths or helminth products to alleviate inflammatory bowel disease. *Parasit Vectors* 2011; **4**: 186 [PMID: 21943110 DOI: 10.1186/1756-3305-4-186]
138 **Bernstein CN**, Blanchard JF, Rawsthorne P, Collins MT. Population-based case control study of seroprevalence of Mycobacterium paratuberculosis in patients with Crohn's disease and ulcerative colitis. *J Clin Microbiol* 2004; **42**: 1129-1135 [PMID: 15004064 DOI: 10.1128/JCM.42.3.1129-1135.2004]
139 **Momotani E**, Ozaki H, Hori M, Yamamoto S, Kuribayashi T, Eda S, Ikegami M. Mycobacterium avium subsp. paratuberculosis lipophilic antigen causes Crohn's disease-type necrotizing colitis in Mice. *Springerplus* 2012; **1**: 47 [PMID: 23519342 DOI: 10.1186/2193-1801-1-47]
140 **Nazareth N**, Magro F, Machado E, Ribeiro TG, Martinho A, Rodrigues P, Alves R, Macedo GN, Gracio D, Coelho R, Abreu C, Appelberg R, Dias C, Macedo G, Bull T, Sarmento A. Prevalence of Mycobacterium avium subsp. paratuberculosis and Escherichia coli in blood samples from patients with inflammatory bowel disease. *Med Microbiol Immunol* 2015; **204**: 681-692 [PMID: 25994082 DOI: 10.1007/s00430-015-0420-3]
141 **Feller M**, Huwiler K, Stephan R, Altpeter E, Shang A, Furrer H, Pfyffer GE, Jemmi T, Baumgartner A, Egger M. Mycobacterium avium subspecies paratuberculosis and Crohn's disease: a systematic review and meta-analysis. *Lancet Infect Dis* 2007; **7**: 607-613 [PMID: 17714674 DOI: 10.1016/S1473-3099(07)70211-6]
142 **Wang ZK**, Yang YS, Chen Y, Yuan J, Sun G, Peng LH. Intestinal microbiota pathogenesis and fecal microbiota transplantation for inflammatory bowel disease. *World J Gastroenterol* 2014; **20**: 14805-14820 [PMID: 25356041 DOI: 10.3748/wjg.v20.i40.14805]
143 **Liang J**, Sha SM, Wu KC. Role of the intestinal microbiota and fecal transplantation in inflammatory bowel diseases. *J Dig Dis* 2014; **15**: 641-646 [PMID: 25389085 DOI: 10.1111/1751-2980.12211]
144 **Bosca-Watts MM**, Tosca J, Anton R, Mora M, Minguez M, Mora F. Pathogenesis of Crohn's disease: Bug or no bug. *World J Gastrointest Pathophysiol* 2015; **6**: 1-12 [PMID: 25685606 DOI: 10.4291/wjgp.v6.i1.1]
145 **Forbes A**, Kalantzis T. Crohn's disease: the cold chain hypothesis. *Int J Colorectal Dis* 2006; **21**: 399-401 [PMID: 16059694 DOI: 10.1007/s00384-005-0003-7]
146 **Korzenik JR**. Past and current theories of etiology of IBD: toothpaste, worms, and refrigerators. *J Clin Gastroenterol* 2005; **39**: S59-S65 [PMID: 15758661 DOI: 10.1097/01.mcg.0000155553.28348.fc]
147 **Hugot JP**, Alberti C, Berrebi D, Bingen E, Cézard JP. Crohn's disease: the cold chain hypothesis. *Lancet* 2003; **362**: 2012-2015 [PMID: 14683664 DOI: 10.1016/S0140-6736(03)15024-6]
148 **Eckburg PB**, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA. Diversity of the human intestinal microbial flora. *Science* 2005; **308**: 1635-1638 [PMID: 15831718 DOI: 10.1126/science.1110591]
149 **O'Hara AM**, Shanahan F. The gut flora as a forgotten organ. *EMBO Rep* 2006; **7**: 688-693 [PMID: 16819463 DOI: 10.1038/sj.embor.7400731]
150 **Lotz M**, Ménard S, Hornef M. Innate immune recognition on the intestinal mucosa. *Int J Med Microbiol* 2007; **297**: 379-392 [PMID: 17459768 DOI: 10.1016/j.ijmm.2007.03.010]
151 **Qin J**, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C,

Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Doré J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, Bork P, Ehrlich SD, Wang J. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 2010; **464**: 59-65 [PMID: 20203603 DOI: 10.1038/nature08821]

152 **Adlerberth I**, Wold AE. Establishment of the gut microbiota in Western infants. *Acta Paediatr* 2009; **98**: 229-238 [PMID: 19143664 DOI: 10.1111/j.1651-2227.2008.01060.x]

153 **Fallani M**, Young D, Scott J, Norin E, Amarri S, Adam R, Aguilera M, Khanna S, Gil A, Edwards CA, Doré J. Intestinal microbiota of 6-week-old infants across Europe: geographic influence beyond delivery mode, breast-feeding, and antibiotics. *J Pediatr Gastroenterol Nutr* 2010; **51**: 77-84 [PMID: 20479681 DOI: 10.1097/MPG.0b013e3181d1b11e]

154 **Gevers D**, Kugathasan S, Denson LA, Vázquez-Baeza Y, Van Treuren W, Ren B, Schwager E, Knights D, Song SJ, Yassour M, Morgan XC, Kostic AD, Luo C, González A, McDonald D, Haberman Y, Walters T, Baker S, Rosh J, Stephens M, Heyman M, Markowitz J, Baldassano R, Griffiths A, Sylvester F, Mack D, Kim S, Crandall W, Hyams J, Huttenhower C, Knight R, Xavier RJ. The treatment-naive microbiome in new-onset Crohn's disease. *Cell Host Microbe* 2014; **15**: 382-392 [PMID: 24629344 DOI: 10.1016/j.chom.2014.02.005]

155 **Morgan XC**, Tickle TL, Sokol H, Gevers D, Devaney KL, Ward DV, Reyes JA, Shah SA, LeLeiko N, Snapper SB, Bousvaros A, Korzenik J, Sands BE, Xavier RJ, Huttenhower C. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome Biol* 2012; **13**: R79 [PMID: 23013615 DOI: 10.1186/gb-2012-13-9-r79]

156 **Matsuoka K**, Kanai T. The gut microbiota and inflammatory bowel disease. *Semin Immunopathol* 2015; **37**: 47-55 [PMID: 25420450 DOI: 10.1007/s00281-014-0454-4]

157 **Manichanh C**, Rigottier-Gois L, Bonnaud E, Gloux K, Pelletier E, Frangeul L, Nalin R, Jarrin C, Chardon P, Marteau P, Roca J, Dore J. Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. *Gut* 2006; **55**: 205-211 [PMID: 16188921 DOI: 10.1136/gut.2005.073817]

158 **Frank DN**, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci USA* 2007; **104**: 13780-13785 [PMID: 17699621]

159 **Sokol H**, Seksik P, Furet JP, Firmesse O, Nion-Larmurier I, Beaugerie L, Cosnes J, Corthier G, Marteau P, Doré J. Low counts of Faecalibacterium prausnitzii in colitis microbiota. *Inflamm Bowel Dis* 2009; **15**: 1183-1189 [PMID: 19235886 DOI: 10.1002/ibd.20903]

160 **Miquel S**, Martín R, Rossi O, Bermúdez-Humarán LG, Chatel JM, Sokol H, Thomas M, Wells JM, Langella P. Faecalibacterium prausnitzii and human intestinal health. *Curr Opin Microbiol* 2013; **16**: 255-261 [PMID: 23831042 DOI: 10.1016/j.mib.2013.06.003]

161 **Furusawa Y**, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, Nakanishi Y, Uetake C, Kato K, Kato T, Takahashi M, Fukuda NN, Murakami S, Miyauchi E, Hino S, Atarashi K, Onawa S, Fujimura Y, Lockett T, Clarke JM, Topping DL, Tomita M, Hori S, Ohara O, Morita T, Koseki H, Kikuchi J, Honda K, Hase K, Ohno H. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* 2013; **504**: 446-450 [PMID: 24226770 DOI: 10.1038/nature12721]

162 **Ohkusa T**, Koido S. Intestinal microbiota and ulcerative colitis. *J Infect Chemother* 2015; **21**: 1-8 [DOI: 10.1016/j.jiac.2015.07.010]

163 **Atarashi K**, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, Fukuda S, Saito T, Narushima S, Hase K, Kim S, Fritz JV, Wilmes P, Ueha S, Matsushima K, Ohno H, Olle B, Sakaguchi S, Taniguchi T, Morita H, Hattori M, Honda K. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature* 2013; **500**: 232-236 [PMID: 23842501 DOI: 10.1038/nature12331]

164 **Ott SJ**, Plamondon S, Hart A, Begun A, Rehman A, Kamm MA, Schreiber S. Dynamics of the mucosa-associated flora in ulcerative colitis patients during remission and clinical relapse. *J Clin Microbiol* 2008; **46**: 3510-3513 [PMID: 18701655 DOI: 10.1128/jcm.01512-08]

165 **Rolhion N**, Darfeuille-Michaud A. Adherent-invasive Escherichia coli in inflammatory bowel disease. *Inflamm Bowel Dis* 2007; **13**: 1277-1283 [PMID: 17476674 DOI: 10.1002/ibd.20176]

166 **Garrett WS**, Gallini CA, Yatsunenko T, Michaud M, DuBois A, Delaney ML, Punit S, Karlsson M, Bry L, Glickman JN, Gordon JI, Onderdonk AB, Glimcher LH. Enterobacteriaceae act in concert with the gut microbiota to induce spontaneous and maternally transmitted colitis. *Cell Host Microbe* 2010; **8**: 292-300 [PMID: 20833380 DOI: 10.1016/j.chom.2010.08.004]

167 **Mukhopadhya I**, Hansen R, El-Omar EM, Hold GL. IBD-what role do Proteobacteria play? *Nat Rev Gastroenterol Hepatol* 2012; **9**: 219-230 [PMID: 22349170 DOI: 10.1038/nrgastro]

168 **Lupp C**, Robertson ML, Wickham ME, Sekirov I, Champion OL, Gaynor EC, Finlay BB. Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of Enterobacteriaceae. *Cell Host Microbe* 2007; **2**: 204 [PMID: 18030708 DOI: 10.1016/j.chom.2007.08.002]

169 **Kostic AD**, Xavier RJ, Gevers D. The microbiome in inflammatory bowel disease: current status and the future ahead. *Gastroenterology* 2014; **146**: 1489-1499 [PMID: 24560869 DOI: 10.1053/j.gastro.2014.02.009]

170 **Garg M**, Lubel JS, Sparrow MP, Holt SG, Gibson PR. Review article: vitamin D and inflammatory bowel disease--established concepts and future directions. *Aliment Pharmacol Ther* 2012; **36**: 324-344 [PMID: 22686333 DOI: 10.1111/j.1365-2036.2012.05181.x]

171 **Wöbke TK**, Sorg BL, Steinhilber D. Vitamin D in inflammatory diseases. *Front Physiol* 2014; **5**: 244 [PMID: 25071589 DOI: 10.3389/fphys.2014.00244]

172 **Cantorna MT**, Mahon BD. D-hormone and the immune system. *J Rheumatol Suppl* 2005; **76**: 11-20 [PMID: 16142846]

173 **Cantorna MT**, Mahon BD. Mounting evidence for vitamin D as an environmental factor affecting autoimmune disease prevalence. *Exp Biol Med* (Maywood) 2004; **229**: 1136-1142 [PMID: 15564440]

174 **Cantorna MT**, Zhu Y, Froicu M, Wittke A. Vitamin D status, 1,25-dihydroxyvitamin D3, and the immune system. *Am J Clin Nutr* 2004; **80**: 1717S-1720S [PMID: 15585793]

175 **Jørgensen SP**, Agnholt J, Glerup H, Lyhne S, Villadsen GE, Hvas CL, Bartels LE, Kelsen J, Christensen LA, Dahlerup JF. Clinical trial: vitamin D3 treatment in Crohn's disease - a randomized double-blind placebo-controlled study. *Aliment Pharmacol Ther* 2010; **32**: 377-383 [PMID: 20491740 DOI: 10.1111/j.1365-2036.2010.04355.x]

176 **Palmer MT**, Weaver CT. Linking vitamin d deficiency to inflammatory bowel disease. *Inflamm Bowel Dis* 2013; **19**: 2245-2256 [PMID: 23591600 DOI: 10.1097/MIB.0b013e31828a3b6f]

177 **Mouli VP**, Ananthakrishnan AN. Review article: vitamin D and inflammatory bowel diseases. *Aliment Pharmacol Ther* 2014; **39**: 125-136 [PMID: 24236989 DOI: 10.1111/apt.12553]

178 **Levin AD**, Wadhera V, Leach ST, Woodhead HJ, Lemberg DA, Mendoza-Cruz AC, Day AS. Vitamin D deficiency in children with inflammatory bowel disease. *Dig Dis Sci* 2011; **56**: 830-836 [PMID: 21222159 DOI: 10.1007/s10620-010-1544-3]

179 **Ulitsky A**, Ananthakrishnan AN, Naik A, Skaros S, Zadvornova Y, Binion DG, Issa M. Vitamin D deficiency in patients with inflammatory bowel disease: association with disease activity and quality of life. *JPEN J Parenter Enteral Nutr* 2011; **35**: 308-316 [PMID: 21527593 DOI: 10.1177/0148607110381267]

180 **Nerich V**, Jantchou P, Boutron-Ruault MC, Monnet E, Weill A, Vanbockstael V, Auleley GR, Balaire C, Dubost P, Rican S, Allemand H, Carbonnel F. Low exposure to sunlight is a risk factor for Crohn's disease. *Aliment Pharmacol Ther* 2011; **33**: 940-945 [PMID: 21332762 DOI: 10.1111/j.1365-2036.2011.04601.x]

181 **Khalili H**, Huang ES, Ananthakrishnan AN, Higuchi L, Richter JM, Fuchs CS, Chan AT. Geographical variation and incidence of

inflammatory bowel disease among US women. *Gut* 2012; **61**: 1686-1692 [PMID: 22241842 DOI: 10.1136/gutjnl-2011-301574]
182 **Ananthakrishnan AN**, Khalili H, Higuchi LM, Bao Y, Korzenik JR, Giovannucci EL, Richter JM, Fuchs CS, Chan AT. Higher predicted vitamin D status is associated with reduced risk of Crohn's disease. *Gastroenterology* 2012; **142**: 482-489 [PMID: 22155183 DOI: 10.1053/j.gastro.2011.11.040]
183 **Ananthakrishnan AN**, Cagan A, Gainer VS, Cai T, Cheng SC, Savova G, Chen P, Szolovits P, Xia Z, De Jager PL, Shaw SY, Churchill S, Karlson EW, Kohane I, Plenge RM, Murphy SN, Liao KP. Normalization of plasma 25-hydroxy vitamin D is associated with reduced risk of surgery in Crohn's disease. *Inflamm Bowel Dis* 2013; **19**: 1921-1927 [PMID: 23751398 DOI: 10.1097/MIB.0b013e3182902ad9]
184 **Zhu Y**, Mahon BD, Froicu M, Cantorna MT. Calcium and 1 alpha,25-dihydroxyvitamin D3 target the TNF-alpha pathway to suppress experimental inflammatory bowel disease. *Eur J Immunol* 2005; **35**: 217-224 [PMID: 15593122 DOI: 10.1002/eji.200425491]
185 **Zhu T**, Liu TJ, Shi YY, Zhao Q. Vitamin D/VDR signaling pathway ameliorates 2,4,6-trinitrobenzene sulfonic acid-induced colitis by inhibiting intestinal epithelial apoptosis. *Int J Mol Med* 2015; **35**: 1213-1218 [PMID: 25813397 DOI: 10.3892/ijmm.2015.2150]
186 **Limketkai BN**, Bayless TM, Brant SR, Hutfless SM. Lower regional and temporal ultraviolet exposure is associated with increased rates and severity of inflammatory bowel disease hospitalisation. *Aliment Pharmacol Ther* 2014; **40**: 508-517 [PMID: 24943863 DOI: 10.1111/apt.12845]
187 **Shaw SY**, Nugent Z, Targownik LE, Singh H, Blanchard JF, Bernstein CN. Association between spring season of birth and Crohn's disease. *Clin Gastroenterol Hepatol* 2014; **12**: 277-282 [PMID: 23924874 DOI: 10.1016/j.cgh.2013.07.028]
188 **Disanto G**, Chaplin G, Morahan JM, Giovannoni G, Hyppönen E, Ebers GC, Ramagopalan SV. Month of birth, vitamin D and risk of immune-mediated disease: a case control study. *BMC Med* 2012; **10**: 69 [PMID: 22764877 DOI: 10.1186/1741-7015-10-69]
189 **Kaplan GG**, Hubbard J, Korzenik J, Sands BE, Panaccione R, Ghosh S, Wheeler AJ, Villeneuve PJ. The inflammatory bowel diseases and ambient air pollution: a novel association. *Am J Gastroenterol* 2010; **105**: 2412-2419 [PMID: 20588264 DOI: 10.1038/ajg.2010.252]
190 **Ananthakrishnan AN**, McGinley EL, Binion DG, Saeian K. Ambient air pollution correlates with hospitalizations for inflammatory bowel disease: an ecologic analysis. *Inflamm Bowel Dis* 2011; **17**: 1138-1145 [PMID: 20806342 DOI: 10.1002/ibd.21455]
191 **Salim SY**, Kaplan GG, Madsen KL. Air pollution effects on the gut microbiota: a link between exposure and inflammatory disease. *Gut Microbes* 2014; **5**: 215-219 [PMID: 24637593 DOI: 10.4161/gmic.27251]
192 **Rice KM**, Walker EM, Wu M, Gillette C, Blough ER. Environmental mercury and its toxic effects. *J Prev Med Public Health* 2014; **47**: 74-83 [PMID: 24744824 DOI: 10.3961/jpmph.2014.47.2.74]
193 **Mawdsley JE**, Rampton DS. Psychological stress in IBD: new insights into pathogenic and therapeutic implications. *Gut* 2005; **54**: 1481-1491 [PMID: 16162953 DOI: 10.1136/gut.2005.064261]
194 **Reber SO**. Stress and animal models of inflammatory bowel disease--an update on the role of the hypothalamo-pituitary-adrenal axis. *Psychoneuroendocrinology* 2012; **37**: 1-19 [PMID: 21741177 DOI: 10.1016/j.psyneuen.2011.05.014]
195 **Ennaifer R**, Elleuch N, Cheikh M, Hefaiedh R, Romdhane H, Ben Nejma H, Belhadj N. Risk factors of psychological disorders in inflammatory bowel disease in a tunisian survey. Results of a cross-sectional study. *Tunis Med* 2014; **92**: 723-726 [PMID: 25879596]
196 **Bannaga AS**, Selinger CP. Inflammatory bowel disease and anxiety: links, risks, and challenges faced. *Clin Exp Gastroenterol* 2015; **8**: 111-117 [PMID: 25848313 DOI: 10.2147/CEG.S57982]
197 **Goodhand JR**, Wahed M, Mawdsley JE, Farmer AD, Aziz Q, Rampton DS. Mood disorders in inflammatory bowel disease: relation to diagnosis, disease activity, perceived stress, and other factors. *Inflamm Bowel Dis* 2012; **18**: 2301-2309 [PMID: 22359369 DOI: 10.1002/ibd.22916]
198 **Long MD**, Kappelman MD, Martin CF, Chen W, Anton K, Sandler RS. Risk factors for depression in the elderly inflammatory bowel disease population. *J Crohns Colitis* 2014; **8**: 113-119 [PMID: 23932782 DOI: 10.1016/j.crohns.2013.07.002]
199 **Szigethy EM**, Youk AO, Benhayon D, Fairclough DL, Newara MC, Kirshner MA, Bujoreanu SI, Mrakotsky C, Bousvaros A, Srinath AI, Keljo DJ, Kupfer DJ, DeMaso DR. Depression subtypes in pediatric inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 2014; **58**: 574-581 [PMID: 24345836 DOI: 10.1097/MPG.0000000000000262]
200 **Ananthakrishnan AN**, Gainer VS, Perez RG, Cai T, Cheng SC, Savova G, Chen P, Szolovits P, Xia Z, De Jager PL, Shaw SY, Churchill S, Karlson EW, Kohane I, Perlis RH, Plenge RM, Murphy SN, Liao KP. Psychiatric co-morbidity is associated with increased risk of surgery in Crohn's disease. *Aliment Pharmacol Ther* 2013; **37**: 445-454 [PMID: 23289600 DOI: 10.1111/apt.12195]
201 **Collins SM**. Stress and the Gastrointestinal Tract IV. Modulation of intestinal inflammation by stress: basic mechanisms and clinical relevance. *Am J Physiol Gastrointest Liver Physiol* 2001; **280**: G315-G318 [PMID: 11171612]
202 **Levenstein S**, Prantera C, Varvo V, Scribano ML, Andreoli A, Luzi C, Arcà M, Berto E, Milite G, Marcheggiano A. Stress and exacerbation in ulcerative colitis: a prospective study of patients enrolled in remission. *Am J Gastroenterol* 2000; **95**: 1213-1220 [PMID: 10811330 DOI: 10.1111/j.1572-0241.2000.02012.x]
203 **Bitton A**, Dobkin PL, Edwardes MD, Sewitch MJ, Meddings JB, Rawal S, Cohen A, Vermeire S, Dufresne L, Franchimont D, Wild GE. Predicting relapse in Crohn's disease: a biopsychosocial model. *Gut* 2008; **57**: 1386-1392 [PMID: 18390994 DOI: 10.1136/gut.2007.134817]
204 **Bernstein CN**, Singh S, Graff LA, Walker JR, Miller N, Cheang M. A prospective population-based study of triggers of symptomatic flares in IBD. *Am J Gastroenterol* 2010; **105**: 1994-2002 [PMID: 20372115 DOI: 10.1038/ajg.2010.140]
205 **Kaname S**, Uchida S, Ogata E, Kurokawa K. Autocrine secretion of transforming growth factor-beta in cultured rat mesangial cells. *Kidney Int* 1992; **42**: 1319-1327 [PMID: 1474764 DOI: 10.1038/ki.1992.423]
206 **Graff LA**, Walker JR, Bernstein CN. Depression and anxiety in inflammatory bowel disease: a review of comorbidity and management. *Inflamm Bowel Dis* 2009; **15**: 1105-1118 [PMID: 19161177 DOI: 10.1002/ibd.20873]
207 **Ananthakrishnan AN**, Long MD, Martin CF, Sandler RS, Kappelman MD. Sleep disturbance and risk of active disease in patients with Crohn's disease and ulcerative colitis. *Clin Gastroenterol Hepatol* 2013; **11**: 965-971 [PMID: 23376797 DOI: 10.1016/j.cgh.2013.01.021]
208 **Boye B**, Lundin KE, Jantschek G, Leganger S, Mokleby K, Tangen T, Jantschek I, Pripp AH, Wojniusz S, Dahlstroem A, Rivenes AC, Benninghoven D, Hausken T, Roseth A, Kunzendorf S, Wilhelmsen I, Sharpe M, Blomhoff S, Malt UF, Jahnsen J. INSPIRE study: does stress management improve the course of inflammatory bowel disease and disease-specific quality of life in distressed patients with ulcerative colitis or Crohn's disease? A randomized controlled trial. *Inflamm Bowel Dis* 2011; **17**: 1863-1873 [PMID: 21287660 DOI: 10.1002/ibd.21575]
209 **Ng V**, Millard W, Lebrun C, Howard J. Low-intensity exercise improves quality of life in patients with Crohn's disease. *Clin J Sport Med* 2007; **17**: 384-388 [PMID: 17873551]
210 **Packer N**, Hoffman-Goetz L, Ward G. Does physical activity affect quality of life, disease symptoms and immune measures in patients with inflammatory bowel disease? A systematic review. *J Sports Med Phys Fitness* 2010; **50**: 1-18 [PMID: 20308966]
211 **Khalili H**, Ananthakrishnan AN, Konijeti GG, Liao X, Higuchi LM, Fuchs CS, Spiegelman D, Richter JM, Korzenik JR, Chan AT. Physical activity and risk of inflammatory bowel disease: prospective study from the Nurses' Health Study cohorts. *BMJ* 2013; **347**: f6633 [PMID: 24231178 DOI: 10.1136/bmj.f6633]
212 **Ali T**, Madhoun MF, Orr WC, Rubin DT. Assessment of the

relationship between quality of sleep and disease activity in inflammatory bowel disease patients. *Inflamm Bowel Dis* 2013; **19**: 2440-2443 [PMID: 23945186 DOI: 10.1097/MIB.0b013e3182a0ea54]

213 **Nimmo ER**, Prendergast JG, Aldhous MC, Kennedy NA, Henderson P, Drummond HE, Ramsahoye BH, Wilson DC, Semple CA, Satsangi J. Genome-wide methylation profiling in Crohn's disease identifies altered epigenetic regulation of key host defense mechanisms including the Th17 pathway. *Inflamm Bowel Dis* 2012; **18**: 889-899 [PMID: 22021194 DOI: 10.1002/ibd.21912]

214 **Saito S**, Kato J, Hiraoka S, Horii J, Suzuki H, Higashi R, Kaji E, Kondo Y, Yamamoto K. DNA methylation of colon mucosa in ulcerative colitis patients: correlation with inflammatory status. *Inflamm Bowel Dis* 2011; **17**: 1955-1965 [PMID: 21830274 DOI: 10.1002/ibd.21573]

215 **Karatzas PS**, Mantzaris GJ, Safioleas M, Gazouli M. DNA methylation profile of genes involved in inflammation and autoimmunity in inflammatory bowel disease. *Medicine* (Baltimore) 2014; **93**: e309 [PMID: 25526479 DOI: 10.1097/MD.0000000000000309]

216 **Ventham NT**, Kennedy NA, Nimmo ER, Satsangi J. Beyond gene discovery in inflammatory bowel disease: the emerging role of epigenetics. *Gastroenterology* 2013; **145**: 293-308 [PMID: 23751777 DOI: 10.1053/j.gastro.2013.05.050]

217 **Tsaprouni LG**, Ito K, Powell JJ, Adcock IM, Punchard N. Differential patterns of histone acetylation in inflammatory bowel diseases. *J Inflamm* (Lond) 2011; **8**: 1 [PMID: 21272292 DOI: 10.1186/1476-9255-8-1]

218 **Stylianou E**. Epigenetics: the fine-tuner in inflammatory bowel disease? *Curr Opin Gastroenterol* 2013; **29**: 370-377 [PMID: 23743674 DOI: 10.1097/MOG.0b013e328360bd12]

219 **Wu F**, Zikusoka M, Trindade A, Dassopoulos T, Harris ML, Bayless TM, Brant SR, Chakravarti S, Kwon JH. MicroRNAs are differentially expressed in ulcerative colitis and alter expression of macrophage inflammatory peptide-2 alpha. *Gastroenterology* 2008; **135**: 1624-1635.e24 [PMID: 18835392 DOI: 10.1053/j.gastro.2008.07.068]

220 **Wu F**, Guo NJ, Tian H, Marohn M, Gearhart S, Bayless TM, Brant SR, Kwon JH. Peripheral blood microRNAs distinguish active ulcerative colitis and Crohn's disease. *Inflamm Bowel Dis* 2011; **17**: 241-250 [PMID: 20812331 DOI: 10.1002/ibd.21450]

P- Reviewer: Efthymiou A, Kopylov U, Spisni E, Tsai HH
S- Editor: Qi Y **L- Editor**: A **E- Editor**: Wang CH

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i1.126

World J Gastrointest Pharmacol Ther 2016 February 6; 7(1): 126-132
ISSN 2150-5349 (online)

MINIREVIEWS

Helicobacter pylori and its reservoirs: A correlation with the gastric infection

Spencer Luiz Marques Payão, Lucas Trevizani Rasmussen

Spencer Luiz Marques Payão, Laboratório de Genética, Hemocentro, Marília Medical School, FAMEMA, São Paulo, CEP 17519-050, Brazil

Spencer Luiz Marques Payão, Lucas Trevizani Rasmussen, Department of Graduate-Level Research Sacred Heart University, Universidade do Sagrado Coração, Bauru, SP 17011-160, Brazil

Author contributions: Payão SLM and Rasmussen LT contributed equally to this work.

Supported by São Paulo Research Foundation (FAPESP), Sacred Heart University of Bauru Marília School of Medicine, No. 2012/18333-3.

Conflict-of-interest statement: The authors declare no conflict of interest.

Correspondence to: Spencer Luiz Marques Payão, Professor, Laboratório de Genética, Hemocentro, Marília Medical School, FAMEMA, Rua Lourival Freire, 240, Bairro Fragata, Marília, São Paulo, CEP 17519-050, Brazil. slmpayao@famema.br
Telephone: +55-14-34021856
Fax: +55-14-34330148

Received: April 29, 2015
Peer-review started: April 30, 2015
First decision: July 25, 2015
Revised: October 16, 2015
Accepted: November 10, 2015
Article in press: November 11, 2015
Published online: February 6, 2016

Abstract

Helicobacter pylori (*H. pylori*) has long been found to cause gastric diseases such as gastritis, gastric ulcers and gastric cancer. The transmission medium of this bacterium has yet to be determined, though several studies have speculated that the oral cavity is a reservoir for *H. pylori*. Others have also reported that the oral cavity may be a source of both transmission and gastric reinfection; however, such results are controversial. We reviewed the literature and selected studies that report an association among *H. pylori* detections in the oral cavity (dental plaque, saliva, tongue, tonsil tissue, root canals, oral mucosa) in humans and in animals, as well as in the human stomach. The oral cavity may be considered the main reservoir for *H. pylori*. There are a correlations between *H. pylori* infection in the oral cavity and periodontal disease, oral tissue inflammation, *H. pylori* transmission, and gastric reinfection. We believe that the mouth is a reservoir and that it plays a crucial role in both *H. pylori* transmission and gastric infection.

Key words: *Helicobacter pylori*; Reservoirs; Oral cavity; Infection; Gastric disease

Core tip: This review focuses on some aspects of infection and reinfection by *Helicobacter pylori* (*H. pylori*), particularly in the possible reservoirs of this bacterium. It also explores the association between gastric infection and these reservoirs. In addition, this review highlights possible reservoirs in animals and some routes of infection, and it considers the techniques used to diagnose this bacterium in different environments. The difficulty in accessing bacteria in reservoirs is a problem for *H. pylori* eradication

in particular, and new discoveries in this field will contribute to the understanding of *H. pylori* infection mechanisms.

Payão SLM, Rasmussen LT. *Helicobacter pylori* and its reservoirs: A correlation with the gastric infection. *World J Gastrointest Pharmacol Ther* 2016; 7(1): 126-132 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i1/126.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i1.126

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a gram-negative and microaerophilic bacterium that has been associated with some certain diseases, including chronic gastritis, peptic ulcers, gastric cancer, and mucosa-associated lymphoid tissue (MALT) lymphoma. In 1994, the International Agency for Research on Cancer of the World Health Organization defined *H. pylori* as a Group 1 carcinogen[1,2].

It is estimated that the *H. pylori* is present in the stomachs of 50% of the world's population, but despite this high prevalence, we do not yet clearly understand its transmission. Possible routes are oral-oral and fecal-oral, but no consensus has been reached[3].

Currently, the main area of research into natural reservoirs for *H. pylori* has included oral *H. pylori* with gastric infection and the presence of *H. pylori* in the stomach[1,4]. Many studies have suggested that the primary reservoir for *H. pylori* is the oral cavity, but overall, the results have been very inconsistent[1-7].

Several previous studies reported success in diagnosing *H. pylori* from oral samples from dental plaque, saliva, tongue, tonsil tissue and root canals. These other researchers have raised the question of whether the mouth is a common source of stomach reinfection, even after treatment is received[4-8].

Miyabayashi *et al*[9] were the first to investigate the influence of oral *H. pylori* on the stomach. In short, their patients with oral *H. pylori* were found to be at a significantly higher risk for gastric reinfection after having received successful treatment. The authors also determined that drugs recommended for the eradication of the *H. pylori* from the stomach seem to have no effect on oral *H. pylori*[10].

At this stage of research, the crucial questions are whether the oral cavity is a reservoir and whether it plays a role in *H. pylori* transmission. Therefore, this review includes all relevant studies that report the detection of *H. pylori* in a reservoir and the possible relationship between gastric and extra-gastric infection.

EXTRA-GASTRIC RESERVOIR FOR *H. PYLORI*

Saliva and dental plaque

Krajden *et al*[11] were the first authors to perform *H. pylori* isolation in *H. pylori*-positive patients with gastric symptoms of infection[1]. Twenty-six years later, researchers have yet to reach a conclusion about the role of the oral cavity in gastric infection by *H. pylori.*

In 2015, two relevant studies were published on this topic. In the first, Ismail *et al*[12] developed a new nested-PCR assay that can be used to identify *H. pylori* DNA in dental plaque samples. These authors report that this new nested PCR is as sensitive as tests that rely on histology, and also that it may be useful when patients must be tested for *H. pylori* but are unable to undergo endoscopic examinations. In the second study, Amiri *et al*[13] analyzed 45 samples of dental plaque and detected *H. pylori* in 44% (20/45), 66.67% (30/45) and 77.78% (35/45) using PCR, LAMP reaction, and positivity for both the PCR test and the LAMP test, respectively. In addition to their reports of a high frequency *of H. pylori* in the dental plaque samples, they also determined that dental plaque may be one of the main causes of reinfection, as well as the cause of oral-oral transmission.

Ahmed *et al*[5] used PCR for 16S rRNA to establish the presence of *H. pylori*. They analyzed saliva and gastric biopsy samples from 400 symptomatic subjects and detected *H. pylori* in 246 (61.5%) and 240 (60%) samples from gastric biopsies and saliva, respectively. They also found that *H. pylori* prevalence in both samples was higher in older populations, and their report highlighted the oral-oral transmission.

Liu *et al*[4] analyzed 443 symptomatic patients and verified the presence of *H. pylori* in 59.4% (263/443) of their dental plaque samples and in 61.6% (273/443) of their stomach samples. These results corroborate the findings reported by Liu *et al*[14], who found 126 (58.9%) of 214 children's dental plaque samples to be *H. pylori* positive and who also reported a statistically significant correlation between the *H. pylori* infection and dental caries.

In addition, Souto and Colombo[15] studied 225 adult subjects who sought dental treatment and detected *H. pylori* in 24% of the samples. More sub-gingival biofilm samples (33.3%) than saliva samples (20%) tested positive for *H. pylori.* The same authors also offered the theory that "periodontal pocketing and inflammation may favor the colonization by (*H. pylori*)".

Rasmussen *et al*[16] used southern blots on DNA extracts from saliva and dental plaque samples in order to detect *H. pylori* in 78 patients with gastric disease. A total of 33 saliva samples (42%) were found to be positive for *H. pylori*, and 37 dental plaque samples (47.4%) were found to be positive. In another study by our group, Rasmussen *et al*[17] reported significant genotypic diversity among *H. pylori* cytotoxins that had been found in stomach, saliva, and dental plaque samples. The study revealed the strains from the stomach to be more virulent than those from the oral cavity.

Results described by Silva *et al*[7] are more

convincing. They detected *H. pylori* in 30/30 (100%) of their patients' gastric biopsies, in 16/30 (53.3%) of their saliva samples, and in 11/30 (36.6%) of their dental plaque samples. These researchers also detected the *cag*A gene in 13/30 (43.3%) of their patients' gastric biopsies, in 7/16 (43.8%) of their saliva samples, and in 3/11 (27.3%) of their dental plaque samples. Similar results found by Assumpção *et al*[18] who found that 96% of their patients' gastric mucosa samples and 72% of their dental plaque samples were positive for *H. pylori*. Furthermore, they determined 63/71 (89%) of their patients' dental plaque samples to be both positive for *H. pylori* and to possess identical *vac*A and *cag*A genotypes in gastric mucosa.

All authors mentioned above suggest that dental plaque and saliva may be an important reservoir for *H. pylori* and that the presence of *H. pylori* in the oral cavity may contribute to oral diseases, oral transmission, gastric reinfection, and also virulence strains.

Meanwhile, Silva Rossi-Aguiar *et al*[19] analyzed saliva, tongue dorsum, and supra gingival dental plaque samples from 43 patients with gastric disease and did not detect *H. pylori* in these oral samples. Their results that consistent with those of Olivier *et al*[20], who also failed to detect *H. pylori* in dental samples. Both studies question the theory of the oral cavity serving as a reservoir for *H. pylori* in patients with symptoms of gastric disease.

There is a large variability in the oral cavity *H. pylori* frequency rates described in the literature. These differences could be explained by variations in sample demographics, the use of different sampling procedures or detection methods, the patients' oral health statuses, differences in *H. pylori* infection statuses, the type and number of clinical samples used, and varying complexities among the oral microbiota samples used across the studies[7,15-17,21].

Tongue

Gastric heterotopia (GH) can occur throughout the digestive tract; however, the involvement of the tongue is rare, and fewer than 40 cases have been reported to date. When GH is found in the head and neck region, it is frequently in cases of children or young adults, and it is more prominent among males[22,23].

A histologic evaluation was given to a 21-year-old man who reported the growth of a mass on his tongue 4 years prior to the exam and who presented ulcerations in the last 3 mo. The evaluation revealed the presence of gastric tissue that extended into the striated muscle layer of the tongue. There were scattered intestinal metaplasia foci containing Goblet cells. Toluidine-blue-stained sections suggested the presence of *H. pylori* in the lumina of the glandular epithelium. Colonization by *H. pylori* was determined by the immunohistochemical method using polyclonal *H. pylori* antibody, and the finding was confirmed using the PCR method[23].

There is also a great diversity in the different biological surfaces of the oral cavity that are subject to colonization by different bacterial species[24]. Different studies have isolated *H. pylori* from samples taken from oral cavities, dental plaque (supragingival and subgingival plaque), dorsa of tongue, and salivary secretions. *H. pylori* findings in oral cavities and dental plaque are conflicting. The differing results of tests to determine the prevalence of *H. pylori* in oral cavities are partly due to the use of different detection methods[25,26].

Clinical presentation varies depending on the site considered and on the extent of the lesion. An GH issue of note is the colonization by *H. pylori* and its association with complications. In their study, Berber *et al*[23] reports the first case of a peptic ulcer and intestinal metaplasia associated with the colonization of *H. pylori* in a case of GH of the tongue[23].

Tonsillar tissues

Although the oral cavity has been suggested as a reservoir for *H. pylori* infection, the findings have not been definitive. Some authors have proposed the theory that in cases of gastroesophageal reflux disease (GERD), gastric fluid that is contaminated with *H. pylori* enters the nasopharyngeal cavity, thus allowing the bacterium to colonize in dental plaque and adenotonsillar tissue[27]. Other researchers have also recently offered evidence of *H. pylori* in gastric mucosa that was bound to MALT. Because of these findings, more efforts have recently been placed on detecting *H. pylori* in adenotonsillar tissue[6].

Some studies have used different detection methods to determine the presence of *H. pylori* in tonsil and adenoid tissue[28-30].

Nártová *et al*[31] detected and genotyped *H. pylori* and found data supporting the possible role played by *H. pylori* in the etiologies of both chronic tonsillitis and sleep apnoea syndrome (SAS). *H. pylori* was detected through the use of real-time polymerase chain reaction. A total of 89 patients were tested, 60 of whom had received a diagnosis of chronic tonsillitis and 29 of whom had SAS. In the chronic tonsillitis group, *H. pylori* was detected in 48 (80%) of the samples, the *cag*A gene was detected in 12 samples (25%), and 12 samples were negative. In the SAS group, *H. pylori* was found in 24 samples (82.76%), *cag*A gene was detected in 5 samples (20.83%), and 5 samples (17.24%) were negative.

Nártová *et al*[31]'s study shows that the oropharynx represents a reservoir for *H. pylori* infection that could be an etiopathogenetic factor in chronic tonsillitis and tonsillar hyperplasia caused by SAS. No conclusion has been drawn regarding the mechanisms of the process.

In another study, Abdel-Monem *et al*[28] tested 30 adenotonsillectomy specimens (20 tonsils and 10 adenoids). RUT results were positive in 16 of their samples (12 tonsils and 4 adenoids; 53.3%). The authors report that, "according to the 'gold standard', 11/16 were considered a false positive, yielding a sensitivity of 100% and specificity of 56%". The authors also used PCR, and the ureC gene sequence was detected in 5 specimens (3 tonsils and 2 adenoids; 16.6%), all of which also tested positive when RUT was used. In these cases, the patients were considered to be infected by *H. pylori*. For this reason, the authors reported PCR sensitivity and specificity to be 100%. Serology testing results were positive for *H. pylori* IgG antibodies in 4/20 patients (20%), only two of whom were found to have *H. pylori*-infected adenotonsillar tissue.

On the other hand, Aliakbari *et al*[32] reported that neither gastrointestinal symptoms nor *H. pylori* seropositivity were correlated with the presence of *H. pylori* or *H. hepaticus* in adenotonsillar tissues. The findings did not support the idea that adenotonsills is a reservoir for *H. pylori* or *H. hepaticus*. The study included 90 patients (36% female and 64% male) who had been diagnosed with chronic tonsillitis and adenoid hypertrophy; the average age of the study group was 36 ± 22 years. In their study, Aliakbari *et al*[32] detected *H. pylori* and *H. hepaticus* using *glmM* gene and 16S rRNA-specific primers, respectively. Out of all of their patients, 58 (65%) were found to be seropositive for the *H. pylori* IgG, though only 7 (8%) patients presented any gastrointestinal symptoms and all 7 were cases of gastritis. According to the authors, neither *H. pylori* nor *H. hepaticus* was detected in any of the patients when PCR was used[32].

In conclusion, there are inconsistent results regarding the detection of *H. pylori* in the tonsils and adenoids; however, we believe that there is enough evidence to support the theory that such tissues can be considered a reservoir of such bacteria.

Root canals and oral mucosa

Most of the studies considered in this review analyzed dental plaque, saliva, and/or oral mucosa samples. In these studies, several *H. pylori* markers were identified through the use of various tests, including the urea breath test, the rapid urease test, the *Campylobacter*-like organism test, and/or polymerase chain reaction (PCR). Some PCR studies found *H. pylori* DNA in samples from oral cavities, but overall, reports of live *H. pylori* are very rare and inconclusive[33].

In light of this literature review, we agree with Zou and Li[34] and their report that *H. pylori* can be identified unequivocally only through the use of direct cultures. This limitation exists because erroneous PCR results can result from the presence of transient *H. pylori* in the mouth. This transient presence occurs in cases of interference from food or from acid that includes *H. pylori* or its DNA that reaches the mouth *via* reflux from the stomach[33,35,36]. Hirsch *et al*[8] "erroneous results can also arise from the misclassification of other urease-producing microorganisms. Thus, it is still unclear whether *H. pylori* can indeed survive in the oral environment".

In another study by Hirsch *et al*[8], electron microscopy, selective growth techniques, urease assays, 16S rRNA PCR, and western blotting were used to determine whether live *H. pylori* were present in 10 root canal and corresponding plaque samples taken from endodontic-infected deciduous teeth from three children. In their study, they report that PCR was able to identifiy *H. pylori* DNA in several plaque and root canal samples. However, bacterial colonies were successfully grown from two root canals, but not from plaque. As the authors report, "these colonies were unequivocally identified as *H. pylori* by microscopic, genetic, and biochemical approaches"[8]. The authors showed that root canals performed on endodontic-infected teeth may create a reservoir for live *H. pylori*, and that this reservoir may serve as a potential source of transmission.

Genomic DNA was isolated from samples taken during 25 root canals of teeth from patients with asymptomatic and chronic apical periodontitis and from 25 patients with aspirates from acute apical abscess. These DNA samples were first amplified using the multiple displacement amplification approach and were then used as a template in species-specific PCR in order to determine whether *H. pylori* and *C. pneumoniae* were present. Neither *H. pylori* nor *C. pneumoniae* were found in samples from primary endodontic infections. These findings suggest that these species are not possible endodontic pathogens and that the necrotic root canal does not serve as a reservoir for these human pathogens in healthy patients[37].

In another study by Correia-Silva Jde *et al*[38], many *H. pylori*-positive results were found in the oral mucosa of 46 haematopoietic stem cell transplantation (HSCT) patients. The authors report that their findings may be due to the patients' poor oral hygiene during the transplantation and/or immunosuppression procedures involved in HSCT therapy[38]. Other authors note that, because "the oral cavity is a frequent site of local infections and an important port of entry for systemic infections in HSCT recipients..., the presence of *H. pylori* in the oral cavity may be a risk factor for infection or reinfection of the stomach of these patients"[9,38]. Though this literature review shows that the exact role played by *H. pylori* in oral cavity infections has not been confirmed, these findings may be relevant to the gastrointestinal pathology of HSCT patients.

H. PYLORI IN ANIMMALS: A POSSIBLE RESERVOIR

According to Momtaz *et al*[39], there is a possibility that zoonotic transmission of *H. pylori* occurred, but this transmission has not been proven in non-primate reservoirs.

In the first report of infection by *H. pylori* in animals in 1990, Jones and Elridge[40] isolated a strain of *H. pylori* from a pig stomach and suggested that pigs may be a possible reservoir for this bacterium. Eaton *et al*[41] and Engstrand *et al*[42] supported this hypothesis; both authors have succeeded in infecting pigs, specimens which subsequently developed gastritis. However, De Groote *et al*[43] more recently sequenced the 16S of rDNA of *H. pylori* and then named the bacterium *Helicobacter suis*. Mégraud and Broutet[44] analyzed all of these studies and concluded that pigs are not a reservoir for *H. pylori*. They suggest the bacterium isolated by Jones and Elridge[40] was probably acquired from human beings.

Four years later, Jones and Elridge[40] detected *H. pylori* in a pig stomach sample. Handt *et al*[45] diagnosed the *H. pylori* in six cats - their identification was confirmed through the use of 16S rDNA sequencing. However, no other studies confirm these results. These studies argue that cats serve as a reservoir for *H. pylori,* but additional studies are necessary to determine whether cats are actually an important route of transmission. Based on the overall data available, having a cat as a pet does not put owners at risk of acquiring *H. pylori* infection.

More recently, Momtaz *et al*[39] analyzed 800 samples, 200 of which were from human beings and 600 of which came from healthy animals (200 cows, 200 sheep and 200 goats). They detected *H. pylori* and main virulence markers (gene *cag*A and *vac*A) using PCR and selected 6 *H. pylori*-positive samples (3 samples from cows and 3 samples from sheep) for DNA sequencing analysis.

They reported that the *H. pylori* was detected in 0/200 goat samples, 6/200 (3%) cow samples and in 32/200 (16%) sheep samples. Out of 200 human samples, 164 (82%) were infected with the bacterium. They also considered the virulence markers: A high prevalence of the *cag*A gene and of s1/m1 genotypes of the *vac*A gene were found in all of the samples. When the sequences of *H. pylori* isolates of sheep and humans were compared, 3.4%-8.4% variability and a 92.9%-98.5% homology were found. However, the greatest sequence similarity (98.5%) was found between the *H. pylori* isolates from Iranian sheep and those from German humans (FN598874), while the weakest relationship observed (91.6%) was between the Iranian cow and the South African population (NC017361).

According to Momtaz *et al*[39], cows and sheep were found to have *H. pylori* in their gastric tissue. The authors also theorize that sheep may be the natural reservoir for the bacteria and may be the source of *H. pylori* in human populations.

CONCLUSION

This literature review provides information to help determine the relevance of the several *H. pylori* reservoirs wherein each one possesses specific characteristics that favor or hinder the presence of *H. pylori*. Failure to eradicate and detection of *H. pylori* in reservoirs, may suggests an important route of reinfection and transmission, in additional to increase a risk of gastrointestinal disease.

REFERENCES

1 **Adler I**, Muiño A, Aguas S, Harada L, Diaz M, Lence A, Labbrozzi M, Muiño JM, Elsner B, Avagnina A, Denninghoff V. Helicobacter pylori and oral pathology: relationship with the gastric infection. *World J Gastroenterol* 2014; **20**: 9922-9935 [PMID: 25110422 DOI: 10.3748/wjg.v20.i29.9922]

2 **Al Sayed A**, Anand PS, Kamath KP, Patil S, Preethanath RS, Anil S. Oral Cavity as an Extragastric Reservoir of Helicobacter pylori. *ISRN Gastroenterol* 2014; **2014**: 261369 [PMID: 24701355 DOI: 10.1155/2014/261369]

3 **García A**, Salas-Jara MJ, Herrera C, González C. Biofilm and Helicobacter pylori: from environment to human host. *World J Gastroenterol* 2014; **20**: 5632-5638 [PMID: 24914322 DOI: 10.3748/wjg.v20.i19.5632]

4 **Liu Y**, Yue H, Li A, Wang J, Jiang B, Zhang Y, Bai Y. An epidemiologic study on the correlation between oral Helicobacter pylori and gastric H. pylori. *Curr Microbiol* 2009; **58**: 449-453 [PMID: 19139956 DOI: 10.1007/s00284-008-9341-3]

5 **Ahmed KS**, Khan AA, Ahmed I, Tiwari SK, Habeeb MA, Ali SM, Ahi JD, Abid Z, Alvi A, Hussain MA, Ahmed N, Habibullah CM. Prevalence study to elucidate the transmission pathways of Helicobacter pylori at oral and gastroduodenal sites of a South Indian population. *Singapore Med J* 2006; **47**: 291-296 [PMID: 16572240]

6 **Lukes P**, Astl J, Pavlík E, Potuzníková B, Sterzl I, Betka J. Helicobacter pylori in tonsillar and adenoid tissue and its possible role in oropharyngeal carcinogenesis. *Folia Biol* (Praha) 2008; **54**: 33-39 [PMID: 18498719]

7 **Silva DG**, Stevens RH, Macedo JM, Albano RM, Falabella ME, Veerman EC, Tinoco EM. Detection of cytotoxin genotypes of Helicobacter pylori in stomach, saliva and dental plaque. *Arch Oral Biol* 2009; **54**: 684-688 [PMID: 19442963 DOI: 10.1016/j.archoralbio.2009.04.006]

8 **Hirsch C**, Tegtmeyer N, Rohde M, Rowland M, Oyarzabal OA, Backert S. Live Helicobacter pylori in the root canal of endodontic-infected deciduous teeth. *J Gastroenterol* 2012; **47**: 936-940 [PMID: 22722905 DOI: 10.1007/s00535-012-0618-8]

9 **Miyabayashi H**, Furihata K, Shimizu T, Ueno I, Akamatsu T. Influence of oral Helicobacter pylori on the success of eradication therapy against gastric Helicobacter pylori. *Helicobacter* 2000; **5**: 30-37 [PMID: 10672049 DOI: 10.1046/j.1523-5378.2000.00004.x]

10 **Loster BW**, Majewski SW, Cześnikiewicz-Guzik M, Bielanski W, Pierzchalski P, Konturek SJ. The relationship between the presence of Helicobacter pylori in the oral cavity and gastric in the stomach. *J Physiol Pharmacol* 2006; **57** Suppl 3: 91-100 [PMID: 17033108]

11 **Krajden S**, Fuksa M, Anderson J, Kempston J, Boccia A, Petrea C, Babida C, Karmali M, Penner JL. Examination of human stomach biopsies, saliva, and dental plaque for Campylobacter pylori. *J Clin*

Microbiol 1989; **27**: 1397-1398 [PMID: 2754008]
12 **Ismail H**, Morgan C, Griffiths P, Williams J, Jenkins G. A Newly Developed Nested PCR Assay for the Detection of Helicobacter pylori in the Oral Cavity. *J Clin Gastroenterol* 2016; **50**: 17-22 [PMID: 25811111 DOI: 10.1097/MCG.0000000000000310]
13 **Amiri N**, Abiri R, Eyvazi M, Zolfaghari MR, Alvandi A. The frequency of Helicobacter pylori in dental plaque is possibly underestimated. *Arch Oral Biol* 2015; **60**: 782-788 [PMID: 25766471 DOI: 10.1016/j.archoralbio.2015.02.006]
14 **Liu Y**, Lin H, Bai Y, Qin X, Zheng X, Sun Y, Zhang Y. Study on the relationship between Helicobacter pylori in the dental plaque and the occurrence of dental caries or oral hygiene index. *Helicobacter* 2008; **13**: 256-260 [PMID: 18665933 DOI: 10.1111/j.1523-5378.2008.00602.x]
15 **Souto R**, Colombo AP. Detection of Helicobacter pylori by polymerase chain reaction in the subgingival biofilm and saliva of non-dyspeptic periodontal patients. *J Periodontol* 2008; **79**: 97-103 [PMID: 18166098 DOI: 10.1902/jop.2008.070241]
16 **Rasmussen LT**, Labio RW, Gatti LL, Silva LC, Queiroz VF, Smith Mde A, Payão SL. Helicobacter pylori detection in gastric biopsies, saliva and dental plaque of Brazilian dyspeptic patients. *Mem Inst Oswaldo Cruz* 2010; **105**: 326-330 [PMID: 20512249 DOI: 10.1590/S0074-02762010000300015]
17 **Rasmussen LT**, de Labio RW, Neto AC, Silva LC, Queiroz VF, Smith MAC, Payão SLM. Detection of Helicobacter pylori in gastric biopsies, saliva and dental plaques of dyspeptic patients from Marília, São Paulo, Brazil: presence of vacA and cagA genes. *JVATiTD* 2012; **18**: 7 [DOI: 10.1590/S1678-91992012000200008]
18 **Assumpção MB**, Martins LC, Melo Barbosa HP, Barile KA, de Almeida SS, Assumpção PP, Corvelo TC. Helicobacter pylori in dental plaque and stomach of patients from Northern Brazil. *World J Gastroenterol* 2010; **16**: 3033-3039 [PMID: 20572307 DOI: 10.3748/wjg.v16.i24.3033]
19 **Silva Rossi-Aguiar VP**, Navarro-Rodriguez T, Mattar R, Siqueira de Melo Peres MP, Correa Barbuti R, Silva FM, Carrilho FJ, Eisig JN. Oral cavity is not a reservoir for Helicobacter pylori in infected patients with functional dyspepsia. *Oral Microbiol Immunol* 2009; **24**: 255-259 [PMID: 19416457 DOI: 10.1111/j.1399-302X.2008.00491.x]
20 **Olivier BJ**, Bond RP, van Zyl WB, Delport M, Slavik T, Ziady C, Terhaar Sive Droste JS, Lastovica A, van der Merwe SW. Absence of Helicobacter pylori within the oral cavities of members of a healthy South African community. *J Clin Microbiol* 2006; **44**: 635-636 [PMID: 16455932 DOI: 10.1128/JCM.44.2.635-636.2006]
21 **Graham DY**. Helicobacter pylori update: gastric cancer, reliable therapy, and possible benefits. *Gastroenterology* 2015; **148**: 719-731.e3 [PMID: 25655557 DOI: 10.1053/j.gastro.2015.01.040]
22 **Erdem E**, Tüz HH, Günhan O. Gastric mucosal choristoma of the tongue and floor of the mouth. *J Oral Maxillofac Surg* 2001; **59**: 210-212 [PMID: 11213992 DOI: 10.1053/joms.2001.20497]
23 **Berber U**, Yılmaz İ, Erkul BE, Kaplan M. Peptic ulcer and intestinal metaplasia associated with Helicobacter pylori colonization in gastric heterotopia of the tongue. *Turk J Gastroenterol* 2014; **25**: 224-225 [PMID: 25003690 DOI: 10.5152/tjg.2014.4847]
24 **Mager DL**, Haffajee AD, Socransky SS. Effects of periodontitis and smoking on the microbiota of oral mucous membranes and saliva in systemically healthy subjects. *J Clin Periodontol* 2003; **30**: 1031-1037 [PMID: 15002888 DOI: 10.1046/j.0303-6979.2003.00418.x]
25 **Czesnikiewicz-Guzik M**, Bielanski W, Guzik TJ, Loster B, Konturek SJ. Helicobacter pylori in the oral cavity and its implications for gastric infection, periodontal health, immunology and dyspepsia. *J Physiol Pharmacol* 2005; **56** Suppl 6: 77-89 [PMID: 16340041]
26 **Gebara EC**, Pannuti C, Faria CM, Chehter L, Mayer MP, Lima LA. Prevalence of Helicobacter pylori detected by polymerase chain reaction in the oral cavity of periodontitis patients. *Oral Microbiol Immunol* 2004; **19**: 277-280 [PMID: 15210000 DOI: 10.1111/j.1399-302X.2004.00153.x]
27 **Kurtaran H**, Uyar ME, Kasapoglu B, Turkay C, Yilmaz T, Akcay A, Kanbay M. Role of Helicobacter pylori in pathogenesis of upper respiratory system diseases. *J Natl Med Assoc* 2008; **100**: 1224-1230 [PMID: 18942285]
28 **Abdel-Monem MH**, Magdy EA, Nour YA, Harfoush RA, Ibreak A. Detection of Helicobacter pylori in adenotonsillar tissue of children with chronic adenotonsillitis using rapid urease test, PCR and blood serology: a prospective study. *Int J Pediatr Otorhinolaryngol* 2011; **75**: 568-572 [PMID: 21324534 DOI: 10.1016/j.ijporl.2011.01.021]
29 **Cirak MY**, Ozdek A, Yilmaz D, Bayiz U, Samim E, Turet S. Detection of Helicobacter pylori and its CagA gene in tonsil and adenoid tissues by PCR. *Arch Otolaryngol Head Neck Surg* 2003; **129**: 1225-1229 [PMID: 14623755 DOI: 10.1001/archotol.129.11.1225]
30 **Eyigor M**, Eyigor H, Gultekin B, Aydin N. Detection of Helicobacter pylori in adenotonsiller tissue specimens by rapid urease test and polymerase chain reaction. *Eur Arch Otorhinolaryngol* 2009; **266**: 1611-1613 [PMID: 19130070 DOI: 10.1007/s00405-008-0903-6]
31 **Nártová E**, Kraus J, Pavlík E, Lukeš P, Katra R, Plzák J, Kolářová L, Sterzl I, Betka J, Astl J. Presence of different genotypes of Helicobacter pylori in patients with chronic tonsillitis and sleep apnoea syndrome. *Eur Arch Otorhinolaryngol* 2014; **271**: 607-613 [PMID: 23864246 DOI: 10.1007/s00405-013-2607-9]
32 **Aliakbari I**, Noohi S, Safavi SA, Tabrizi AG, Bolfion M, Razaghi M, Goudarzi H, Dabiri H. The role of adenotonsillar tissues as a reservoir for Helicobacter pylori and Helicobacter hepaticus. *Gastroenterol Hepatol Bed Bench* 2011; **4**: 153-158 [PMID: 24834175]
33 **Gharst G**, Oyarzabal OA, Hussain SK. Review of current methodologies to isolate and identify Campylobacter spp. from foods. *J Microbiol Methods* 2013; **95**: 84-92 [PMID: 23899774]
34 **Zou QH**, Li RQ. Helicobacter pylori in the oral cavity and gastric mucosa: a meta-analysis. *J Oral Pathol Med* 2011; **40**: 317-324 [PMID: 21294774 DOI: 10.1111/j.1600-0714.2011.01006.x]
35 **Nguyen AM**, el-Zaatari FA, Graham DY. Helicobacter pylori in the oral cavity. A critical review of the literature. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1995; **79**: 705-709 [PMID: 7621027]
36 **Nguyen AM**, Engstrand L, Genta RM, Graham DY, el-Zaatari FA. Detection of Helicobacter pylori in dental plaque by reverse transcription-polymerase chain reaction. *J Clin Microbiol* 1993; **31**: 783-787 [PMID: 8463387]
37 **Rôças IN**, Siqueira JF. Searching for Helicobacter pylori and Chlamydia pneumoniae in primary endodontic infections. *Eur J Dent* 2012; **6**: 158-162 [PMID: 22509118]
38 **Correia-Silva Jde F**, Neto Victória JM, Guimarães AL, de Sá AR, Gomes CC, de Almeida HC, Gomez RS. Helicobacter pylori in the oral mucosa of patients submitted to allogeneic haematopoietic stem cell transplantation. *Braz Oral Res* 2006; **20**: 191-195 [PMID: 17119699]
39 **Momtaz H**, Dabiri H, Souod N, Gholami M. Study of Helicobacter pylori genotype status in cows, sheep, goats and human beings. *BMC Gastroenterol* 2014; **14**: 61 [PMID: 24708464 DOI: 10.1186/1471-230X-14-61]
40 **Jones DM**, Elridge J. Gastric campylobacter-like organisms from man (C. pyloridis) compared with GCLO strains from the pig baboon and ferret. In: Campylobacter IV, Kaijser B, Falsen E, editors. Göteborg, Sweden: University of Göteborg, 1990: 44
41 **Eaton KA**, Morgan DR, Krakowka S. Persistence of Helicobacter pylori in conventionalized piglets. *J Infect Dis* 1990; **161**: 1299-1301 [PMID: 2345307]
42 **Engstrand L**, Gustavsson S, Jörgensen A, Schwan A, Scheynius A. Inoculation of barrier-born pigs with Helicobacter pylori: a useful animal model for gastritis type B. *Infect Immun* 1990; **58**: 1763-1768 [PMID: 2341177]
43 **De Groote D**, van Doorn LJ, Ducatelle R, Verschuuren A, Haesebrouck F, Quint WG, Jalava K, Vandamme P. 'Candidatus

Helicobacter suis', a gastric helicobacter from pigs, and its phylogenetic relatedness to other gastrospirilla. *Int J Syst Bacteriol* 1999; **49** Pt 4: 1769-1777 [PMID: 10555359]

44 **Mégraud F**, Broutet N. Review article: have we found the source of Helicobacter pylori? *Aliment Pharmacol Ther* 2000; **14** Suppl 3: 7-12 [PMID: 11050482]

45 **Handt LK**, Fox JG, Dewhirst FE, Fraser GJ, Paster BJ, Yan LL, Rozmiarek H, Rufo R, Stalis IH. Helicobacter pylori isolated from the domestic cat: public health implications. *Infect Immun* 1994; **62**: 2367-2374 [PMID: 8188360]

P- Reviewer: Zhou LY **S- Editor**: Ji FF **L- Editor**: A
E- Editor: Wang CH

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i1.133

World J Gastrointest Pharmacol Ther 2016 February 6; 7(1): 133-138
ISSN 2150-5349 (online)

MINIREVIEWS

Dendritic cell-based vaccine for pancreatic cancer in Japan

Masato Okamoto, Masanori Kobayashi, Yoshikazu Yonemitsu, Shigeo Koido, Sadamu Homma

Masato Okamoto, Department of Advanced Immunotherapeutics, Kitasato University School of Pharmacy, Tokyo 108-8641, Japan

Masato Okamoto, Oncology Cencer, Kitasato Institute Hospital, Tokyo 108-8641, Japan

Masanori Kobayashi, Seren Clinic Nagoya, Nagoya, Aichi 460-0008, Japan

Yoshikazu Yonemitsu, R and D Laboratory for Innovative Biotherapeutics, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka 812-8582, Japan

Shigeo Koido, Division of Gastroenterology and Hepatology, Department of Internal Medicine, the Jikei University School of Medicine, Kashiwa, Chiba 277-8567, Japan

Sadamu Homma, Department of Oncology, the Jikei University School of Medicine, Tokyo 105-8461, Japan

Author contributions: Okamoto M designed the aim of the editorial, wrote the manuscript and generated the figure; Kobayashi M, Yonemitsu Y, Koido S and Homma S contributed to the writing of the manuscript.

Conflict-of-interest statement: Yonemitsu Y is a previous member of the Board of Directors on Science and Medicine at tella Inc; all remaining authors have declared no conflicts of interest.

Correspondence to: Masato Okamoto, DDS, PhD, Professor, Department of Advanced Immunotherapeutics, Kitasato University School of Pharmacy, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan. okamotom@pharm.kitasato-u.ac.jp
Telephone: +81-3-57916495
Fax: +81-3-34469036

Received: July 7, 2015
Peer-review started: July 7, 2015
First decision: July 31, 2015
Revised: August 28, 2015
Accepted: November 13, 2015
Article in press: November 17, 2015
Published online: February 6, 2016

Abstract

"Vaccell" is a dendritic cell (DC)-based cancer vaccine which has been established in Japan. The DCs play central roles in deciding the direction of host immune reactions as well as antigen presentation. We have demonstrated that DCs treated with a streptococcal immune adjuvant OK-432, produce interleukin-12, induce Th1-dominant state, and elicit anti-tumor effects, more powerful than those treated with the known DC-maturating factors. We therefore decided to mature DCs by the OK-432 for making an effective DC vaccine, Vaccell. The 255 patients with inoperable pancreatic cancer who received standard chemotherapy combined with DC vaccines, were analyzed retrospectively. Survival time of the patients with positive delayed type hypersensitivity (DTH) skin reaction was significantly prolonged as compared with that of the patients with negative DTH. The findings strongly suggest that there may be "Responders" for the DC vaccine in advanced pancreatic cancer patients. We next conducted a small-scale prospective clinical study. In this trial, we pulsed HLA class Ⅱ-restricted WT1 peptide (WT1-Ⅱ) in addition to HLA class Ⅰ-restricted peptide (WT1-Ⅰ) into the DCs. Survival of the patients received WT1-Ⅰ and -Ⅱ pulsed DC vaccine was significantly extended as compared to that of the patients received DCs pulsed with WT1-Ⅰ or WT1-Ⅱ alone. Furthermore, WT1-specific DTH positive patients showed significantly improved the overall survival as well as progression-free survival as compared to the DTH negative patients. The activation of antigen-specific immune responses by DC vaccine in combination with standard chemotherapy

may be associated with a good clinical outcome in advanced pancreatic cancer. We are now planning a pivotal study of the Vaccell in appropriate protocols in Japan.

Key words: Dendritic cell; Cancer vaccine; Pancreatic cancer; Cancer immunotherapy; Anti-cancer immunity

Core tip: Dendritic cell (DC)-based cancer vaccine is expected as a strategy to augment the antigen-specific anti-cancer immune response. Vaccell, a DC vaccine which was optimized to fight against a cancer, has been developed in Japan. Here, we reviewed the development and clinical effects of the "Vaccell". We conducted large-scale retrospective observations as well as prospective clinical trials, and obtained the findings strongly suggesting that there are "Responders" that the clinical benefits are provided by the DC vaccine. We are now planning a pivotal study of the Vaccell in Japan.

Okamoto M, Kobayashi M, Yonemitsu Y, Koido S, Homma S. Dendritic cell-based vaccine for pancreatic cancer in Japan. *World J Gastrointest Pharmacol Ther* 2016; 7(1): 133-138 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i1/133.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i1.133

INTRODUCTION

Recently, a clinical benefit of certain immunotherapies for cancer was proved. Especially, anti-cancer effect of the blocking antibodies for checkpoint molecules such as PD-1 and CTLA-4[1-4], is promising, and editors of the Science journal selected the "Cancer Immunotherapy" as a top of breakthrough of the year 2013[5].

On the other hand, although cancer vaccine that is one of the antigen-specific immunotherapy, has been expected for the patients with malignancies resistant to standard treatment, the therapeutic effect has not been evidenced. Even the certain cancer vaccines which showed clinical effects in early phase clinical studies[6-9], has not demonstrated clear clinical benefits in pivotal phase Ⅲ trials.

The dendritic cell (DC)-based vaccine is expected as a strategy to augment the effects of cancer vaccine. On July 16, 2007, the Swiss Institute of Public Health has approved the world's first therapeutic vaccine for brain cancer DCVax®-Brain (Northwest Biotherapeutics Inc.), and on April 29, 2010, the United States Food and Drug Administration (FDA) has approved sipuleucel-T (Dendreon Corp.) which is a cancer vaccine for the treatment of hormone refractory prostate cancer. Sipuleucel-T is the only vaccine approved so far by the US FDA to treat cancer. Phase Ⅲ studies of several DC vaccine for cancer are now ongoing.

Here, we review the development and clinical effects of the DC vaccine "Vaccell" which has been established in Japan.

A Japanese DC vaccine "Vaccell"

Vaccell, a Japanese dendritic cell-based vaccine, is declared with "the vaccine which was optimized to fight against a cancer". What is "optimized"?

The DCs play central roles in deciding the direction of host immune reactions as well as antigen presentation. The DCs are "headquarters" of antigen-specific host immune responses[10,11]. The antigen presentation have to be done under the helper T-cell 1 (Th1) condition that is interferon (IFN)- and interleukin (IL)-12-rich condition for inducing antigen-specific cytotoxic T lymphocytes (CTLs) and eliciting anti-cancer effects (Figure 1A), however it is difficult to predict what kinds of immune responses, *e.g.,* Th1, Th2, Th17 and regulatory T cells (Treg), will be induced by the dendritic cells in patients' bodies. This is the serious problem of the conventional cancer vaccines which are the methods only using cancer antigens such as peptides, proteins and whole cells without DCs. The strategy to perform "optimization" of DCs in Th1-inducing type by processing DCs *ex vivo* should be reasonable.

We have reported that DCs stimulated with a streptococcal immune adjuvant OK-432 which has been developed in Japan 1970'[12], produce IL-12, induce Th1-dominant state, and elicit anti-tumor effect, that these effects are more powerful than those of the known DC-maturating factors such as tumor necrosis factor (TNF)- and LPS, and that these reactions are caused *via* Toll-like receptor signaling (Figure 1B)[13-17].

A Th1-inducing type of DC vaccine, Vaccell is made as follows. Peripheral blood monocytes were cultured in medium containing granulocyte macrophage-colony stimulating factor (GM-CSF) and IL-4 to generate immature DCs. Five days later, the DCs were stimulated with the OK-432 and prostaglandin E2 for 24 h. The DCs were then pulsed with peptide antigens according to the HLA pattern. Based on previous reports, Several antigens such as WT1 and MUC1, were selected to be pulsed into DCs[18] (Figure 2A).

The DCs were cryopreserved and kept until the day of administration. The phenotype $CD14^{-/low}$/$HLA\text{-}DR^{+}$/$HLA\text{-}ABC^{+}$/$CD80^{+}$/$CD83^{+}$/$CD86^{+}$/$CD40^{+}$/$CCR7^{+}$ was taken to define mature DCs. The DCs were prepared by well-trained technical staff in each institutional cell processing center under the Standard Operating Procedure (SOP). Regarding release criteria, testing for sterility, mycoplasma (PCR method), and endotoxin was done using the supernatant or cell suspension just before the tube filling.

Vaccell has become to be served for lots of patients with various malignancies, and then not only large-

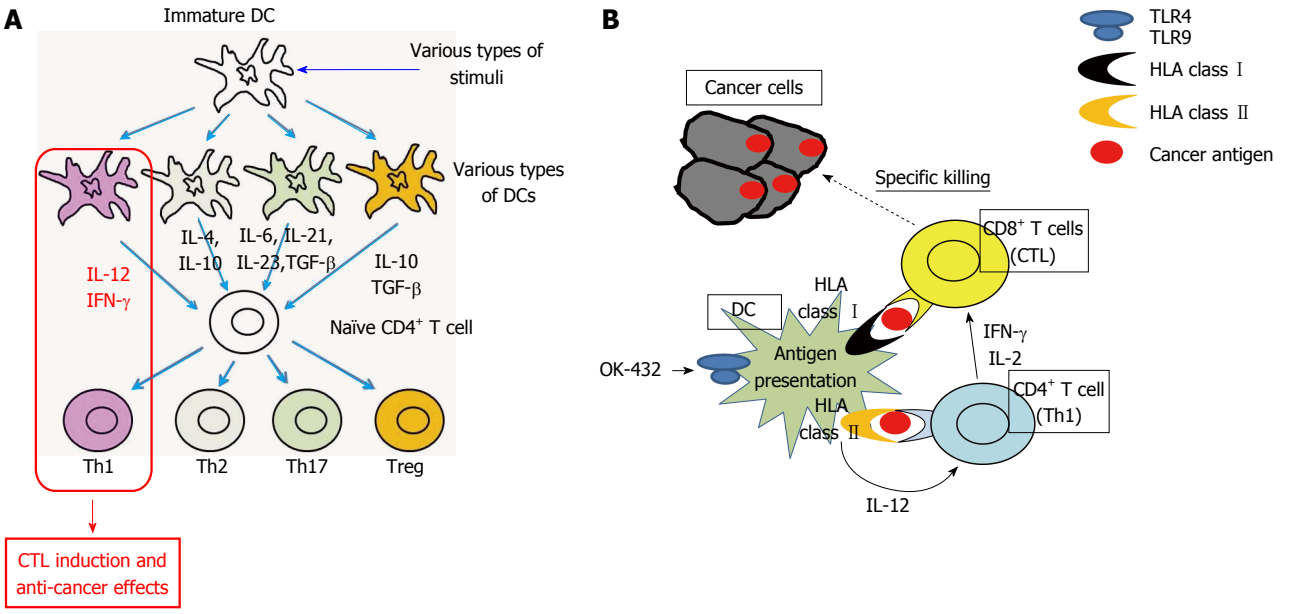

Figure 1 The dendritic cells play central roles in deciding the direction of host immune reactions as well as antigen presentation. A: Role of various dendritic cells in CD4+ T-cell differentiation; B: Antigen presentation and CTL induction under Th1 condition. CTL: Cytotoxic T lymphocytes; DC: Dendritic cell.

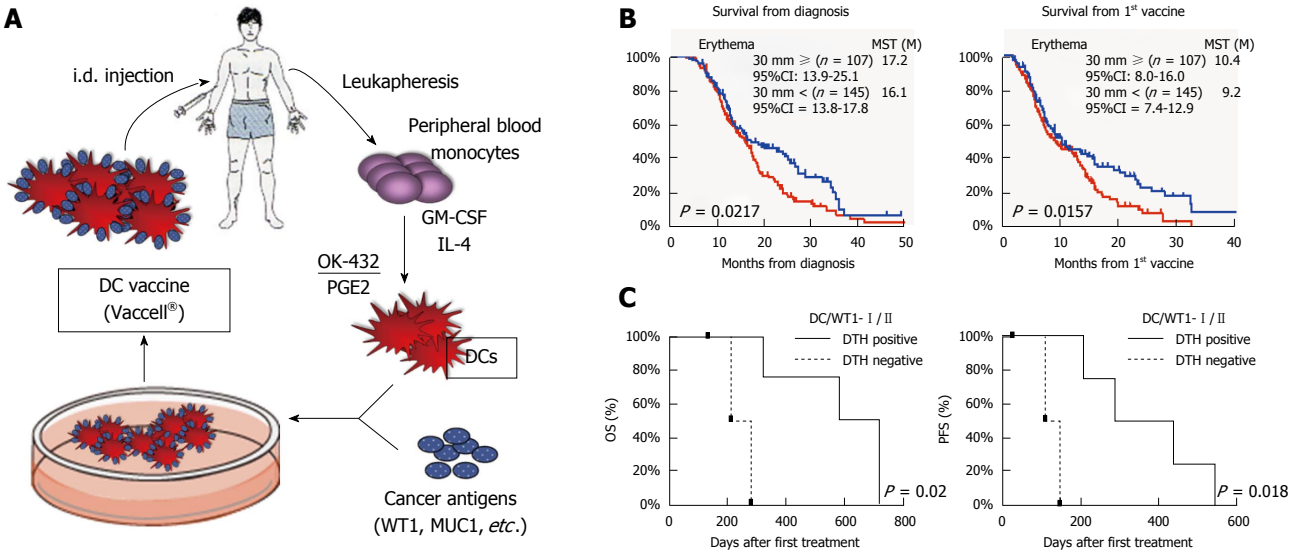

Figure 2 A Th1-inducing type of dendritic cell vaccine, Vaccell is made as follows. A: Preparation of the DC vaccine "Vaccell"; B and C: Vaccell for pancreatic cancer patients; B: Two hundred fifty five patients who received standard chemotherapy combined with DC vaccines were analyzed. DTH skin reaction after vaccination was an independent prognostic factor for better survival; B: The WT1-specific DTH positive patients showed significantly improved OS and PFS compared with the DTH negative patients[20,21]. DC: Dendritic cell; DTH: Delayed type hypersensitivity; PFS: Progressive-free survival; OS: Overall survival.

scale retrospective observations but also prospective clinical trials have been done or are ongoing.

Clinical effects of the Vaccell mainly in pancreatic cancer

At the point of writing, we have published 13 original articles related to the clinical application of Vaccel. Five articles described for pancreatic cancer[19-23], 2 for biliary tract cancer[24,25], 1 for lung cancer[26], 1 for ovarian cancer[27], 1 for pediatric patient with relapsed leukemia[28], 1 for gastric cancer[29], 1 for malignant glioma[30], and 1 for several types of advanced cancers treated with radiation therapy in combination with DC vaccine[31]. Here, we introduce the Vaccell's effects in pancreatic cancer patients in which the analysis has been progressed to most among these cases.

First, the clinical and immunological evaluation of DC-based immunotherapy in combination with standard chemotherapy mainly gemcitabine and S-1, in 49 patients with inoperable, advanced pancreatic carcinoma has been done retrospectively[19]. Prolongation of survival in this cohort was highly likely (median OS: 360 d from the 1st vaccination). There were the patients whose numbers of Tregs were

decreased in peripheral blood, and the overall survival (OS) from 1st vaccination tended to be prolonged in these patients.

We have conducted the next retrospective observation by expanding sample size as a multicenter analysis[20]. The 255 patients with inoperable pancreatic cancer who received standard chemotherapy combined with peptide-pulsed DC vaccines, were analyzed. Relapse cases were excluded. The median OS from diagnosis was 16.5 mo and that from the 1st vaccination was 9.9 mo. Interestingly, The median survival time (MST) of the patients with positive delayed type hypersensitivity (DTH) skin reaction was significantly prolonged compared to that of the patients with negative DTH (P = 0.0157 by log-rank test) (Figure 2B). These findings strongly suggest that there may be "Responders" for the DC vaccine in advanced pancreatic patients. This is the first report of a multicenter clinical study suggesting the feasibility and possible clinical benefit of an add-on DC vaccine in patients with advanced pancreatic combined with standard chemotherapy.

Based on the results of the above retrospective analysis, we conducted 2 small-scale prospective clinical studies for advanced pancreatic cancer patients. One protocol is the combination therapy with gemcitabine and DC vaccine pulsed with only HLA class I-restricted WT1 peptide[23]. In 10 patients, the disease control associated with a low neutrophil/lymphocyte (N/L) ratio was observed in all 3 patients with DTH positivity. In another protocol of gemcitabine in combination with DC vaccine for pancreatic cancer, we loaded the WT1-specific HLA class Ⅱ-restricted epitope (WT1-Ⅱ) as well as the HLA class I-restricted epitope (WT1-Ⅰ) into the DCs[21,22]. Ten stage IV patients with pancreatic ductal adenocarcinoma who showed HLA-positive for A*02:01, A*02:06, A*24:02, DRB1*04:05, DRB1*08:03, DRB1*15:01, DRB1*15:02, DPB1*05:01, or DPB1*09:01 were enrolled. The survival of 7 patients received WT1-Ⅰ and -Ⅱ pulsed DC vaccine was significantly extended compared to that of the 3 patients received DC vaccine pulsed with WT1-Ⅰ or WT1-Ⅱ alone [P = 0.036 in OS, P = 0.010 in Progressive-free survival (PFS)]. WT1-specific DTH positive patients showed significantly improved OS and PFS as compared to the DTH negative patients (P = 0.021 in OS, P = 0.018 in PFS) (Figure 2C). In particular, all 3 patients with strong DTH reactions (erythema > 5 mm) had a median OS of 717 d. In addition, it was also observed in this protocol that the decreased N/L ratio may be prognostic markers of longer survival (P = 0.018).

The activation of WT1-specific immune responses by DC vaccine pulsed with WT1-Ⅰ as well as with WT1-Ⅱ in combination with standard chemotherapy may be associated with disease stability in advanced pancreatic cancer.

DTH skin reaction was associated with good clinical outcome or clinical response in pancreatic cancer patients received DC vaccine in a large-scale retrospective observation as well as in 2 small-scale prospective studies. Moreover, N/L ratio was a marker for clinical benefit both in 2 prospective studies. In retrospective analysis of 255 cases, N/L ratio was not statistically significant, but tended to be a good prognostic marker. It is a notable thing that the much similar result was provided in a large-scale retrospective observation and in the 2 independent prospective trials. Additionally, a number granulocytic myeloid-derived suppressor cells (MDSCs) was a marker for poor clinical response[23]. Rodriguez *et al*[32] reported that MDSCs are a subpopulation of activated granulocytes. We have also observed that the infiltration of CD66b-positive neutrophils in a tumor site may be a negative prognostic marker (authors' personal observation, manuscript in preparing). Granulocytic MDSCs may be a core population of neutrophils which may suppress anti-tumor immunity.

Previously, there have been some reports describing the advantage of the use of HLA-class Ⅱ-restricted antigen epitope(s)[33], while it is also suggested the possibility that HLA-class Ⅱ-restricted epitope may induce suppressive immune responses such as Th2, Th17 and Tregs. I believe that it is reasonable and useful to make a cancer vaccine using the DCs optimized *ex vivo* for Th1-inducing type.

CONCLUSION

To provide more effective cancer immunotherapy for patients: (1) development of the strategy and technology for cancellation of immunosuppression such as checkpoint inhibitors; and (2) establishment of biomarkers to discriminate between responder and non-responder of the certain immunotherapy in addition to development of more powerful cancer vaccine. Cancer vaccine may play a significant role for enhancing the anti-cancer effect of checkpoint inhibitory antibodies[34,35]. The combination therapy using cancer vaccine and checkpoint inhibitors is a promising strategy. Moreover, searching of the biomarkers is a significant theme not only for the prediction of the therapeutic effects of immunotherapies but also for the development of the novel strategy of cancellation of the immunosuppression.

Furthermore, as described above, the results of retrospective observation of past cases are extremely useful to prepare an appropriate prospective protocol.

I believe that there are some populations (relatively large populations) of cancer patients who can obtain clinical benefit by DC vaccine. DC vaccine has a potential to elicit anti-tumor effect, and we therefore have to make DC vaccine a standard treatment for cancer patients.

We will be able to make the DC vaccine much more effective by expanding DC number, by identifying more effective antigens, by developing predictive

biomarkers, and by combining with the therapies for cancelling an immunosuppressive condition as well as for tumor mass reduction.

We are now planning phase Ⅱ/Ⅲ studies of Vaccell in appropriate protocols.

ACKNOWLEDGMENTS

We thank all of the members of the DC Vaccine Study Group at the J-SICT (the Japan Society of Immunotherapy and Cell Therapy) for carrying out clinical studies, and for giving me critical comments for the DC vaccines.

REFERENCES

1 **Pardoll DM**. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* 2012; **12**: 252-264 [PMID: 22437870 DOI: 10.1038/nrc3239]

2 **Hodi FS**, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, Akerley W, van den Eertwegh AJ, Lutzky J, Lorigan P, Vaubel JM, Linette GP, Hogg D, Ottensmeier CH, Lebbé C, Peschel C, Quirt I, Clark JI, Wolchok JD, Weber JS, Tian J, Yellin MJ, Nichol GM, Hoos A, Urba WJ. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* 2010; **363**: 711-723 [PMID: 20525992 DOI: 10.1056/NEJMoa1003466]

3 **Topalian SL**, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming PD, Spigel DR, Antonia SJ, Horn L, Drake CG, Pardoll DM, Chen L, Sharfman WH, Anders RA, Taube JM, McMiller TL, Xu H, Korman AJ, Jure-Kunkel M, Agrawal S, McDonald D, Kollia GD, Gupta A, Wigginton JM, Sznol M. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* 2012; **366**: 2443-2454 [PMID: 22658127 DOI: 10.1056/NEJMoa1200690]

4 **Wolchok JD**, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, Segal NH, Ariyan CE, Gordon RA, Reed K, Burke MM, Caldwell A, Kronenberg SA, Agunwamba BU, Zhang X, Lowy I, Inzunza HD, Feely W, Horak CE, Hong Q, Korman AJ, Wigginton JM, Gupta A, Sznol M. Nivolumab plus ipilimumab in advanced melanoma. *N Engl J Med* 2013; **369**: 122-133 [PMID: 23724867 DOI: 10.1056/NEJMoa1302369]

5 **Couzin-Frankel J**. Breakthrough of the year 2013. Cancer immunotherapy. *Science* 2013; **342**: 1432-1433 [PMID: 24357284 DOI: 10.1126/science.342.6165.1432]

6 **Vansteenkiste J**, Zielinski M, Linder A, Dahabreh J, Gonzalez EE, Malinowski W, Lopez-Brea M, Vanakesa T, Jassem J, Kalofonos H, Perdeus J, Bonnet R, Basko J, Janilionis R, Passlick B, Treasure T, Gillet M, Lehmann FF, Brichard VG. Adjuvant MAGE-A3 immunotherapy in resected non-small-cell lung cancer: phase II randomized study results. *J Clin Oncol* 2013; **31**: 2396-2403 [PMID: 23715567 DOI: 10.1200/JCO.2012.43.7103]

7 **Kruit WH**, Suciu S, Dreno B, Mortier L, Robert C, Chiarion-Sileni V, Maio M, Testori A, Dorval T, Grob JJ, Becker JC, Spatz A, Eggermont AM, Louahed J, Lehmann FF, Brichard VG, Keilholz U. Selection of immunostimulant AS15 for active immunization with MAGE-A3 protein: results of a randomized phase II study of the European Organisation for Research and Treatment of Cancer Melanoma Group in Metastatic Melanoma. *J Clin Oncol* 2013; **31**: 2413-2420 [PMID: 23715572 DOI: 10.1200/JCO.2012.43.7111]

8 **Butts C**, Maksymiuk A, Goss G, Soulières D, Marshall E, Cormier Y, Ellis PM, Price A, Sawhney R, Beier F, Falk M, Murray N. Updated survival analysis in patients with stage IIIB or IV non-small-cell lung cancer receiving BLP25 liposome vaccine (L-BLP25): phase IIB randomized, multicenter, open-label trial. *J Cancer Res Clin Oncol* 2011; **137**: 1337-1342 [PMID: 21744082 DOI: 10.1007/s00432-011-1003-3]

9 **Butts C**, Socinski MA, Mitchell PL, Thatcher N, Havel L, Krzakowski M, Nawrocki S, Ciuleanu TE, Bosquée L, Trigo JM, Spira A, Tremblay L, Nyman J, Ramlau R, Wickart-Johansson G, Ellis P, Gladkov O, Pereira JR, Eberhardt WE, Helwig C, Schröder A, Shepherd FA. Tecemotide (L-BLP25) versus placebo after chemoradiotherapy for stage III non-small-cell lung cancer (START): a randomised, double-blind, phase 3 trial. *Lancet Oncol* 2014; **15**: 59-68 [PMID: 24331154 DOI: 10.1016/S1470-2045(13)70510-2]

10 **Steinman RM**. The dendritic cell system and its role in immunogenicity. *Annu Rev Immunol* 1991; **9**: 271-296 [PMID: 1910679 DOI: 10.1146/annurev.iy.09.040191.001415]

11 **Banchereau J**, Steinman RM. Dendritic cells and the control of immunity. *Nature* 1998; **392**: 245-252 [PMID: 9521319 DOI: 10.1038/32588]

12 **Okamoto H**, Shoin S, Koshimura S, Shimizu R. Studies on the anticancer and streptolysin S-forming abilities of hemolytic streptococci. *Jpn J Microbiol* 1967; **11**: 323-326 [PMID: 4875331 DOI: 10.1111/j.1348-0421.1967.tb00350.x]

13 **Okamoto M**, Oshikawa T, Tano T, Ohe G, Furuichi S, Nishikawa H, Ahmed SU, Akashi S, Miyake K, Takeuchi O, Akira S, Moriya Y, Matsubara S, Ryoma Y, Saito M, Sato M. Involvement of Toll-like receptor 4 signaling in interferon-gamma production and antitumor effect by streptococcal agent OK-432. *J Natl Cancer Inst* 2003; **95**: 316-326 [PMID: 12591988 DOI: 10.1093/jnci/95.4.316]

14 **Okamoto M**, Furuichi S, Nishioka Y, Oshikawa T, Tano T, Ahmed SU, Takeda K, Akira S, Ryoma Y, Moriya Y, Saito M, Sone S, Sato M. Expression of toll-like receptor 4 on dendritic cells is significant for anticancer effect of dendritic cell-based immunotherapy in combination with an active component of OK-432, a streptococcal preparation. *Cancer Res* 2004; **64**: 5461-5470 [PMID: 15289356 DOI: 10.1158/0008-5472.CAN-03-4005]

15 **Ahmed SU**, Okamoto M, Oshikawa T, Tano T, Sasai A, Kan S, Hiroshima T, Ohue H, Moriya Y, Ryoma Y, Saito M, Sato M. Anti-tumor effect of an intratumoral administration of dendritic cells in combination with TS-1, an oral fluoropyrimidine anti-cancer drug, and OK-432, a streptococcal immunopotentiator: involvement of toll-like receptor 4. *J Immunother* 2004; **27**: 432-441 [PMID: 15534487 DOI: 10.1097/00002371-200411000-00003]

16 **Okamoto M**, Oshikawa T, Tano T, Ahmed SU, Kan S, Sasai A, Akashi S, Miyake K, Moriya Y, Ryoma Y, Saito M, Sato M. Mechanism of anticancer host response induced by OK-432, a streptococcal preparation, mediated by phagocytosis and Toll-like receptor 4 signaling. *J Immunother* 2006; **29**: 78-86 [PMID: 16365603 DOI: 10.1097/01.cji.0000192106.32206.30]

17 **Oshikawa T**, Okamoto M, Tano T, Sasai A, Kan S, Moriya Y, Ryoma Y, Saito M, Akira S, Sato M. Antitumor effect of OK-432-derived DNA: one of the active constituents of OK-432, a streptococcal immunotherapeutic agent. *J Immunother* 2006; **29**: 143-150 [PMID: 16531815 DOI: 10.1097/01.cji.0000189028.18288.6f]

18 **Cheever MA**, Allison JP, Ferris AS, Finn OJ, Hastings BM, Hecht TT, Mellman I, Prindiville SA, Viner JL, Weiner LM, Matrisian LM. The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. *Clin Cancer Res* 2009; **15**: 5323-5337 [PMID: 19723653 DOI: 10.1158/1078-0432.CCR-09-0737]

19 **Kimura Y**, Tsukada J, Tomoda T, Takahashi H, Imai K, Shimamura K, Sunamura M, Yonemitsu Y, Shimodaira S, Koido S, Homma S, Okamoto M. Clinical and immunologic evaluation of dendritic cell-based immunotherapy in combination with gemcitabine and/or S-1 in patients with advanced pancreatic carcinoma. *Pancreas* 2012; **41**: 195-205 [PMID: 21792083 DOI: 10.1097/MPA.0b013e31822398c6]

20 **Kobayashi M**, Shimodaira S, Nagai K, Ogasawara M, Takahashi H, Abe H, Tanii M, Okamoto M, Tsujitani S, Yusa S, Ishidao T, Kishimoto J, Shibamoto Y, Nagaya M, Yonemitsu Y. Prognostic factors related to add-on dendritic cell vaccines on patients with inoperable pancreatic cancer receiving chemotherapy: a multicenter analysis. *Cancer Immunol Immunother* 2014; **63**: 797-806 [PMID: 24777613 DOI: 10.1007/s00262-014-1554-7]

21 **Koido S**, Homma S, Okamoto M, Takakura K, Mori M, Yoshizaki S, Tsukinaga S, Odahara S, Koyama S, Imazu H, Uchiyama K,

Kajihara M, Arakawa H, Misawa T, Toyama Y, Yanagisawa S, Ikegami M, Kan S, Hayashi K, Komita H, Kamata Y, Ito M, Ishidao T, Yusa S, Shimodaira S, Gong J, Sugiyama H, Ohkusa T, Tajiri H. Treatment with chemotherapy and dendritic cells pulsed with multiple Wilms' tumor 1 (WT1)-specific MHC class I/II-restricted epitopes for pancreatic cancer. *Clin Cancer Res* 2014; **20**: 4228-4239 [PMID: 25056373 DOI: 10.1158/1078-0432.CCR-14-0314]

22 **Takakura K**, Koido S, Kan S, Yoshida K, Mori M, Hirano Y, Ito Z, Kobayashi H, Takami S, Matsumoto Y, Kajihara M, Misawa T, Okamoto M, Sugiyama H, Homma S, Ohkusa T, Tajiri H. Prognostic markers for patient outcome following vaccination with multiple MHC Class I/II-restricted WT1 peptide-pulsed dendritic cells plus chemotherapy for pancreatic cancer. *Anticancer Res* 2015; **35**: 555-562 [PMID: 25550602]

23 **Mayanagi S**, Kitago M, Sakurai T, Matsuda T, Fujita T, Higuchi H, Taguchi J, Takeuchi H, Itano O, Aiura K, Hamamoto Y, Takaishi H, Okamoto M, Sunamura M, Kawakami Y, Kitagawa Y. Phase I pilot study of Wilms tumor gene 1 peptide-pulsed dendritic cell vaccination combined with gemcitabine in pancreatic cancer. *Cancer Sci* 2015; **106**: 397-406 [PMID: 25614082 DOI: 10.1111/cas.12621]

24 **Koido S**, Kan S, Yoshida K, Yoshizaki S, Takakura K, Namiki Y, Tsukinaga S, Odahara S, Kajihara M, Okamoto M, Ito M, Yusa S, Gong J, Sugiyama H, Ohkusa T, Homma S, Tajiri H. Immunogenic modulation of cholangiocarcinoma cells by chemoimmunotherapy. *Anticancer Res* 2014; **34**: 6353-6361 [PMID: 25368235]

25 **Kobayashi M**, Sakabe T, Abe H, Tanii M, Takahashi H, Chiba A, Yanagida E, Shibamoto Y, Ogasawara M, Tsujitani S, Koido S, Nagai K, Shimodaira S, Okamoto M, Yonemitsu Y, Suzuki N, Nagaya M. Dendritic cell-based immunotherapy targeting synthesized peptides for advanced biliary tract cancer. *J Gastrointest Surg* 2013; **17**: 1609-1617 [PMID: 23877328 DOI: 10.1007/s11605-013-2286-2]

26 **Takahashi H**, Okamoto M, Shimodaira S, Tsujitani S, Nagaya M, Ishidao T, Kishimoto J, Yonemitsu Y. Impact of dendritic cell vaccines pulsed with Wilms' tumour-1 peptide antigen on the survival of patients with advanced non-small cell lung cancers. *Eur J Cancer* 2013; **49**: 852-859 [PMID: 23245331 DOI: 10.1016/j.ejca.2012.11.005]

27 **Kobayashi M**, Chiba A, Izawa H, Yanagida E, Okamoto M, Shimodaira S, Yonemitsu Y, Shibamoto Y, Suzuki N, Nagaya M. The feasibility and clinical effects of dendritic cell-based immunotherapy targeting synthesized peptides for recurrent ovarian cancer. *J Ovarian Res* 2014; **7**: 48 [PMID: 25298213 DOI: 10.1186/1757-2215-7-48]

28 **Saito S**, Yanagisawa R, Yoshikawa K, Higuchi Y, Koya T, Yoshizawa K, Tanaka M, Sakashita K, Kobayashi T, Kurata T, Hirabayashi K, Nakazawa Y, Shiohara M, Yonemitsu Y, Okamoto M, Sugiyama H, Koike K, Shimodaira S. Safety and tolerability of allogeneic dendritic cell vaccination with induction of Wilms tumor 1-specific T cells in a pediatric donor and pediatric patient with relapsed leukemia: a case report and review of the literature. *Cytotherapy* 2015; **17**: 330-335 [PMID: 25484308 DOI: 10.1016/j.jcyt.2014.10.003]

29 **Kobayashi M**, Sakabe T, Chiba A, Nakajima A, Okamoto M, Shimodaira S, Yonemitsu Y, Shibamoto Y, Suzuki N, Nagaya M. Therapeutic effect of intratumoral injections of dendritic cells for locally recurrent gastric cancer: a case report. *World J Surg Oncol* 2014; **12**: 390 [PMID: 25526950 DOI: 10.1186/1477-7819-12-390]

30 **Sakai K**, Shimodaira S, Maejima S, Udagawa N, Sano K, Higuchi Y, Koya T, Ochiai T, Koide M, Uehara S, Nakamura M, Sugiyama H, Yonemitsu Y, Okamoto M, Hongo K. Dendritic cell-based immunotherapy targeting Wilms' tumor 1 in patients with recurrent malignant glioma. *J Neurosurg* 2015; **123**: 989-997 [PMID: 26252465]

31 Shibamoto Y, Okamoto M, Kobayashi M, Ayakawa S, Iwata H, Sugie C, Mitsuishi Y, Takahashi H. Immune-maximizing (IMAX) therapy for cancer: Combination of dendritic cell vaccine and intensity-modulated radiation. *Mol Clin Oncol* 2013; **1**: 649-654 [PMID: 24649223 DOI: 10.3892/mco.2013.108]

32 **Rodriguez PC**, Ernstoff MS, Hernandez C, Atkins M, Zabaleta J, Sierra R, Ochoa AC. Arginase I-producing myeloid-derived suppressor cells in renal cell carcinoma are a subpopulation of activated granulocytes. *Cancer Res* 2009; **69**: 1553-1560 [PMID: 19201693 DOI: 10.1158/0008-5472.CAN-08-1921]

33 **Rosa DS**, Ribeiro SP, Cunha-Neto E. CD4+ T cell epitope discovery and rational vaccine design. *Arch Immunol Ther Exp* (Warsz) 2010; **58**: 121-130 [PMID: 20155490 DOI: 10.1007/s00005-010-0067-0]

34 **Lutz ER**, Wu AA, Bigelow E, Sharma R, Mo G, Soares K, Solt S, Dorman A, Wamwea A, Yager A, Laheru D, Wolfgang CL, Wang J, Hruban RH, Anders RA, Jaffee EM, Zheng L. Immunotherapy converts nonimmunogenic pancreatic tumors into immunogenic foci of immune regulation. *Cancer Immunol Res* 2014; **2**: 616-631 [PMID: 24942756 DOI: 10.1158/2326-6066.CIR-14-0027]

35 **Lutz ER**, Kinkead H, Jaffee EM, Zheng L. Priming the pancreatic cancer tumor microenvironment for checkpoint-inhibitor immunotherapy. *Oncoimmunology* 2014; **3**: e962401 [PMID: 25941589 DOI: 10.4161/21624011.2014.962401]

P- Reviewer: De Berardinis P **S- Editor**: Qiu S **L- Editor**: A
E- Editor: Wang CH

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i1.139

World J Gastrointest Pharmacol Ther 2016 February 6; 7(1): 139-144
ISSN 2150-5349 (online)

ORIGINAL ARTICLE

Retrospective Study

Under-diagnosing and under-treating iron deficiency in hospitalized patients with gastrointestinal bleeding

Mustapha M El-Halabi, Michael S Green, Christopher Jones, William J Salyers Jr

Mustapha M El-Halabi, Michael S Green, Christopher Jones, William J Salyers Jr, Department of Internal Medicine, University of Kansas School of Medicine - Wichita, Wichita, KS 67214, United States

William J Salyers Jr, Division of Gastroenterology, University of Kansas School of Medicine - Wichita, Wichita, KS 67214, United States

Author contributions: Salyers Jr WJ was the guarantor of the study and contributed study idea and conception; El-Halabi MM and Salyers Jr WJ designed the study; El-Halabi MM, Green MS and Jones C acquired data; El-Halabi MM, Green MS, Jones C and Salyers Jr WJ analyzed and interpreted data; El-Halabi MM drafted the manuscript; Green MS, Jones C and Salyers Jr WJ revised the manuscript and appraised it critically for important intellectual content; all authors approved the final version of the manuscript.

Institutional review board statement: The study was reviewed and approved by the University of Kansas School of Medicine in Wichita Institutional Review Board and by the Via Christi Hospital Institutional Review Board.

Informed consent statement: Informed consents were not obtained from the patients. The IRB waived the need for informed consents based on the fact that our study was a minimal risk retrospective chart review study.

Conflict-of-interest statement: All authors declare that there is no conflict of interests regarding the publication of this article.

Data sharing statement: No additional data are available.

Correspondence to: William J Salyers Jr, MD, MPH, Assistant Professor, Head, Program Director, Department of Internal Medicine, University of Kansas School of Medicine - Wichita, 1010 N. Kansas, Wichita, KS 67214, United States. wsalyers@kumc.edu
Telephone: +1-316-2932650
Fax: +1-316-2931878

Received: July 13, 2015
Peer-review started: July 15, 2015
First decision: November 11, 2015
Revised: November 27, 2015
Accepted: December 29, 2015
Article in press: January 4, 2016
Published online: February 6, 2016

Abstract

AIM: To determine whether patients hospitalized with gastrointestinal (GI) blood loss anemia are being checked and treated for iron deficiency.

METHODS: Retrospective chart review was conducted for all patients admitted to a single tertiary care hospital between 11/1/2011 and 1/31/2012 for any type of GI bleeding. The primary endpoint was the percentage of patients who had their iron studies checked during a hospitalization for GI blood loss anemia. Secondary outcomes included percentage of anemic GI bleeders who had adequate documentation of anemia and iron deficiency, and those who were treated for their iron deficiency. Then we tried to identify possible predictors of checking iron studies in an attempt to understand the thought process that physicians go through when managing these patients. Iron deficiency was defined as Iron saturation less than 15% or ferritin level less than 45 μg/L. Anemia was defined as hemoglobin level less than 13 g/dL for males and 12 g/dL for females.

RESULTS: Three hundred and seven GI bleeders were hospitalized during the study period, and 282 of those (91.9%) had anemia during their hospital stay. Ninety-five patients (30.9%) had iron studies performed during hospitalization, and 45 of those (47.4%) were actually found to be iron deficient. Only 29 of those 45 iron deficient patients were discharged home on iron supplements. Of the 282 patients that had anemia during hospitalization, 50 (17.7%) had no documentation of the anemia in their hospital chart. Of the 45 patients that had lab proven iron deficiency anemia (IDA), only 22 (48.5%) had documentation of IDA in at least one note in their chart. Predictors of checking iron studies in anemic GI bleeders were lower mean corpuscular volume, documentation of anemia, having fecal occult blood testing, not having hematemesis or past history of GI bleeding. There were no significant differences between the teaching and non-teaching services in any patient characteristics or outcomes.

CONCLUSION: Iron deficiency is under-diagnosed, under-recognized even when iron studies were checked, and under-treated in hospitalized patients with GI bleeding.

Key words: Gastrointestinal bleeding; Iron deficiency anemia; Acute blood loss anemia; Iron supplements; Documentation

Core tip: Iron deficiency anemia (IDA) is under-diagnosed and under treated in hospitalized gastrointestinal (GI) bleeders. Less than a third of our patients had evaluation of their anemia to detect IDA. Around half of these investigated patients had lab proven IDA. Less than two thirds of those patients with proven IDA received iron supplementation, which means that IDA was either under-recognized or disregarded on purpose. In an attempt to understand the reasoning of physicians leading to this discrepancy, we analyzed predictors of checking iron studies on these hospitalized GI bleeders and the main predictors were lower mean corpuscular volume and early documentation of anemia in the chart.

El-Halabi MM, Green MS, Jones C, Salyers Jr WJ. Under-diagnosing and under-treating iron deficiency in hospitalized patients with gastrointestinal bleeding. *World J Gastrointest Pharmacol Ther* 2016; 7(1): 139-144 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i1/139.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i1.139

INTRODUCTION

Gastrointestinal (GI) bleeding is the cause of iron deficiency anemia (IDA) in the majority of men and post-menopausal women[1-5]. However, in our experience, only a small percentage of anemic patients admitted to the hospital with GI bleeding undergo appropriate evaluation to detect IDA and to treat it if present. This observation has also been documented in one observational and one retrospective study on hospitalized anemic patients without GI bleeding[6,7] but such data on hospitalized GI bleeders is not available in the literature. Our observation was puzzling because tests to detect iron deficiency are noninvasive, readily available at the hospital, inexpensive, and finally highly reliable.

Patients admitted to the hospital for GI bleed and anemia have either been chronically losing small amounts of blood or had a severe acute loss of large amounts of blood. In both cases, being iron deficient because of blood loss is highly probable. Losing 4 units of blood, chronically or acutely, will deplete completely the body iron stores[8]. However, losing much less than 4 units is enough to start depleting the iron stores and affect erythropoiesis. From here actually arises the necessity to test any GI bleeder with anemia for iron deficiency since anemia reflects a loss of significant amount of blood that would have probably affected iron stores. This concept was made even more plausible recently after a Danish group proved in a randomized controlled trial that iron supplementation significantly improved hemoglobin levels in GI bleeders over placebo[9]. However, there have been no studies yet assessing the degree of compliance of physicians with investigating anemia looking for IDA in GI bleeders and then treating these patients with iron supplements.

Therefore, we designed this study to determine whether patients admitted to the hospital with acute blood loss anemia secondary to GI losses are being worked up for concomitant, highly prevalent, IDA and adequately treated for their iron deficiency prior to or at discharge. Then, we tried to find possible predictors that led physicians to evaluate for iron deficiency in our anemic GI bleeders in an effort to try to explain why physicians would check some GI bleeders for iron deficiency and not others in order to hopefully be able to raise physicians' awareness about the topic and ultimately affect patient outcomes.

MATERIALS AND METHODS

This study was a retrospective chart review conducted at a single tertiary care hospital. Patients included in the study were all patients hospitalized with any kind of GI bleeding between 11/1/2011 and 1/31/2012. GI bleeding was not necessarily the primary reason for hospitalization. Excluded patients were those who were less than 18 years of age and those who were transferred from another hospital without complete medical records in the hospital electronic medical records system. The study was approved by our Institutional Review Board.

Table 1 Percentage of patients on iron supplements *n* (%)

	GI bleeders (*n* = 307)	GI bleeders with proven iron deficiency[1] (*n* = 45)
Prior to hospitalization	44 (14.3)	7 (15.6)
During hospitalization	71 (23.1)	29 (64.4)
After hospitalization (discharge instructions)	68 (22.1)	29 (64.4)

[1]Iron deficiency by laboratory results during the current hospitalization defined as either iron saturation < 15% or ferritin < 45 μg/L.

The primary endpoint of this study was the percentage of patients with iron studies performed in the hospital during a hospitalization for GI blood loss anemia. Secondary endpoints included prevalence of iron deficiency in acute GI bleeders, percentage of iron deficient patients that received treatment for their iron deficiency, percentage of anemic patients hospitalized for GI bleeding who had adequate documentation of anemia and iron deficiency in their charts. Secondary endpoints also included identifying possible predictors of checking iron studies or in other terms what were the patients' characteristics or laboratory or radiologic findings that made physicians check for iron deficiency in patients with acute blood loss anemia secondary to GI bleeding.

After identifying all ICD9 codes associated with any kind of GI bleeding, encounters that had any of these ICD9 codes within our study timeline were included in the review process. The authors then reviewed electronic medical records that were associated with these encounters. Each encounter represented one hospitalization.

Iron deficiency was defined as iron saturation less than 15% or ferritin level less than 45 μg/L. These cutoffs were used as criteria for iron deficiency in many studies in the literature[3,6,10,11]. Serum ferritin level of less than 45 μg/L has been calculated to have a specificity of 92% and a positive likelihood ratio of 11 in diagnosing iron deficiency, and an iron saturation of less than 15% is around the 12th percentile of the iron saturation in the general population[12,13]. Anemia was defined as hemoglobin level less than 13 g/dL for males and 12 g/dL for females as per the WHO definition of anemia[14].

Variables obtained from the medical records for each patient included demographics like age, gender and race, and hospital team; medical history including history of anemia, transfusion, GI bleeds in the past, chronic diseases and GI diseases; medications including antiplatelets, anticoagulants and anti-acids; GI bleeding symptoms; complete blood count (CBC) result on hospital admission and lowest hemoglobin recorded in the chart during hospitalization; fecal occult blood testing (FOBT) result; iron panel including iron level, total iron binding capacity (TIBC), Transferrin, iron saturation, and ferritin; reticulocyte count, peripheral blood smear results and bone marrow aspirate results if available; any radiology and endoscopy results; final diagnosis for the GI bleed; presence or absence and type of documentation of anemia and IDA in the electronic chart; iron treatment before, during or after hospitalization.

Statistical analysis was performed using SPSS 20.0 (SPSS, Chicago, IL, United States). Means and Frequencies were used to identify and summarize patients' characteristics and results for all continuous and categorical variables, respectively. To check for predictors of ordering iron studies in anemic patients with GI blood losses a univariate analysis was performed first comparing patients that had their iron studies done and those that did not have their iron studies done for each variable separately. Means and *t* tests were used to compare continuous variables. Crosstabs and χ^2 tests were used to compare categorical variables. All variables that were significantly different between the 2 groups on univariate analysis were included in the multivariable analysis. The multivariable analysis was performed using backward logistic regression model then confirmed with Forward and Enter models. *P*-value < 0.05 was considered to be significant.

RESULTS

Three hundred and seven charts of patients hospitalized with GI bleeding during the study period were identified and reviewed. Mean age was 66.2 ± 18.6 years. There was a slight female predominance with 177 (57.7%) females and 130 (42.3%) males. Two hundred and sixty one patients (85%) were Caucasians, 26 (8.5%) were African American or Black, and 14 (4.6%) were Hispanics. Of those 307 GI bleeders, 236 patients (76.9%) had anemia on admission to the hospital, while 282 (91.9%) had anemia at some point during their hospital stay.

As for our primary outcome, only 95 patients (30.9%) had iron studies performed during hospitalization. Additionally, 4 patients (1.3%) were discharged home with recommendations to their primary care physician to check iron studies.

We also had many secondary outcomes. Of the 95 patients that had iron studies performed, 45 were actually found to be iron deficient (47.4%). However, only 29 of those 45 iron deficient patients were discharged home on iron supplements. Iron supplementation data is summarized in Table 1.

Ninety patients (29.3%) were admitted to a residency teaching service and 217 (70.7%) to a hospitalist non-teaching service. There were no significant differences between the 2 services in any patient characteristics or outcomes.

Of the 282 patients that had anemia during hospitalization, 50 (17.7%) had no formal documentation of the anemia diagnosis on their discharge summary, 30 (9.8%) had a documented IDA, and 202 (71.6%) had a documented anemia diagnosis

Table 2 Predictors of iron deficiency anemia investigation *n* (%)

Variable		Univariate analysis[1]		Multivariate analysis	
		No iron studies (*n* = 188)	Iron studies (*n* = 94)	*P* value	OR (95%CI)
Anemia documentation[2]	None	35 (92.1)	3 (7.9)		Reference
	Before discharge summary	153 (62.7)	91 (37.3)	0.001	8.45 (2.35-30.33)
History of GI bleed	None	150 (63.0)	88 (37.0)		Reference
	Yes	38 (86.4)	6 (13.6)	0.001	0.19 (0.07-0.50)
Hematemesis	None	130 (61.6)	81 (38.4)		Reference
	Yes	58 (81.7)	13 (18.3)	0.003	0.32 (0.15-0.68)
FOBT	Negative	56 (62.9)	33 (37.1)	< 0.001[3]	Reference
	Positive	59 (55.7)	47 (44.3)	0.07	1.87 (0.95-3.69)
	Not performed	73 (83.9)	14 (16.1)	0.005	0.32 (0.14-0.71)
MCV[4]		90.5 ± 9.4 fL	82.9 ± 19.2 fL	< 0.001	0.95 (0.93-0.98)

[1]All variables included in multivariable analysis were statistically significant on univariate analysis, including all the variables shown in this table; [2]Anemia documentation on any note including admission, consultation or progress notes but excluding discharge summary; [3]*P* value for trend; [4]MCV is a continuous variable, so a OR < 1 means that higher MCV was inversely associated with iron deficiency investigation (with every 1 fL increase in MCV there was a 5% decrease in probability of getting iron studies). GI: Gastrointestinal; IDA: Iron deficiency anemia; FOBT: Fecal occult blood testing; MCV: Mean corpuscular volume. Variables included in the multivariable logistic regression but excluded from the table above for being not statistically significant are: Iron supplements on admission, occult bleeding, and lowest hemoglobin. All patients' characteristics that were not included in the multivariate regression were not significantly different on univariate analysis.

without specification whether it was iron deficient or not. Of the 45 patients that had lab proven IDA, only 22 (48.5%) had documentation of IDA in at least one note in their chart.

On multivariable analysis, using logistic regression modeling, predictors of checking iron studies in anemic patients with GI blood losses are presented in Table 2.

DISCUSSION

Our results confirmed our original hypothesis. IDA is under-diagnosed and under treated in hospitalized GI bleeders. Less than a third of our patients had evaluation of their anemia to detect IDA. Around half of them had lab proven IDA and less than two thirds of those patients with proven IDA received iron supplementation.

In an attempt to understand the reasoning that was leading physicians not to check iron studies and then sometimes to not prescribe iron supplements for patients that did turn out to have IDA, we designed the study with our secondary outcomes in mind, too. These results we think were able to explain this problem partially but not completely by any means.

According to our results, lower mean corpuscular volume (MCV) was associated with higher probability of checking iron studies. This reflects the current teaching that IDA is a microcytic anemia. However, this finding also means that physicians might be getting deterred from checking iron studies when there is no microcytosis and forgetting or dismissing the fact that there might be an overlap with another kind of anemia like anemia of chronic disease or B12 deficiency that keeps the MCV within normal range.

Documenting anemia as a diagnosis on any note including History and Physical, consult note, or progress note, but excluding discharge summary note, was also associated with ordering iron studies. This finding reflects the fact that when physicians were aware of the anemia and hence documented it in their notes they were more likely to investigate it. This reasoning might lead us to say that in the cases where patients did not get their iron studies checked, physicians in some of these instances, at least, might have been unaware of a mild anemia or did not consider that it was important or worthy enough to spend time documenting it or working it up, especially when patients had many other comorbidities. The documentation of anemia or IDA on the discharge summary note was not part of that variable because it would be more of a consequence rather than a predictor of IDA since discharge summaries are generally written after discharge.

Our search for iron deficiency investigation predictors also resulted in 3 other predictors that were all inversely associated with ordering iron studies: Past history of GI bleeding, hematemesis as one of the symptoms of the current GI bleeding episode, and not having a FOBT done during this current hospitalization.

It was especially surprising to find that having a past history of GI bleeding was a deterrent for physicians to check their patients iron storage levels since these patients were probably even more iron deficient than others since they have lost at least part of their iron stores in the previous bleeding event. A possible explanation for this finding could be that physicians consider patients presenting with anemia in the setting of a GI bleed, especially recurrent GI bleed, to have an explained anemia that does not need further workup and forget about the iron losses that accompany the blood losses which require replacement for a quicker hemoglobin recovery.

Hematemesis was the only symptom that was associated in any way with predicting iron level evaluations. It was most likely a confounding variable for something else that we did not assess in our multivariate analysis because there is no clear clinical reasoning that might explain why hematemesis would be associated with iron stores evaluation while melena, for example, was not evaluated.

Like any retrospective study, our study has its own limitations. We could not follow up patients after their discharge from the hospital. We could not contact them or check their outpatient records. However, we checked discharge summaries for any recommendations from the hospitalist to the patient's primary care physician regarding anything related to anemia or IDA in an attempt to try to minimize this specific limitation in the study design that could not have been otherwise overcome given that it is a retrospective chart review study. In the absence of a prospective randomized trial on the subject, our study is still the best evidence on the topic so far.

Our study had a good representative sample of GI bleeders that get admitted to our hospital. It included all patients hospitalized with any GI bleed as a primary or a secondary diagnosis for a whole 3 mo period. We believe that 3 mo is a long enough period to represent the usual population and be able to capture the usual practices of our physicians. Our study also included patients on 2 different types of services, the residency teaching services and the hospitalist non-teaching service. All patients' characteristics were the same between the 2 services, which was expected since patients were admitted usually randomly to either the residency team or the hospitalist team. However, patient outcomes were also not different and being on one team instead of the other was not a predictor of a different outcome regarding iron studies or iron supplementation.

In conclusion, Iron deficiency was under-diagnosed and under-treated in hospitalized GI bleeders. Possible reasons could be that physicians are dismissing iron deficiency when there is no microcytosis, forgetting about the possibility of overlap with another type of anemia, or when anemia is not very severe they might be deeming it unimportant or unworthy of investigation in a sick patient.

We believe that investigating GI bleeders for iron deficiency is important, especially that there is evidence in the literature that upper GI bleeders treated with iron had significantly higher hemoglobin with 2.0 g/dL difference on average compared to placebo as early as 4 wk from hospital discharge[9]. Therefore, we believe that physicians need to be made aware of our results and about that topic in general for better patient care and outcomes. We understand that our study is a retrospective study and that it has its limitations, therefore a future prospective trial might add strength to the evidence.

COMMENTS

Background

From the authors' experience, only a small percentage of anemic patients admitted to the hospital with gastrointestinal (GI) bleeding undergo appropriate evaluation to detect iron deficiency anemia (IDA) and to treat it if present. This observation has also been documented in two other studies on hospitalized anemic patients without GI bleeding but such data on hospitalized GI bleeders is not available in the literature.

Research frontiers

One recent randomized controlled trial proved that iron supplementation significantly improved hemoglobin levels in GI bleeders over placebo. However, there have been no studies yet assessing the degree of compliance of physicians with investigating anemia looking for IDA in GI bleeders and then treating these patients with iron supplements.

Innovations and breakthroughs

This is the first study to address this specific question. The authors designed this study to determine whether patients admitted to the hospital with acute blood loss anemia secondary to gastrointestinal losses are being worked up for concomitant, highly prevalent, IDA and adequately treated for their iron deficiency prior to or at discharge. The results indicated that IDA was under-diagnosed and under-treated in this group of patients.

Applications

The authors hope that our study will help in raising physicians' awareness about this topic of IDA in GI bleeders for a better recognition and treatment of IDA and ultimately affect patient outcomes.

Terminology

IDA was defined as hemoglobin less than 13 g/dL in males and 12 g/dL in females with iron saturation less than 15% or ferritin level less than 45 μg/L.

Peer-review

The paper is very interesting and important because it highlights the fact that IDA is underestimated even in the United States.

REFERENCES

1 **Rockey DC**. Occult gastrointestinal bleeding. *N Engl J Med* 1999; **341**: 38-46 [PMID: 10387941 DOI: 10.1056/NEJM199907013410107]

2 American Gastroenterological Association medical position statement: evaluation and management of occult and obscure gastrointestinal bleeding. *Gastroenterology* 2000; **118**: 197-201 [PMID: 10611169 DOI: 10.1016/S0016-5085(00)70429-X]

3 **Joosten E**, Ghesquiere B, Linthoudt H, Krekelberghs F, Dejaeger E, Boonen S, Flamaing J, Pelemans W, Hiele M, Gevers AM. Upper and lower gastrointestinal evaluation of elderly inpatients who are iron deficient. *Am J Med* 1999; **107**: 24-29 [PMID: 10403349 DOI: 10.1016/S0002-9343(99)00162-X]

4 **Gordon SR**, Smith RE, Power GC. The role of endoscopy in the evaluation of iron deficiency anemia in patients over the age of 50. *Am J Gastroenterol* 1994; **89**: 1963-1967 [PMID: 7942718]

5 **Rockey DC**, Cello JP. Evaluation of the gastrointestinal tract in patients with iron-deficiency anemia. *N Engl J Med* 1993; **329**: 1691-1695 [PMID: 8179652 DOI: 10.1056/NEJM199312023292303]

6 **Ioannou GN**, Spector J, Rockey DC. Predictors of endoscopic and laboratory evaluation of iron deficiency anemia in hospitalized patients. *South Med J* 2007; **100**: 976-984 [PMID: 17943040 DOI: 10.1097/SMJ.0b013e3181520392]

7 **Smieja MJ**, Cook DJ, Hunt DL, Ali MA, Guyatt GH. Recognizing and investigating iron-deficiency anemia in hospitalized elderly people. *CMAJ* 1996; **155**: 691-696 [PMID: 8823214]

8 **Zhu A**, Kaneshiro M, Kaunitz JD. Evaluation and treatment of iron deficiency anemia: a gastroenterological perspective. *Dig*

Dis Sci 2010; **55**: 548-559 [PMID: 20108038 DOI: 10.1007/s10620-009-1108-6]

9 **Bager P**, Dahlerup JF. Randomised clinical trial: oral vs. intravenous iron after upper gastrointestinal haemorrhage--a placebo-controlled study. *Aliment Pharmacol Ther* 2014; **39**: 176-187 [PMID: 24251969 DOI: 10.1111/apt.12556]

10 **Looker AC**, Dallman PR, Carroll MD, Gunter EW, Johnson CL. Prevalence of iron deficiency in the United States. *JAMA* 1997; **277**: 973-976 [PMID: 9091669 DOI: 10.1001/jama.1997.03540360041028]

11 **Bini EJ**, Micale PL, Weinshel EH. Evaluation of the gastrointestinal tract in premenopausal women with iron deficiency anemia. *Am J Med* 1998; **105**: 281-286 [PMID: 9809688 DOI: 10.1016/S0002-9343(98)00260-5]

12 **Guyatt GH**, Oxman AD, Ali M, Willan A, McIlroy W, Patterson C. Laboratory diagnosis of iron-deficiency anemia: an overview. *J Gen Intern Med* 1992; **7**: 145-153 [PMID: 1487761 DOI: 10.1007/BF02598003]

13 **Guyatt GH**, Patterson C, Ali M, Singer J, Levine M, Turpie I, Meyer R. Diagnosis of iron-deficiency anemia in the elderly. *Am J Med* 1990; **88**: 205-209 [PMID: 2178409 DOI: 10.1016/0002-9343(90)90143-2]

14 **World Health Organization**. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System. Geneva, World Health Organization, 2011 (WHO/NMH/NHD/MNM/11.1). [accessed Jun 30 2015]. Available from: URL: http://www.who.int/vmnis/indicators/haemoglobin.pdf

P- Reviewer: D'Elios MM, Ivanovski P, Kurtoglu E, Rodrigo L
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Wang CH

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i1.145

World J Gastrointest Pharmacol Ther 2016 February 6; 7(1): 145-155
ISSN 2150-5349 (online)

ORIGINAL ARTICLE

Prospective Study

Effect of electrical stimulation of the lower esophageal sphincter in gastroesophageal reflux disease patients refractory to proton pump inhibitors

Edy Soffer, Leonardo Rodríguez, Patricia Rodriguez, Beatriz Gómez, Manoel G Neto, Michael D Crowell

Edy Soffer, Division of Gastroenterology, Keck School of Medicine, University of Southern California, Los Angeles, CA 90033, United States

Leonardo Rodríguez, Patricia Rodriguez, Beatriz Gómez, Surgery, CCO Centro Clínico de Obesidad, Diabetes y Reflujo, Las Condes, Santiago 7550000, Chile

Manoel G Neto, Surgery, Gastro Obeso Center, Bela Vista, São Paolo 1308-000, Brazil

Michael D Crowell, Department of Gastroenterology and Hepatology, Mayo Clinic, Scottsdale, AZ 85259, United States

Author contributions: Soffer E acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis; Rodríguez L analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis; obtained funding; technical, or material support; study supervision); Rodriguez P acquisition of data; analysis and interpretation of data; critical revision of the manuscript for important intellectual content; technical or material support; Gómez B acquisition of data; analysis and interpretation of data; critical revision of the manuscript for important intellectual content; technical or material support; Neto MG acquisition of data; analysis and interpretation of data; critical revision of the manuscript for important intellectual content; technical or material support; Crowell MD study concept and design; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis; technical, or material support.

Institutional review board statement: The study was approved by the ethics committee of the eastern metropolitan health service in Santiago, Chile "Comite de Etica Cientifico, Servicio de Salud Metropolitano Oriente".

Clinical trial registration statement: The study was registered in www.clinicaltrials.gov. The registration identification is NCT01578642.

Informed consent statement: All study participants provided informed written consent prior to study enrollment.

Conflict-of-interest statement: EndoStim Inc., St. Louis, MO, United States provided funding for the trial. The sponsor helped with monitoring, collection and tabulation of the data. The data analyses were independently performed by the investigators. EndoStim Inc, St. Louis, MO, United States provided funding for the trial. Professor Michael D Crowell is a consultant for EndoStim Inc. and the chair of its data monitoring committee. Professor Edy Soffer is a shareholder in EndoStim Inc. Remaining study authors or their immediate family have no financial relationship with the sponsor that would constitute a conflict of interest.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at edy.soffer@med.usc.edu. Participants gave informed consent for data sharing

Correspondence to: Edy Soffer, MD, Division of Gastroenterology, Keck School of Medicine, University of Southern California, 1510 San Pablo Street, HCC One, Los Angeles, CA 90033, United States. esoffer@usc.edu
Telephone: +1-323-4425833

Received: July 21, 2015
Peer-review started: July 30, 2015
First decision: September 14, 2015
Revised: October 9, 2015
Accepted: December 3, 2015
Article in press: December 4, 2015
Published online: February 6, 2016

Abstract

AIM: To evaluate the efficacy of lower esophageal sphincter (LES)-electrical stimulation therapy (EST) in a subgroup of patients that reported only partial response to proton pump inhibitors (PPIs) therapy, compared to a group of patient with complete response.

METHODS: Bipolar stitch electrodes were laparoscopically placed in the LES and connected to an implantable pulse generator (EndoStim BV, the Hague, the Netherlands), placed subcutaneously in the anterior abdominal wall. Stimulation at 20 Hz, 215 μsec, 3-8 mAmp in 30 min sessions was delivered starting on day 1 post-implant. Patients were evaluated using gastroesophageal reflux disease (GERD)-HRQL, symptom diaries; esophageal pH and esophageal manometry before and up to 24 mo after therapy and results were compared between partial and complete responders.

RESULTS: Twenty-three patients with GERD on LES-EST were enrolled and received continuous per-protocol stimulation through 12 mo and 21 patients completed 24 mo of therapy. Of the 23 patients, 16 (8 male, mean age 52.1 ± 12 years) had incomplete response to PPIs prior to LES-EST, while 7 patients (5 male, mean age 52.7 ± 4.7) had complete response to PPIs. In the sub-group with incomplete response to PPIs, median (IQR) composite GERD-HRQL score improved significantly from 9.5 (9.0-10.0) at baseline on-PPI and 24.0 (20.8-26.3) at baseline off-PPI to 2.5 (0.0-4.0) at 12-mo and 0.0 (0.0-2.5) at 24-mo follow-up (P < 0.05 compared to on-and off-PPI at baseline). Median (IQR) % 24-h esophageal pH < 4.0 at baseline in this sub-group improved significantly from 9.8% (7.8-11.5) at baseline to 3.0% (1.9-6.3) at 12 mo (P < 0.001) and 4.6% (2.0-5.8) at 24 mo follow-up (P < 0.01). At their 24-mo follow-up, 9/11 patients in this sub-group were completely free of PPI use. These results were comparable to the sub-group that reported complete response to PPI therapy at baseline. No unanticipated implantation or stimulation-related adverse events, or any untoward sensation due to stimulation were reported in either group and LES-EST was safely tolerated by both groups.

CONCLUSION: LES-EST is safe and effective in controlling symptoms and esophageal acid exposure in GERD patients with incomplete response to PPIs. These results were comparable to those observed PPI responders.

Key words: Refractory gastroesophageal reflux disease; Gastroesophageal reflux; Electrical stimulation; Lower esophageal sphincter; Proton pump inhibitors

Core tip: Proton pump Inhibitors (PPI) are the main medical therapy for gastroesophageal reflux disease (GERD). However, 30%-40% of patients are unsatisfied with PPI therapy. Traditional antireflux surgery is effective but is associated with adverse effects and its numbers are declining, resulting in an unmet need for alternative therapies. Electrical stimulation therapy (EST) of the LES has been shown to significantly improve GERD symptoms and esophageal acid exposure in patients with GERD. The current study shows that patients who respond to PPI but are concerned about the drugs, as well as those with incomplete response to PPI respond equally to EST of the LES.

Soffer E, Rodríguez L, Rodriguez P, Gómez B, Neto MG, Crowell MD. Effect of electrical stimulation of the lower esophageal sphincter in gastroesophageal reflux disease patients refractory to proton pump inhibitors. *World J Gastrointest Pharmacol Ther* 2016; 7(1): 145-155 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i1/145.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i1.145

INTRODUCTION

Gastroesophageal reflux disease (GERD) is a common and widespread condition[1-3], impacting patients' wellbeing and driving health care cost[4]. Acid suppression agents and surgical therapy with laparoscopic Nissen fundoplication have been shown to significantly improve the care of many patients with GERD[5]. However, acid suppression therapy targets acid secretion rather than a dysfunctional lower esophageal sphincter and reflux, with inadequate control of symptoms resulting in poor quality of life in approximately 30%-40% of patients with GERD[6]. While fundoplication is effective in expert hands, results are inferior in community centers as compared to high volume ones[7], and the intervention can be associated with significant long-term adverse effects including dysphagia and gas bloat[8]. These limitations have led to a decline of traditional anti-reflux procedures and to a search for alternative treatment modalities of GERD[9].

Lower esophageal sphincter (LES)-electrical stimulation therapy (EST) has been shown to increase resting LES pressure in both acute and chronic animal models[10-12]. Short term LES-EST, using temporary leads implanted in the LES of subjects with GERD has confirmed these results and demonstrated a significant enhancement in LES tone without impairing swallow-induced LES relaxation and without inducing any adverse sensation[13,14]. These results suggested that LES-EST might be an effective method of restoring the anti-reflux function of the LES in GERD patients and led to the development of the EndoStim® LES Stimulation System for treatment of GERD.

We have previously reported the safety and efficacy of 1 and 2-year LES-EST therapy in patients with GERD enrolled in an open-label, single -enter trial, using a

permanently implanted stimulation system[15,16]. The objectives of this post-hoc analysis of this open-label human trial were to compare the effects of LES-EST on GERD symptoms and medication use between GERD patients with partial response to daily PPI medications and those reporting a complete response at their baseline evaluation. Additionally, esophageal acid exposure, esophageal motor function and healing of erosive esophagitis, evaluated at 12 and 24 mo of LES-EST, were compared between the groups.

MATERIALS AND METHODS

This is a post-hoc analysis of a prospective, single-center, open-label, and treatment only trial that evaluated the safety and efficacy of LES stimulation for the treatment of GERD. The objective of this post-hoc analysis was to assess the response to LES-EST in a sub-group of patients with incomplete response to PPIs, defined as bothersome symptoms of heartburn at least 1 d a week while ON-PPIs[17] and compared to the sub-group of complete responders. The incidence of serious device- and/or procedure-related adverse effects (AEs) was the primary safety endpoint of the trial, while the incidence of non-serious device- and procedure-related AEs was the secondary safety endpoint. The primary efficacy endpoint was a reduction in the GERD-HRQL composite score on LES-EST compared with baseline score while on and OFF-PPIs. Additional efficacy endpoints included improvement in esophageal acid exposure, GERD symptoms reported in daily symptom diaries and medication use on LES-EST compared with baseline, and improvement in esophagitis grade and mean respiratory and end-expiratory LES pressures.

The open-label trial was approved by the Servicio de Salud Metropolitano Oriente, Santiago, Chile ethics committee and all subjects signed informed consent.

All patients enrolled in the trial suffered from chronic GERD symptoms, were not satisfied with their medical therapy or were concerned about long-term acid suppression therapy, and contemplated surgical intervention for GERD. Key inclusion criteria included subjects between 21 and 65 years of age with a history of heartburn, regurgitation or both for > 6 mo prompting physician recommendation of chronic daily use of PPI before study entry, and a baseline GERD-HRQL heartburn score of $\geqslant$ 20 OFF-PPI with a symptomatic response to a course of GERD therapy ($\geqslant$ 2 wk) and a GERD-HRQL heartburn score improvement of $\geqslant$ 10 on therapy. Subjects had to exhibit excessive esophageal acid exposure during 24-h pH-measurement off antisecretory therapy defined as pH < 4 for $\geqslant$ 5% of total or $\geqslant$ 3% of supine time. Exclusion criteria were: A resting LES end expiratory pressure $\leqslant$ 5mm Hg on a high resolution manometry; esophageal body contraction amplitude $\leqslant$ 30 mmHg for $\geqslant$ 70% of swallows; $\leqslant$ 50% peristaltic contractions on high resolution manometry; esophagitis > Grade C (LA classification) on upper endoscopy performed within 6 mo prior to enrollment; Barrett's epithelium (> M2; > C1) with any grade of dysplasia; hiatus hernia greater than 3 cm; BMI > than 35 kg/m^2; uncontrolled Type 2 diabetes mellitus (T2DM) defined as HbA1c > 9.5 in the previous 6 mo; a history of T2DM for > 10 years or Type 1 diabetes mellitus. Detailed inclusion, exclusion criteria and study details have been previously reported[15,16].

LES stimulation system

The LES stimulation system is similar to traditional neurostimulators with three components: a bipolar stimulation lead with two stitch electrodes, an implantable pulse generator (IPG) and an external programmer (Figure 1A), and the details were described previously[16]. In brief, the IPG and stimulation lead were all implanted by laparoscopy, using 4-5 ports. The anterior right aspect of the abdominal esophagus was exposed through dissection of the paraesophageal fat and pars flaccida of the hepatogastric ligament. A rectangular longitudinal area of approximately 3 cm × 1 cm is needed in which the electrodes are implanted. This approach minimized dissection of the phreno-esophageal attachment and damage to the anterior vagal nerve. The 2 stitch electrodes were implanted *via* a superficial bite into the LES muscle along the main esophageal axis with approximately 10 mm between the electrodes. Each electrode was then secured by a clip on the proximal edge of the electrode on to the nylon suture wire and also by suturing the distal anchoring "butterfly" present on the back end of the electrode. Upper gastrointestinal endoscopy was performed to verify electrode position in the LES and to confirm that no perforation of the esophageal lumen had occurred with the needle or electrode. The lead delivered through the abdominal wall and secured to the IPG located in a subcutaneous pocket in the left upper quadrant (Figure 1B). Interrogation and programming of the IPG were provided *via* a wireless external programmer and computer software.

The LES stimulation system delivers therapy personalized to individual patient needs. The stimulation pulse is monophasic followed by a charge-balancing phase. The pulse is 215 μsec wide and nominally 5 mA in amplitude (range 3-8 mA). The stimulation pulse is delivered at a rate of 20 Hz and continues for a period of 30 min. Up to twelve 30-min sessions per day were delivered. Electrical stimulation could be optimized using the external programmer to tailor therapy to individual patients' needs. Therapy could be adjusted at follow-up to address residual symptoms or acid events seen on pH testing by altering stimulation parameters such as number or timing of stimulation sessions, electrode polarity and stimulation amplitude.

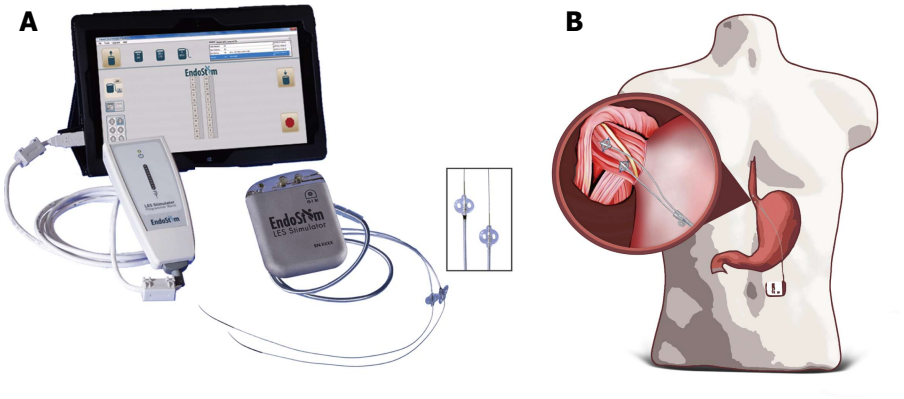

Figure 1 Lower esophageal sphincter stimulation system. A: EndoStim® wireless Programmer, Implantable Pulse Generator and Bipolar Stimulation Lead. Inset shows the two stimulation electrodes and the butterfly used for anchoring the electrode at the LES; B: Schematic of the EndoStim® System Implant in a Patient: Electrode position and IPG implant location. Bipolar stitch electrodes are placed in the abdominal esophagus anteriorly in an inline configuration 1 cm apart. The lead is connected to the IPG that is implanted in the subcutaneous pocket in the anterior abdomen. IPG: Implantable pulse generator; LES: Lower esophageal sphincter.

After signing an informed consent, symptoms were assessed while on-PPIs and after a period of 2 wk off-PPIs. High resolution esophageal manometry and ambulatory esophageal pH test were performed 2 wk after being OFF-PPIs. Data on GERD medication usage was recorded. Patients fulfilling entry criteria underwent a laparoscopic LES stimulation system implant procedure, EST was started immediately post-procedure and PPI therapy was discontinued. Patients were allowed to use antacid or antisecretory medications for control of breakthrough symptoms on LES-EST. Patients were evaluated at regular intervals after implantation per-protocol.

Symptom assessment and physiological tests

GERD symptoms were assessed by the validated GERD-Health Related Quality of Life (GERD-HRQL) questionnaire[17], which provides a composite score based on the frequency and severity of symptoms. Patients' GERD-HRQL scores were assessed at baseline while ON-PPI therapy and at 10-14 d off-PPI, before initiation of LES-EST and at follow-up periods of 1, 3, 6, 12, 15, 18 and 24 mo. Symptoms of heartburn and regurgitation and medication use were assessed by 14-d daily diary, and overall quality of life was assessed by the SF-12 Physical and Mental Health Surveys at each follow-up time point. High resolution esophageal manometry at baseline was performed using the Medical Measurement System (MMS, Dover, NH) in 6 patients and the Sierra Scientific Instruments system (Given Imaging, Los Angeles, California) in 18 patients. Due to equipment availability, all 12-mo manometries were performed with the MMS system.

Esophageal acid exposure was assessed with 24-h esophageal pH-metry at baseline and at 3, 6, and 12 and 24 mo of follow-up with patients off-PPI for at least 5 d (AL1 Sistema de pH-Metria, Ver 1.26, Alacer Biomedica, Brazil).

The degree of esophagitis was assessed by upper endoscopy performed within 6 mo prior to enrollment, and at 12-mo follow-up.

Statistical analysis

Safety evaluation was descriptive and included the incidence, severity, and type of AEs, and clinically significant changes or abnormalities in each patient's physical examination, vital signs, clinical tests and EKG results, for patients in both sub-groups. All reported adverse events were adjudicated by an independent Data Monitoring Committee for relatedness to the procedure, device and/or therapy.

The effect of LES-EST on patient symptoms was assessed by comparing patients' GERD-HRQL at 12 and 24-mo follow-up with baseline scores (both on and 2-wk off-PPI). Frequency and severity of symptoms and medication use were assessed by 14 d daily diary and compared between 12 and 24-mo follow-up and baseline. The impact of GERD symptoms on global quality of life measured by SF-12 was also compared at 12 and 24-mo follow-up *vs* baseline.

Esophageal acid exposure was expressed as the proportion of time during 24-h pH-metry with distal esophageal pH < 4.0. Esophageal acid exposure was compared between baseline and 12 and 24 mo follow-up. A reviewer (MDC), blinded to all identifying patient and visit data, independently analyzed all pH data. Manometry was performed as described above. Due to the change in equipment only descriptive manometry findings are presented. Esophagitis, if present by endoscopy, was classified using the Los Angeles (LA) Classification scheme.

Data were analyzed and presented as mean ± SD, or median and quartiles. All comparisons between the two groups and changes from baseline were made at the P <0.05 level using related-samples Wilcoxon Sign Rank test (for continuous or scale measures) or McNemar's test (for categorical measures), SAS version 9.4 (SAS Institute, Cary, NC, United States).

RESULTS

Twenty-five patients were enrolled in the LES stimulation study. One patient withdrew consent 2

wk post-implant due to the demanding nature of the protocol and underwent an uneventful explant of the stimulator 6 wk after implantation. One patient quit the study for an elective surgical procedure for control of diabetes. Twenty three and 21 patients respectively completed the 1 and 2 years follow up.

Of the 23 patients that were enrolled in the study, a subgroup of 16 patients (8 male, mean age 52.1 ± 12 years) reported incomplete response to PPI therapy and 7 patients (5 male, mean age 52.7 ± 4.7) reported complete response to PPI therapy prior to LES-EST. All patients completed 12 mo follow up, while 14/16 incomplete responders and all 7 complete responders completed the 24 mo follow up.

Patients with incomplete response had a mean duration of GERD of 12.9 ± 9.0 years and all were on chronic PPI therapy prior to implantation for a mean duration of 6.3 ± 3.4 years (QD = 12, BID = 4). Patients with complete response had a mean duration of GERD of 8.6 ± 4.3 years and all were on chronic PPI therapy prior to implantation for a mean duration of 4.7 ± 3.4 years (QD = 6, BID = 1). Baseline characteristics and signal optimization were comparable among the two groups.

Safety

During the 24 mo following implant 11 out of the 16 patients who were incomplete responders to PPI reported 28 AEs. One serious not related adverse event was reported; a diagnosis of nodular thyroid disease requiring hospitalization and surgery. The remaining 27 AEs were non-serious. Two events in 1 patient were reported as probably or possibly related to the device or the laparoscopic implant procedure (implant site pain = 1, abdominal pain and excess salivation = 1). Additionally, 1 event (post-operative nausea/vomiting) in 1 patient was reported as possibly procedure related, which was resolved with medication.

During the 24 mo following implant, 6 out of the 7 patients who were complete responders to PPI reported 25 AEs. One not related serious adverse event was reported; an episode of non-cardiac chest pain not related to LES stimulation. Similar episodes were experienced by the patient prior to starting LES-EST. The patient was hospitalized and cardiac evaluation, including cardiac catheterization, was normal. Chest X-ray revealed stable lead position without any evidence of migration. The episode resolved spontaneously and stimulation was restarted. One event in one patient was reported as probably device related (implant site pain = 1) and 6 events were procedure related (implant site pain = 1, post-op nausea = 2, hypertensive crisis = 1, acute shoulder pain = 1, localized infection = 1).

None of the patients in either group reported any GI side effects of bloating, inability to belch or new dysphagia associated with LES-EST. Rates of AE/SAE were comparable among the two groups.

GERD-HRQL and daily symptom diaries

GERD symptoms improved significantly in both groups following treatmet and improvement persisted over the next 2 years. There was a significant reduction of GERD-HRQL composite and individual symptom scores compared to baseline (Table 1).

Composite GERD-HRQL scores are presented in Table 1. Median (IQR) scores of the incomplete responders patients at baseline on-and-OFF-PPI were 9.5 (9-10) and 24 (20.8-26.3) respectively. Scores significantly improved at both 12- and 24-mo of treatment to 2.5 (0-4) and 0 (0-2.5) respectively ($P < 0.01$). Median (IQR) composite GERD-HRQL scores for the sub-group of patients responding completely to PPI improved from 4 (2.5-6) at baseline on-PPI and 21 (21-23) at baseline off-PPI to 1 (0.0-2.5) and 0.0 (0.0-3.0) at 12- and 24-mo of treatment ($P = 0.02$). The percentage improvement in GERD-HRQL scores from baseline on-PPI and off- PPI scores was greater in the incomplete responder subgroup than the complete responder subgroup at both 12- and 24-mo of LES-EST.

Dysphagia In the incomplete responder group was reported in 14/16 and 6/16 patients at baseline off-PPI and on-PPI repectively, while only 2 patients reported dysphagia at 24-mo ($P = 0.01$ *vs* baseline). In the complete responder group, 7/7 patients reported dysphagia at baseline off-PPI and 2/7 reported dysphagia at baseline on-PPI, while only one patient reported dysphagia at 24-mo follow-up ($P = 0.18$ *vs* baseline on-PPI; $P = 0.002$ *vs* baseline off-PPI).

In the incomplete responder group, reflux affecting sleep was reported by 15/16 patients at baseline off-PPI and 13/16 patients on-PPI, while two patients reported reflux affecting sleep at 24-mo follow-up ($P < 0.01$ *vs* baseline). In the complete responder group, nocturnal reflux was reported by 7/7 patients at baseline off-PPI and 3/7 patients at baseline on-PPI, while one patient reported reflux affecting sleep at 24-mo follow-up ($P = 0.05$ *vs* baseline).

Fourteen-day symptom diaries evaluating heartburn and regurgitation symptoms were completed in 14 of the 16 patients in the incomplete responder subgroup and in 7 of 7 patients in the complete responder subgroup (Figure 2 and Table 2). Frequency and severity of heartburn and regurgitation, both during the day and night, improved significantly over time with LES-EST.

At 24 mo, 14/16 (88%) incomplete responders were completely OFF-PPI and only 2 patients (13%) were still using PPIs. At 24 mo, 4/7 (57%) patients in the responder sub-group were completely OFF-PPI and one (14%) patient used the medication occasionally (< 50% of diary days). Two out of seven (29%) used PPI regularly (100% of diary days).

Patients in both groups reported improvement of

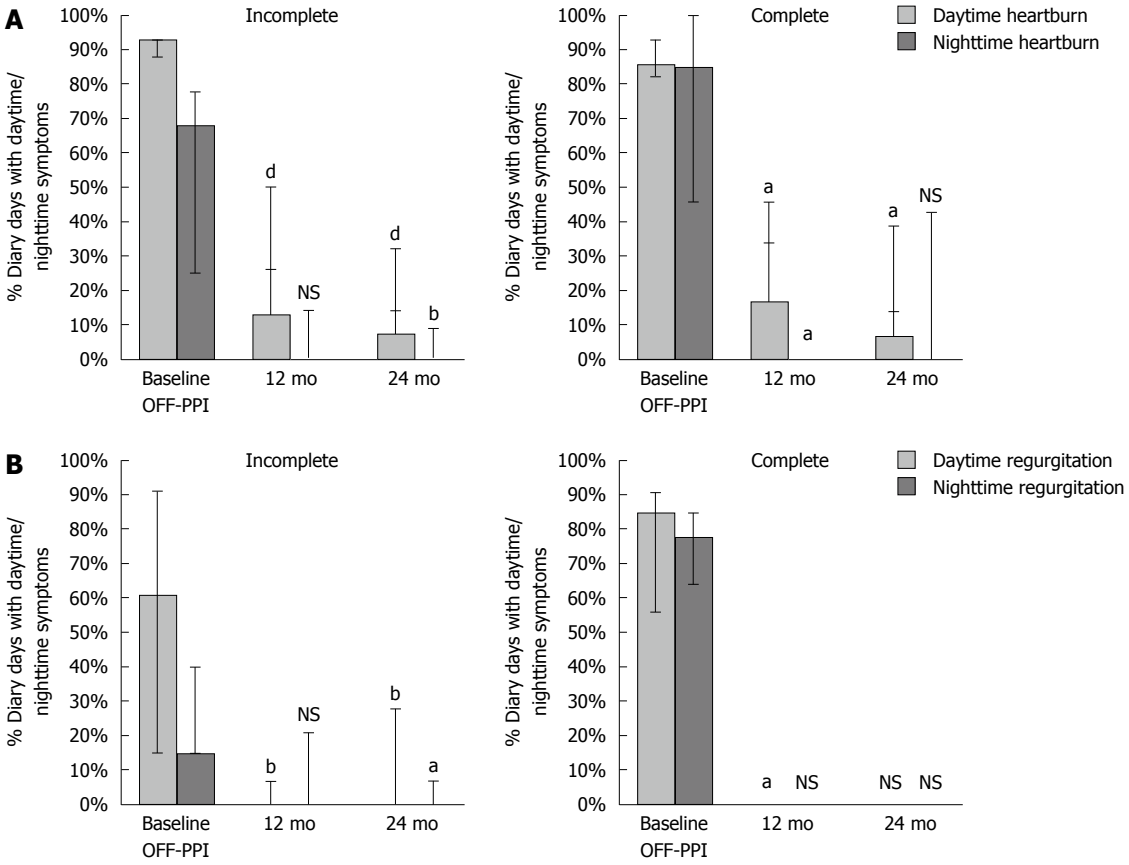

Figure 2 Frequency of daytime and nighttime symptoms of (A) heartburn and (B) regurgitation at baseline and with lower esophageal sphincter - electrical stimulation therapy at 12 and 24 mo. Data are presented as median and interquartile range (IQR). Absence of median value or IQR bars indicate a value of zero for the listed variables. NS = Not significant, [a]*P* < 0.05, [b]*P* < 0.01, [d]*P* < 0.001. A: Percent days with Heartburn at baseline, 12- and 24-mo following LES-EST. There was a significant reduction in reported daytime and nighttime with heartburn at both time points compared to baseline, in both groups; B: Percent of days with Regurgitation at baseline, 12- and 24-mo following LES-EST. There was a marked reduction in reported daytime and nighttime with regurgitation at both time points compared to baseline, in the incomplete responder group and a marked reduction in these variables in the responder group. LES: Lower esophageal sphincter; EST: Electrical stimulation therapy.

both SF-12 Physical Component Score and the SF-12 Mental Component Score over time, compared to baseline, and most scores were significantly improved (Table 1).

At baseline none of the patients in the incomplete responder group were satisfied with their condition, while at 12 mo 69% (11/16) were (*P* < 0.01). In the complete responder group all patients were satisfied at one year (7/7), as compared to only 1 patients satisfied at baseline (*P* = 0.12).

Esophageal acid exposure

Variables of esophageal acid exposure improved in both groups, and significantly so in the subgroup of patients with incomplete response to PPI (Table 1). In the incomplete responder group, 24-h esophageal pH-metry was completed in all 16 patients at baseline and 12 mo, and 10 of the 16 patients at 24-mo who received continuous stimulation through 24-mo follow-up. Median (IQR) percent time with pH < 4.0 improved from 9.8% (7.8-11.5) at baseline to 3.0% (1.9-6.3) at 12 mo (*P* < 0.001) and 4.6% (2.0-5.8) at 24 mo (*P* < 0.01). Esophageal acid exposure was normalized in 7/10 (70%) of patients at their 24 mo follow-up. In the complete responder group, 24-h esophageal pH-metry was performed in all 7 patients at baseline, 6 out of 7 at 12 mo, and 4 out of 7 at 24 mo. Esophageal acid exposure was normalized in 67% (4/ 6) patients and > 50% improvement was observed in an additional 16% (1/6) patients at 12 mo. At 24 mo, 50% (2/4) of patients were normalized or improved by at least 50%. Improvement in esophageal pH was comparable between the groups.

High-resolution manometry

High-resolution manometry revealed no substantial change in end expiratory LES pressure following stimulation therapy. Importantly, LES-EST had no effect on Swallow-induced LES residual pressure with 6.5 mmHg (2.9-10.9), 8.0 mmHg (4.0-11.0) and 7.0 mmHg (4.5-11.5) at baseline, 12- and 24-mo respectively in the incomplete responder group and 8.9 mmHg (7.4-10.9), 7.0 mmHg (1.5-8.0) and 5.0 mmHg (4.0-10.0) in the responder group.

Healing of erosive esophagitis

At baseline endoscopy, all patients had esophagitis, for the most part mild. In the incomplete responder

Table 1 Gastroesophageal reflux disease - health related quality of life, esophageal acid exposure and esophageal manometry at baseline and 12 and 24-mo post-lower esophageal sphincter - electrical stimulation therapy

	Incomplete responders				Complete responders			
	Visit interval (subject number)	Median (IQR)	*P* value		Visit interval (subject number)	Median (IQR)	*P* value	
			Baseline ON-PPI	Baseline OFF-PPI			Baseline ON-PPI	Baseline OFF-PPI
GERD-HRQL scores	Baseline on PPI (16)	9.5 (9-10)	0.012		Baseline ON PPI (7)	4 (2.5-6)	0.13	0.02
	Baseline off PPI (16)	24 (20.8-26.3)	0.0013	< 0.001	Baseline OFF PPI (7) (16)	21 (21-23)	0.40	0.02
	12 mo (16)	2.5 (0-4)		0.0011	12 mo (7)	1 (0-2.5)		
	24 mo (14)	0 (0-2.8)			24 mo (7)	0 (0-3)		
Percent of 24-h esophageal pH < 4.0	Baseline off PPI (16)	9.8 (7.8-11.5)		< 0.001	Baseline OFF PPI (7)	16.7 (7.5-17.8)		0.09
Total	12 mo (16)	3.0 (1.9-6.3)		0.003	12 mo (6)	3.5 (2.1-6.1)		0.12
	24 mo (13)	4.6 (1.6-5.1)			24 mo (5)	7.5 (5.1-12.6)		
Percent 24-h esophageal pH < 4.0	Baseline off PPI (16)	9.4 (7.3-13.6)		< 0.001	Baseline OFF PPI (7)	10.4 (10.1-15.2)		0.03
Upright	12 mo (16)	3.9 (3.1-5.2)		< 0.001	12 mo (6)	5.0 (2.3-7.8)		0.06
	24 mo (13)	5.5 (1.8-7.2)			24 mo (5)	5.0 (4.3-6.8)		
% 24-h esophageal pH < 4.0	Baseline off PPI (16)	5.8 (1.7-10.3)		0.10	Baseline OFF PPI (7)	8.3 (3.2-22)		0.30
Supine	12 mo (16)	0.4 (0.0-5.9)		0.56	12 mo (6)	1.0 (0.0-3)		1.00
	24 mo (13)	0.5 (0.2-4.1)			24 mo (5)	11.1 (0.6-18.9)		
DeMeester score	Baseline off PPI (16)	34.8 (29.6-43.8)		< 0.001	Baseline OFF PPI (7)	67 (31.9-70.2)		0.09
	12 mo (16)	10.8 (5.9-28.3)		0.02	12 mo (6)	17.6 (10.2-24.5)		0.13
	24 mo (13)	15.4 (8-20)			24 mo (5)	30.0 (18.1-54.5)		
Percent patients with abnormal distal esophageal pH (pH < 4.0 for > 4%)	Baseline off PPI (16)	94%		0.008	Baseline OFF PPI (7)	100%		0.13
	12 mo (16)	38%		0.021	12 mo (6)	33%		0.33
	24 mo (13)	54%			24 mo (5)	80%		
Percent 24-h proximal esophageal pH < 4.0	Baseline off PPI (16)	0.2 (0.1-1.2)		0.006	Baseline OFF PPI (7)	0.6 (0.4-2)		0.06
	12 mo (16)	0.0 (0-0)		0.006	12 mo (6)	0.0 (0-0.1)		0.13
	24 mo (13)	0.0 (0-0)			24 mo (5)	0.0 (0-0.1)		
Percent 24-h proximal esophageal pH < 4.0	Baseline off PPI (16)	0.4 (0.2-1.9)		0.006	Baseline OFF PPI (7)	1.1 (0.6-2.6)		
	12 mo (16)	0.0 (0-0)		0.008	12 mo (6)	0.0 (0-0.1)		0.06
	24 mo (13)	0.0 (0-0.1)			24 mo (5)	0.0 (0-0.1)		0.13
Percent 24-h proximal esophageal pH < 4.0	Baseline off PPI (16)	0.0 (0-0.1)		0.20	Baseline OFF PPI (7)	0.1 (0.0-1.3)		0.37
Supine	12 mo (16)	0.0 (0-0)		0.37	12 mo (6)	0.0 (0-0)		0.37
	24 mo (13)	0.0 (0-0)			24 mo (5)	0.0 (0-0)		
Percent patients with abnormal proximal esophageal pH (pH < 4.0 for > 1.1%)	Baseline OFF PPI (16)	29%		0.13	Baseline OFF PPI (7)	33%		0.50
	12 mo (16)	0%		0.18	12 mo (6)	0%		0.14
	24 mo (13)	0%			24 mo (5)	0%		
LES end expiratory pressure (mmHg)	Baseline OFF PPI (16)	8.2 (6.8-9.8)			Baseline OFF PPI (7)	8.8 (5.7-10.5)		
	12 mo (16)	12 (7.8-18)			12 mo (6)	9.5 (4.5-14.5)		
	24 mo (13)	6 (4-10)			24 mo (5)	6 (4-9)		
LES respiratory mean pressure (mmHg)	Baseline OFF PPI (16)	17.2 (16.1-20.9)			Baseline OFF PPI (7)	2 (1.9-2.5)		
	12 mo (16)	12 (17.5-29)			12 mo (6)	2.7 (2.2-3.0)		
	24 mo (13)	19 (16-22)			24 mo (5)	2.5 (2.4-2.6)		
LES length (cm)	Baseline off PPI (16)	2.1 (1.8-2.6)			Baseline OFF PPI (7)	2.0 (1.9-2.5)		
	12 mo (16)	2.6 (2.3-2.9)			12 mo (6)	2.7 (2.2-3)		
	24 mo (11)	2.3 (2.0-2.5)			24 mo (5)	2.5 (2.4-2.6)		
Percent peristaltic swallows	Baseline OFF PPI (16)	100 (95-100)			Baseline OFF PPI (7)	100 (98-100)		
	12 mo (16)	100 (91-100)			12 mo (6)	98 (70-100)		
	24 mo (11)	100 (85-100)			24 mo (5)	86 (85-100)		
Distal esophageal contraction amplitude (mmHg)	Baseline OFF PPI (16)	78.3 (67-95)			Baseline OFF PPI (7)	46.1 (39.9-54.5)		
	12 mo (16)	71.5 (48.5-80.5)			12 mo (6)	40.5 (34.5-46.5)		
	24 mo (11)	53.0 (42.5-69.5)			24 mo (5)	45.0 (41.0-53.0)		

Number of esophageal contractions > 30 mmHg	Baseline OFF PPI (16)	18 (14-20)			Baseline OFF PPI (7)	19 (11.5-19.5)		
	12 mo (16)	19.5 (18.5-20.0)			12 mo (6)	16 (9.3-19.8)		
	24 mo (11)	18.0 (8.0-20.0)			24 mo (5)	11.0 (6.0-15.0)		
Residual LES pressure (mmHg)	Baseline OFF PPI (16)	6.5 (2.9-10.9)			Baseline OFF PPI (7)	8.9 (7.4-10.9)		
	12 mo (16)	8.0 (4.0-11.0)			12 mo (6)	7.0 (1.5-8.0)		
	24 mo (11)	7.0 (4.5-11.5)			24 mo (5)	5.0 (4.0-10.0)		
SF-12 PCS	Baseline ON PPI (16)	45 (41.5-49.5)	0.38	0.28	Baseline ON PPI (7)	50 (46-52.5)	0.34	0.03
	Baseline OFF PPI (16)	46.5 (41.2-53)	0.004	0.002	Baseline OFF PPI (7) (16)	45 (40.5-47)	0.09	0.02
	12 mo (16)	51.0 (42.8-55.2)			12 mo (7)	55 (47-55)		
	24 mo (14)	54.5 (52.2-56.5)			24 mo (7)	55 (53-58.5)		
SF-12 MCS	Baseline ON PPI (16)	49 (42.5-59)	0.68	0.18	Baseline ON PPI (16)	43.0 (40.5-47.5)	0.38	0.83
	Baseline OFF PPI (16)	45 (36.5-54.2)	0.01	0.002	Baseline OFF PPI (16)	54 (51-55.5)	0.61	0.16
	12 mo (16)	52.5 (43.8-58.0)			12 mo (16)	50 (47.5-53.5)		
	24 mo (14)	60.5 (54.5-62)			24 mo (14)	44 (38.5-50)		

There was no statistically significant difference between the 2 groups at any time point. PPI: Proton pump inhibitor.

group, 11 patients had LA grade A esophagitis, 4 LA grade B and 1 LA grade C. At 12 mo, esophagitis resolved in 6 patients, 7 had LA grade A, 2 LA grade B and 1 LA grade C. Esophagitis improved by at least 1 grade in 63% of patients at 12-mo (P = 0.02). In the complete responder group, 4 patients had LA grade A esophagitis, 2 LA grade B and 1 LA grade C. At 12 mo, esophagitis resolved in 1 patient, 5 had LA grade A, 1 LA grade B and none with LA grade C. Esophagitis improved by at least 1 grade in 43 % of patients at 12-mo (P = 0.15).

DISCUSSION

Our post-hoc analysis shows that LES electrical stimulation was as effective in incomplete responders as it was in complete responders. LES stimulation was not associated with any adverse effects or sensations that are typically observed with traditional anti-reflux surgery (as dysphagia, gas-bloat, and diarrhea), in either group. In both groups, LES electrical stimulation safely improved symptoms, significantly reduced esophageal acid exposure, and almost completely eliminated the need for daily acid suppression medications (PPIs). Swallow-induced LES relaxation or peristaltic activity was not affected by electrical stimulation in either group.

The definition of GERD by the Montreal consensus emphasizes both subjective complaints as well as complications of GERD, defining it as "a condition that develops when the reflux of stomach contents causes troublesome symptoms and/or complications"[18]. GERD symptoms are common and affect 10%-20% of adults in Western countries[19] and up to 40% in the United States[20]. Proton pump inhibitors are potent suppressors of gastric acid secretion and have revolutionized the treatment of GERD, however, a substantial number of patients remain symptomatic in spite of maximal medical therapy[6].

There has been increasing awareness of partial responders to medical therapy. A meta-analysis of randomized, controlled studies conducted in the secondary care practices showed that 10%-40% of GERD patients reported partial- or nonresponse of their reflux symptoms to PPI therapy[21]. Comparable rates were recently reported in a systematic review of persistent reflux symptoms in patients ON-PPI therapy evaluated in primary care and community studies[22]. In this study, the persistence of GERD symptoms was associated with decreased psychological and physical well-being. A recent observational study, conducted in primary care as well as specialized settings, supported these findings, reporting that incomplete response to PPI therapy was associated with considerable direct and indirect costs, and substantial impairment in HRQL and productivity of such patients[23]. Failure of PPI treatment to resolve GERD-related symptoms has become the most common presentation of GERD in gastrointestinal practice, and presents a significant therapeutic challenge to the practicing physician.

Both the "AGA position statement on the management of GERD" and "SAGES guidelines for surgical treatment of GERD" recommend anti-reflux surgery for GERD patients with incomplete response to PPI, with persistent and troublesome GERD symptoms despite medical therapy, or in those intolerant to PPIs[24,25]. Other recommended reasons for surgery include the lifelong need for medication intake, expense of medications, and side-effects[24,25]. Indeed, almost 50% of patients chose to undergo surgery because inadequate control of GERD symptoms in spite of acid suppression therapy[7]. The patients enrolled in our study met these two sets of indications, with 2/3 selecting a surgical intervention because of continuing bothersome symptoms despite daily single or double-dose PPI therapy, while a 1/3 chose surgical therapy because of quality of life concerns with continuous use of daily medications.

Table 2 Severity of heartburn and regurgitation was significantly improved in both groups during the study, with significant difference between the 2 subgroups

	Incomplete responders				Complete responders			
	Visit interval (subject number)	Median (IQR)	*P* value		Visit interval (subject number)	Median (IQR)	*P* value	
			Baseline ON-PPI	Baseline OFF-PPI			Baseline ON-PPI	Baseline OFF-PPI
Percent diary days with heartburn severity	Baseline OFF PPI (14)				Baseline OFF PPI (6)			
	12 mo (15)			< 0.001	12 mo (6)			0.03
	24 mo (12)			< 0.001	24 mo (7)			0.03
None	Baseline OFF PPI (14)	0 (0-10)			Baseline OFF PPI (6)	0 (0-14)		
	12 mo (15)	88 (42-100)			12 mo (6)	83 (54-100)		
	24 mo (12)	86 (73-100)			24 mo (7)	79 (32-93)		
Mild	Baseline OFF PPI (14)	19 (14-29)			Baseline OFF PPI (6)	14 (0-14)		
	12 mo (15)	0 (0-32)			12 mo (6)	0 (0-25)		
	24 mo (12)	7 (0-14)			24 mo (7)	7 (0-18)		
Moderate	Baseline OFF PPI (14)	46 (31-66)			Baseline OFF PPI (6)	62 (46-68)		
	12 mo (15)	0 (0-7)			12 mo (6)	0 (0-11)		
	24 mo (12)	0 (0-7)			24 mo (7)	7 (0-29)		
	Baseline OFF PPI (14)	11 (0-29)			Baseline OFF PPI (6)	8 (4-21)		
Severe	12 mo (15)	0 (0-0)			12 mo (6)	0 (0-1)		
	24 mo (12)	0 (0-0)			24 mo (7)	0 (0-4)		
% Diary days with regurgitation severity	Baseline OFF PPI (14)				Baseline OFF PPI (6)		0.03	0.03
	12 mo (15)			< 0.003	12 mo (6)			
	24 mo (12)			< 0.03	24 mo (7)			
None	Baseline OFF PPI (14)	35 (7-70)			Baseline OFF PPI (6)	4 (0-8)		
	12 mo (15)	100 (100-100)			12 mo (6)	100 (100-100)		
	24 mo (12)	100 (95-100)			24 mo (7)	100 (86-100)		
Mild	Baseline OFF PPI (14)	8 (7-20)			Baseline OFF PPI (6)	19 (9-54)		
	12 mo (15)	0 (0-0)			12 mo (6)	0 (0-0)		
	24 mo (12)	0 (0-0)			24 mo (7)	0 (0-4)		
Moderate	Baseline OFF PPI (14)	30 (4-57)			Baseline OFF PPI (6)	29 (11-46)		
	12 mo (15)	0 (0-0)			12 mo (6)	0 (0-0)		
	24 mo (12)	0 (0-0)			24 mo (7)	0 (0-4)		
	Baseline OFF PPI (14)	0 (0-21)			Baseline OFF PPI (6)	0 (0-16)		
Severe	12 mo (15)	0 (0-0)			12 mo (6)	0 (0-0)		
	24 mo (12)				24 mo (7)	0 (0-0)		

PPI: Proton pump inhibitor.

A number of mechanisms are thought to contribute to incomplete response, an important one being the persistent reflux of weakly acidic or non-acidic gastric content while ON-PPIs since PPIs target acid secretion rather than reflux of gastric contents into the esophagus[26]. In a group of 145 patients with refractory symptoms on twice daily PPI; only 13% of persistent heartburn events and 8% of persistent regurgitation events were associated with acid reflux; meanwhile, 27% of heartburn events and 58% of regurgitation events were associated with weakly acid reflux[6]. In fact, PPIs are effective in control of the symptom of heartburn but not regurgitation, as highlighted by a recent study showing a higher therapeutic gain with PPI therapy for heartburn over regurgitation[27], highlighting the important role of regurgitation in PPI failure[28], and its being one of the indications for antireflux surgery. Accordingly, in our study, regurgitation reported by 21/23 patients at baseline. Regurgitation was reported by 3/15 patients with incomplete response to PPI at 12 mo and by 3/14 at 12 mo when OFF-PPI (data not reported in 1 and 2 patients respectively). In the complete responder group, none of the 7 patients had regurgitation at 12 mo and only 1/7 reported regurgitation at 24 mo, thus supporting the notion that LES-EST is truly an antireflux therapy.

Anti-reflux surgery has been the main alternative to medical therapy. However, complications and the need for continuous GERD medications despite surgery remain a concern, resulting in a declining number of anti-reflux surgeries in the United States[29]. Moreover, data suggest that laparoscopic fundoplication is less effective at reducing symptoms in partial responders to medical therapy than in complete responders[30]. Consequently, surgical therapy is generally not recommended in patients who are complete non-responders to PPI therapy. This highlights the need for an alternative therapy in this challenging group of patients.

Our results demonstrate that a comparable, significant and sustained improvement in esophageal acid exposure and symptoms, and almost completely eliminated the need for daily PPIs in patients with incomplete response to PPI, who may have been less than optimal candidates for anti-reflux surgery. The

improvement in symptoms was sustained over 24 mo. Symptoms continued to improve over time which was likely the result of the ability to non-invasively optimize therapy to individual needs, a feature not available with any of the endoscopic or surgical interventions.

Our results also demonstrate the safety of this intervention and its excellent adverse effects profile, without the symptoms that are commonly observed after surgery[29], reflecting the "non-disruptive" nature of this intervention on the GE junction anatomy. No device or procedure-related SAEs were reported with LES-EST during the study, only minor anticipated AEs typically observed in the postoperative state. All events resolved without a need for any significant intervention, in line with previous experience with gastric electrical stimulation[31]. Importantly, EST had no effect on LES residual pressure in response to swallows, and none of the patients in this trial reported dysphagia or any other gastrointestinal symptoms associated with stimulation. The technical simplicity of this intervention is likely to result in less procedural variability and more uniform long-term outcomes, unlike laparoscopic fundoplication where outcomes from low-volume centers are less favorable compared to those reported from high-volume centers[7].

There are limitations to this study. The open label design cannot control for placebo effect and a "regression to mean", affecting subjective variables such as GERD-HRQL and other patient reported outcomes. However, objective variables, such as esophageal acid exposure are much less likely to be influenced by placebo. Also, because of the selective enrollment criteria, the results may not be generalizable to these patients. The efficacy of LES-EST in patients with large hernia, combined with hernia repair, remains to be established. Finally, these are small numbers and some of the comparisons are susceptible to a type-Ⅱ error.

In conclusion, the results of the study support the safety and efficacy of electrical stimulation of the LES in the treatment of GERD patients with incomplete response to PPI therapy. These results were comparable to those seen in patients with complete response to PPI at baseline.

COMMENTS

Background

A substantial number of gastroesophageal reflux disease (GERD) patients are not satisfied with medical therapy, and are reluctant to consider fundoplication, as evident by the declining number of such intervention.

Research frontiers

Surgical therapy for GERD.

Innovations and breakthroughs

A novel therapy for GERD, using application of electrical stimulation to the lower esophageal sphincter.

Applications

Patients with GERD who are not controlled or are not unsatisfied with medical therapy and are seeking an alternative to established surgical interventions.

Peer-review

This paper is concerning an interesting topic, *i.e.*, alternative treatment to proton pump inhibitors (PPI) for patients poorly responding to PPI. Obviously, as pointed by the authors, the paper has two limits: the very low number of patients in both arms (one arm include 7 patients), which strongly reduces its power, and the "open label" design. Likely more data should be elaborated in order to support the final conclusions.

REFERENCES

1 **Sandler RS**, Everhart JE, Donowitz M, Adams E, Cronin K, Goodman C, Gemmen E, Shah S, Avdic A, Rubin R. The burden of selected digestive diseases in the United States. *Gastroenterology* 2002; **122**: 1500-1511 [PMID: 11984534 DOI: 10.1053/gast.2002.32978]

2 **Everhart JE**. Digestive diseases in the United States: Epidemiology and Impact. NIDDK: NIH Publication, 1994: 94-1447

3 **El-Serag HB**. Time trends of gastroesophageal reflux disease: a systematic review. *Clin Gastroenterol Hepatol* 2007; **5**: 17-26

4 **Wahlqvist P**, Reilly MC, Barkun A. Systematic review: the impact of gastro-oesophageal reflux disease on work productivity. *Aliment Pharmacol Ther* 2006; **24**: 259-272 [PMID: 16842452 DOI: 10.1111/j.1365-2036.2006.02996.x]

5 **McQuaid KR**, Laine L. Early heartburn relief with proton pump inhibitors: a systematic review and meta-analysis of clinical trials. *Clin Gastroenterol Hepatol* 2005; **3**: 553-563 [PMID: 15952097 DOI: 10.1016/S1542-3565(05)00023-6]

6 **Fass R**, Shapiro M, Dekel R, Sewell J. Systematic review: proton-pump inhibitor failure in gastro-oesophageal reflux disease--where next? *Aliment Pharmacol Ther* 2005; **22**: 79-94 [PMID: 16011666 DOI: 10.1111/j.1365-2036.2005.02531.x]

7 **Vakil N**, Shaw M, Kirby R. Clinical effectiveness of laparoscopic fundoplication in a U.S. community. *Am J Med* 2003; **114**: 1-5 [PMID: 12543281 DOI: 10.1016/S0002-9343(02)01390-6]

8 **Richter JE**. Gastroesophageal reflux disease treatment: side effects and complications of fundoplication. *Clin Gastroenterol Hepatol* 2013; **11**: 465-471; quiz e39 [PMID: 23267868 DOI: 10.1016/j.cgh.2012.12.006]

9 **Vassiliou MC**, von Renteln D, Rothstein RI. Recent advances in endoscopic antireflux techniques. *Gastrointest Endosc Clin N Am* 2010; **20**: 89-101, vii [PMID: 19951796 DOI: 10.1016/j.giec.2009.08.002]

10 **Ellis F**, Berne TV, Settevig K. The prevention of experimentally induced reflux by electrical stimulation of the distal esophagus. *Am J Surg* 1968; **115**: 482-487 [PMID: 5642728 DOI: 10.1016/0002-9610(68)90191-8]

11 **Clarke JO**, Jagannath SB, Kalloo AN, Long VR, Beitler DM, Kantsevoy SV. An endoscopically implantable device stimulates the lower esophageal sphincter on demand by remote control: a study using a canine model. *Endoscopy* 2007; **39**: 72-76 [PMID: 17252464 DOI: 10.1055/s-2006-945102]

12 **Sanmiguel CP**, Hagiike M, Mintchev MP, Cruz RD, Phillips EH, Cunneen SA, Conklin JL, Soffer EE. Effect of electrical stimulation of the LES on LES pressure in a canine model. *Am J Physiol Gastrointest Liver Physiol* 2008; **295**: G389-G394 [PMID: 18687754 DOI: 10.1152/ajpgi.90201.2008]

13 **Banerjee R**, Pratap N, Kalpala R, Reddy DN. Effect of electrical stimulation of the lower esophageal sphincter using endoscopically implanted temporary stimulation leads in patients with reflux disease. *Surg Endosc* 2014; **28**: 1003-1009 [PMID: 24170067 DOI: 10.1007/s00464-013-3271-2]

14 **Rodríguez L**, Rodríguez P, Neto MG, Ayala JC, Saba J, Berel D, Conklin J, Soffer E. Short-term electrical stimulation of the lower esophageal sphincter increases sphincter pressure in

patients with gastroesophageal reflux disease. *Neurogastroenterol Motil* 2012; **24**: 446-450, e213 [PMID: 22292889 DOI: 10.1111/j.1365-2982.2012.01878.x]

15 **Rodríguez L**, Rodriguez P, Gómez B, Ayala JC, Oksenberg D, Perez-Castilla A, Netto MG, Soffer E, Crowell MD. Long-term results of electrical stimulation of the lower esophageal sphincter for the treatment of gastroesophageal reflux disease. *Endoscopy* 2013; **45**: 595-604 [PMID: 23881803 DOI: 10.1055/s-0033-1344213]

16 **Rodríguez L**, Rodriguez P, Gómez B, Ayala JC, Oxenberg D, Perez-Castilla A, Netto MG, Soffer E, Boscardin WJ, Crowell MD. Two-year results of intermittent electrical stimulation of the lower esophageal sphincter treatment of gastroesophageal reflux disease. *Surgery* 2015; **157**: 556-567 [PMID: 25726315 DOI: 10.1016/j.surg.2014.10.012]

17 **Velanovich V**. The development of the GERD-HRQL symptom severity instrument. *Dis Esophagus* 2007; **20**: 130-134 [PMID: 17439596 DOI: 10.1111/j.1442-2050.2007.00658.x]

18 **Vakil N**, van Zanten SV, Kahrilas P, Dent J, Jones R. The Montreal definition and classification of gastroesophageal reflux disease: a global evidence-based consensus. *Am J Gastroenterol* 2006; **101**: 1900-1920; quiz 1943 [PMID: 16928254 DOI: 10.1111/j.1572-0241.2006.00630.x]

19 **El-Serag HB**, Sweet S, Winchester CC, Dent J. Update on the epidemiology of gastro-oesophageal reflux disease: a systematic review. *Gut* 2014; **63**: 871-880 [PMID: 23853213 DOI: 10.1136/gutjnl-2012-304269]

20 **Locke GR**, Talley NJ, Fett SL, Zinsmeister AR, Melton LJ. Prevalence and clinical spectrum of gastroesophageal reflux: a population-based study in Olmsted County, Minnesota. *Gastroenterology* 1997; **112**: 1448-1456 [PMID: 9136821 DOI: 10.1016/S0016-5085(97)70025-8]

21 **Crawley JA**, Schmitt CM. How satisfied are chronic heartburn sufferers with their prescription medications? Results of the patient unmet needs study. *J Clin Outcomes Manag* 2000; **7**: 29

22 **El-Serag H**, Becher A, Jones R. Systematic review: persistent reflux symptoms on proton pump inhibitor therapy in primary care and community studies. *Aliment Pharmacol Ther* 2010; **32**: 720-737 [PMID: 20662774 DOI: 10.1111/j.1365-2036.2010.04406.x]

23 **Stålhammar N,** Spiegel BM, Löfman HG, Karlsson M, Wahlqvist P, Næsdal J, Nelson MT, Despiége N. Partial response to proton pump inhibitor therapy for GERD: observational study of patient characteristics, burden of disease, and costs in the USA. *Pragm Obser Res* 2012: **3:** 57-67 [DOI: 10.2147/POR.S36704]

24 **Kahrilas PJ**, Shaheen NJ, Vaezi MF, Hiltz SW, Black E, Modlin IM, Johnson SP, Allen J, Brill JV. American Gastroenterological Association Medical Position Statement on the management of gastroesophageal reflux disease. *Gastroenterology* 2008; **135**: 1383-1391, 1391.e1-5 [PMID: 18789939 DOI: 10.1053/j.gastro.2008.08.045]

25 **Stefanidis D**, Hope WW, Kohn GP, Reardon PR, Richardson WS, Fanelli RD. Guidelines for surgical treatment of gastroesophageal reflux disease. *Surg Endosc* 2010; **24**: 2647-2669 [PMID: 20725747 DOI: 10.1007/s00464-010-1267-8]

26 **Sifrim D**, Zerbib F. Diagnosis and management of patients with reflux symptoms refractory to proton pump inhibitors. *Gut* 2012; **61**: 1340-1354 [PMID: 22684483 DOI: 10.1136/gutjnl-2011-301897]

27 **Kahrilas PJ**, Howden CW, Hughes N. Response of regurgitation to proton pump inhibitor therapy in clinical trials of gastroesophageal reflux disease. *Am J Gastroenterol* 2011; **106**: 1419-1425; quiz 1426 [PMID: 21537361 DOI: 10.1038/ajg.2011.146]

28 **Kahrilas PJ**, Jonsson A, Denison H, Wernersson B, Hughes N, Howden CW. Regurgitation is less responsive to acid suppression than heartburn in patients with gastroesophageal reflux disease. *Clin Gastroenterol Hepatol* 2012; **10**: 612-619 [PMID: 22343515 DOI: 10.1016/j.cgh.2012.01.022]

29 **Wang YR**, Dempsey DT, Richter JE. Trends and perioperative outcomes of inpatient antireflux surgery in the United States, 1993-2006. *Dis Esophagus* 2011; **24**: 215-223 [PMID: 21073616 DOI: 10.1111/j.1442-2050.2010.01123.x]

30 **Lundell L**. Borderline indications and selection of gastroesophageal reflux disease patients: 'Is surgery better than medical therapy'? *Dig Dis* 2014; **32**: 152-155 [PMID: 24603401 DOI: 10.1159/000357182]

31 **McCallum RW**, Lin Z, Forster J, Roeser K, Hou Q, Sarosiek I. Gastric electrical stimulation improves outcomes of patients with gastroparesis for up to 10 years. *Clin Gastroenterol Hepatol* 2011; **9**: 314-319.e1 [PMID: 21185396 DOI: 10.1016/j.cgh.2010.12.013]

P- Reviewer: Contini S, Luo HS, Mann O **S- Editor**: Qi Y
L- Editor: A **E- Editor**: Wang CH

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i1.156

World J Gastrointest Pharmacol Ther 2016 February 6; 7(1): 156-161
ISSN 2150-5349 (online)

ORIGINAL ARTICLE

Randomized Controlled Trial

Therapeutic effect of melatonin on pediatric functional dyspepsia: A pilot study

Katherine Zybach, Craig A Friesen, Jennifer V Schurman

Katherine Zybach, Craig A Friesen, Division of Gastroenterology, Hepatology and Nutrition, Children's Mercy Kansas City, Kansas City, MO 64108, United States

Jennifer V Schurman, Division of Developmental and Behavioral Sciences, Children's Mercy Kansas City, Kansas City, MO 64108, United States

Author contributions: Zybach K was the primary contributor to study design, study management, data analysis, and manuscript writing and revision; Friesen CA contributed to study design, analysis, and manuscript writing and revision; Schurman JV contributed to analysis, manuscript writing, and critical review.

Institutional review board statement: The study was reviewed and approved by the Children's Mercy Hospital Institutional Review Board.

Clinical trial registration statement: This study is registered at ClinicalTrials.gov (NCT00148603).

Informed consent statement: The legal guardians of all study participants provided informed written consent and all study participants provided assent prior to study enrollment.

Conflict-of-interest statement: The authors have no conflicts of interest.

Correspondence to: Craig A Friesen, MD, Division of Gastroenterology, Hepatology and Nutrition, Children's Mercy Kansas City, 2401 Gillham Road, Kansas City, MO 64108, United States. cfriesen@cmh.edu
Telephone: +1-816-2343016
Fax: +1-816-8021465

Received: August 8, 2015
Peer-review started: August 11, 2015
First decision: September 11, 2015
Revised: October 6, 2015
Accepted: November 23, 2015
Article in press: November 25, 2015
Published online: February 6, 2016

Abstract

AIM: To study the effectiveness of melatonin *vs* placebo in children with functional dyspepsia (FD).

METHODS: The study was conducted as a double blind, randomized, placebo controlled crossover trial. Subjects were aged 8-17 years and diagnosed with FD based on Rome Ⅲ criteria. All subjects had failed to respond to 4 wk of acid suppression. Subjects receive a continuous two weeks of placebo and a continuous two weeks of melatonin in an order blinded to the participant and the study team. A Global Clinical Score was obtained to assess changes in abdominal pain. Pain was self-reported to be worse (grade 1), no change (grade 2), moderate improvement (grade 3), good (grade 4; minimal pain and not interfering with daily activities), or excellent (grade 5; no pain), respectively. A positive clinical response was defined as a grade 3 or greater response. Subjects wore an actigraph to assess sleep during a one week baseline period and during each treatment period. Subjects' sleep latency and total sleep time were recorded throughout the duration of the study.

RESULTS: Fourteen subjects were enrolled and 12 completed the study. One withdrew prior to starting both melatonin and placebo and the other before starting melatonin. A positive clinical response (grade 3-5) was achieved in 42% of subjects on melatonin *vs* 50% of subjects on placebo (NS). Effect size was calculated

and revealed a Cohen's D of 0.343 which demonstrates a medium effect favoring placebo. A grade 4 or grade 5 response was seen in 4 patients on melatonin and 5 patients on placebo. Baseline sleep parameters were in the healthy range with the longest sleep latency being just over 20 min (mean 7.46 ± 8.53 min) and the shortest sleep duration just over 7 h (mean 10.09 ± 2.72 h). The mean latency did not differ between periods of treatment with melatonin as compared to placebo (4.48 ± 6.45 min *vs* 3.58 ± 4.24 min; NS). The mean sleep duration did not differ between periods of treatment with melatonin as compared to placebo (9.90 ± 3.53 h *vs* 9.41 ± 2.70 h; NS).

CONCLUSION: Melatonin does not appear to have efficacy in relieving pain in unselected pediatric FD. Future studies should consider FD subtypes, pathophysiologic mechanisms, and baseline sleep disturbances.

Key words: Melatonin; Abdominal pain; Functional dyspepsia; Sleep latency; Sleep duration; Actigraphy

Core tip: Medical therapy is limited in children with functional dyspepsia. This creates a challenging clinical dilemma with regards to managing their symptoms. Melatonin has been shown to have a positive effect on pain in adults with functional dyspepsia or irritable bowel syndrome, independent of its effects on sleep. To date, there have been no studies to evaluate the effect of melatonin on abdominal pain in children. In the current study, melatonin did not result in improvement in abdominal pain or sleep parameters in children with functional dyspepsia.

Zybach K, Friesen CA, Schurman JV. Therapeutic effect of melatonin on pediatric functional dyspepsia: A pilot study. *World J Gastrointest Pharmacol Ther* 2016; 7(1): 156-161 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i1/156.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i1.156

INTRODUCTION

Pediatric functional dyspepsia (FD) is defined as persistent or recurrent pain or discomfort centered in the upper abdomen (above the umbilicus) that is unrelated to a change in stool frequency or form and not exclusively relieved by defecation[1]. It is a common diagnosis in children with population prevalence estimates of 3.5% to 27%[1,2]. A number of biologic contributors to the generation of FD have been identified and include inflammation, electromechanical dysfunction (*e.g.*, altered gastric emptying) and visceral hyperalgesia[3,4].

Melatonin, a hormone produced, in part, by the pineal gland, has a well-known role in regulation of sleep-wake cycles through its sleep promoting effects. Melatonin is commonly used to treat sleep disturbances related to sleep-onset latency in children and has been shown to be effective at a dose of 5 mg[5]. Parent-reported sleep disturbances occur in nearly half of children with FD with the most common problems being related to sleep onset and maintenance[6]. These sleep disturbances are positively associated with functional disability in FD, with this association mediated through physical symptoms, including pain[6]. Thus, melatonin may be a useful treatment in this population to decrease pain indirectly through improvement in sleep.

Less widely recognized is melatonin's production in parts of the body other than the pineal gland, such as in the digestive system and in immune cells including mast cells[7]. The total amount of melatonin in the digestive tract exceeds that of the pineal gland and blood. Melatonin is produced at high levels in the gastrointestinal mucosa in response to food. It exhibits a large number of biologic effects which may be relevant to FD including both excitatory and inhibitory effects on the enteric nervous system, anti-inflammatory properties, and both anxiolytic and antidepressant effects[8-10]. At pharmacologic doses, melatonin delays gastric emptying, in part acting as a partial 5-HT antagonist[10]. Thus, melatonin may also have a direct impact on visceral sensitivity.

One study of melatonin in adult patients with irritable bowel syndrome (IBS), another functional gastrointestinal disorder which shares common pathophysiologic mechanisms with FD, provides support for this secondary pathway of action[9]. In this study, two weeks of treatment with melatonin resulted in decreased mean abdominal pain scores and an increased mean rectal pain threshold without influencing sleep, anxiety, or depression scores[9]. To date, melatonin efficacy has been demonstrated in reducing pain in adults with FD in a single study[8]. Specifically, twelve weeks of melatonin (5 mg taken at bedtime) resulted in complete resolution of pain in 56.6% of patients as compared to only 6.7% of the patients who received placebo[8]. However, the specific mechanism of action was not entirely clear, given limited information provided related to methods of measurement on key variables. Although significant differences for complete resolution of pain did not emerge until the second month of treatment, participants receiving placebo took a significantly larger number of "sham" pain pills, nearly doubling those take by the melatonin group in the first month of treatment. This would suggest that they may have had a partial response during the first month that was not captured by the primary measurement strategy. Taken together, these studies suggest that treatment with melatonin may have a beneficial effect on pain in children with FD within a relatively short window of time.

Of note, there have been only a few placebo controlled trials of medications in children specifically with FD. This holds true for melatonin, as well, which has not yet been studied in children with abdominal pain in general or with FD specifically. This paucity of studies reflects the difficulty with conducting such trials in children, as well as concerns regarding the ethics of witholding treatment for prolonged periods (during the placebo phase) in a vulnerable population. These issues might be addressed, in part, by small n trials which create the potential for establishing preliminary efficacy of an intervention and establishing benefit and feasibility of completing a similar trial with a larger sample size[11]. The length of treatment can be based on the suspected mechanism of action to minimize study duration and the associated potential for unnecessary delay in treatment modification if/when a treatment is found to be ineffective. In a small n preliminary trial for FD, a cross-over design is important to not only control for the large number of biopsychosocial factors which may affect treatment response but also to evaluate the effects of treatment withdrawal, an important aspect of small n trials.

The aim of the current study was to assess the effectiveness of melatonin *vs* placebo in children with FD. The study was designed as a preliminary small n cross-over trial to establish an anticipated effect size from which a larger, adequately powered study could be based if results appeared promising. The specific study duration was selected based on previous work in adults with IBS and FD suggesting the beneficial impact of melatonin may be seen in as little as 2-4 wk. Thus, a 1-mo cross-over trial was deemed appropriate at this stage both from a scientific and ethical perspective.

MATERIALS AND METHODS

Study design

The study was conducted as a single site, double blind, randomized, placebo-controlled crossover trial. The study involved a one week baseline sleep data collection period followed by four weeks of sleep data collection and medication administration. The four weeks of medication administration involved a continuous two weeks of placebo and a continuous two weeks of melatonin in an order blinded to the participant and the study team. The study protocol was approved by the Children's Mercy Kansas City Institutional Review Board. Informed parental consent and participant assent were obtained prior to completion of any study procedures.

Subjects

Patients ages 8-17 seen in the pediatric gastroenterology clinic with a diagnosis of FD defined by Rome Ⅲ criteria and persistent pain despite a minimum of 4 wk of acid suppression were included. Patients were excluded if they received opiates, tramadol, gabapentin or benzodiazepines in the 4 wk prior to enrollment.

Methods

Melatonin and placebo were compounded by the Children's Mercy Hospital Investigational Drug Services (CMH IDS) Pharmacy in liquid form. Melatonin bottles contained 70 mg of melatonin (5 mg/dose × 14 d). Bottles were labeled A or B and d 8-21 or d 22-35 with dosing instructions of "take 20 mL at bedtime per measurement on dropper".

At enrollment, each participant received both treatment bottles and each participant was given a sleep diary with instructions and an actigraph watch with instruction on its usage. On D 1-7, in order to obtain baseline sleep parameters, participants were instructed to wear the actigraph and complete the sleep diary (sleep latency and sleep duration each day). On D 8-21, participants were instructed to wear the actigraph, complete the sleep diary and take the medication labeled "D 8-21". On D 22, a follow up phone call was made and a standardized history was utilized to assign a Global Clinical Score (see Measures). On D 22-35, participants were instructed to wear the actigraph, complete the sleep diary and take the medication labeled "D 22-35". Between day 36-40, participants returned to clinic and turned in the sleep diary and actigraph. A standardized history was utilized to obtain a second Global Clinical Score.

Measures

Clinical response: A Global Clinical Score was determined by interview to measure changes in abdominal pain as follows: (1) Grade 1: Worse - clinical deterioration with increasing pain intensity and/or frequency; (2) Grade 2: No change - no increase or decrease in pain intensity or frequency; (3) Grade 3: Moderate improvement - partial clinical response with definite improvement in pain, but not meeting criteria for Grade 4 response; (4) Grade 4: Good - nearly complete relief of symptoms with minimal residual pain and not interfering with daily activities; and (5) Grade 5: Excellent - complete relief of pain. A positive clinical response was defined as a response grade of 3 or greater, with subjects dichotomized into "responders" and "non-responders" following each 2-wk medication trial.

Sleep parameters: Time in bed and out of bed were taken from the sleep diaries and uploaded to the actigraph data. Sleep latency and duration were calculated from actigraphy data. The ActiGraph brand accelerometry monitor watches and its accompanying software were used for this study. The accelerometer measures movement several times per second. Scoring programs show activity levels for specified time periods and determine when an individual is

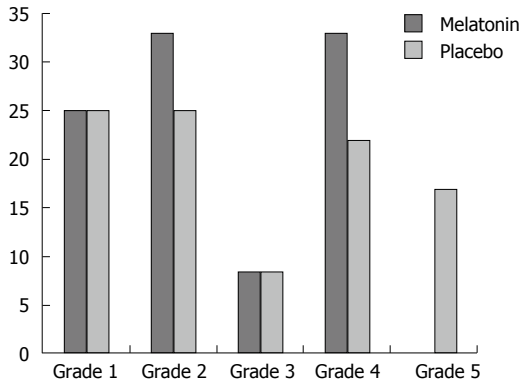

Figure 1 Overall % of patients with each global response grade on melatonin *vs* placebo.

asleep or awake. This methodology has been validated in studies comparing actigraphy to polysomnography (the gold standard for evaluating sleep)[12,13].

Statistical analysis

SPSS version 18 was utilized. The response rate on melatonin was compared to placebo using Fischer's exact test. The mean sleep latency and sleep duration, respectively, on melatonin *vs* placebo were compared using Wilcoxon Signed Rank Test.

RESULTS

Subjects

Fourteen subjects were enrolled and 12 completed the study. One withdrew prior to starting both melatonin and placebo. The other withdrew after completing placebo and before starting melatonin (this participant did not respond to placebo). The two participants who withdrew were not included in analysis. Fifty-eight percent of the participants were female and 83% were Caucasian. Patients ranged in age from 11 to 16 years old with a mean age of 13.8 ± 1.6 years. Pain was the predominant symptom in each patient and associated symptoms included nausea in 67%, bloating in 42%, early satiety in 33%, and vomiting in 25%. All participants met criteria for FD, while three participants also met criteria for IBS.

Clinical response

A positive clinical response (Grade 3-5) was achieved in 42% of subjects on melatonin *vs* 50% of subjects on placebo (NS). Effect size was calculated and revealed a Cohen's D of 0.343 which demonstrates a medium effect favoring placebo. A summary of the post treatment pain relief grades are shown in Figure 1. Individual global clinical scores for melatonin and placebo are shown in Table 1. Of the three patients who also met criteria for IBS, two responded to placebo and not melatonin. The third responded to both.

Table 1 Global clinical scores for participants on melatonin and placebo

Participant	Melatonin	Placebo
1	2[1]	2
2	1	4[1]
3	2[1]	2
4	4[1]	5
5	2[1]	1
6	1[1]	5
7	4	1[1]
8	3	4
9	2	1[1]
10	4	3[1]
11	4	2[1]
12	1[1]	4

[1]Indicates initial treatment.

Sleep

Baseline sleep parameters were in the healthy range with the longest sleep latency being just over 20 min (mean 7.46 ± 8.53 min) and the shortest sleep duration just over 7 h (mean 10.09 ± 2.72 h). The mean latency did not differ between periods of treatment with melatonin as compared to placebo (4.48 ± 6.45 min *vs* 3.58 ± 4.24 min; NS). The mean sleep duration did not differ between periods of treatment with melatonin as compared to placebo (9.90 ± 3.53 h *vs* 9.41 ± 2.70 h; NS).

Just one participant had improved sleep (50% reduction in sleep latency and 20% increase in total sleep time), and this was while taking melatonin. However, this participant was classified as a non-responder, with a clinical grade of 2 following the melatonin treatment phase. Therefore, improved sleep could not be correlated to improved pain in this case.

DISCUSSION

In contrast to the previous study of FD in adults, melatonin had no significant impact on pain in children with FD in the current study[8]. In fact, the study was discontinued following interim analyses which indicated no beneficial response of melatonin and, in fact, appeared to favor placebo. The 50% placebo response rate in the current study was similar to what has been reported in other pediatric abdominal pain trials where placebo response has generally been 32%-58%[14]. These discordant results may be a function of different pathophysiologic processes being more or less important at different ages or may be a result of differences in study design which target different pathophysiologic factors.

One potential differential factor relates to FD subtyping across the lifespan. In adults, there are two recognized FD subtypes, epigastric pain syndrome (EPS) and postprandial distress syndrome (PDS)[2]. The pediatric FD criteria do not contain these subtypes.

EPS is defined by pain or burning confined to the epigastrium whereas PDS is defined by the presence of early satiety and/or postprandial bloating[2]. The participants in the one adult FD study previously noted consisted of patients with symptoms of EPS. EPS is rare in pediatric FD and, in fact, none of the subjects in our study had pain localized only to the epigastrium[15]. Most of the participants in the current study would have met adult criteria for PDS. This may be important in that it has been suggested that adults with EPS may have altered melatonin production[16]. Thus, differences in response between the two studies may indicate different mechanisms of symptom generation for EPS as compared to PDS.

A second factor that may have led to different results is duration of treatment. As noted previously, we chose the treatment duration for the current study based on initial findings in adults with IBS and FD that suggested a treatment response of melatonin could be detected in as little as 2-4 wk[8,9]. In the previous adult FD study, although a partial response arguably was seen within the first month of treatment, significant differences in pain resolution did not emerge between the melatonin and placebo groups until the second month of treatment[8]. This overall delay in treatment response suggests that the observed benefits of melatonin treatment may have been due to improvement in some factor other than visceral hypersensitivity, possibly mediated through improvement in sleep over time. In short, treatment duration in our study may simply not have been long enough to test an indirect effect of melatonin on pain through improvement in sleep.

Third, and related to the above, is the relative composition of samples. The adult study contained patients with sleep disturbances and these disturbances frequently improved or resolved with melatonin. However, the current study utilized an unselected group of children and adolescents presenting for evaluation of FD who all ultimately demonstrated sleep parameters in the normal range at baseline and which did not change significantly on melatonin. The composition of our sample is both a strength and limitation for our study. On the one hand, there was limited ability to test the impact of melatonin on pain *via* a sleep improvement pathway. On the other hand, having a group of participants without discernible sleep problems at baseline allowed us to more clearly assess the effects of melatonin independent from sleep, *i.e.*, *via* a visceral hypersensitivity pathway.

In conclusion, the short-term (*i.e.*, 2 wk) use of melatonin does not appear to have efficacy in relieving pain in an unselected pediatric FD population. The strengths of our study are the cross-over design and that we were able to study melatonin in a group of patients without sleep disturbances so we were able to assess efficacy independent from effects on sleep. In contrast, we studied a relatively small population and did not address whether melatonin might be effective over a longer duration of treatment and/or in patients with comorbid sleep issues. Future studies involving melatonin may take into consideration FD subtypes and whether melatonin has effects on specific potential pathophysiologic factors including the presence of inflammation, electromechanical dysfunction, or the presence of baseline sleep disturbances.

COMMENTS

Background

Pediatric functional dyspepsia (FD) is defined as persistent or recurrent pain or discomfort centered in the upper abdomen (above the umbilicus) that is unrelated to a change in stool frequency or form and not exclusively relieved by defecation.

Research frontiers

Melatonin and placebo were compounded by the Children's Mercy Hospital Investigational Drug Services Pharmacy in liquid form.

Innovations and breakthroughs

The mean sleep latency and sleep duration, respectively, on melatonin *vs* placebo were compared using Wilcoxon Signed Rank Test.

Applications

Subjects wore an actigraph to assess sleep during a one week baseline period and during each treatment period. Subjects' sleep latency and total sleep time were recorded throughout the duration of the study.

Terminology

The study was conducted as a double blind, randomized, placebo controlled crossover trial.

Peer-review

This is an original study looking for a new therapeutic indication for melatonin in children with FD.

REFERENCES

1 **Rasquin A**, Di Lorenzo C, Forbes D, Guiraldes E, Hyams JS, Staiano A, Walker LS. Childhood functional gastrointestinal disorders: child/adolescent. *Gastroenterology* 2006; **130**: 1527-1537 [PMID: 16678566]

2 **Tack J**, Talley NJ, Camilleri M, Holtmann G, Hu P, Malagelada JR, Stanghellini V. Functional gastroduodenal disorders. *Gastroenterology* 2006; **130**: 1466-1479 [PMID: 16678560]

3 **Friesen CA**, Schurman JV, Colombo JM, Abdel-Rahman SM. Eosinophils and mast cells as therapeutic targets in pediatric functional dyspepsia. *World J Gastrointest Pharmacol Ther* 2013; **4**: 86-96 [PMID: 24199024 DOI: 10.4292/wjgpt.v4.i4.86]

4 **Rosen JM**, Cocjin JT, Schurman JV, Colombo JM, Friesen CA. Visceral hypersensitivity and electromechanical dysfunction as therapeutic targets in pediatric functional dyspepsia. *World J Gastrointest Pharmacol Ther* 2014; **5**: 122-138 [PMID: 25133041 DOI: 10.4292/wjgpt.v5.i3.122]

5 **van Geijlswijk IM**, Korzilius HP, Smits MG. The use of exogenous melatonin in delayed sleep phase disorder: a meta-analysis. *Sleep* 2010; **33**: 1605-1614 [PMID: 21120122]

6 **Schurman JV**, Friesen CA, Dai H, Danda CE, Hyman PE, Cocjin JT. Sleep problems and functional disability in children with functional gastrointestinal disorders: an examination of the potential mediating effects of physical and emotional symptoms. *BMC Gastroenterol* 2012; **12**: 142 [PMID: 23067390 DOI: 10.1186/1471-230x-12-142]

7 **Maldonado MD**, Mora-Santos M, Naji L, Carrascosa-Salmoral

MP, Naranjo MC, Calvo JR. Evidence of melatonin synthesis and release by mast cells. Possible modulatory role on inflammation. *Pharmacol Res* 2010; **62**: 282-287 [PMID: 19963060 DOI: 10.1016/j.phrs.2009.11.014]

8 **Klupińska G**, Poplawski T, Drzewoski J, Harasiuk A, Reiter RJ, Blasiak J, Chojnacki J. Therapeutic effect of melatonin in patients with functional dyspepsia. *J Clin Gastroenterol* 2007; **41**: 270-274 [PMID: 17426465]

9 **Song GH**, Leng PH, Gwee KA, Moochhala SM, Ho KY. Melatonin improves abdominal pain in irritable bowel syndrome patients who have sleep disturbances: a randomised, double blind, placebo controlled study. *Gut* 2005; **54**: 1402-1407 [PMID: 15914575]

10 **Mozaffari S**, Rahimi R, Abdollahi M. Implications of melatonin therapy in irritable bowel syndrome: a systematic review. *Curr Pharm Des* 2010; **16**: 3646-3655 [PMID: 21128901]

11 **Cushing CC**, Walters RW, Hoffman L. Aggregated N-of-1 randomized controlled trials: modern data analytics applied to a clinically valid method of intervention effectiveness. *J Pediatr Psychol* 2014; **39**: 138-150 [PMID: 24284134 DOI: 10.1093/jpepsy/jst083]

12 **Cellini N**, Buman MP, McDevitt EA, Ricker AA, Mednick SC. Direct comparison of two actigraphy devices with polysomnographically recorded naps in healthy young adults. *Chronobiol Int* 2013; **30**: 691-698 [PMID: 23721120 DOI: 10.3109/07420528.2013.782312]

13 **Sadeh A**, Sharkey KM, Carskadon MA. Activity-based sleep-wake identification: an empirical test of methodological issues. *Sleep* 1994; **17**: 201-207 [PMID: 7939118]

14 **Friesen CA**, Schurman JV, Abdel-Rahman SM. Present state and future challenges in pediatric abdominal pain therapeutics research: Looking beyond the forest. *World J Gastrointest Pharmacol Ther* 2015; **6**: 96-104 [PMID: 26558142]

15 **Schurman JV**, Singh M, Singh V, Neilan N, Friesen CA. Symptoms and subtypes in pediatric functional dyspepsia: relation to mucosal inflammation and psychological functioning. *J Pediatr Gastroenterol Nutr* 2010; **51**: 298-303 [PMID: 20479684 DOI: 10.1097/MPG.0b013e3181d1363c]

16 **Chojnacki C**, Poplawski T, Blasiak J, Chojnacki J, Klupinska G. Does melatonin homeostasis play a role in continuous epigastric pain syndrome? *Int J Mol Sci* 2013; **14**: 12550-12562 [PMID: 23771022 DOI: 10.3390/ijms140612550]

P- Reviewer: Chiarioni G, Dumitrascu DL, Rodella LF
S- Editor: Qiu S **L- Editor**: A **E- Editor**: Wang CH

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i1.162

World J Gastrointest Pharmacol Ther 2016 February 6; 7(1): 162-170
ISSN 2150-5349 (online)

SYSTEMATIC REVIEWS

Economic evaluations in gastroenterology in Brazil: A systematic review

Luciana Bertocco de Paiva Haddad, Tassia Cristina Decimoni, Jose Antonio Turri, Roseli Leandro, Patrícia Coelho de Soárez

Luciana Bertocco de Paiva Haddad, Jose Antonio Turri, Department of Transplantation, Gastroenterology Division, University of São Paulo School of Medicine Hospital das Clínicas, São Paulo 05403-010, Brazil

Tassia Cristina Decimoni, Roseli Leandro, Patrícia Coelho de Soárez, Department of Preventive Medicine, University of São Paulo School of Medicine, São Paulo 01246-903, Brazil

Author contributions: Decimoni TC and de Soárez PC designed the research; Decimoni TC, Leandro R and de Soárez PC performed the research; de Paiva Haddad LB, Decimoni TC, Turri JA, Leandro R and de Soárez PC analyzed the data; de Paiva Haddad LB and de Soárez PC wrote the paper.

Supported by The Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, São Paulo Research Foundation), as part of a larger project entitled "Systematic review of health economic evaluations conducted in Brazil, 1980-2013", carried out under the leadership of Professor Patricia Coelho de Soárez, No. 2012/22126-3.

Conflict-of-interest statement: None of the authors has received fees for serving as a speaker, consultant or advisory board member for any organization that might have a stake in the results of this study; nor do any of the authors owns stocks in or shares of any such organization. None of the authors owns any patents related to the materials, devices or procedures mentioned in the manuscript.

Data sharing statement: No additional data are available.

Correspondence to: Patrícia Coelho de Soárez, PhD, Department of Preventive Medicine, University of São Paulo School of Medicine, Av. Dr. Arnaldo 455, 2 Andar Sala 2228, São Paulo 01246-903, Brazil. patricia.soarez@usp.br
Telephone: +55-11-30617290
Fax: +55-11-30618466

Received: April 14, 2015
Peer-review started: April 16, 2015
First decision: July 1, 2015
Revised: November 13, 2015
Accepted: December 1, 2015
Article in press: December 2, 2015
Published online: February 6, 2016

Abstract

AIM: To systematically review economic evaluations in gastroenterology, relating to Brazil, published between 1980 and 2013.

METHODS: We selected full and partial economic evaluations from among those retrieved by searching the following databases: MEDLINE (PubMed); *Excerpta Medica*; the Latin American and Caribbean Health Sciences Literature database; the Scientific Electronic Library Online; the database of the Centre for Reviews and Dissemination; the National Health Service (NHS) Economic Evaluation Database; the NHS Health Technology Assessment database; the Health Economics database of the Brazilian Virtual Library of Health; Scopus; Web of Science; and the Brazilian Network for the Evaluation of Health Technologies. Two researchers, working independently, selected the studies and extracted the data.

RESULTS: We identified 535 health economic evaluations relating to Brazil and published in the 1980-2013 period. Of those 535 articles, only 40 dealt with gastroenterology. Full and partial economic evaluations respectively accounted for 23 (57.5%) and

17 (42.5%) of the 40 studies included. Among the 23 full economic evaluations, there were 11 cost-utility analyses, seven cost-effectiveness analyses, four cost-consequence analyses, and one cost-minimization analysis. Of the 40 studies, 25 (62.5%) evaluated medications; 7 (17.5%) evaluated procedures; and 3 (7.5%) evaluated equipment. Most (55%) of the studies were related to viral hepatitis, and most (63.4%) were published after 2010. Other topics included gastrointestinal cancer, liver transplantation, digestive diseases and hernias. Over the 33-year period examined, the number of such economic evaluations relating to Brazil, especially of those evaluating medications for the treatment of hepatitis, increased considerably.

CONCLUSION: Further studies are needed in order to ensure that expenditures on health care in Brazil are made as fairly and efficiently as possible.

Key words: Costs and cost analysis; Health care costs; Cost-benefit analysis; Gastroenterology; Brazil

Core tip: The volume and scope of economic evaluations relating to Brazil remain unknown. To improve understanding of what studies are available as inputs for resource-allocation decisions, as well as of how that body of knowledge can be expanded, we conducted a systematic review of such economic evaluations. Although there have been many economic evaluations related to gastroenterology in Brazil, most have analyzed medications for the treatment of viral hepatitis. In most cases, decisions to incorporate new technologies into the public health care system were made before such studies were conducted and were therefore not based on local cost-effectiveness analyses.

de Paiva Haddad LB, Decimoni TC, Turri JA, Leandro R, de Soárez PC. Economic evaluations in gastroenterology in Brazil: A systematic review. *World J Gastrointest Pharmacol Ther* 2016; 7(1): 162-170 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i1/162.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i1.162

INTRODUCTION

In addition to safety and efficacy, economic considerations have increasingly been taken into account in decisions regarding the use of health care technologies. Health economic evaluations typically draw comparisons between and among alternative treatments, devices and health programs, in terms of their costs and consequences. Such studies analyze data regarding clinical effectiveness, in relation to cost. Although most health economic evaluations examine only the direct costs of a technology-those related to medications, services and hospitalization-some take a broader approach, also evaluating the indirect costs-those related to lost productivity (on the part of the patients and caregivers), as well as to other aspects[1].

In a number of countries, the decision-making process regarding the reimbursement for and incorporation of new technologies have been informed by health economic evaluations. In Brazil, the performance of such evaluations has been an obligatory part of the process of incorporating new technologies into the Brazilian *Sistema Único de Saúde* (SUS, Unified Health Care System), as mandated by Federal Law no. 12401, since 2011. However, health economic evaluations have been conducted in Brazil since the 1980s. One recent study demonstrated that, as of 2009, Brazil had produced more health economic evaluations than had any other country in South America[2].

In the field of gastroenterology, economic evaluations are performed in order to evaluate or compare new alternative medications, surgical techniques, diagnostic tests or procedures. Now-classic studies include that in which omeprazole was shown to be more cost-effective than ranitidine in the treatment of gastroesophageal reflux[3], as well as those demonstrating that it was more cost-effective to eradicate *Helicobacter pylori* (*H. pylori*) than to provide symptomatic treatment to *H. pylori*-positive patients with suspected peptic ulcer disease[4,5].

Knowledge of economic evaluations related to gastroenterology can help health care professionals make better choices from among the available technologies and can help researchers identify segments in which there is a need for further studies. It is incumbent upon researchers to identify technologies that have already been assessed and to understand the relationship between the emergence of new technologies (clinical or surgical) and the need for specific studies. Such knowledge also provides health care managers with additional input that can be used in the decision-making processes related to the incorporation of new technologies into a health care system.

The volume and scope of economic evaluations relating to gastroenterology in Brazil remain unknown. To gain a better understanding of what kind of research is available as input for resource-allocation decisions, as well as of how that body of knowledge can be expanded, we conducted a systematic review of such economic evaluations.

MATERIALS AND METHODS

We conducted this review, which is specific to gastroenterology and to Brazil, in accordance with the guidelines for systematic review of economic evaluations published by the UK National Health Service (NHS) Centre for Reviews and Dissemination[6]. The methodology of the systematic review and the search strategy have been detailed elsewhere[7].

In brief, we searched multiple databases, including MEDLINE (PubMed); *Excerpta Medica*; the Latin American and Caribbean Health Sciences Literature database; the Scientific Electronic Library Online; the database of the Centre for Reviews and Dissemination; the NHS Economic Evaluation Database; the NHS Health Technology Assessment database; and Health Economics database of the Brazilian Virtual Library of Health. The last three sources are repositories of economic evaluations. We searched the citation indexes: Scopus; Web of Science; and the Brazilian Network for the Evaluation of Health Technologies, a bibliographic database of Brazilian health technology assessment studies. We also performed hand searches of the Brazilian Journal of Health Economics, which is not indexed for any of the previously mentioned databases. All searches were limited to the 1980-2013 period.

Articles were included if they were partial or full economic evaluations, according to the classification devised by Drummond *et al*[8], if they dealt with gastroenterology, were conducted in or related to Brazil, and if at least one of the authors was affiliated with an institution in Brazil. We defined partial economic evaluations as those that examined only costs (cost description studies), described the costs of a particular disease to society (cost-of-illness studies), described the costs and consequences of a single service or program (cost-outcome description studies), or compared two or more interventions only in terms of their costs (cost analyses). Studies were considered full economic evaluations if they compared the costs and consequences of two or more health care interventions or alternatives, designs including cost-consequences analysis, cost-minimization analysis, cost-effectiveness analysis, cost-utility analysis and cost-benefit analysis.

The titles and abstracts of identified citations were screened for relevance by two independent reviewers. The full texts of "relevant" and "potentially relevant" articles were retrieved and evaluated independently by both reviewers. From each of the selected studies, two reviewers independently extracted data on the year and journal of publication; type of economic evaluation; the category of technology assessed (medications, vaccines, equipment, clinical practices, surgical techniques, diagnostic procedures, public health programs or health promotion programs); the purpose of the technology assessed (treatment, prevention, screening or diagnosis); the category of health problem studied (according to the tenth revision of the International Classification of Diseases); the type of affiliation of the first author (academia, government, research institute, health organization, consultancy, pharmaceutical industry, equipment industry or international body); the geographical location of the first author; and conflicts of interest, as defined by Valachis *et al*[9]. Disagreements regarding the extracted data were resolved by consensus or through consultation with a third reviewer. We summarized the characteristics of the selected articles and evaluated them using narrative synthesis. The statistical review of the study was performed by a biomedical statistician.

RESULTS

Characteristics of the studies

We identified 535 health economic evaluations relating to Brazil and published in the 1980-2013 period (Figure 1). Of those 535 articles, only 40 dealt with gastroenterology, the first of those having been published in 1982. Partial and full economic evaluations respectively accounted for 17 (42.5%) and 23 (57.5%) of the articles selected. Of the 23 full evaluations, 11 were cost-utility analyses, seven were cost-effectiveness analyses, four were cost-consequences analyses and one was a cost-minimization analysis. Of the 17 partial evaluations, nine were cost analyses, seven were cost-description studies, and one was a cost-outcome description study (Figure 2). Over the period under study, there was an increase in the frequency of such economic evaluations (Figure 3), five articles being published in the 1980s, only one being published in the 1990s, 14 being published in the 2000s and 19 being published after 2010.

The majority (90%) of the selected studies evaluated technologies that are employed in the treatment of diseases of the gastrointestinal tract. There were two studies evaluating diagnostic technologies and one evaluating a technology for the prevention of viral hepatitis. Twenty-five studies evaluated medications; seven evaluated clinical or surgical procedures; three evaluated equipment; and one evaluated vaccines. There were four studies that evaluated more than one technology simultaneously.

The first authors of the selected articles had the following types of affiliations: Academia, in 33 studies; health care facilities, in four; consultancy, in two; and industry, in one. There were only four articles in which the authors declared conflicts of interest, all related to funding provided by industry or consultancy sources. However, when we evaluated the articles using the criteria proposed by Valachis *et al*[9], we identified conflicts of interest in four other studies. In three of those four articles, the authors had declared no conflicts, whereas the remaining article contained no conflict of interest statement. According to the published statements of financial support, six of the 40 studies received financial support from industry sources, seven received financial support from funding agencies, and two received no funding from external sources. The remaining 25 articles contained no information regarding financial support.

Of the 40 institutions at which the studies were conducted, 30 were located in Southeastern Brazil, nine were located in Southern Brazil and one was located in Northeastern Brazil. The majority (67.5%)

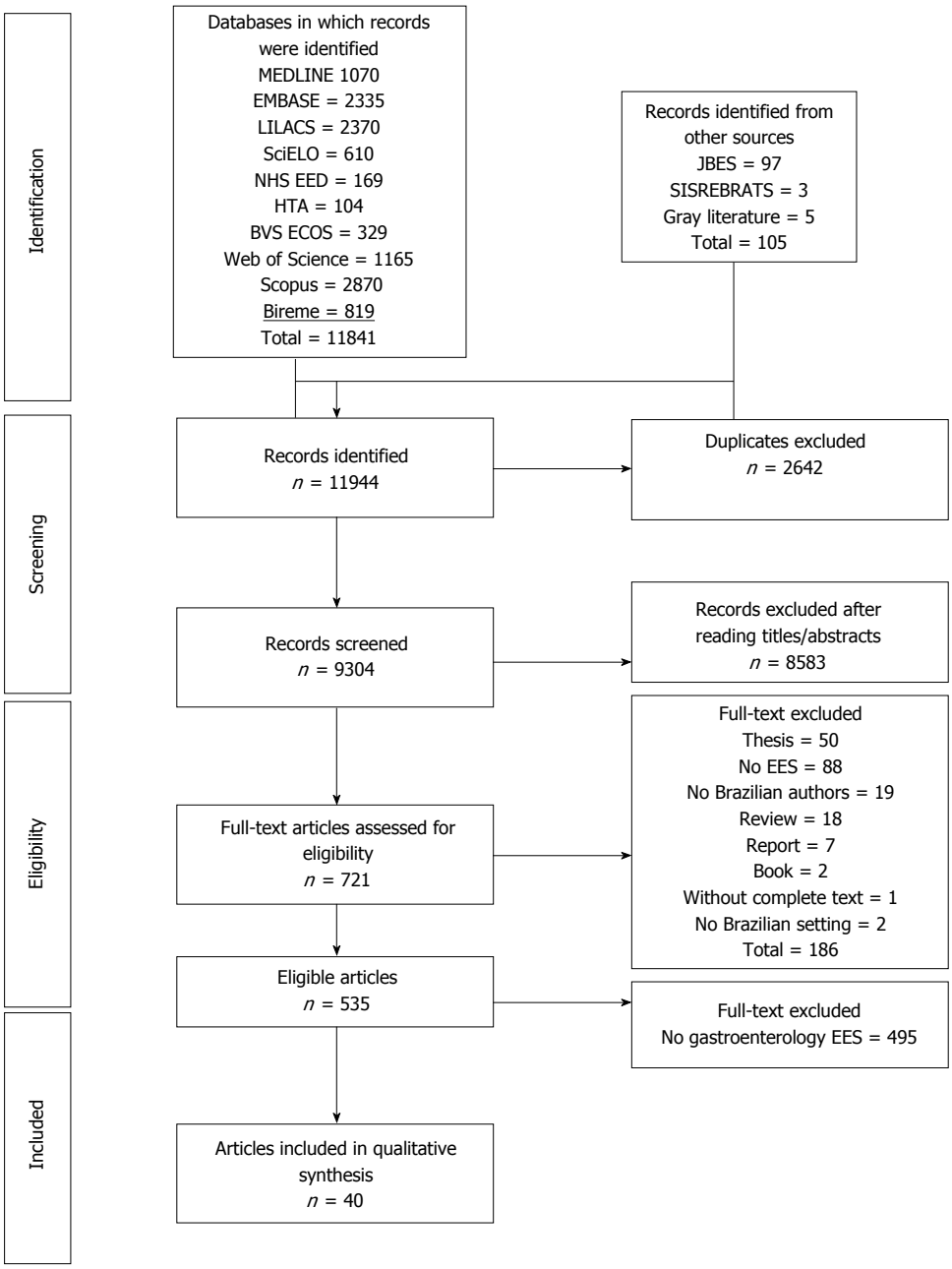

Figure 1 Flow diagram of the process for the selection of economic evaluations in gastroenterology related to Brazil, 1980-2013. EMBASE: *Excerpta Medica*; LILACS: Latin American and Caribbean Health Sciences Literature; SciELO: Scientific Electronic Library Online; NHS EED: (United Kingdom) National Health Service Economic Evaluation Database; HTA: Health Technology Assessment; BVS ECOS: *Biblioteca Virtual em Saúde Economia da Saúde* [Health Economics (database) of the (Brazilian) Virtual Library of Health]; Bireme: *Biblioteca Regional de Medicina* (Regional Library of Medicine); JBES: *Jornal Brasileiro de Economia da Saúde* (Brazilian Journal of Health Economics); SISREBRATS: *Sistema de Informação da Rede Brasileira de Avaliação de Tecnologias em Saúde* (Brazilian Network for the Evaluation of Health Technologies); EES: Economic evaluation study.

of the selected studies were published in national journals. For the period evaluated, as a whole, the mean impact factor of the journals in which the studies were published was 1.57 ± 0.66. For the 2000-2005 period, the mean impact factor was 2:09, compared with 1.38 for the 2006-2010 period and 1.53 for the 2011-2013 period (Figure 4).

Subtypes of economic evaluations in gastroenterology

We analyzed the type of disease addressed in the economic evaluations under study. On that basis, we divided the studies into three main groups, by topic (Figure 3): Gastrointestinal cancer; viral hepatitis; and other.

Gastrointestinal cancer

We identified five economic evaluations related to gastrointestinal cancer: Four related to colorectal cancer (three dealing with the metastatic form); and one related to esophageal cancer. All five studies evaluated treatments, and three were full economic evaluations. Funding from the pharmaceutical industry

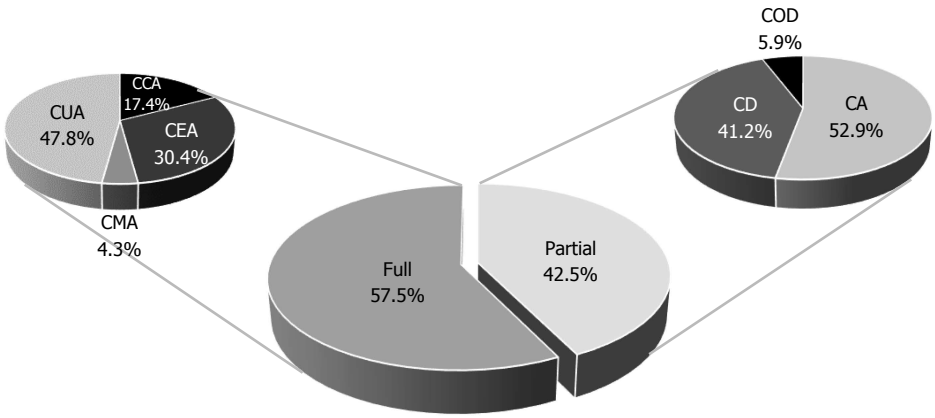

Figure 2 Number of economic evaluations in gastroenterology related to Brazil, by type, 1982-2013. CUA: Cost-utility analysis; CCA: Cost-consequence analysis; CEA: Cost-effectiveness analysis; CMA: Cost-minimization analysis; CD: Cost description; CA: Cost analysis; COD: Cost-outcome description.

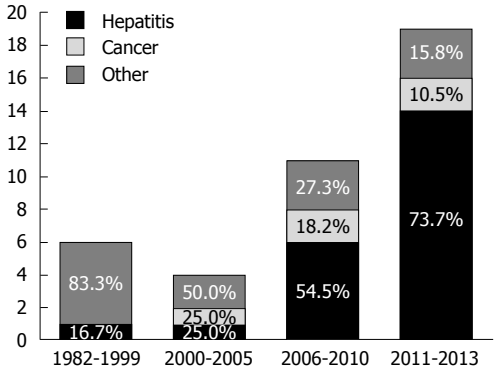

Figure 3 Number and proportional distribution of economic evaluations in gastroenterology related to Brazil, by publication date and theme, 1982-2013.

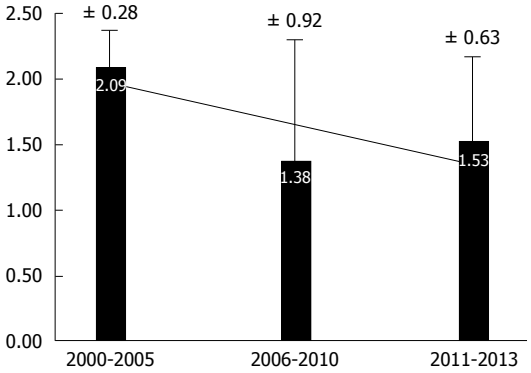

Figure 4 Means and standard deviations for Journal Citation Report impact factors among economic evaluations in gastroenterology related to Brazil, by year of publication, 2000-2013.

was reported in only one of the five studies.

The first of the five studies was published in 2001 and evaluated palliative treatment for advanced esophageal cancer, comparing the costs of using a self-expanding metallic stent with those of esophageal bypass[10]. The authors concluded that endoscopic treatment of dysphagia was as effective as was surgical bypass and could be performed at a lower cost.

A cost-description study, published in 2010, evaluated the costs of hospitalizations related to colorectal cancer in Brazil, drawing upon data obtained from the Information Technology Department of the Brazilian Unified Health Care System[11] The study showed that such costs increased from US$16.5 million in 1996 to US$33.5 million in 2008.

The first of the three economic evaluations of metastatic colorectal cancer in Brazil, published in 2008, was a partial evaluation, a cost analysis comparing the various first-line chemotherapy treatments. The authors of that study concluded that chemotherapy regimens containing capecitabine, especially capecitabine plus oxaliplatin, are less expensive than are those containing 5-fluorouracil and leucovorin[12]. The next of those studies, published in 2012, also compared first-line chemotherapy treatments for metastatic colorectal cancer[13]. That study was a cost-effectiveness analysis comparing 5-fluorouracil plus leucovorin, followed by irinotecan, *vs* oxaliplatin, 5-fluorouracil and leucovorin, followed by irinotecan, 5-fluorouracil and leucovorin. The two strategies provided estimated gains of 0.17 and 0.91 life-years (LYs), respectively, in comparison with support, the incremental cost-effectiveness ratios (ICERs), in Brazilian reals (R$), being R$50504 and R$73626, respectively, per LY gained. When comparing the new and previous strategies, the authors found the gain to be 0.74 LYs, with an ICER of R$78188 per LY gained[13].

The last of the studies dealing with the treatment of metastatic colorectal cancer was a cost-utility analysis comparing two chemotherapy regimens, modified 5-fluorouracil, leucovorin and oxaliplatin (mFLOX) and modified 5-fluorouracil, folinic acid and oxaliplatin (mFOLFOX6). Over a 20-wk period of treatment, the cost of the mFLOX regimen was R$9000, compared with R$22000 for the mFOLFOX6 regimen. The effective gain for the mFOLFOX6 regimen was 0.117 QALYs, with an ICER of R$110344 per QALY gained[14].

Viral hepatitis

Of the 40 studies evaluated, 19 dealt with technologies related to the treatment of viral hepatitis. Those studies were published more recently phase, the first in 2007 and the majority (14 of the 19) after 2010. Of those 19 studies, 14 evaluated medications for the treatment of hepatitis, one evaluated a vaccine, one evaluated a piece of diagnostic equipment and three evaluated multiple technologies (combinations of procedures, medications and diagnostic equipment). Most of the studies (15 of the 19) were full economic evaluations. Eleven of the articles dealt with hepatitis C, and eight dealt with hepatitis B.

The study published in 2007 was a cost-of-illness study showing that, if hepatitis B was left untreated, the related health care expenditures rose in parallel with advances in the stage of the disease[15]. Of the studies evaluated, the first to evaluate the treatment of hepatitis B was a cost-utility analysis, published in 2008, comparing lamivudine and entecavir[16]. The authors concluded that entecavir reduced the incremental cost per QALY gained, making it the more cost-effective option for the treatment of hepatitis B. A cost-effectiveness analysis published in 2011 compared interferon and lamivudine[17], the result being that interferon proved to be the more cost-effective therapeutic option for the treatment of hepatitis B. Another economic evaluation of hepatitis B treatments compared the cost-effectiveness of telbivudine with that of lamivudine[18], showing that the latter was more cost-effective. Studies evaluating the use of nucleoside analogues as treatments for hepatitis B in Brazil began to be published in 2012[19], evaluating the cost-effectiveness of entecavir and tenofovir. Only one of those studies was a cost-utility analysis, the findings of which were favorable to tenofovir[20]. However, a cost-effectiveness analysis, published in the same year, showed that lamivudine was the more cost-effective option[21].

Of the 11 economic evaluations related to hepatitis C in Brazil, the first, published in 2008, analyzed the cost-effectiveness of diagnostic methods[22]. The first such article dealing with treatment, published the following year, evaluated the cost-effectiveness of treatment with pegylated interferon (peginterferon) alpha-2b in comparison with that of ribavirin[23]. There were a number of subsequent economic evaluations related to the treatment of hepatitis C in Brazil, all of which were based on the use of peginterferon[24-29]. In all of those studies, treatment with peginterferon proved cost-effective. A budget impact analysis, published in 2011, evaluated the treatment of hepatitis C in patients who are candidates for kidney transplantation[25]. In 2012, Blatt *et al*[27] published a study evaluating the use of ribavirin in combination with interferon alpha, peginterferon alpha-2a or peginterferon alpha-2b, comparing the three combinations in terms of the microeconomics of their use in the treatment of hepatitis C[27]. In addition, there were two studies that analyzed the cost-effectiveness of including hepatitis C patients who are slow responders to treatment with peginterferon, both studies showing that to be a cost-effective strategy for the public health care system in Brazil[28,29].

Other topics

The first economic evaluations related to gastroenterology in Brazil, published in the 1980s, dealt with therapeutic approaches to epigastric hernia[30] and peptic ulcers[31,32]. More recently, there were three economic evaluations that dealt with liver transplantation[33-35]. Of those three studies, two were cost-description studies based on retrospective analyses of medical records; and one was a cost analysis comparing the costs of living donor liver transplantation with those of deceased donor liver transplantation[34]. The remaining studies were related to a variety of topics: Inflammatory bowel disease[36,37]; colonic diseases[38,39]; the equipment used in patients undergoing colostomy or ileostomy[40]; appendicitis[41]; and peritonitis[42]. There was only one study comparing the costs of surgical procedures-laparoscopic *vs* conventional appendectomy[41]. As would be expected, the authors found that the minimally invasive (laparoscopic) procedure had a higher cost. One study, comparing the costs of nutritional therapy provided in the hospital with those of that provided in the home environment[43], showed that the home therapy model allowed a savings of US$3132 per patient.

DISCUSSION

Overview

In various countries, economic evaluation has become an integral part of the decision-making processes related to the incorporation of and reimbursement for the use of new technologies, primarily new medications. In gastroenterology, the medications with the highest costs are chemotherapy agents used for the treatment of gastrointestinal cancer, antiviral agents used for the treatment of hepatitis and immunosuppressants used in solid organ transplant recipients. Therefore, it is expected that these technologies would give rise to further studies seeking evidence to support the incorporation of these high-cost medications into the public health care system and to justify their use in the private system.

Hepatitis

In Brazil, the reported prevalence of hepatitis C in the general population is 0.28%-1.42%[44], and 75%-85% of all cases progress to the chronic form. The successful treatment of hepatitis C (achieving a sustained virological response) is associated with a better prognosis, reducing the rate of evolution to end-stage liver disease and consequently the need for liver transplantation[29]. Initially, the treatments available for hepatitis C were based on the use of conventional

interferon in combination with ribavirin. With the introduction of peginterferon and the advent of studies demonstrating its greater efficacy[27]. Because it is an expensive medication, petitions were filed with the judicial system in Brazil, seeking public funding for its use. In 2006, peginterferon alpha-2 was the fifth most requested medication through such petitions[45]. Soon thereafter, the use of peginterferon alpha-2 was incorporated into the standard practices of the SUS. Although economic evaluations of treatment with peginterferon alpha-2 in Brazil were published only after its incorporation into the standard practices of the public health care system, those studies have shown that it is cost-effective at the national level.

Treatment of hepatitis C with the protease inhibitors telaprevir and boceprevir has now also been incorporated into the standard practices of the SUS. Triple therapy-the association of one of those protease inhibitors with peginterferon and ribavirin-is becoming the standard treatment for viral hepatitis, further increasing the cost of treatment. To our knowledge, there have as yet been no analyses of the cost-effectiveness of this new treatment option in Brazil.

Cancer

In the field of oncology in Brazil, we observed a predominance of publications related to chemotherapy in patients with metastatic colorectal cancer, as was expected, given the prevalence of the disease. Colorectal cancer is the second most prevalent type of cancer worldwide. In Brazil, approximately 30000 new cases are diagnosed each year, and 24% of those cases are already metastatic at diagnosis[46]. The use of the various chemotherapy regimens, all aimed at increasing survival in such patients, often results in a significant increase in costs. Oncology studies have attempted to determine the most cost-effective options for the treatment of metastatic colorectal cancer to be incorporated into the standard practices of the SUS.

In Brazil, chemotherapy is provided at hospitals and accredited oncology clinics, which provide the medications to the patients and are subsequently reimbursed by the SUS. Beginning in 2010, the reimbursement values for the treatment of metastatic colorectal cancer were revised and new therapeutic regimens were incorporated[13]. It then became possible to prescribe regimens such as FOLFOX and the combination of folinic acid, 5-fluorouracil and irinotecan performed were after that date, comparing the new arrangements with existing ones. It is unlikely that the decision to incorporate the new treatments was based on local cost-effectiveness analyses, given that there were no economic evaluations of such treatments published before 2010.

Other topics

The studies on the costs of liver transplantation in Brazil identified here were partial economic evaluations. Transplantation is the only therapeutic option for the treatment of end-stage liver disease. It is highly complex procedure, associated with high hospital costs, implying high costs to the public health care system. The economic evaluations performed were the basis for setting the reimbursement values for this procedure.

The remaining studies were heterogeneous in their focus and reflect the evolution of technologies, from the initial studies for the treatment of peptic ulcer to more recent studies evaluating minimally invasive therapies.

Although there have been a significant number of economic evaluations related to gastroenterology in Brazil, most have focused on the analysis of medications for the treatment of viral hepatitis. In addition, a temporal evaluation of the implementation of the studies showed that these were published after the incorporation of those technologies into the public health care system, which should, ideally, be based on prior economic evaluation.

In the field of gastroenterology, there are currently a number of new technologies that merit evaluation in order to shape the direction of future investments. Minimally invasive surgery is a rapidly expanding area and involves the use of expensive, increasingly specialized, equipment, including the use of robotics. With increasing frequency, such equipment is employed in simple procedures such as cholecystectomy and hernia surgery, although its use is advancing at a more rapid pace in procedures that are more complex and costly, such as cancer and liver surgery.

Another area in which there is a lack of economic evaluations is that of endoscopy. Endoscopic procedures are no longer used solely for diagnosis, having a variety of therapeutic applications, such as the resection of polyps and the palliative treatment of cancer. To our knowledge, there have been no economic evaluations is the field of endoscopy in Brazil.

Therefore, it is evident that, although there have been a number of economic evaluations related to gastroenterology in Brazil, there should be a push for further studies of this kind, so that expenditures on health care in Brazil are made as fairly and efficiently as possible.

COMMENTS

Background

Economic evaluations in gastroenterology evaluate or compare medications, surgical techniques, diagnostic tests or procedures. The volume and scope of economic evaluations relating to gastroenterology in Brazil remain unknown.

Research frontiers

New technologies in the field of gastroenterology, such as minimally invasive surgery (including the use of robotics), merit detailed evaluation in terms of their cost-effectiveness in simple procedures, such as cholecystectomy and hernia surgery, as well as in those that are more complex and costly, such as surgical procedures used in the treatment of cancer and liver disease.

Innovations and breakthroughs

The authors identified certain trends among economic evaluations related to gastroenterology in Brazil, most of which have focused on the analysis of medications for the treatment of viral hepatitis. In addition, a temporal evaluation of the implementation of the studies showed that many were published after the incorporation of those technologies into the public health care system, which should, ideally, be based on prior economic evaluation. Furthermore, studies on the costs of liver transplantation in Brazil appear to have been predominantly partial, rather than full, economic evaluations. Moreover, although triple therapy-the combination of a protease inhibitor with peginterferon and ribavirin-is becoming the standard treatment for viral hepatitis in Brazil, there have as yet been no analyses of the cost-effectiveness of this new treatment option in the country.

Applications

The data should prompt researchers in Brazil to conduct additional economic evaluations related to gastroenterology, attempting to bridge the knowledge gaps that exist at the present time.

Terminology

The authors considered two broad categories of economic evaluations, partial and full. Partial economic evaluations were designated "cost description studies" (those that examined only costs), "cost-of-illness studies" (those that described the costs of a particular disease to society), "cost-outcome description studies" (those that described the costs and consequences of a single service or program) or "cost analyses" (those that compared two or more interventions only in terms of their costs). Full economic evaluations (those that compared the costs and consequences of two or more health care interventions or alternatives) were designated "cost-consequences analyses", "cost-minimization analyses", "cost-effectiveness analyses", "cost-utility analyses", or "cost-benefit analyses".

Peer-review

The manuscript investigates the economic evaluations in gastroenterology in Brazil. The authors tried to bring a reference to the decision-making processes in gastroenterology by analyses the cost-effectiveness of different treatments.

REFERENCES

1 **Drummond MF**. Economic evaluation of treatment strategies in gastroenterology. *Am J Gastroenterol* 2005; **100**: 2143-2145 [PMID: 16181359 DOI: 10.1111/j.1572-0241.2005.00299.x]

2 **Augustovski F**, Iglesias C, Manca A, Drummond M, Rubinstein A, Martí SG. Barriers to generalizability of health economic evaluations in Latin America and the Caribbean region. *Pharmacoeconomics* 2009; **27**: 919-929 [PMID: 19888792 DOI: 10.2165/11313670-000000000-00000]

3 **Kleinman L**, McIntosh E, Ryan M, Schmier J, Crawley J, Locke GR, De Lissovoy G. Willingness to pay for complete symptom relief of gastroesophageal reflux disease. *Arch Intern Med* 2002; **162**: 1361-1366 [PMID: 12076234]

4 **Ladabaum U**, Fendrick AM, Glidden D, Scheiman JM. Helicobacter pylori test-and-treat intervention compared to usual care in primary care patients with suspected peptic ulcer disease in the United States. *Am J Gastroenterol* 2002; **97**: 3007-3014 [PMID: 12492183 DOI: 10.1111/j.1572-0241.2002.07118.x]

5 **Ford AC**, Delaney BC, Forman D, Moayyedi P. Eradication therapy in Helicobacter pylori positive peptic ulcer disease: systematic review and economic analysis. *Am J Gastroenterol* 2004; **99**: 1833-1855 [PMID: 15330927 DOI: 10.1111/j.1572-0241.2004.40014.x]

6 **Centre for Reviews and Disseminations**. Systematic reviews of economic evaluations. CRD' guidance for undertaking reviews in health care: Centre for Reviews and Disseminations. University of York, 2009

7 **Decimoni TC**, Leandro R, Soarez PC, Craig D. Systematic Review of Economic Evaluation of Health Technologies Developed In Brazil From 1980-2013. *Value Health* 2014; **17**: A438

8 **Drummond MF**, Sculpher M, Torrance G, O'Brien B. Methods for the Economic Evaluation of Health Care Programmes. Third edition. Oxford University Press, 2005

9 **Valachis A**, Polyzos NP, Nearchou A, Lind P, Mauri D. Financial relationships in economic analyses of targeted therapies in oncology. *J Clin Oncol* 2012; **30**: 1316-1320 [PMID: 22430267 DOI: 10.1200/JCO.2011.38.6078]

10 **Hourneaux G**, de Moura E, Sakai P, Cecconello I, Ishioka S. [Palliative treatment of advanced esophageal cancer. Comparative study: auto-expandable metal stent and isoperistaltic esophagogastric bypass]. *Acta Gastroenterol Latinoam* 2001; **31**: 13-22 [PMID: 11370175]

11 **Torres Udos S**, Almeida TE, Netinho JG. Increasing hospital admission rates and economic burden for colorectal cancer in Brazil, 1996-2008. *Rev Panam Salud Publica* 2010; **28**: 244-248 [PMID: 21152711]

12 **Caponero R**, Ribeiro Rde A, Santos E, Cirrincione A, Saggia M. Medical resource use and cost of different first-line treatments for metastatic colorectal cancer in Brazil. *J Med Econ* 2008; **11**: 311-325 [PMID: 19450088 DOI: 10.3111/13696990802160817]

13 **de Carvalho AC**, Sasse EC, Sasse AD. Cost-effectiveness analysis of FOLFOX followed by FOLFIRI versus 5-FU/LV followed by irinotecan in patients with metastatic colorectal cancer within the Unified Health Care System of Brazil. In Portuguese. *J Bras Econ Saúde* 2012; **4**: 413-419

14 **Mak M**. Modified FLOX as first-line chemotherapy for metastatic colorectal cancer patients in the public health system in Brazil: Effectiveness and cost-utility analysis. *Mol Clin Oncol* 2013; **1**: 175-179 [PMID: 24649143]

15 **Castelo A**, Pessôa MG, Barreto TC, Alves MR, Araújo DV. [Cost estimates of chronic hepatitis B virus for the Brazilian unified health system in 2005]. *Rev Assoc Med Bras* 2007; **53**: 486-491 [PMID: 18157360]

16 **Costa AM**, L 'italien G, Nita ME, Araujo ES. Cost-effectiveness of entecavir versus lamivudine for the suppression of viral replication in chronic hepatitis B patients in Brazil. *Braz J Infect Dis* 2008; **12**: 368-373 [PMID: 19219274 DOI: 10.1590/S1413-86702008000500005]

17 **Almeida AM**, da Silva AL, Cherchiglia ML, Andrade EI, de Oliveira GL, Acurcio Fde A. Chronic hepatitis B treatment: the cost-effectiveness of interferon compared to lamivudine. *Value Health* 2011; **14**: S24-S28 [PMID: 21839893 DOI: 10.1016/j.jval.2011.05.011]

18 **Wiens A**, Venson R, Correr CJ, Pontarolo R. Cost-effectiveness of telbivudine versus lamivudine for chronic hepatitis B. *Braz J Infect Dis* 2011; **15**: 225-230 [PMID: 21670922]

19 **Almeida AM**, Silva AL, Brandão CM, Cherchiglia ML, Andrade EI, Araújo de Oliveira GL, Carmo RA, Acurcio Fde A. [Cost-effectiveness of nucleoside/nucleotide analogues in chronic hepatitis B]. *Rev Saude Publica* 2012; **46**: 942-949 [PMID: 23503535]

20 **Wiens A**, Lenzi L, Venson R, Pedroso ML, Correr CJ, Pontarolo R. Economic evaluation of treatments for chronic hepatitis B. *Braz J Infect Dis* 2013; **17**: 418-426 [PMID: 23849851 DOI: 10.1016/j.bjid.2012.12.005]

21 **Mosegui G**, Silva V, Mello MD. Assessment of Interferon, Peginterferon, Tenofovir, Entecavir, Lamivudine and Adefovir for the Treatment of Chronic Hepatitis B in HBEAG-Positive Patients Without Cirrhosis. *Int J Pharm Pharm Sc* 2013; **5**: 208-212

22 **Barreto AM**, Takei K, E C S, Bellesa MA, Salles NA, Barreto CC, Nishiya AS, Chamone DF. Cost-effective analysis of different algorithms for the diagnosis of hepatitis C virus infection. *Braz J Med Biol Res* 2008; **41**: 126-134 [PMID: 18235967 DOI: 10.1128/JCM.01045-08]

23 **Fonseca MC**, Araújo GT, Araújo DV. Cost effectiveness of peginterferon alfa-2B combined with ribavirin for the treatment of chronic hepatitis C in Brazil. *Braz J Infect Dis* 2009; **13**: 191-199 [PMID: 20191195 DOI: 10.1590/S1413-86702009000300007]

24 **Massad E**, Coutinho FA, Chaib E, Burattini MN. Cost-effectiveness analysis of a hypothetical hepatitis C vaccine compared to antiviral therapy. *Epidemiol Infect* 2009; **137**: 241-249 [PMID: 18631422 DOI: 10.1017/S0950268808000873]

25 **Silva F**, Peregrino A, Costa-Couto MH. Treatment of hepatitis C

infection in renal transplant candidates undergoing dialysis: budget impact on the SUS (Unified Health Care System of Brazil). In Portuguese. *Physis RSC* 2011; **21**: 437-448

26 **Mosegui G**, Vianna C, Rodrigues M. Peginterferon alpha-2a and ribavirin versus peginterferon alpha-2b and ribavirin: cost-effectiveness and budget impact of the treatment of chronic hepatitis C, genotype 1. In Portuguese. *Physis RSC* 2011; **21**: 377-393

27 **Blatt CR**, da Cunha Bernardo NLM, Rosa JA, Bagatini F, Alexandre RF, Neto GB, Siebert U, Farias MR. An Estimate of the Cost of Hepatitis C Treatment for the Brazilian Health System. *Value in Health Regional Issues* 2012; **1**: 129-135 [DOI: 10.1016/j.vhri.2012.10.001]

28 **de Mello Vianna CM**, Gonzalez Mosegui GB, Costa e Silva FV, Freitas Peregrino AA, da Silva Rodrigues MP, Nagib Jardim F. Economic evaluation of pegylated interferon alpha-2a in combination with ribavirin for the treatment of chronic infection with hepatitis C, genotype 1: comparison of 48 weeks of treatment and extended therapy. *Value in Health Regional Issues* 2013; **2**: 342-346 [DOI: 10.1016/j.vhri.2013.09.003]

29 **Barros FM**, Cheinquer H, Tsuchiya CT, Santos EA. Cost-effectiveness analysis of treatment with peginterferon-alfa-2a versus peginterferon-alfa-2b for patients with chronic hepatitis C under the public payer perspective in Brazil. *Cost Eff Resour Alloc* 2013; **11**: 25 [PMID: 24103591 DOI: 10.1186/1478-7547-11-25]

30 **Pimenta LG**, Silva AL. [Epigastric hernia (xipho-umbilical lipoma): classification, frequency, repercussion on work leave time and operating costs. Valorization of the outpatient surgical system]. In Portuguese. *Rev Assoc Med Bras* 1983; **29**: 162-165

31 **Castro LP**, Souza ZP, Ribeiro TC. Financial costs of treating peptic ulcers. In Portuguese. *Rev Assoc Med* 1986; **37**: 26-29

32 **Verceze AV**, Troncon L. Analysis of buffering power and concentration of sodium, and the cost of some antacids. In Portuguese. *Medicina* (Ribeirao Preto) 1982; **15**: 211-216

33 **Coelho JCU**, Weiderkehr M, Campos AC, Matias J, Campos G. Cost of liver transplantation at the Clinical Hospital of the (Brazilian) Federal University of Paraná. In Portuguese. *Rev Assoc Med Bras* 2000; **43**: 1-5

34 **Coelho JC**, Trubian PS, Freitas AC, Parolin MB, Schulz GJ, Martins EL. Cost comparison of cadaveric liver transplantation with living-donor transplantation. *Rev Assoc Med Bras* 2005; **51**: 158-163 [PMID: 16007302]

35 **Portela MP**, Neri ED, Fonteles MM, Garcia JH, Fernandes ME. [The cost of liver transplantation at a university hospital of Brazil]. *Rev Assoc Med Bras* 2010; **56**: 322-326 [PMID: 20676541]

36 **Kotze PG**, Albuquerque IC, Moraes AC, Vieira A. Cost-minimization analysis of infliximab (IFX) versus adalimumab (ADA) in the treatment of Crohn's disease (CD). In Portuguese. *Rev Bras Coloproct* 2009; **29**: 158-162

37 **Nishikawa AM**, Paladini L, Delfini R, Kotze PG, Clark O. Decision tree construction and cost-effectiveness analysis of treatment of ulcerative colitis with pentasa® mesalazine 2 g sachet. *Arq Gastroenterol* 2013; **50**: 297-303 [PMID: 24474233 DOI: 10.1590/S0004-28032013000400011]

38 **Takemoto ML**, Fernandes RA, Almeida GR, Monteiro RD, Colombini-Neto M, Bertola-Neto A. Health care resource use and costs in opioid-treated patients with and without constipation in Brazil. *Value Health* 2011; **14**: S78-S81 [PMID: 21839904 DOI: 10.1016/j.jval.2011.05.019]

39 **Britto M**, Fillmann L, Seabra MK. Comparative study of mannitol and polyethylene glycol in bowel preparation for colonoscopy. In Portuguese. *J Coloproctol* (Rio J) 2009; **29**: 226-230

40 **Santos VL**, de Paula CA, Secoli SR. [Adult ostomy patients in the city of São Paulo: a study of specialized equipment costs]. *Rev Esc Enferm USP* 2008; **42**: 249-255 [PMID: 18642735]

41 **Bresciani C**, Perez RO, Habr-Gama A, Jacob CE, Ozaki A, Batagello C, Proscurshim I, Gama-Rodrigues J. Laparoscopic versus standard appendectomy outcomes and cost comparisons in the private sector. *J Gastrointest Surg* 2005; **9**: 1174-1180; discussion 1180-1181 [PMID: 16269389 DOI: 10.1016/j.gassur.2005.06.001]

42 **de Araujo A**, de Barros Lopes A, Trucollo Michalczuk M, Stifft J, Nardelli E, Escobar G, Rossi G, Alvares-da-Silva MR. Is there yet any place for reagent strips in diagnosing spontaneous bacterial peritonitis in cirrhotic patients? An accuracy and cost-effectiveness study in Brazil. *J Gastroenterol Hepatol* 2008; **23**: 1895-1900 [PMID: 19120878 DOI: 10.1111/j.1440-1746.2008.05571.x]

43 **Baxter YC**, Dias MC, Maculevicius J, Cecconello I, Cotteleng B, Waitzberg DL. Economic study in surgical patients of a new model of nutrition therapy integrating hospital and home vs the conventional hospital model. *JPEN J Parenter Enteral Nutr* 2005; **29**: S96-105 [PMID: 15709552 DOI: 10.1177/01486071050290S1S96]

44 **Ribeiro JE**. Clinical protocol and treatment guidelines for treating viral hepatitis C and co-infections. Portuguese: Brasília DF, 2011

45 **Chieffi AL**, Barata RCB. Litigation: the pharmaceutical industry's strategy for introducing new drugs. In Portuguese. *Rev Saude Publica* 2010; **44**: 1-9

46 INCA - Instituto Nacional do Câncer [Internet]. 2014. Available from: URL: http://www2.inca.gov.br/wps/wcm/connect/inca/portal/home

P- Reviewer: Guo YM **S- Editor**: Ji FF **L- Editor**: A
E- Editor: Wang CH

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

ISSN 2150-5349 (online)

World Journal of *Gastrointestinal Pharmacology and Therapeutics*

World J Gastrointest Pharmacol Ther 2016 May 6; 7(2): 171-342

Published by Baishideng Publishing Group Inc

WJGPT

World Journal of Gastrointestinal Pharmacology and Therapeutics

A peer-reviewed, online, open-access journal of gastrointestinal pharmacology and therapeutics

Editorial Board

2016-2019

The *World Journal of Gastrointestinal Pharmacology and Therapeutics* Editorial Board consists of 394 members, representing a team of worldwide experts in surgery research. They are from 45 countries, including Argentina (3), Australia (12), Austria (3), Belarus (1), Belgium (4), Brazil (10), Canada (10), China (39), Czech Repoublic (1), Egypt (3), Estonia (1), Finland (1), France (6), Germany (19), Greece (7), Hungary (4), India (17), Iran (5), Ireland (1), Israel (5), Italy (32), Japan (34), Lebanon (1), Lithuania (3), Mexico (2), Netherlands (10), New Zealand (2), Norway (2), Pakistan (1), Philippines (1), Poland (3), Portugal (2), Romania (1), Russia (1), Saudi Arabia (3), Singapore (3), Slovenia (1), South Africa (1), South Korea (14), Spain (13), Sweden (3), Thailand (4), Turkey (6), United Kingdom (19) and United States (80).

Duo Li, *Hangzhou*
Yu-Yuan Li, *Guangzhou*
Xiong Ma, *Shanghai*
Qin Pan, *Shanghai*
Guo-Ping Sun, *Hefei*
Xue-Ying Sun, *Harbin*
Ming-Fu Wang, *Hong Kong*
Che-Yuen Justin Wu, *Hong Kong*
De Xiang Xu, *Hefei*
Ruian Xu, *Xiamen*
Ming-Xian Yan, *Jinan*
Yong-Feng Yang, *Nanjing*
Thomas Yau, *Hong Kong*
Win-Nei Yeo, *Hong Kong*
Long Yu, *Guangzhou*
Jian-Ping Yuan, *Guangzhou*
Man-Fung Yuen, *Hong Kong*
Liqun Zhang, *Beijing*
Min-Sheng Zhu, *Nanjing*

Czech Republic

Rene Kizek, *Brno*

Denmark

Rene Kizek, *Brno*

Egypt

Omar ME Abdel-Salam, *Cairo*
Ahmed Osman Abdel-Zaher, *Assiut*
Osama Ahmed Badary, *Cairo*

Estonia

Riin Tamm, *Tartu*

Finland

Riitta Korpela, *Helsinki*

France

Tarik Asselah, *Clichy*
Ferrand Audrey, *Toulouse*
Frederic Batteux, *Paris*
Thierry Capiod, *Paris*
Frederic Lagarce, *Angers*
Hang Thi Thu Nguyen, *Clermont-Ferrand*

Germany

Susanne Beckebaum, *Essen*
Jürgen Borlak, *Hannover*
Güralp Onur Ceyhan, *Munich*
Walee Chamulitrat, *Heidelberg*
Anton Gillessen, *Munster*
Dirk Heitzmann, *Muenster*
Jens Michael Heyn, *München*
Joachim Labenz, *Siegen*
Thomas Müller, *Berlin*
Belal Naser, *Salzgitter*
Beate Niesler, *Heidelberg*
Matthias Ocker, *Marburg*
Andreas Marc Palmer, *Konstanz*
Dirk Rades, *Luebeck*
Fuat Hakan Saner, *Essen*
Manfred V Singer, *Mannheim*
Konrad Ludwig Streetz, *Aachen*
Frank Tacke, *Aachen*
Gerhard Treiber, *Balingen*

Greece

Moses S Elisaf, *Ioannina*
Anastasios Koulaouzidis, *Greek*
Ioannis E Koutroubakis, *Heraklion*
Spilios Manolakopoulos, *Athens*
Konstantinos Christou Mountzouris, *Athens*
George V Papatheodoridis, *Athens*
Konstantinos Tziomalos, *Thessaloniki*

Hungary

Zsolt Barta, *Debrecen*
László Czakó, *Szeged*
Bela Molnar, *Budapest*
Gyula Mózsik, *Pecs*

India

Anil Kumar Agarwal, *New Delhi*
Sandip Basu, *Bombay*
Chiranjib Chakraborty, *Vellore*
Rukhsana Chowdhury, *Kolkata*
C M Habibullah, *Hyderabad*
Mohandas K Mallath, *Mumbai*
Balraj Mittal, *Lucknow*
Rama Devi Mittal, *Lucknow*
Asish K Mukhopadhyay, *Kolkata*
Lekha Saha, *Chandigarh*
Darisetty Santosh, *Hyderabad*
Shiv Kumar Sarin, *New Delhi*
Jayshri Ankur Shah, *Mumbai*
Sonu Sundd Singh, *Gurgaon*
Sikta Swarnakar, *Calcutta*
Rakesh Kumar Tandon, *New Delhi*
Asna Urooj, *Mysore*

Iran

Seyed Mohsen Dehghani, *Shiraz*
Ahmad Reza Dehpour, *Tehran*
Sara Farhang, *Tabriz*
Parisa Hasanein, *Hamadan*
Amir Mohammad Mortazavian, *Tehran*

Ireland

Zaid S Heetun, *Dublin*

Israel

Nimer Assy, *Safed*
Simon Bar-Meir, *Tel-Hashomer*
Rami Eliakim, *Haifa*
Tiberiu Hershcovici, *Jerusalem*
Haim Shmuel Odes, *Beer Sheva*

Italy

Giovanni C Actis, *Turin*
Bruno Annibale, *Roma*
Leonardo Baiocchi, *Rome*
Gabrio Bassotti, *San Sisto*
Francesca Borrelli, *Napoli*
Giuseppe Brisinda, *Rome*
Renzo Caprilli, *Rome*
Mauro Antonio Maria Carai, *Cagliari*
Renato Caviglia, *Rome*
Carolina Ciacci, *Naples*
Mario Cottone, *Trabucco*
Roberto De Giorgio, *Bologna*
Luca Elli, *Milano*
Alessandro Granito, *Bologna*
Francesco William Guglielmi, *Bari*
Mario Guslandi, *Milano*
Pietro Invernizzi, *Monza*
Mariano Malaguarnera, *Catania*
Massimo C Mauri, *Milan*
Massimo Montalto, *Rome*
Giovanni Monteleone, *Rome*
Gerardo Nardone, *Naples*
Fabio Pace, *Seriate*
Raffaele Pezzilli, *Bologna*
Rita Rezzani, *Brescia*
Carmelo Scarpignato, *Parma*
Generoso Uomo, *Napoli*
Paolo Usai-Satta, *Cagliari*
Maurizio Vecchi, *Milan*
Massimiliano Veroux, *Catania*

Japan

Akira Andoh, *Otsu*
Yuichiro Eguchi, *Saga*
Munechika Enjoji, *Fukuoka*
Norihiro Furusyo, *Fukuoka*
Naoki Hotta, *Nagoya*
Shigeo Ikegawa, *Higashi-Osaka*
Susumu Ito, *Okinawa*
Satoru Kakizaki, *Maebashi*
Terumi Kamisawa, *Tokyo*
Motoyori Kanazawa, *Miyagi*
Takuma Kato, *Mie*
Takashi Kawai, *Tokyo*
Shirao Kuniaki, *Oita*
Nobuyuki Matsuhashi, *Tokyo*
Tatsuya Matsura, *Yonago*
Teruo Murakami, *Hiroshima*
Yuji Naito, *Kyoto*
Katsuyuki Nakajima, *Maebashi Gunma*
Hiroshi Nakase, *Kyoto*
Nobuhiro Ohkohchi, *Tsukuba*
Shogo Ohkoshi, *Niigata*
Tomohiko Shimatani, *Kure*
Yasuhiko Sugawara, *Tokyo*
Yoshitaka Takuma, *Okayama*
Tatsuhiro Tsujimoto, *Nara*
Takato Ueno, *Fukuoka*
Kenji Watanabe, *Osaka*
Toshiaki Watanabe, *Tokyo*

Jiro Watari, *Nishinomiya*
Satoshi Yamagiwa, *Niigata*
Takayuki Yamamoto, *Mie*
Norimasa Yoshida, *Kyoto*
Hitoshi Yoshiji, *Kashihara*
Katsutoshi Yoshizato, *Higashihiroshima*

Lebanon

AIa Sharara, *Beirut*

Lithuania

Dalia Adukauskiene, *Kaunas*
Giedrius Barauskas, *Kaunas*
Laimas Jonaitis, *Kaunas*

Mexico

Pablo Muriel, *Mexico City*
Guillermo Benito Robles-Díaz, *Mexico City*

Netherlands

Judith Elisabeth Baars, *Rotterdam*
Albert J Bredenoord, *Nieuwegein*
Nanne KH De Boer, *Amsterdam*
Pieter Jan Floris De Jonge, *Rotterdam*
Wouter J de Jonge, *Amsterdam*
Mireille A Edens, *Groningen*
Chris JJ Mulder, *Amsterdam*
Paul E Sijens, *Groningen*
Vera Esther Valkhoff, *Rotterdam*

New Zealand

Momir M Mikov, *Dunedin*
Maxim Petrov, *Auckland*

Norway

Guanglin Cui, *Tromso*
Reidar Fossmark, *Trondheim*

Pakistan

Anwar Hassan Gilani, *Karachi*

Philippines

Mark Anthony Ayuyao De Lusong, *Quezon City*

Poland

Halina Cichoz-Lach, *Lublin*
Jaroslaw Czyz, *Cracow*
Julian Teodor Swierczynski, *Gdansk*

Portugal

Cristina Freire, *Hull*
Ana Isabel Lopes, *Lisbon*

Romania

Dan Lucian Dumitrascu, *Cluj*

Russia

Tatyana A Korolenko, *Novosibirsk*

Saudi Arabia

Mohammad S Khuroo, *Riyadh*
Moamen Salah Refat, *Taif*
Shahab Uddin, *Riyadh*

Singapore

Kok-Ann Gwee, *Singapore*
Khek-Yu Ho, *Singapore*
Kok-Yuen Ho, *Singapore*

Slovenia

Rok Orel, *Ljubljana*

South Africa

Christoffel Van Rensburg, *Cape Town*

South Korea

Ki Baik Hahm, *Inchon*
Seok Joo Han, *Seoul*
Jeong Won Jang, *Seoul*
Dong Joon Kim, *Gangwon-do*
Jae J Kim, *Seoul*
Kyoung Mee Kim, *Seoul*
Nayoung Kim, *Songnam*
Sung-Bae Kim, *Seoul*
Byung-Hoon Lee, *Seoul*
Kwan S Lee, *Seoul*
Sang-Han Lee, *Daegu*
Yun Jeong Lim, *Goyang*
Ji-Young Park, *Seoul*
Uy Dong Sohn, *Seoul*

Spain

Matias Antonio Avila, *Pamplona*
Luis Bujanda, *San Sebastián*
Maria Carmen Collado, *Valencia*
Conrado M Fernandez-Rodriguez, *Madrid*
ángel Lanas, *Zaragoza*
Juan-R Malagelada, *Barcelona*
Jose JG Marin, *Salamanca*
Antonio Ruiz Medina, *Jaén*
Maria J Monte, *Salamanca*
Miguel Munoz, *Seville*
Jesus Prieto, *Pamplona*
Victor Manuel Victor, *Valencia*
Maria D Yago, *Granada*

Sweden

Bodil Ohlsson, *Malmo*
Henrik Thorlacius, *Malmo*
Curt Tysk, *Orebro*

Thailand

Weekitt Kittisupamongkol, *Bangkok*
Abhasnee Sobhonslidsuk, *Bangkok*
Suporn Treepongkaruna, *Bangkok*
Sombat Treeprasertsuk, *Bangkok*

Turkey

Fusun Acarturk, *Etiler-Ankara*
Engin Altintas, *Mersin*
Güldeniz K Cakmak, *Kozlu Zonguldak*
Hayrullah Derici, *Balkesir*
Mukaddes Esrefoglu, *Istanbul*
Ilker Tasci, *Ankara*

United Kingdom

Nadeem Ahmad Afzal, *Hampshire*
Qasim Aziz, *London*
Hugh Barr, *Gloucester*
Ian Leonard Phillip Beales, *Norwich*
Barbara Braden, *Oxford*
Susan J Duthie, *Aberdeen*
Eyad Elkord, *Manchester*
Anton Vignaraj Emmanuel, *London*
Konstantinos C Fragkos, *London*
Nusrat Husain, *Manchester*
Jin-Yong Kang, *London*
Mariusz Madalinski, *Ipswich*
Srinivasan Madhusudan, *Nottingham*
Subramanian Mahadevan, *Birmingham*
John Francis Mayberry, *Leicester*
Chuka Uche Nwokolo, *Coventry*
Ajith Kumar Siriwardena, *Manchester*
Her-Hsin Tsai, *Hull*
Craig LC Williams, *Glasgow*

United States

Zubair H Aghai, *Camden*
Shrikant Anant, *Oklahoma*
Kondala R Atkuri, *Stanford*
Cheryl Hunt Baker, *Orlando*
James M Becker, *Boston*
Qiang Cai, *Atlanta*
Jian-De Chen, *Galveston*
Liang Cheng, *Indianapolis*

WJGPT

World Journal of Gastrointestinal Pharmacology and Therapeutics

Contents

Quarterly Volume 7 Number 2 May 6, 2016

TOPIC HIGHLIGHT

REVIEW

MINIREVIEWS

ORIGINAL ARTICLE

Basic Study

Retrospective Study

World Journal of Gastrointestinal Pharmacology and Therapeutics
Volume 7 Number 2 May 6, 2016

Contents

NAME OF JOURNAL
World Journal of Gastrointestinal Pharmacology and Therapeutics

ISSN
ISSN 2150-5349 (online)

LAUNCH DATE
May 6, 2010

FREQUENCY
Quarterly

World Journal of Gastrointestinal Pharmacology and Therapeutics
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Inc
8226 Regency Drive,
Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLICATION DATE
May 6, 2016

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/bpg/g_info_20160116143427.htm

ONLINE SUBMISSION
http://www.wjgnet.com/esps/

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i2.171

World J Gastrointest Pharmacol Ther 2016 May 6; 7(2): 171-178
ISSN 2150-5349 (online)

TOPIC HIGHLIGHT

2016 *Helicobacter pylori*: Global view

Non-pharmacological treatment of *Helicobacter pylori*

Haim Shmuely, Noam Domniz, Jacob Yahav

Haim Shmuely, Jacob Yahav, Department Medicine D, Kaplan Medical Center, Rehovot 76100, Israel

Noam Domniz, Sheba Medical Center, Tel-Hashomer, Ramat Gan 52506, Israel

Author contributions: Shmuely H, Domniz N and Yahav J contributed equally to this work.

Conflict-of-interest statement: The authors have no conflicts of interest to report.

Correspondence to: Dr. Haim Shmuely, MD, Head, Department Medicine D, Kaplan Medical Center, Pasternak St., Rehovot 76100, Israel. hshmuely@zahav.net.il
Telephone: +972-8-9441996
Fax: +972-8-9441866

Received: April 28, 2015
Peer-review started: May 6, 2015
First decision: September 8, 2015
Revised: January 13, 2016
Accepted: February 14, 2016
Article in press: February 16, 2016
Published online: May 6, 2016

Abstract

Many food and plant extracts have shown *in vitro* anti-*Helicobacter pylori* (*H. pylori*) activity, but are less effective *in vivo*. The anti-*H. pylori* effects of these extracts are mainly permeabilitization of the membrane, anti-adhesion, inhibition of bacterial enzymes and bacterial grown. We, herein, review treatment effects of cranberry, garlic, curcumin, ginger and pistacia gum against *H. pylori* in both *in vitro*, animal studies and *in vivo* studies.

Key words: *Helicobacter pylori*; Cranberry; Garlic; Curcumin; Ginger; Pistacia gum

Core tip: *Helicobacter pylori* (*H. pylori*) infection is difficult to eradicate and therefore, it is necessary to combine several antibiotics as well as administering a proton-pump inhibitor. Many food and plant extracts have demonstrated *in vitro* antibacterial activity, however, in *in vivo*, they are less effective. The food reviewed, herein, can be effective in preventing and/or reducing *H. pylori* infection. A preventive dietary approach can be very inexpensive in areas with poor health care systems.

Shmuely H, Domniz N, Yahav J. Non-pharmacological treatment of *Helicobacter pylori*. *World J Gastrointest Pharmacol Ther* 2016; 7(2): 171-178 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i2/171.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i2.171

INTRODUCTION

The main cause of peptic ulcers, chronic gastritis and gastric neoplasms is *Helicobacter pylori* (*H. pylori*) infection. The International Agency for Research on Cancer[1,2] first classified this bacterium as a group I carcinogen. Several putative virulence-associated factors contribute to its pathogenesis[3]. Virulence markers of *H. pylori* are intermittently associated with diseases. To effectively treat *H. pylori* associated diseases, the need to eradicate *H. pylori* in infected

individuals remains the best option. This infection is difficult to eradicate and therefore it is necessary to administer a proton-pump inhibitor (PPI)[4] and group several antibiotics together. *H. pylori i*s sensitive to several antibiotics, *i.e.,* clarithromycin, amoxicillin, metronidazole and tetracycline[5], however, alone these antibiotics cannot eradicate the microorganism[6]. The widespread treatment of amoxicillin, clarithromycin and omeprazole at present, is hardly effective due to increasing resistance to antibiotics. The efficacy of a particular therapy may vary due to patient compromise, age, local antibiotic guidelines, food and hygiene[7].

CRANBERRY

Vaccinium macrocarpon, also known as cranberry is a natural fruit. Studies have shown drinking cranberry juice can in part attenuate *H. pylori* infection. Cranberries are indigenous to North America and have been widely developed commercially in states, *i.e.,* Wisconsin, Massachusetts, and New Jersey. Cranberry juice is successful in inhibiting or treating urinary tract infections (UTIs) due to its capability to avoid adhesion to the lining of the UT. This bacteriostatic characteristic is attributable to proanthocyanidins[8]. Cranberries, a resource of vitamin C may also provide a bacteriostatic effect.

A previous study demonstrated that an integral part of elevated molecular weight of cranberry juice can prevent *H. pylori* adhesion *in vitro* to the human gastric mucosa[9,10] and act on specific adhesions. Other adhesions such as BabA, may also be affected[11].

Animal model studies have demonstrated the importance of BabA in associated *H. pylori* diseases, influencing the severity of the disease[12]. A recent study illustrated that when cranberry juice was fed to mice infected with *H. pylori*, 80% were cured 24 h following treatment, with an eradication rate of 20%, 4 wk post-treatment[13]. However, the actual process by which cranberry juice affects the colonization of *H. pylori* and its suppression deserves further exploration.

Several mechanisms have been postulated as causing the inhibitory action of cranberries against *H. pylori*; among them are adhesion, biofilm formation blocking[14], anti-oxidative and anti-carcinogen activity[15], proliferation suppression[16,17] due to high concentrations of proanthocyanidins[17], urease inhibition[18], inhibition of the *H. pylori* adhesion to human gastric mucus[19] and even a cytotoxic effect against the germ[20].

Significant positive results in treating *H. pylori* infections with cranberry juice have been shown in human *in vivo* studies. Almost a decade ago, cranberries were tested in combination with traditional anti-*H. pylori* antibiotics such as metronidazole and clarithromycin[21,22] and proved effective in improving eradication rates and suppressing infections in endemic populations. Nevertheless, very few studies have evaluated the possible beneficial effect of cranberries in healing *H. pylori* infection.

Zhang *et al*[23]'s 90 d trial of cranberry juice compared to placebo in 189 patients, exhibited an increase in eradication rates of *H. pylori*. Shmuely *et al*[24] suggested, following a double-blind randomized clinical study of several hundreds of subjects, that the inclusion of cranberry juice into a standard therapy protocol of amoxicillin, clarithromycin and omeprazole, may improve eradication rates of *H. pylori* in females. A recent *in vivo* study[17] showed that the consumption of cranberry juice may assist in managing colonization among asymptomatic children. Further *in vivo* studies are needed to advance our knowledge of these mechanisms.

GARLIC

The action of oxidation of fresh *Allium sativum L*. (garlic) has been established. It is mainly due to unpredictable and irritating organosulphur compounds. Fresh garlic kept for a protracted period (until 20 mo) yields an odorless aged garlic extract comprised of unchanging water soluble organosulphur compounds that deter oxidative damage by scavenging free radicals. Garlic, comparable to allium vegetables, includes a wide range of thiosulphinates, *i.e.,* allicin believed to be accountable for antibacterial activity[25]. It has been shown that the discriminate elimination of thiosulphinates or the avoidance of their creation by obstructing alliinase, destroys the garlic's antibacterial activity[25].

Several studies have revealed that extracts from raw garlic[26] or garlic powder tablets[27] maintains *in vitro* activity against *H. pylori*, *i.e.*, steam-distilled garlic oil.

In Cañizares *et al*[28]'s study of allium sativum extracts; the authors used purple garlic of the "Las Pedroñeras" variety. By using the solvents ethanol and acetone in a stirred tank, it was shown that garlic extracts inhibit *H. pylori* comparable to commercial materials. The extracted material can be directly applied thus, necessitating an extraction procedure which is simple and economical.

Allicin, associated with Allium sativum is believed accountable for garlic's bacteriostatic properties. The existence or lack of allicin is critical in inhibiting *in-vitro* growth of *H. pylori*[27].

Several studies have proven a diminished gastric cancer risk with a rise in the intake of allium vegetables[29], perhaps producing a positive influence on *H. pylori.* You *et al*[30] in a randomized trial of 3365 subjects randomly selected from villages in the Shandong Province of China, a district with high gastric cancer death rates and an occurrence of approximately 67% in individuals infected with *H. pylori,* tested the outcomes of short-term (once) *H. pylori* treatment and continuous vitamin or garlic supplements (long-term) in the incidence of progressive precancerous gastric lesions. Individuals aged 35-64 years were randomly assigned to three interventions or placebos: Amoxicillin

and omeprazole for 14 d (*H. pylori* treatment); vitamin C, vitamin E, and selenium for 7.3 years (vitamin supplement); and aged garlic extract and steam-distilled garlic oil for 7.3 years (garlic supplement)[30]. The patients endured an esophagogastroduodenoscopy and biopsy. The frequency of the appearance of precancerous gastric lesions was established by a histopathologic examination of seven biopsy sites[30]. Treatment for *H. pylori* did not diminish the occurrence of dysplasia or gastric cancer. However, a smaller number of patients receiving treatment for *H. pylori* rather than a placebo developed gastric cancer. There were no significant favorable disparities when garlic or vitamin supplements were consumed.

In a recent study[31], permanent residents of West China underwent a ^{14}C-urea breath test (^{14}C-UBT) used to diagnose *H. pylori* infection. Of the 8365 participants, 53.1% were diagnosed with *H. pylori* infection. Those who ate raw garlic had a statistically significant lower level of *H. pylori* infection than those who did not eat the raw garlic. In this region, raw garlic seemed to reduce the infection.

Salih *et al*[32] reported that in a Turkish population, consumption of garlic for long periods of time did not affect the occurrence of *H. pylori* infection. Those ingesting garlic demonstrated a significantly lower antibody titer than the non-garlic groups, suggesting an unintended inhibitory effect on the generation of *H. pylori* and a possible advancement to more acute diseases. McNulty *et al*[33]'s *in vivo* pilot study, failed to show that steam distilled garlic oil, inhibits *H. pylori* based on *in vitro* activity. In this study, 20 dyspeptic patients aged 18-75 years, exhibiting *H. pylori* positive serology, verified by a ^{13}C urea breath test, were treated with a 4 mg garlic oil capsule taken with meals, 4 times a day for two weeks.

Negative UBT indicated *H. pylori* eradication. A 50% fall in ^{13}C excess between baseline and follow-up was defined as suppression. There was no verification that by ingesting garlic oil, *H. pylori* was either eradicated, suppressed or improvement of symptoms.

Aydin *et al*[34] also reported negative results in a trial using "Ortis" brand "garlic oil" produced by mixing ground garlic cloves with vegetable oil. These negative *in vivo* results show that garlic oil at these doses does not inhibit *H. pylori*. Further exploration of the possible beneficial outcomes of garlic oil against *H. pylori,* is necessary.

CURCUMIN

Curcumin (diferuloylmethane) was first chemically classified in 1910 and is generally considered the most active component of the Curcuma longa herb (turmeric). Due to its distinguishing flavor and yellow color similar to curry, it is used as a spice[35]. Its anti-inflammatory, antimutagen, antioxidant, and anti- infectious properties have been previously studied[36-41]. The significance of curcumin has been established in *in vitro* and *in vivo* studies. Curcumin has been used in healing peptic ulcers as well as preventing *H. pylori* growth[42-44].

Kundu *et al*[45] demonstrated that curcumin is capable of eradicating *H. pylori* in mice. In *H. pylori* infected human gastric epithelial cells, a dose of curcumin suppressed MMP-3 and -9 expression. Eliminating *H. pylori* using curcumin, entails significant down regulation of MMP-3 and -9 activities in addition to expression in the cytotoxic associated gene (*cag*) positive and cag-negative *H. pylori*-infected gastric tissues. These data indicate that curcumin healing of *H. pylori* infection includes regulating MMP-3 and -9 activities.

Han *et al*[46] confirmed that the growth inhibitory activity of curcumin *via H. pylori* infection is a result of inhibition of the shikimate pathway essential for the production of aromatic amino acids in bacteria, but not in humans. The shikimate pathway is vital for the production of metabolites in bacteria, *i.e.,* aromatic amino acids, folic acid and ubiquinone[47]. The enzymes affected include shikimate dehydrogenase which are innovative drug targets in the development of nontoxic antimicrobial agents[48].

Recently, the effect of curcumin on the formulation of interleukin (IL)-8, IL-1β, tumor necrosis factor (TNF)-α and cyclooxygenase (COX)-2 in gastric mucosa taken from *H. pylori*-infected gastritis subjects, was investigated by Koosirirat *et al*[49]. Patients were assigned at random to either a treatment course of Omeprazole, Amoxicillin and Metronidazole (OAM) or curcumin. Gastric biopsies were collected pre and post-treatment. In addition, the level of inflammatory cytokines mRNA were measured using semi-quantitative reverse transcription polymerase chain reaction.

Patients who received OAM treatment found that the eradication rate was significantly higher in these patients than those who ingested curcumin (78.9% *vs* 5.9%). In the OAM group, the levels of IL-8 mRNA expression significantly worsened after treatment, however, no alterations of other cytokines were found. Thus, only curcumin may have a reduced *in-vivo* anti-bactericidal effect on *H. pylori* and on the generation of inflammatory cytokines.

Prucksunand *et al*[50]'s phase Ⅱ clinical trial reporting on the results of healing peptic ulcers with long turmeric (Curcuma longa Linn), examined patients with peptic ulcer symptoms. While performing an endoscopy, ulcers measuring 0.5 to 1.5 cm in diameter were found in the duodenal bulb and stomach. An oral dose of 300 mg, 5 times daily of capsule-filled turmeric was given. Treatment after 4 wk, revealed no ulcers in 48% and after another 12 wk of treatment, 76% had no ulcers. Abdominal pain and discomfort sufficiently lessened during the first and second week. The subjects were able to ingest normal foods instead of soft meals. New insights as to the therapeutic effect of curcumin in the treatment of peptic ulcers, encourages the use of curcumin as an alternative therapy. Yet *in-vivo* evidence

that curcumin is active against *H. pylori* infection is still lacking.

GINGER

Ginger root (Zingiber officinale) is traditionally designed for treating gastrointestinal ailments, *i.e.,* hyperemesis gravidarum, dyspepsia, peptic ulcer, motion sickness and inflammatory disorders[51]. The proximate chemical composition of ginger contains volatile oils (1%-4%), medically active elements of ginger.

Ginger employs anti-oxidant and anti-ulcer[52], anti-inflammatory, anti-tumor[53], carminative, diaphonic and digestive, expectorant actions[54]. The phenols found in solvent extracts of ginger are mainly gingerol and zingerone.

Siddaraju *et al*[55] found that an aqueous extract of ginger can protect the gastric mucosa from stress-induced mucosal lesions and inhibit gastric acid secretion, which can be done by blocking H^+, K^+-ATPase action, thus restricting H. *pylori* growth. Ginger produces anti-oxidant protection against oxidative stress-induced gastric damage, thus, exhibiting anti-oxidative properties *in vitro*.

Li *et al*[56] validated and strengthened the association between hyperemesis gravidarum (HG) and *H. pylori* infection in normal pregnant control subjects and pregnant women with HG. They found positive *H. pylori* in 1289 (69.6%) HG cases and 1045 (46.2%) *H. pylori*-positive in the control group. The infection rate of *H. pylori* was considerably higher in pregnant women with HG compared to the non-HG normal pregnant controls. Analysis of a subgroup revealed that *H. pylori* infection was a risk factor of HG in other countries, *i.e.*, Oceania, Asia and especially Africa. Karaca *et al*[57] stated that lower socio-economic status was an important risk factor for *H. pylori* infected pregnant women with an HG factor.

Other studies have found that certain agents active against *H. pylori* are very effective in the treatment of hyperemesis[58,59]. The human Chorionic Gonadotropin (hCG) when elevated in pregnancy, concurrently alters the pH in pregnancy. hCG was found to induce gastrointestinal dysmotility, altered humoral as well as cell mediated immunity in pregnancy believed to be the basis for infection.

Several preclinical studies suggest that ginger, an agent linked to gastric and colon carcinogenesis, generates a protective effect against *H. pylori*[57,58]. Ginger phenolic fractions provide inhibitory effects on the growth of *H. pylori,* scavenge free radicals, reduce power abilities, protect DNA and inhibit lipid peroxidation[59,60].

Mahady *et al*[58] reported on the chemo-preventative effects of ginger which directly impede *H. pylori* growth, particularly CagA+ strains. The authors showed that gingerols and ginger extracts inhibit the development of *H. pylori in vitro* of 19 clinical strains. In addition, the fraction comprising the gingerols and 6-shogoal was very successful in inhibiting the growth of *H. pylori* CagA+ strains. This documentation suggests that specific ginger extracts containing gingerols may assist in treating or preventing *H. pylori* CagA and strains *in vivo*.

Researchers studying Mongolian gerbils noted that ginger extract prevented and treated *H. pylori*-induced infection and inflammation[61]. Moreover, additional research was implemented to clarify the *in vitro* mechanism of the ginger extract. These results confirm the medicinal properties of ginger in Ayurveda and folklore medicines and further advocate that ginger be considered a new therapeutic approach in the treatment of gastric disorders.

PISTACIA GUM

A resin called Chios mastic gum (CMG), produced by the Pistacia lentiscus var. chia. plant, is nurtured predominantly in the southern part of the Greek island of Chios and other Mediterranean countries. However, this plant can be planted or re-planted in other locations around the world, including the northern part of Chios, however, it will not produce resin.

The first mention of mastic was noted by Herodotus in the 5th century BC. Since 3000 BC, CMG has been used by the Greeks in cooking, cosmetics, and treating gastric illnesses.

In the 1980s, CMG was found to be a potential agent in treating duodenal ulcers in humans[62]. The antibacterial action of CMG was assessed and compared to clinical isolates of *H. pylori*[63]. Transmission electron microscopy determined CMG's influence on *H. pylori* morphology. CMG presents with anti-*H. pylori* activity due its inducement of protrusions, morphological abnormalities and cellular fragmentation in *H. pylori* cells[64].

A 2011 study presented proof that CMG prevents *H. pylori* inflammation by inhibiting neutrophil activation *in vitro*[65]. Dabos *et al*[66] confirmed these observations by examining the influence of CMG on *H. pylori* eradication in *H. pylori* patients. Mastic gum was well tolerated and the mild side effects were reversible.

It was determined that CMG has bactericidal action against *H. pylori in vivo*[66]. Paraschos *et al*[67] found that extracts and elements of CMG were active against *H. pylori*. After the insoluble polymer was removed, a total mastic extract without polymer was prepared, thus improving solubility and enhancing *in vivo* activity. The acid fraction generated major triterpenic acids after chromatographic separation, while the neutral fraction generated several triterpenic alcohols and aldehydes.

Employing a panel of 11 *H. pylori* clinical strains, CMG extracts and isolated pure triterpenic acids were tested for *in vitro* action. The authors demonstrated that

Table 1 Suggested anti-*Helicobacter pylori* mechanisms of the foods and plant extracts

Agent administered	Major mechanisms	Ref.
Cranberry	Bacteriostatic properties of proanthocyanidins	Howell[8], Gotteland *et al*[17]
	Inhibition of adhesion to the human gastric mucosa *in vitro*	Burger *et al*[9], Parente *et al*[10], Burger *et al*[19]
	Inhibition of adhesion and biofilm formation blocking	Shmuely *et al*[14]
	Anti-oxidative and anti-carcinogen activity	Côté *et al*[15]
	Proliferation suppression	Matsushima *et al*[16], Gotteland *et al*[17]
	Urease inhibition	Lin *et al*[18]
	Cytotoxic effect	Zafra-Stone *et al*[20]
Garlic	Antibacterial activity by thiosulphinates	Farbman *et al*[25]
Curcumin	Suppression of Matrix Metalloproteinase-3 and -9 expression in *H. pylori* infected human gastric epithelial cells	Kundu *et al*[45]
	Inhibition of the shikimate pathway, necessary for synthesis of aromatic amino acids	Han *et al*[46]
	Effect upon the production of IL-8, IL-1β, tumor necrosis factor-α and cyclooxygenase-2 in gastric mucosa	Koosirirat *et al*[49]
Ginger	Anti-oxidant and anti-ulcer activity	Yoshikawa *et al*[52]
	Anti-inflammatory and anti-tumor activity	Kim *et al*[53]
	Blocking H^+, K^+-ATPase action, inhibitory effects on the growth of *H. pylori*, DNA protection and inhibition of lipid peroxidation	Siddaraju *et al*[55]
	6-gingerol enhances the tumor necrosis factor-related apoptosis by inhibiting nuclear factor kappa B	Ishiguro *et al*[60]
	Directly inhibiting the growth of *H. pylori*, particularly the CagA+ strains	Mahady *et al*[58]
Pistacia Gum	Induction of protrusions, morphological abnormalities, and cellular fragmentation in *H. pylori* cells	Marone *et al*[64]
	Inhibition of neutrophil activation	Choli-Papadopoulou *et al*[65]
	Triterpenic acids present in the acid extract	Paraschos *et al*[67]

H. pylori: *Helicobacter pylori*; IL: Interleukin.

administration of CMG may reduce *H. pylori* settlement. In addition, the major triterpenic acids found in the acid extract may be responsible for this activity[68].

Other animal studies reported that CMG has no effect on *H. pylori*[68,69]. Monotherapy of CMG was administered to prove its ability to eliminate *H. pylori* infection in mice. The results showed that CMG was unable to eradicate *H. pylori* infection in mice. Also, Loughlin *et al*[69] reported that CMG failed to suppress or destroy *H. pylori* infection in humans. Patients with *H. pylori* infection were treated with 1g of CMG, 4 times daily for 14 d. CMG was found to have no effect on *H. pylori* status; they all remained *H. pylori*-positive. It was resolved that despite the anti-*H. pylori* action *in vitro*, there seems to be no effect on *H. pylori* in humans due to CMG[69].

All *H. pylori*-positive patients, treated with mastic capsules for 7 d remained *H. pylori* positive[70]. Miyamoto *et al*[70] and Huwez *et al*[71] observed that no "antibiotic-like" activity should be anticipated from crude mastic.

It has been shown that mastic has definite antibacterial action *via H. pylori.* This may partially explain the anti-peptic-ulcer mastic's properties[62,71]. By examining the effect of anti-*H. pylori* of the various elements of mastic, researchers may in the future, identify the participating ingredient.

Mastic is inexpensive and widely accessible in third world countries, hence, more *in-vivo* studies should be performed in developing countries.

CONCLUSION

Compared with the use of antibiotic and PPI treatment, a preventive dietary approach can be very inexpensive in areas with poor health care systems. The food reviewed can be effective in preventing and/or reducing *H. pylori* infection due to their potent anti-inflammatory activity. The rapid uptake by cells (Table 1) provides the suggested anti-*H. pylori* mechanisms of the foods and plant extracts.

REFERENCES

1 **Covacci A**, Telford JL, Del Giudice G, Parsonnet J, Rappuoli R. Helicobacter pylori virulence and genetic geography. *Science* 1999; **284**: 1328-1333 [PMID: 10334982 DOI: 10.1126/science.284.5418.1328]

2 **Westblom TU**, Czinn SJ, Nedrud JG. Current topics in microbiology and immunology, Gastroduodenal disease and Helicobacter pylori: pathophysiology, diagnosis and treatment. Berlin Heidelberg: Springer, 1999: 57-69 [DOI: 10.1007/978-3-642-60013-5]

3 **Kundu P**, Mukhopadhyay AK, Patra R, Banerjee A, Berg DE, Swarnakar S. Cag pathogenicity island-independent up-regulation of matrix metalloproteinases-9 and -2 secretion and expression in mice by Helicobacter pylori infection. *J Biol Chem* 2006; **281**: 34651-34662 [PMID: 16966323 DOI: 10.1074/jbc.M604574200]

4 **Graham DY**. Efficient identification and evaluation of effective Helicobacter pylori therapies. *Clin Gastroenterol Hepatol* 2009; **7**: 145-148 [PMID: 19026766 DOI: 10.1016/j.cgh.2008.10.024]

5 **Mégraud F**, Lehours P. Helicobacter pylori detection and antimicrobial susceptibility testing. *Clin Microbiol Rev* 2007; **20**: 280-322 [PMID: 17428887 DOI: 10.1128/CMR.00033-06]

6 **Peterson WL**, Graham DY, Marshall B, Blaser MJ, Genta RM,

Klein PD, Stratton CW, Drnec J, Prokocimer P, Siepman N. Clarithromycin as monotherapy for eradication of Helicobacter pylori: a randomized, double-blind trial. *Am J Gastroenterol* 1993; **88**: 1860-1864 [PMID: 8237933]
7 **Calvet X**, Gisbert JP, Suarez D. Key points for designing and reporting Helicobacter pylori therapeutic trials: a personal view. *Helicobacter* 2011; **16**: 346-355 [PMID: 21923680 DOI: 10.1111/j.1523-5378.2011.00890.x]
8 **Howell AB**. Cranberry proanthocyanidins and the maintenance of urinary tract health. *Crit Rev Food Sci Nutr* 2002; **42**: 273-278 [PMID: 12058985 DOI: 10.1080/10408390209351915]
9 **Burger O**, Ofek I, Tabak M, Weiss EI, Sharon N, Neeman I. A high molecular mass constituent of cranberry juice inhibits helicobacter pylori adhesion to human gastric mucus. *FEMS Immunol Med Microbiol* 2000; **29**: 295-301 [PMID: 11118911 DOI: 10.1111/j.1574-695X.2000.tb01537.x]
10 **Parente F**, Cucino C, Anderloni A, Grandinetti G, Bianchi Porro G. Treatment of Helicobacter pylori infection using a novel antiadhesion compound (3'sialyllactose sodium salt). A double blind, placebo-controlled clinical study. *Helicobacter* 2003; **8**: 252-256 [PMID: 12950597 DOI: 10.1046/j.1523-5378.2003.00152.x]
11 **Ilver D**, Arnqvist A, Ogren J, Frick IM, Kersulyte D, Incecik ET, Berg DE, Covacci A, Engstrand L, Borén T. Helicobacter pylori adhesin binding fucosylated histo-blood group antigens revealed by retagging. *Science* 1998; **279**: 373-377 [PMID: 9430586 DOI: 10.1126/science.279.5349.373]
12 **Guruge JL**, Falk PG, Lorenz RG, Dans M, Wirth HP, Blaser MJ, Berg DE, Gordon JI. Epithelial attachment alters the outcome of Helicobacter pylori infection. *Proc Natl Acad Sci USA* 1998; **95**: 3925-3930 [PMID: 9520469]
13 **Xiao SD**, Shi T. Is cranberry juice effective in the treatment and prevention of Helicobacter pylori infection in mice? *Chinese J Dig Dis* 2003; **4**: 136-139 [DOI: 10.1046/j.1443-9573.2003.00127.x]
14 **Shmuely H**, Ofek I, Weiss EI, Rones Z, Houri-Haddad Y. Cranberry components for the therapy of infectious disease. *Curr Opin Biotechnol* 2012; **23**: 148-152 [PMID: 22088310 DOI: 10.1016/j.copbio.2011.10.009]
15 **Côté J**, Caillet S, Doyon G, Sylvain JF, Lacroix M. Bioactive compounds in cranberries and their biological properties. *Crit Rev Food Sci Nutr* 2010; **50**: 666-679 [PMID: 20694928 DOI: 10.1080/10408390903044107]
16 **Matsushima M**, Suzuki T, Masui A, Kasai K, Kouchi T, Takagi A, Shirai T, Mine T. Growth inhibitory action of cranberry on Helicobacter pylori. *J Gastroenterol Hepatol* 2008; **23** Suppl 2: S175-S180 [PMID: 19120894 DOI: 10.1111/j.1440-1746.2008.05409.x]
17 **Gotteland M**, Andrews M, Toledo M, Muñoz L, Caceres P, Anziani A, Wittig E, Speisky H, Salazar G. Modulation of Helicobacter pylori colonization with cranberry juice and Lactobacillus johnsonii La1 in children. *Nutrition* 2008; **24**: 421-426 [PMID: 18343637 DOI: 10.1016/j.nut.2008.01.007]
18 **Lin YT**, Kwon YI, Labbe RG, Shetty K. Inhibition of Helicobacter pylori and associated urease by oregano and cranberry phytochemical synergies. *Appl Environ Microbiol* 2005; **71**: 8558-8564 [PMID: 16332847 DOI: 10.1128/AEM.71.12.8558-8564.2005]
19 **Burger O**, Weiss E, Sharon N, Tabak M, Neeman I, Ofek I. Inhibition of Helicobacter pylori adhesion to human gastric mucus by a high-molecular-weight constituent of cranberry juice. *Crit Rev Food Sci Nutr* 2002; **42**: 279-284 [PMID: 12058986 DOI: 10.1080/10408390209351916]
20 **Zafra-Stone S**, Yasmin T, Bagchi M, Chatterjee A, Vinson JA, Bagchi D. Berry anthocyanins as novel antioxidants in human health and disease prevention. *Mol Nutr Food Res* 2007; **51**: 675-683 [PMID: 17533652 DOI: 10.1002/mnfr.200700002]
21 **Chatterjee A**, Yasmin T, Bagchi D, Stohs SJ. Inhibition of Helicobacter pylori in vitro by various berry extracts, with enhanced susceptibility to clarithromycin. *Mol Cell Biochem* 2004; **265**: 19-26 [PMID: 15543930 DOI: 10.1023/B:MCBI.0000044310.92444.ec]
22 **Shmuely H**, Burger O, Neeman I, Yahav J, Samra Z, Niv Y, Sharon N, Weiss E, Athamna A, Tabak M, Ofek I. Susceptibility of Helicobacter pylori isolates to the antiadhesion activity of a high-molecular-weight constituent of cranberry. *Diagn Microbiol Infect Dis* 2004; **50**: 231-235 [PMID: 15582295 DOI: 10.1016/j.diagmicrobio.2004.08.011]
23 **Zhang L**, Ma J, Pan K, Go VL, Chen J, You WC. Efficacy of cranberry juice on Helicobacter pylori infection: a double-blind, randomized placebo-controlled trial. *Helicobacter* 2005; **10**: 139-145 [PMID: 15810945 DOI: 10.1111/j.1523-5378.2005.00301]
24 **Shmuely H**, Yahav J, Samra Z, Chodick G, Koren R, Niv Y, Ofek I. Effect of cranberry juice on eradication of Helicobacter pylori in patients treated with antibiotics and a proton pump inhibitor. *Mol Nutr Food Res* 2007; **51**: 746-751 [PMID: 17487928 DOI: 10.1002/mnfr.200600281]
25 **Farbman KS**, Barnett ED, Bolduc GR, Klein JO. Antibacterial activity of garlic and onions: a historical perspective. *Pediatr Infect Dis J* 1993; **12**: 613-614 [PMID: 8346006 DOI: 10.1097/00006454-199307000-00013]
26 **Cañizares P**, Gracia I, Gómez LA, Martín de Argila C, de Rafael L, García A. Optimization of Allium sativum solvent extraction for the inhibition of in vitro growth of Helicobacter pylori. *Biotechnol Prog* 2002; **18**: 1227-1232 [PMID: 12467456 DOI: 10.1021/bp025592z]
27 **Lawson LD**, Wang ZJ, Papadimitriou D. Allicin release under simulated gastrointestinal conditions from garlic powder tablets employed in clinical trials on serum cholesterol. *Planta Med* 2001; **67**: 13-18 [PMID: 11270714 DOI: 10.1055/s-2001-10624]
28 **Cañizares P**, Gracia I, Gómez LA, García A, Martín De Argila C, Boixeda D, de Rafael L. Thermal degradation of allicin in garlic extracts and its implication on the inhibition of the in-vitro growth of Helicobacter pylori. *Biotechnol Prog* 2004; **20**: 32-37 [PMID: 14763820 DOI: 10.1021/bp034135v]
29 **Dorant E**, van den Brandt PA, Goldbohm RA, Sturmans F. Consumption of onions and a reduced risk of stomach carcinoma. *Gastroenterology* 1996; **110**: 12-20 [PMID: 8536847 DOI: 10.1053/gast.1996.v110.pm8536847]
30 **You WC**, Brown LM, Zhang L, Li JY, Jin ML, Chang YS, Ma JL, Pan KF, Liu WD, Hu Y, Crystal-Mansour S, Pee D, Blot WJ, Fraumeni JF, Xu GW, Gail MH. Randomized double-blind factorial trial of three treatments to reduce the prevalence of precancerous gastric lesions. *J Natl Cancer Inst* 2006; **98**: 974-983 [PMID: 16849680 DOI: 10.1093/jnci/djj264]
31 **Tang HR**, Fan YJ, Liu S. Helicobacter pylori infection and associated risk factors in Chengdu. *Sichuan Daxue Xuebao Yixueban* 2014; **45**: 823-826 [PMID: 25341349]
32 **Salih BA**, Abasiyanik FM. Does regular garlic intake affect the prevalence of Helicobacter pylori in asymptomatic subjects? *Saudi Med J* 2003; **24**: 842-845 [PMID: 12939668]
33 **McNulty CA**, Wilson MP, Havinga W, Johnston B, O'Gara EA, Maslin DJ. A pilot study to determine the effectiveness of garlic oil capsules in the treatment of dyspeptic patients with Helicobacter pylori. *Helicobacter* 2001; **6**: 249-253 [PMID: 11683929 DOI: 10.1046/j.1523-5378.2001.00036.x]
34 **Aydin A**, Ersöz G, Tekesin O, Akçiçek E, Tuncyurek M, Batur Y. Does garlic oil have a role in the treatment of Helicobacter pylori infection? *Turk Gastroenteroloji Dergisi* 1997; **8**: 181-184
35 **Sharma RA**, Gescher AJ, Steward WP. Curcumin: the story so far. *Eur J Cancer* 2005; **41**: 1955-1968 [PMID: 16081279 DOI: 10.1016/j.ejca.2005.05.009]
36 **Egan ME**, Pearson M, Weiner SA, Rajendran V, Rubin D, Glöckner-Pagel J, Canny S, Du K, Lukacs GL, Caplan MJ. Curcumin, a major constituent of turmeric, corrects cystic fibrosis defects. *Science* 2004; **304**: 600-602 [PMID: 15105504 DOI: 10.1126/science.1093941]
37 **Bengmark S**. Curcumin, an atoxic antioxidant and natural NFkappaB, cyclooxygenase-2, lipooxygenase, and inducible nitric oxide synthase inhibitor: a shield against acute and chronic

diseases. *JPEN J Parenter Enteral Nutr* 2006; **30**: 45-51 [PMID: 16387899 DOI: 10.1177/014860710603000145]
38 **Punithavathi D**, Venkatesan N, Babu M. Protective effects of curcumin against amiodarone-induced pulmonary fibrosis in rats. *Br J Pharmacol* 2003; **139**: 1342-1350 [PMID: 12890714 DOI: 10.1038/sj.bjp.0705362]
39 **Ringman JM**, Frautschy SA, Cole GM, Masterman DL, Cummings JL. A potential role of the curry spice curcumin in Alzheimer's disease. *Curr Alzheimer Res* 2005; **2**: 131-136 [PMID: 15974909 DOI: 10.2174/1567205053585882]
40 **Duvoix A**, Blasius R, Delhalle S, Schnekenburger M, Morceau F, Henry E, Dicato M, Diederich M. Chemopreventive and therapeutic effects of curcumin. *Cancer Lett* 2005; **223**: 181-190 [PMID: 15896452 DOI: 10.1016/j.canlet.2004.09.041]
41 **Holt PR**, Katz S, Kirshoff R. Curcumin therapy in inflammatory bowel disease: a pilot study. *Dig Dis Sci* 2005; **50**: 2191-2193 [PMID: 16240238 DOI: 10.1007/s10620-005-3032-8]
42 **Swarnakar S**, Ganguly K, Kundu P, Banerjee A, Maity P, Sharma AV. Curcumin regulates expression and activity of matrix metalloproteinases 9 and 2 during prevention and healing of indomethacin-induced gastric ulcer. *J Biol Chem* 2005; **280**: 9409-9415 [PMID: 15615723 DOI: 10.1074/jbc.M413398200]
43 **Foryst-Ludwig A**, Neumann M, Schneider-Brachert W, Naumann M. Curcumin blocks NF-kappaB and the motogenic response in Helicobacter pylori-infected epithelial cells. *Biochem Biophys Res Commun* 2004; **316**: 1065-1072 [PMID: 15044093]
44 **Santos AM**, Lopes T, Oleastro M, Gato IV, Floch P, Benejat L, Chaves P, Pereira T, Seixas E, Machado J, Guerreiro AS. Curcumin inhibits gastric inflammation induced by Helicobacter pylori infection in a mouse model. *Nutrients* 2015; **7**: 306-320 [PMID: 25569625 DOI: 10.3390/nu7010306]
45 **Kundu P**, De R, Pal I, Mukhopadhyay AK, Saha DR, Swarnakar S. Curcumin alleviates matrix metalloproteinase-3 and -9 activities during eradication of Helicobacter pylori infection in cultured cells and mice. *PLoS One* 2011; **6**: e16306 [PMID: 21283694 DOI: 10.1371/journal.pone.0016306]
46 **Han C**, Wang L, Yu K, Chen L, Hu L, Chen K, Jiang H, Shen X. Biochemical characterization and inhibitor discovery of shikimate dehydrogenase from Helicobacter pylori. *FEBS J* 2006; **273**: 4682-4692 [PMID: 16972983]
47 **Cheng WC**, Chen YF, Wang HJ, Hsu KC, Lin SC, Chen TJ, Yang JM, Wang WC. Structures of Helicobacter pylori shikimate kinase reveal a selective inhibitor-induced-fit mechanism. *PLoS One* 2012; **7**: e33481 [PMID: 22438938 DOI: 10.1371/journal.pone.0033481]
48 **Coggins JR**, Abell C, Evans LB, Frederickson M, Robinson DA, Roszak AW, Lapthorn AP. Experiences with the shikimate-pathway enzymes as targets for rational drug design. *Biochem Soc Trans* 2003; **31**: 548-552 [PMID: 12773154]
49 **Koosirirat C**, Linpisarn S, Changsom D, Chawansuntati K, Wipasa J. Investigation of the anti-inflammatory effect of Curcuma longa in Helicobacter pylori-infected patients. *Int Immunopharmacol* 2010; **10**: 815-818 [PMID: 20438867 DOI: 10.1016/j.intimp.2010.04.021]
50 **Prucksunand C**, Indrasukhsri B, Leethochawalit M, Hungspreugs K. Phase II clinical trial on effect of the long turmeric (Curcuma longa Linn) on healing of peptic ulcer. *Southeast Asian J Trop Med Public Health* 2001; **32**: 208-215 [PMID: 11485087]
51 **Farnsworth RF**, Fong HHS, Mahady GB. WHO monographs on selected medicinal plants. Geneva, Switzerland: WHO Publications, 1999
52 **Yoshikawa M**, Yamaguchi S, Kunimi K, Matsuda H, Okuno Y, Yamahara J, Murakami N. Stomachic principles in ginger. III. An anti-ulcer principle, 6-gingesulfonic acid, and three monoacyldigalactosylglycerols, gingerglycolipids A, B, and C, from Zingiberis Rhizoma originating in Taiwan. *Chem Pharm Bull* (Tokyo) 1994; **42**: 1226-1230 [PMID: 8069973 DOI: 10.1248/cpb.42.1226]
53 **Kim EC**, Min JK, Kim TY, Lee SJ, Yang HO, Han S, Kim YM, Kwon YG. [6]-Gingerol, a pungent ingredient of ginger, inhibits angiogenesis in vitro and in vivo. *Biochem Biophys Res Commun* 2005; **335**: 300-308 [PMID: 16081047 DOI: 10.1016/j.bbrc.2005.07.076]
54 **al-Yahya MA**, Rafatullah S, Mossa JS, Ageel AM, Parmar NS, Tariq M. Gastroprotective activity of ginger zingiber officinale rosc., in albino rats. *Am J Chin Med* 1989; **17**: 51-56 [PMID: 2589236 DOI: 10.1142/S0192415X89000097]
55 **Siddaraju MN**, Dharmesh SM. Inhibition of gastric H+, K+-ATPase and Helicobacter pylori growth by phenolic antioxidants of Zingiber officinale. *Mol Nutr Food Res* 2007; **51**: 324-332 [PMID: 17295419 DOI: 10.1002/mnfr.200600202]
56 **Li L**, Li L, Zhou X, Xiao S, Gu H, Zhang G. Helicobacter pylori Infection Is Associated with an Increased Risk of Hyperemesis Gravidarum: A Meta-Analysis. *Gastroenterol Res Pract* 2015; **2015**: 278905 [PMID: 25861257 DOI: 10.1155/2015/278905]
57 **Karaca C**, Güler N, Yazar A, Camlica H, Demir K, Yildirim G. Is lower socio-economic status a risk factor for Helicobacter pylori infection in pregnant women with hyperemesis gravidarum? *Turk J Gastroenterol* 2004; **15**: 86-89 [PMID: 15334316]
58 **Mahady GB**, Pendland SL, Yun GS, Lu ZZ, Stoia A. Ginger (Zingiber officinale Roscoe) and the gingerols inhibit the growth of Cag A+ strains of Helicobacter pylori. *Anticancer Res* 2003; **23**: 3699-3702 [PMID: 14666666]
59 **El Younis CM**, Abulafia O, Sherer DM. Rapid marked response of severe hyperemesis gravidarum to oral erythromycin. *Am J Perinatol* 1998; **15**: 533-534 [PMID: 9890250 DOI: 10.1055/s-2007-994055]
60 **Ishiguro K**, Ando T, Maeda O, Ohmiya N, Niwa Y, Kadomatsu K, Goto H. Ginger ingredients reduce viability of gastric cancer cells via distinct mechanisms. *Biochem Biophys Res Commun* 2007; **362**: 218-223 [PMID: 17706603]
61 **Gaus K,** Huang Y, Israel DA, Pendland SL, Adeniyi BA, Mahady GB. Standardized ginger (Zingiber officinale) extract reduces bacterial load and suppresses acute and chronic inflammation in Mongolian gerbils infected with cagAHelicobacter pylori. *Pharm Biol* 2009; **47**: 92-98 [PMID: 20376296 DOI: 10.1111/j.1440-1681.1984.tb00864.x]
62 **Al-Habbal MJ**, Al-Habbal Z, Huwez FU. A double-blind controlled clinical trial of mastic and placebo in the treatment of duodenal ulcer. *Clin Exp Pharmacol Physiol* 1984; **11**: 541-544 [PMID: 6395994]
63 **Aebischer T**, Meyer TF, Andersen LP. Inflammation, immunity, and vaccines for Helicobacter. *Helicobacter* 2010; **15** Suppl 1: 21-28 [PMID: 21054649 DOI: 10.1111/j.1523-5378.2010.00777.x]
64 **Marone P**, Bono L, Leone E, Bona S, Carretto E, Perversi L. Bactericidal activity of Pistacia lentiscus mastic gum against Helicobacter pylori. *J Chemother* 2001; **13**: 611-614 [PMID: 11806621]
65 **Choli-Papadopoulou T**, Kottakis F, Papadopoulos G, Pendas S. Helicobacter pylori neutrophil activating protein as target for new drugs against H. pylori inflammation. *World J Gastroenterol* 2011; **17**: 2585-2591 [PMID: 21677824 DOI: 10.3748/wjg.v17.i21.2585]
66 **Dabos KJ**, Sfika E, Vlatta LJ, Giannikopoulos G. The effect of mastic gum on Helicobacter pylori: a randomized pilot study. *Phytomedicine* 2010; **17**: 296-299 [PMID: 19879118 DOI: 10.1016/j.phymed.2009.09.010]
67 **Paraschos S**, Magiatis P, Mitakou S, Petraki K, Kalliaropoulos A, Maragkoudakis P, Mentis A, Sgouras D, Skaltsounis AL. In vitro and in vivo activities of Chios mastic gum extracts and constituents against Helicobacter pylori. *Antimicrob Agents Chemother* 2007; **51**: 551-559 [PMID: 17116667]
68 **Bebb JR**, Bailey-Flitter N, Ala'Aldeen D, Atherton JC. Mastic gum has no effect on Helicobacter pylori load in vivo. *J Antimicrob Chemother* 2003; **52**: 522-523 [PMID: 12888582]
69 **Loughlin MF**, Ala'Aldeen DA, Jenks PJ. Monotherapy with mastic does not eradicate Helicobacter pylori infection from mice. *J Antimicrob Chemother* 2003; **51**: 367-371 [PMID: 12562704]
70 **Miyamoto T**, Okimoto T, Kuwano M. Chemical Composition

of the Essential Oil of Mastic Gum and their Antibacterial Activity Against Drug-Resistant Helicobacter pylori. *Nat Prod Bioprospect* 2014; **4**: 227-231 [PMID: 25089241 DOI: 10.1007/s13659-014-0033-3]

71 **Huwez FU**, Al-Habbal MJ. Mastic in treatment of benign gastric ulcers. *Gastroenterol Jpn* 1986; **21**: 273-274 [PMID: 3732760]

P- Reviewer: Ierardi E, Ozen H **S- Editor**: Gong ZM
L- Editor: A **E- Editor**: Wu HL

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i2.179

World J Gastrointest Pharmacol Ther 2016 May 6; 7(2): 179-189
ISSN 2150-5349 (online)

REVIEW

Transoral incisionless fundoplication for gastro-esophageal reflux disease: Techniques and outcomes

Pier Alberto Testoni, Giorgia Mazzoleni, Sabrina Gloria Giulia Testoni

Pier Alberto Testoni, Giorgia Mazzoleni, Sabrina Gloria Giulia Testoni, Division of Gastroenterology and Gastrointestinal Endoscopy, Vita-Salute San Raffaele University, San Raffaele Scientific Institute, 20132 Milan, Italy

Author contributions: Testoni PA, Mazzoleni G and Testoni SGG equally contributed to this paper with regard to conception and design of the study, literature review, analysis and interpretation of data, drafting the article and making critical revisions related to important intellectual content of the manuscript; Testoni PA contributed to final approval of the version of the article to be published.

Conflict-of-interest statement: Authors declare no conflict of interests for this article.

Correspondence to: Pier Alberto Testoni, MD, Division of Gastroenterology and Gastrointestinal Endoscopy, Vita-Salute San Raffaele University, San Raffaele Scientific Institute, via Olgettina 58, 20132 Milan, Italy. testoni.pieralberto@hsr.it
Telephone: +39-2-26432756
Fax: +39-2-26433491

Received: October 17, 2015
Peer-review started: October 17, 2015
First decision: November 24, 2015
Revised: December 24, 2015
Accepted: February 23, 2016
Article in press: February 24, 2016
Published online: May 6, 2016

Abstract

Gastro-esophageal reflux disease (GERD) is a very common disorder that results primarily from the loss of an effective antireflux barrier, which forms a mechanical obstacle to the retrograde movement of gastric content. GERD can be currently treated by medical therapy, surgical or endoscopic transoral intervention. Medical therapy is the most common approach, though concerns have been increasingly raised in recent years about the potential side effects of continuous long-term medication, drug intolerance or unresponsiveness, and the need for high dosages for long periods to treat symptoms or prevent recurrences. Surgery too may in some cases have consequences such as long-lasting dysphagia, flatulence, inability to belch or vomit, diarrhea, or functional dyspepsia related to delayed gastric emptying. In the last few years, transoral incisionless fundoplication (TIF) has proved an effective and promising therapeutic option as an alternative to medical and surgical therapy. This review describes the steps of the TIF technique, using the EsophyX® device and the MUSE™ system. Complications and their management are described in detail, and the recent literature regarding the outcomes is reviewed. TIF reconfigures the tissue to obtain a full-thickness gastro-esophageal valve from inside the stomach, by serosa-to-serosa plications which include the muscle layers. To date the procedure has achieved lasting improvement of GERD symptoms (up to six years), cessation or reduction of proton pump inhibitor medication in about 75% of patients, and improvement of functional findings, measured by either pH or impedance monitoring.

Key words: Gastro-esophageal reflux disease; Transoral incisionless fundoplication; Anterior fundoplication with ultrasonic surgical endostapler; EsophyX; MUSE; Surgical fundoplication

Core tip: Transoral incisionless fundoplication (TIF) has recently emerged as an effective and promising therapeutic option in alternative to medical and surgical

therapy for gastro-esophageal reflux disease (GERD). A number of prospective observational studies for TIF using the EsophyX® device have been published but there is still only limited data for TIF with the MUSE™ system. This review describes the techniques for TIF with both these devices, and is intended to consolidate the current literature, clarifying better the outcomes of TIF in patients with GERD.

Testoni PA, Mazzoleni G, Testoni SGG. Transoral incisionless fundoplication for gastro-esophageal reflux disease: Techniques and outcomes. *World J Gastrointest Pharmacol Ther* 2016; 7(2): 179-189 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i2/179.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i2.179

INTRODUCTION

Gastro-esophageal reflux disease (GERD) is a very common disorder that can be currently treated by medical therapy, surgical or endoscopic transoral intervention. Medical therapy with proton pump inhibitors (PPIs) is the most common approach: However, concerns have increasingly been voiced in recent years regarding the potential PPI-related side effects, intolerance or unresponsiveness, and in some cases the need for a long-term therapy with high dosages to relieve symptoms or prevent recurrences. Surgical therapy too may in some cases have consequences such as long-lasting dysphagia, flatulence, inability to belch or vomit, diarrhea, or functional dyspepsia related to delayed gastric emptying[1-4]. Even for interventions done in centers of excellence, incisional hernias at the site of trocar insertion have been reported in up to 3% of cases[5].

For these reasons several transoral endoscopic techniques have been proposed in the last 15 years as alternatives to medical and surgical therapy; however most of them had disappointing outcomes and have been abandoned.

In the last few years, transoral incisionless fundoplication (TIF) has proved to be an effective and promising therapeutic alternative to medical and surgical therapy; the procedure achieves lasting improvement of GERD symptoms (up to six years) and functional findings, and cessation or reduction of PPI medication in about 75% of patients. TIF reconfigures the tissue to obtain a full-thickness gastro-esophageal valve from inside the stomach, by serosa-to-serosa plications which include the muscle layers; the new valve boosts the barrier function of the LES with potentially fewer procedure-related side effects than surgery.

TIF can be done using the EsophyX® device (EndoGastric Solutions, Redmond, WA, United States) or the Medigus Ultrasonic Surgical Endostapler system (MUSE™, Medigus Ltd., Omer; Israel). EsophyX® device constructs an omega-shaped valve 3-5 cm long, in a 250°-300° circumferential pattern around the gastro-esophageal junction, by deploying non-absorbable polypropylene fasteners through the two layers (esophagus and stomach) under endoscopic vision of the operator. The MUSE™ system staples the fundus of the stomach to the esophagus below the diaphragm using multiple sets of metal stitches placed under an ultrasound-guided technique and creates an anterior fundoplication functionally similar to the standard surgical Dor-Thal operation. In a patient with sliding hiatal hernia, the procedure can be done only if the hernia can be reduced below the diaphragm.

Publications on TIF with the EsophyX® device report the persistence of the newly created valve at six months in all studies and for up to six years in one study, with satisfactory outcomes, assessed by 24-h pH and/or impedance monitoring[6-27]. There is less information so far for TIF with the MUSE™ system: One animal study found the technique safe and feasible and two trials in humans reported good clinical and functional results at six-month and up to five-year follow-up[28-30].

This review describes the techniques for TIF, using the EsophyX® device and the MUSE™ system, pre- and post-procedure patients' management, and complications. Outcomes are reported in detail, and a revision of the literature was performed to assess the efficacy of TIF in patients with GERD. Manuscripts were identified by searching PubMed, Embase and The Cochrane Library databases, using the following key words "gastro-esophageal reflux disease", "transoral incisionless fundoplication", "anterior fundoplication", "medigus ultrasonic surgical endostapler", "EsophyX", "MUSE", and "surgical fundoplication".

TECHNIQUE

Pre-procedure evaluation

Preoperative upper gastrointestinal endoscopy must be done to assess the distance between the incisor teeth and the esophago-gastric junction (EGJ), and the transverse dimension of the diaphragmatic hiatus. With the current TIF technique only a hiatal hernia not more than 3.0 cm long can be reduced below the diaphragm, while a hiatus larger than 3.0 cm may facilitate a cranial displacement of the plication up in the thorax, making ineffective the newly created valve.

Prior to the intervention all the patients should be examined by esophageal manometry to exclude primary motility disorders, and by 24-h pH-impedance monitoring to exclude a functional heartburn. If the MUSE™ system is used, barium swallow should be done to assess the reducibility of the hernia, since irreducibility is a contraindication to the procedure.

Transoral fundoplication with the EsophyX® device

The EsophyX® device is composed of: (1) a handle that houses the controls; (2) an 18-mm diameter chassis that includes operative channels through which a front-view 9-mm diameter endoscope can be inserted; (3)

the tissue invaginator, provided by side holes on the distal part of the chassis, to which external suction can be applied; (4) the tissue mold, which can be brought into retroflection and pushes tissue against the shaft of the device; (5) a helical screw, which is advanced into the tissue so the tissue between the tissue mold and the shaft can be retracted; (6) two stylets, which pass through the plicated tissue and the tissue mold, and H-shaped polypropylene fasteners can be deployed over them; and (7) a cartridge containing 20 fasteners. The device has been recently updated and improved in a new generation instrument: The EsophyX® Z device. The fastener deployment is similar to a surgical stapler firing mechanism with a reduction of control complexity and dual fastener deployment, and is improved by managing trailing leg. The crossing profile has been reduced with elimination of tissue mold elbow and increase of tissue mold lateral stiffness; the tissue mold tip covers stylets during deployment.

Details of the first and second generation devices are illustrated in the Figure 1.

The procedure requires two operators: One handles the device and the other the endoscope.

The device is introduced transorally with the patient in the left lateral or supine position, under general anesthesia. In cases with difficult insertion, the device can be gently rotated during the introduction: This maneuver allows to easily pass the upper esophageal sphincter. In this phase, there is a risk of hypopharyngeal perforation if the instrument is inserted without caution.

During the procedure, air or CO_2 is insufflated to distend the gastric cavity and permit adequate vision of the fundus and EGJ; CO_2 is preferable because it reduces the patient's discomfort and is safer in case of perforation.

With the patient placed in left decubitus and endoscope positioned in retroflexed view, the lesser curve is located at the 12 o'clock position and the greater curve at 6 o'clock. The tissue mold is retroflexed and closed against the Esophyx device; then it is rotated to 11 or 1 o'clock (lesser curve) and pulled back, to have its tip just inside the esophageal lumen. At this point: (1) the helical retractor is advanced to engage tissue under direct vision just below the Z-line; (2) the tissue mold is opened and the helical screw cable is pulled back to retract the tissue; (3) in this phase of the procedure the stomach is being desufflated to engage an adequate amount of tissue for fundoplication; (4) once such a maneuver has been completed, with both the helical retractor and tissue mold locked in place, suction is applied to the tissue invaginator and the device is then advanced into the stomach, which has been re-insufflated. This permits to create the esophago-gastric plication in an intra-abdominal position and reduces any hiatal hernia.

Plication is performed by deploying multiple H-shaped polypropylene fasteners advanced over the two stylets, starting on the far posterior and anterior sides of the esophago-gastric junction; then additional fasteners are deployed along the greater curvature part of the valve by rotating the tissue mold axially to slide the stomach over the esophagus. This maneuver results in circumferential tightening and a new valve circumference of > 240°. In general 14 fasteners are needed to construct an adequate circumferential valve; however, the more fasteners are deployed the more continent is the valve.

Details of the EsophyX® technique are shown in Figure 2. Endoscopic pre- and post-procedural findings are reported in Figure 3.

Beside the standard procedure, two modified techniques have been described over time to create the fundoplication.

The one we use, engages the tissue below the Z-line at 11 and 1 o'clock positions; then a torque is applied by rotating the locked tissue mold clockwise and counterclockwise before inserting the stylet. By this maneuver, part of the fundus is rotated around the esophageal wall and more tissue is engaged by the stylet. Four fasteners for each site are deployed at 1 and 11 o'clock, and two for each site in the middle part of the valve, at 4, 6, and 8 o'clock, to reinforce the plication. This technique increased by 30% the success rate of the procedure, achieving the complete elimination of PPI use at 12 mo in 14/22 patients (63.6%), while with the standard technique only 11/27 patients (40.7%) completely stopped PPIs.

Bell *et al*[19] have developed a so called rotational fundoplication. The helical retractor is engaged at 12 o'clock and the tissue mold is placed at 6 o'clock. Then the tissue mold locked is rotated toward the lesser curve by a radial motion of the handle of the device, to the 12 o'clock position. This maneuver rolls the fundus over and around the distal esophagus to the 1 o'clock position.

At the end of the plication, endoscopy is done to examine the pharynx, esophageal lumen and the gastric fundus, and the fundoplication.

Trans-oral fundoplication with the MUSE™ system

The MUSE™ system includes the endostapler and a console connected with it, containing a controller for the camera, ultrasonic range finder and various sensors, a pump for insufflation and irrigation, a suction system, power and controls for the LED.

The endostapler has: (1) a handle, housing the controls; (2) an insertion tube 15.5 mm in diameter, 66 cm long, containing the suction, insufflation/irrigation channels, and electrical and mechanical cables to operate the device; (3) a rigid section 66 mm long containing the cartridge. Each cartridge holds five standard 4.8-mm titanium staples, the ultrasound mirror, one alignment pin funnel, and two anvil screw funnels; and (4) the distal tip, similar to that of an endoscope, for suction, irrigation, illumination (with a

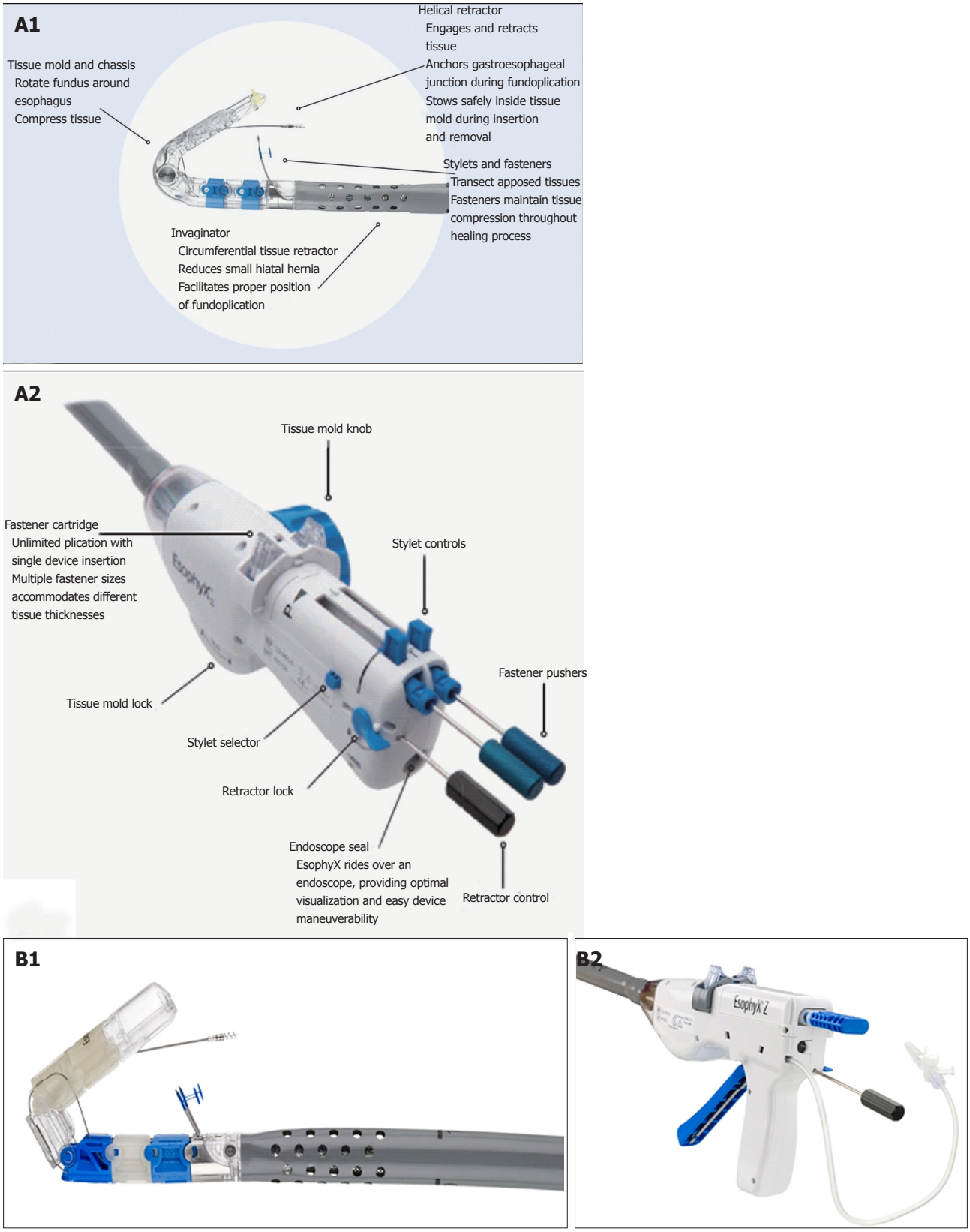

Figure 1 EsophyX® device: First and second generation devices (courtesy of EndoGastric Solutions, Inc. Redmond, WA, United States). A1-A2: The device currently used (©2014 EndoGastric Solutions, Inc); B1-B2: The new generation device (©2014 EndoGastric Solutions, Inc).

LED) and visualization (with a miniature camera). The anvil, alignment pin, anvil screw and ultrasound are all designed to ensure proper alignment and positioning of the device during stapling. The distal tip can be articulated in one direction to align with the rigid section and cartridge, with a bending radius of 26 and 40 mm. Details of the device are illustrated in Figure 4.

The whole procedure can be done by one operator

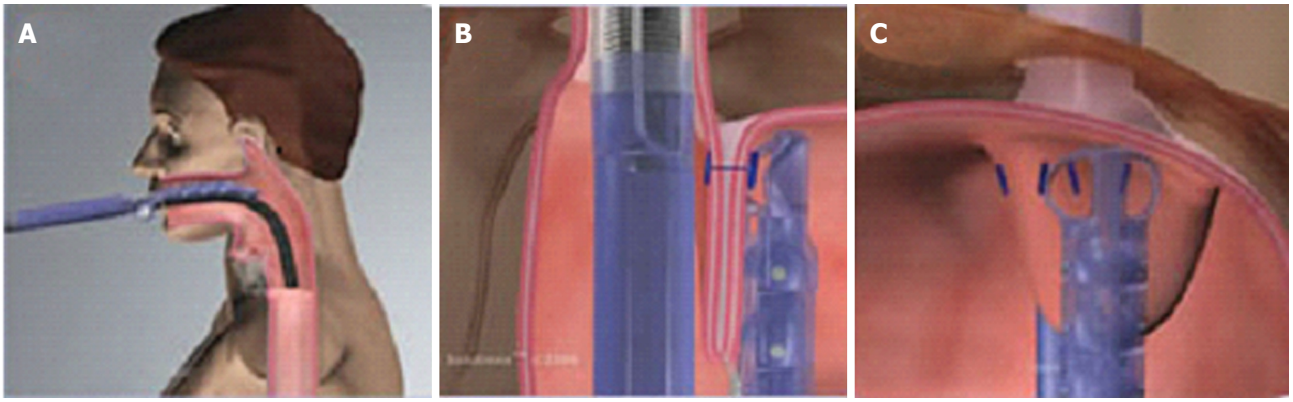

Figure 2 Schematic representation of the procedure with EsophyX® device (Courtesy of EndoGastric Solutions Inc. Redmond, WA, United States). A: The EsophyX® device enters the esophagus through the mouth and is positioned at the gastro-esophageal junction; B: The device wraps the fundus around the distal esophagus and fastens a tissue fold; C: This step is then repeated multiple times to reconstruct a robust, tight valve (©2014 EndoGastric Solutions, Inc).

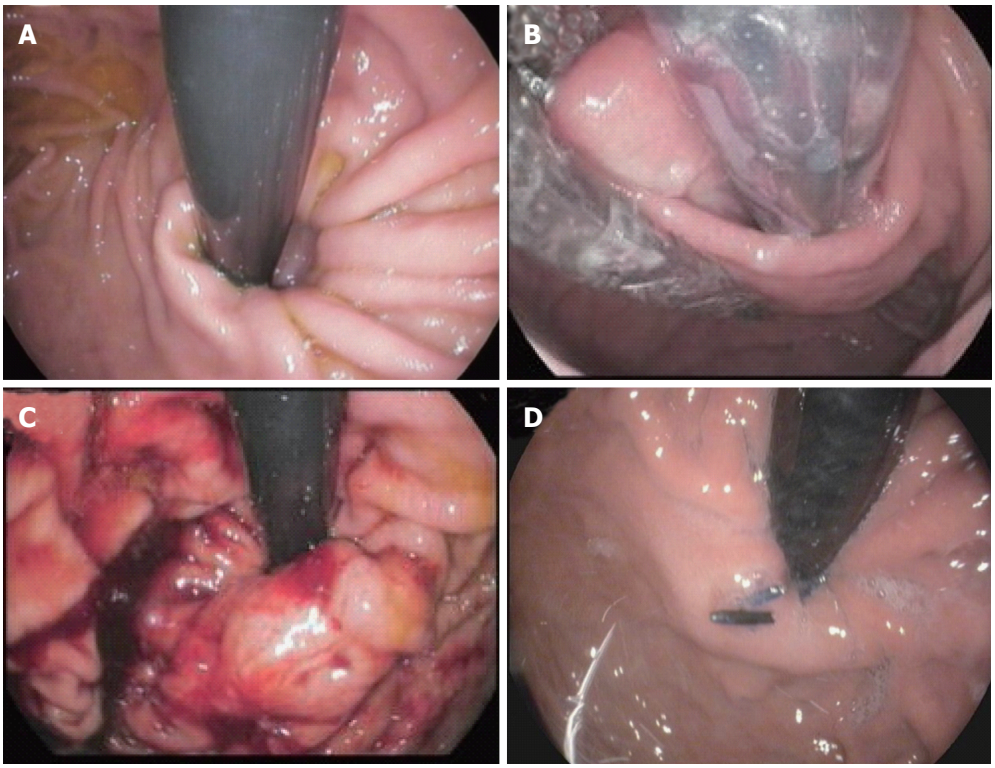

Figure 3 Endoscopic views of the gastro-esophageal valve before and immediately after the transoral incisionless fundoplication procedure by EsophyX® device (Authors' case). A: The gastro-esophageal valve: Before the procedure with the EsophyX® device; B: The "Bell Roll" maneuver to create the new gastro-esophageal valve; C: The gastro-esophageal valve: Immediately after the procedure with the EsophyX® device; D: The gastro-esophageal valve: Six months after the procedure.

in experienced hands. The patient is placed in the supine position, under general anesthesia with tracheal intubation. Positive end-expiratory pressure of at least 5 mmHg (7.5 cmH_2O) is provided. After a preliminary endoscopic assessment of the esophagus and stomach and, as long as once no contraindications are found, an overtube is placed. The endostapler is then inserted transorally through the overtube and gently advanced into the stomach under direct vision; passing the rigid section across the pharyngo-esophageal junction it may encounter some resistance. To avoid having to apply excessive force and risk injury the esophagus, the overtube may be withdrawn about 5 cm and then advanced with the endostapler as a unit. This maneuver can be repeated until the system reaches the esophageal midbody. Flexing the neck may make passage easier.

Once in the stomach, distended by insufflation of air or CO_2, the stapler is advanced until the tip is approximately 5 cm past the EGJ and then retroflexed 180° to obtain adequate vision of the gastric fundus and EGJ so as to select the stapling location. The most important location is the left-most, and is typically done first. This is the anchoring point for the fundus, and should be as far to the left of the esophagus as possible.

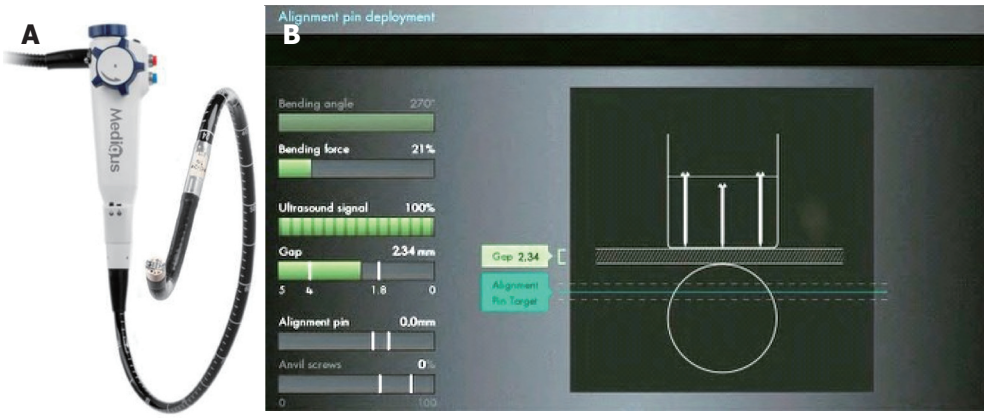

Figure 4 Medigus Surgical Ultrasonic Endostapler system, MUSE™ (Courtesy of Medigus Ltd., Omer, Israel). A: The MUSE™ system (© All rights reserved to Medigus Ltd 2008-2015); B: The console connected with the endostapler, containing a controller for the camera, ultrasonic range finder and various sensors (bending angle, bending force, alignment pin, anvil screws, gap) (© All rights reserved to Medigus Ltd 2008-2015).

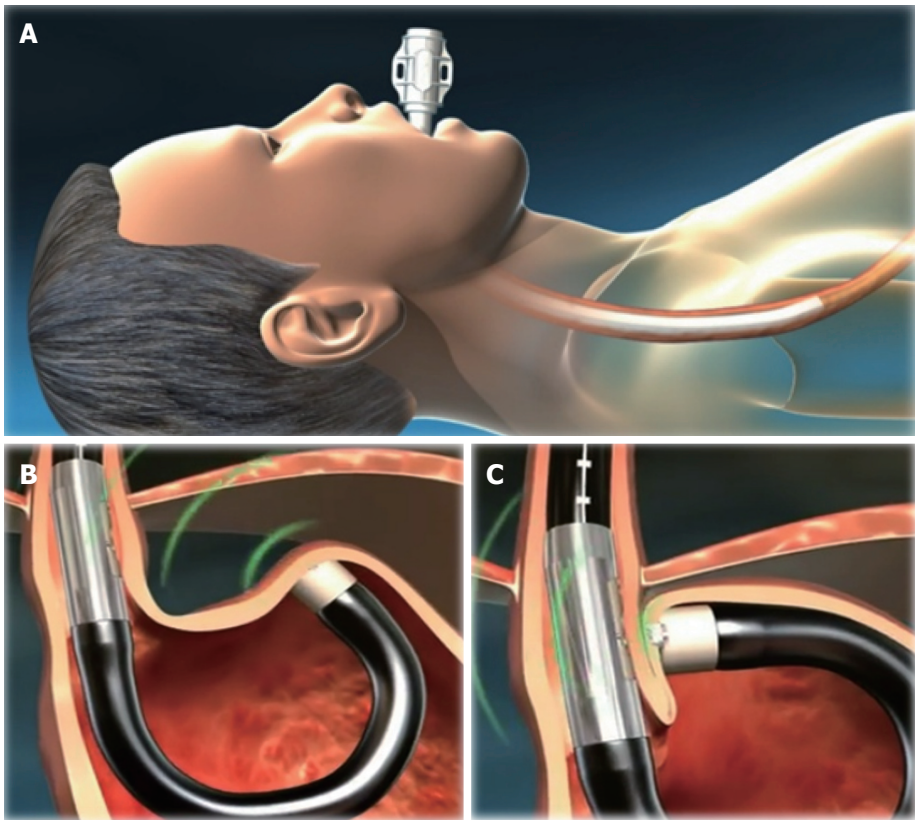

Figure 5 Schematic representation of the Medigus Ultrasonic Surgical Endostapler (MUSE™) procedure (Courtesy of Medigus Ltd., Omer, Israel). A: The endostapler is inserted transorally through the overtube and gently advanced into the stomach under direct vision; B: Once in the stomach, distended by insufflation of air or CO_2, the stapler is advanced until the tip is approximately 5 cm past the EGJ and then retroflexed 180° to give adequate vision of the gastric fundus and EGJ to select the stapling location. Tissue is clamped and stapled under ultrasonic guidance; C: This step is then repeated at least twice to reconstruct a robust, tight valve. Additional stapling locations should be within 60°-180° of the valve circumference (© All rights reserved to Medigus Ltd 2008-2015). EGJ: Esophago-gastric junction.

Sometimes, depending on the anatomy, it may be easier to do the first stapling in a more central position.

Subsequent staplings should be within 60°-180° as long as the right-most stapling is not be on the lesser curve, where it may attach the antrum to the esophagus and open the esophago-gastric junction rather than close it. Further staplings may be placed between the left-most and right-most.

Once the correct locations for stapling have been identified, the rest of the procedure is done under ultrasound guidance. Subsequent phases include clamping tissue, deploying the alignment pin, advancing the anvil screw, stapling, and retrieving anvil screws.

Details of the MUSE™ technique are shown in Figure 5. Endoscopic pre- and post-procedural findings after TIF with this device are reported in Figure 6.

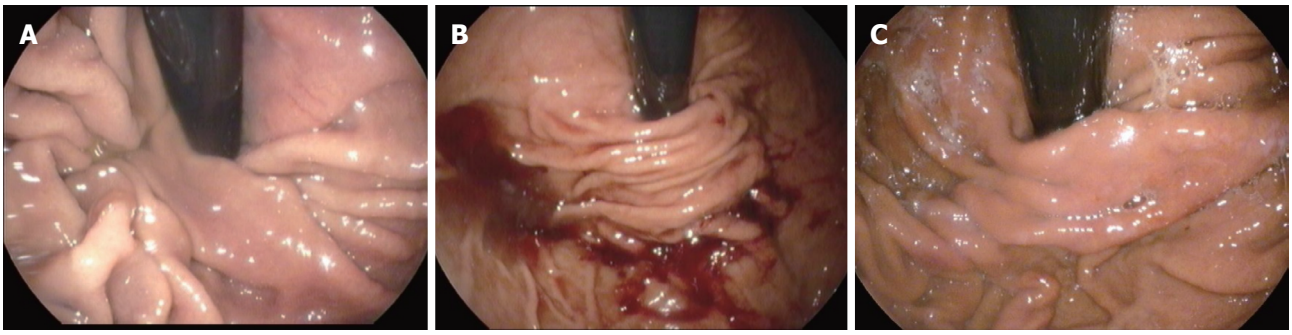

Figure 6 Endoscopic views of the gastro-esophageal valve before and after the transoral incisionless fundoplication procedure with the Medigus Ultrasonic Surgical Endostapler (MUSE™) (authors' case). A: The gastro-esophageal valve: before the transoral incisionless fundoplication (TIF) procedure with the MUSE™ system; B: The gastro-esophageal valve: Immediately after the TIF procedure by MUSE™ system; C: The gastro-esophageal valve: Six months after the TIF procedure by MUSE™ system.

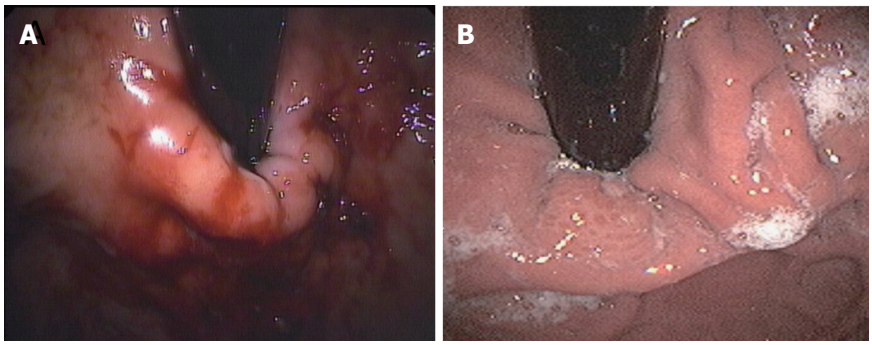

Figure 7 Endoscopic views of the gastro-esophageal valve immediately after and 24 mo after the transoral incisionless fundoplication procedure with EsophyX® device (authors' case). A: The gastro-esophageal valve: Immediately after the transoral incisionless fundoplication (TIF) procedure with EsophyX® device; B: The gastro-esophageal valve: 24 mo after the TIF procedure with EsophyX® device.

Post-operative care

Antiemetic prophylaxis with at least two drugs (according to the ASA recommendations for interventions with high risk of post-procedural nausea and vomiting) and full muscle relaxation throughout the procedure are mandatory for TIF. Antiemetic prophylaxis is maintained intravenously for 24 h, and broad-spectrum antibiotic therapy is continued intravenously for 48 h, then orally for five days.

A transient pharyngeal irritation occurs in most patients, as a result of insertion and manipulation of the device; some patients suffer from a mild to moderate epigastric pain in the six hours after the intervention. If pain persists longer, an esophageal or gastric leak should be considered; in these cases a CT scan and hydrosoluble contrast X-ray investigation should be done. A transient slight elevation of white blood cells count may occur in the 24 h after the intervention.

Patients must follow a liquid diet for the first two weeks and a soft diet for the next four weeks. They are also asked to refrain from vigorous exercise for four weeks. PPIs can be discontinued seven days after the procedure.

COMPLICATIONS

The overall complication rates reported so far for TIF with the EsophyX® device range from 3% to 10%. Major complications arose rarely and were bleeding, mucosal tears or perforation requiring endoscopic intervention or surgery, pneumothorax, and mediastinal abscesses. Bleeding requiring transfusions has been reported in about 3%-5% of cases. Mediastinal abscesses have been reported in less than 2% of cases. No procedure-related deaths have occurred.

Among the two studies so far published on TIF with the MUSE™ system, only one reported complications[28]. Minor side effects such as chest pain, sore throat, transient atelectasia, shoulder pain and belching were reported by 5.5% to 22% of patients. Major complications occurred in 6.2% of cases (4 out of 64 patients): Pneumothorax, pneumothorax and esophageal leak, pneumomediastinum, and severe bleeding. Patients with pneumothorax and esophageal leak and with bleeding required intervention. All major complications occurred in the first 24 patients.

No late complications or lasting side effects have occurred with either TIF technique.

OUTCOMES

To date, 21 prospective studies (3 randomized, controlled) and one retrospective study have been published for TIF using Esophyx® device. Most studies

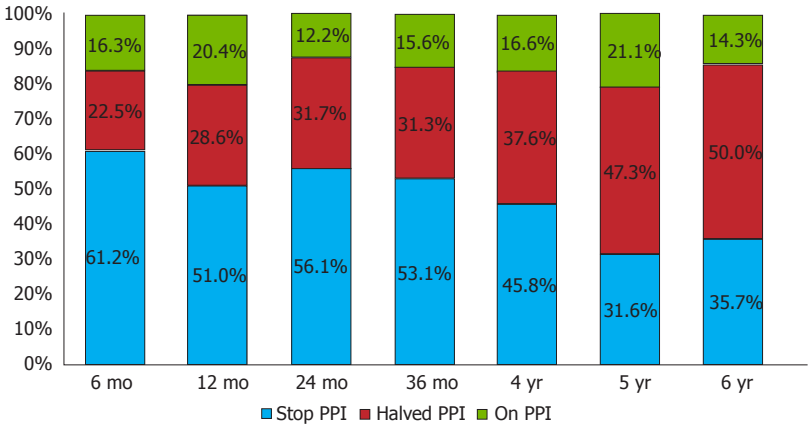

Figure 8 Symptomatic responses six months and 1-6 years after transoral incisionless fundoplication with Esophyx® device, classified according to proton pump inhibitor use. Patients were grouped as complete responders [who completely stopped using proton pump inhibitor (PPI)] or partial responders (who halved the previous PPI dose) and non-responders (who still used the pre-TIF PPI dose): 12 mo *vs* 6 mo after TIF $P = 0.8$; 24 mo *vs* 12 mo, $P = 0.4$; 36 mo *vs* 24 mo, $P = 0.7$; 4 years *vs* 36 mo, $P = 1.0$; 5 years *vs* 4 years, $P = 1.0$; 6 years *vs* 5 years, $P = 1.0$.

were observational and carried out in limited series, with one to three years follow-up. One study reported outcomes up to six years after the procedure. Sixteen studies assessed symptoms using the GERD health-related quality of life (HRQL) questions; 11 assessed pre- and post-procedure pH ± impedance recordings. A multicenter prospective study compared the efficacy of TIF *vs* omeprazole in a randomized controlled trial.

In all, 16 studies found TIF enabled patients to discontinue anti-reflux medications or markedly reduce their doses; four voiced concerns about the effectiveness of the procedure. In successful studies, 6- and 12-mo outcomes after TIF showed that 75%-93% and 72%-85% of patients had either discontinued PPI or halved the dose. Normalization of esophageal acid exposure, in terms of total acidic refluxes, number of refluxates, and De Meester score was reported in 37%-89% of patients. By 24 mo after TIF, daily high-dosage PPI dependence had been eliminated in 75%-93%[8,21,22].

Endoscopic findings comparing fundoplication immediately after the procedure and two years later are reported in Figure 7. In the two series reporting three-year outcomes lasting discontinuation of daily PPI ranged from 74%-84% of cases[22,24].

In the only study that followed patients for six years after TIF (14 out of 50), high-dosage PPI dependence was eliminated in 86% and approximately half completely stopped PPI. Unsuccessful outcomes mainly occurred between 6 and 12 mo after the intervention; results did not change substantially between 12 and 36 mo. The six-year results were similar to those at 36 mo[24], providing evidence of the lasting efficacy of TIF (Figure 8).

These findings show that the patient selection is determinant to achieve clinical success and confirm that failures occur within the first 6-12 mo after the procedure in most patients.

The operator's experience is also important in the outcomes. All TIF failures in our series were in patients who underwent the procedure early in the operator's learning curve. A retrospective study in 124 unselected patients in two community hospitals, reported respectively 75% and 80% of patients free of GER symptoms over a mean follow-up of seven months, confirming that operator's experience markedly affects outcomes[20].

Only three prospective randomized controlled trials have been published so far. Two compared the six-month efficacy of TIF or omeprazole: One found TIF more effective than PPI in treating regurgitation and extra-esophageal symptoms (97% *vs* 50% of patients, respectively, $P = 0.006$)[26]; in the second one intention-to-treat analysis indicated TIF was more effective than PPI in eliminating GERD symptoms (67% *vs* 45%, $P = 0.023$)[27]. These discrepancies require additional randomized studies to clarify the efficacy of TIF in treating GERD. The third study compared 3- and 12-mo results of TIF and Nissen fundoplication, showing TIF as effective and safe as the Nissen method but with significantly shorter hospital stays (2.9 ± 0.8 d *vs* 6.4 ± 0.7 d, $P < 0.0001$)[31]. Symptomatic responses up to six years after TIF with EsophyX® device, in terms of PPI abolition or 50% reduction, in published series (20 studies) are reported in Table 1. Outcomes up to five years after TIF by the MUSE™ system, as regards the effects on PPI use, in published series (two studies) are reported in Table 2.

Unsuccessful outcomes after TIF were reported in three studies. Two series found worsening of distal esophageal acid exposure in 66.7% of cases and persistent GER symptoms in 68% of cases, in small series with a short follow-up (12 mo)[12,13]. A trial comparing TIF with Nissen fundoplication in PPI-refractory GERD patients reported symptom remission and normalization of gastro-esophageal acid reflux in 30% and 100% of patients after TIF and 50% and 100% after surgical fundoplication[16]. These data suggest that in patients unresponsive to PPIs Nissen fundoplication seems more effective than TIF by

Table 1 Symptomatic responses after transoral incisionless fundoplication with EsophyX® device

Ref.	6 mo	12 mo	24 mo	36 mo	6 yr
Cadière *et al*[6], 2008	-	85%	-	-	-
Cadière *et al*[8], 2009	-	-	93%	-	-
Testoni *et al*[9], 2010	82%	76%	-	-	-
Velanovich *et al*[11], 2010	79%	-	-	-	-
Repici *et al*[12], 2010	55%	47%	-	-	-
Demyttenaere *et al*[10], 2010	-	53%	-	-	-
Hoppo *et al*[13], 2010	-	42%	-	-	-
Barnes *et al*[20], 2011	93%	-	-	-	-
Bell *et al*[14], 2011	75%	-	-	-	-
Ihde *et al*[15], 2011	76%	-	-	-	-
Trad *et al*[18], 2012	-	82%	-	-	-
Testoni *et al*[21], 2012	-	-	75%	75%	-
Petersen *et al*[17], 2012	58%	-	-	-	-
Bell *et al*[23], 2012	86%	-	-	-	-
Muls *et al*[22]2013	-	77%	-	65%	-
Bell *et al*[34], 2013	-	82%	-	-	-
Bell *et al*[25], 2014	-	-	77%-80%	-	-
Trad *et al*[26], 2015	93%	-	-	-	-
Hunter *et al*[27], 2015	-	72%	-	-	-
Testoni *et al*[24], 2015	84%	80%	88%	84%	86%

Table 2 Symptomatic responses after transoral incisionless fundoplication by the MUSE™ system

Ref.	6 mo	12 mo	24 mo	36 mo	6 yr
Zacheri *et al*[28], 2015	83%	-	-	-	-
Roy-Shapira *et al*[29], 2015	-	82%	73%	73%	-

EsophyX®.

When TIF fails surgical fundoplication is still feasible, with no technical difficulties or increased morbidity. Surgical revision after TIF failure was reported in 8.1%-18.0% of cases[21,22,32,33]. In two studies (9 and 11 patients) Nissen fundoplication achieved the complete disappearance of symptoms in all cases of TIF failure[32,33]. In our series, however, only one of the four patients who required Nissen fundoplication for persisting GERD symptoms after TIF stopped using acid-suppressive therapy[21]. This may depend on the fact that the patients who underwent TIF in our series had only mild impairment of the gastro-esophageal junction and suffered from GERD-related symptoms that could derive from several mechanisms, including increased esophageal sensitivity to refluxate.

On the other hand, re-intervention after laparoscopic fundoplication has been reported in up to 14% of cases[1] and TIF has been found effective after failed surgery[34].

Only two studies so far have reported outcomes after TIF with the MUSE™ technique (anterior fundoplication): A pilot study with a five-year follow-up and a multicenter prospective study. The pilot study examined GERD-related symptoms and PPI use up to five years after the procedure in 13 patients: The GERD-related symptom score returned to normal in 92% of cases, PPI use was stopped or halved in 77% (54% stopped PPI completely)[29].

Another study reported outcomes after TIF using the MUSE technique in a multicenter, prospective international trial enrolling 66 patients with a six-month follow-up[28]. GERD-related symptoms scores improved by more than 50% in 73% of patients and 64.6% were no longer taking daily PPIs. Among patients who continued to take PPI, 56.5% cut the dose by more than half. At 24-h pH-recording the total time with esophageal pH < 4.0 dropped significantly from baseline. There were none of the post-procedure side effects commonly seen after laparoscopic fundoplication such as gas bloating, inability to belch or vomit, dysphagia or diarrhea.

Factors affecting TIF outcomes

An important issue regarding all new interventional procedures introduced in clinical practice is the recognition of technique- or patient-related factors that can affect the outcomes. Factors affecting TIF outcomes have been reported to date only in EsophyX studies.

In our series, from the technical point of view, the number of fasteners deployed and the rotational technique were associated with a better outcome; a larger number of fasteners increased by four folds the success rate[21]. Another study too reported that the number of fasteners plays a key role for the success of the procedure[19]. The rotational technique increased by half the probability of being a responder, according with other reports[19,23].

Patient-related factors affecting post-operative outcomes in our series were pre-operative Hill grades Ⅲ and Ⅳ, hiatal hernia larger than 2 cm, and ineffective esophageal motility, which were associated with a higher rate of unsuccessful results. An impaired esophageal clearance may induce epithelial sensitization and reflux-related symptoms, even in presence of a low-volume reflux[35].

Univariate and multivariate analysis of preoperative factors influencing symptomatic outcomes of TIF with EsophyX® device was done on data from 158 consecutive patients identified[25]. Predictors of successful outcomes for patients with typical symptoms was age 50 years or more, GERD health-related quality of life score (GERD-HRQL) on PPIs 15 or more, a reflux symptom index > 13 on PPIs, and the gastroesophageal reflux symptom score 18 or more on PPIs. Age and GERD-HRLQ remained significant predictors also in multivariate analysis. For patients with atypical GER symptoms only a GERD-HRQL score 15 or more on PPIs was associated with successful outcomes.

CONCLUSION

In the last few years TIF has only been done in clinical trials enrolling patients with typical gastro-esophageal reflux symptoms responsive or partially responsive to PPI therapy, without or with only small hiatal

hernia (< 3 cm), who refused long-term medication, or were intolerant to PPIs, or required high doses of antisecretory maintenance therapy. Patients with grade C and D esophagitis, according to Los Angeles classification, and Barrett's esophagus were excluded from these studies. The majority of studies used the EsophyX® device, which was effective in the short term in approximately 75% of patients, eliminating their daily dependence on PPIs in half the PPI-responsive GERD patients and markedly reducing the overall dose in the other cases. Similar results were obtained more recently for TIF with the MEDIGUS endostapler, but there are only a few studies.

These results were confirmed in the few studies with follow-up up to three years, and in the single study with up to six years follow-up. None of these reported any troublesome procedure-related persisting side-effects.

Overall outcomes showed that the TIF procedure can be an effective and safe alternative therapeutic option to surgery in selected patients, like those recruited in the published studies. In the series with three- to six-year follow-up, TIF resulted slightly inferior to Nissen fundoplication, but similar to partial posterior (Toupet) or anterior (Dor-Thal) fundoplication[36,37], without surgery-related side effects.

Currently, on the basis of the clinical results, TIF may be offered as an alternative to surgery in patients suffering from gastro-esophageal reflux disease and grade A-B esophagitis, if present, with the sole limitation of the length and reducibility of any hiatal hernia, which at present is the only limiting factor. TIF may also be offered to patients who have some risk of persistent post-surgical side effects. To date, data supporting the efficacy of TIF in the treatment of severe grades of esophagitis or symptoms associated with oro-pharingeal reflux are lacking.

However, as for any new intervention, despite the encouraging short- and medium-term outcomes, the long-term efficacy of TIF needs to be further assessed, mainly for the MUSE™ technique. Therefore, randomized controlled trials are now needed to establish the role of TIF in the management of GERD, and whether one or other of the two techniques is likely to be more effective and safe. Preoperative anatomical and functional findings and technical procedural aspects that will help select patients and predict a successful outcome still need to be identified, too.

REFERENCES

1 **Lundell L**, Miettinen P, Myrvold HE, Pedersen SA, Liedman B, Hatlebakk JG, Julkonen R, Levander K, Carlsson J, Lamm M, Wiklund I. Continued (5-year) followup of a randomized clinical study comparing antireflux surgery and omeprazole in gastroesophageal reflux disease. *J Am Coll Surg* 2001; **192**: 172-179; discussion 179-181 [PMID: 11220717]

2 **Draaisma WA**, Rijnhart-de Jong HG, Broeders IA, Smout AJ, Furnee EJ, Gooszen HG. Five-year subjective and objective results of laparoscopic and conventional Nissen fundoplication: a randomized trial. *Ann Surg* 2006; **244**: 34-41 [PMID: 16794387]

3 **Smith CD**. Surgical therapy for gastroesophageal reflux disease: indications, evaluation, and procedures. *Gastrointest Endosc Clin N Am* 2009; **19**: 35-48, v-vi [PMID: 19232279 DOI: 10.1016/j.giec.2008.12.007]

4 **Broeders JA**, Draaisma WA, Bredenoord AJ, Smout AJ, Broeders IA, Gooszen HG. Impact of symptom-reflux association analysis on long-term outcome after Nissen fundoplication. *Br J Surg* 2011; **98**: 247-254 [PMID: 20960456 DOI: 10.1002/bjs.7296]

5 **Bowrey DJ**, Blom D, Crookes PF, Bremner CG, Johansson JL, Lord RV, Hagen JA, DeMeester SR, DeMeester TR, Peters JH. Risk factors and the prevalence of trocar site herniation after laparoscopic fundoplication. *Surg Endosc* 2001; **15**: 663-666 [PMID: 11591964]

6 **Cadière GB**, Buset M, Muls V, Rajan A, Rösch T, Eckardt AJ, Weerts J, Bastens B, Costamagna G, Marchese M, Louis H, Mana F, Sermon F, Gawlicka AK, Daniel MA, Devière J. Antireflux transoral incisionless fundoplication using EsophyX: 12-month results of a prospective multicenter study. *World J Surg* 2008; **32**: 1676-1688 [PMID: 18443855 DOI: 10.1007/s00268-008-9594-9]

7 **Bergman S**, Mikami DJ, Hazey JW, Roland JC, Dettorre R, Melvin WS. Endolumenal fundoplication with EsophyX: the initial North American experience. *Surg Innov* 2008; **15**: 166-170 [PMID: 18757375 DOI: 10.1177/1553350608321588]

8 **Cadière GB**, Van Sante N, Graves JE, Gawlicka AK, Rajan A. Two-year results of a feasibility study on antireflux transoral incisionless fundoplication using EsophyX. *Surg Endosc* 2009; **23**: 957-964 [PMID: 19288158 DOI: 10.1007/s00464-009-0384-8]

9 **Testoni PA**, Corsetti M, Di Pietro S, Castellaneta AG, Vailati C, Masci E, Passaretti S. Effect of transoral incisionless fundoplication on symptoms, PPI use, and ph-impedance refluxes of GERD patients. *World J Surg* 2010; **34**: 750-757 [PMID: 20091308 DOI: 10.1007/s00268-010-0394-7]

10 **Demyttenaere SV**, Bergman S, Pham T, Anderson J, Dettorre R, Melvin WS, Mikami DJ. Transoral incisionless fundoplication for gastroesophageal reflux disease in an unselected patient population. *Surg Endosc* 2010; **24**: 854-858 [PMID: 19730949 DOI: 10.1007/s00464-009-0676-z]

11 **Velanovich V**. Re: "Endoscopic, endoluminal fundoplication for gastroesophageal reflux disease: initial experience and lessons learned" by Vic Velanovich, MD, Surgery 2010; 148: 646-653. *Surgery* 2011; **149**: 595 [PMID: 21406271 DOI: 10.1016/j.surg.2010.12.009]

12 **Repici A**, Fumagalli U, Malesci A, Barbera R, Gambaro C, Rosati R. Endoluminal fundoplication (ELF) for GERD using EsophyX: a 12-month follow-up in a single-center experience. *J Gastrointest Surg* 2010; **14**: 1-6 [PMID: 19902310 DOI: 10.1007/s11605-009-1077-2]

13 **Hoppo T**, Immanuel A, Schuchert M, Dubrava Z, Smith A, Nottle P, Watson DI, Jobe BA. Transoral incisionless fundoplication 2.0 procedure using EsophyX™ for gastroesophageal reflux disease. *J Gastrointest Surg* 2010; **14**: 1895-1901 [PMID: 20878257 DOI: 10.1007/s11605-010-1331-7]

14 **Bell RC**, Freeman KD. Clinical and pH-metric outcomes of transoral esophagogastric fundoplication for the treatment of gastroesophageal reflux disease. *Surg Endosc* 2011; **25**: 1975-1984 [PMID: 21140170 DOI: 10.1007/s00464-010-1497-9]

15 **Ihde GM**, Besancon K, Deljkich E. Short-term safety and symptomatic outcomes of transoral incisionless fundoplication with or without hiatal hernia repair in patients with chronic gastroesophageal reflux disease. *Am J Surg* 2011; **202**: 740-746; discussion 746-747 [PMID: 22014853 DOI: 10.1016/j.amjsurg.2011.06.035]

16 **Frazzoni M**, Conigliaro R, Manta R, Melotti G. Reflux parameters as modified by EsophyX or laparoscopic fundoplication in refractory GERD. *Aliment Pharmacol Ther* 2011; **34**: 67-75 [PMID: 21539587 DOI: 10.1111/j.1365-2036.2011.04677.x]

17 **Petersen RP**, Filippa L, Wassenaar EB, Martin AV, Tatum R, Oelschlager BK. Comprehensive evaluation of endoscopic fundoplication using the EsophyX™ device. *Surg Endosc* 2012; **26**: 1021-1027 [PMID: 22042587 DOI: 10.1007/s00464-011-1989-2]

18 **Trad KS**, Turgeon DG, Deljkich E. Long-term outcomes after transoral incisionless fundoplication in patients with GERD and

LPR symptoms. *Surg Endosc* 2012; **26**: 650-660 [PMID: 21959689 DOI: 10.1007/s00464-011-1932-6]
19 **Bell RC**, Cadière GB. Transoral rotational esophagogastric fundoplication: technical, anatomical, and safety considerations. *Surg Endosc* 2011; **25**: 2387-2399 [PMID: 21184101 DOI: 10.1007/s00464-010-1528-6]
20 **Barnes WE**, Hoddinott KM, Mundy S, Williams M. Transoral incisionless fundoplication offers high patient satisfaction and relief of therapy-resistant typical and atypical symptoms of GERD in community practice. *Surg Innov* 2011; **18**: 119-129 [PMID: 21307014 DOI: 10.1177/1553350610392067]
21 **Testoni PA**, Vailati C, Testoni S, Corsetti M. Transoral incisionless fundoplication (TIF 2.0) with EsophyX for gastroesophageal reflux disease: long-term results and findings affecting outcome. *Surg Endosc* 2012; **26**: 1425-1435 [PMID: 22170317 DOI: 10.1007/s00464-011-2050-1]
22 **Muls V**, Eckardt AJ, Marchese M, Bastens B, Buset M, Devière J, Louis H, Rajan A, Daniel MA, Costamagna G. Three-year results of a multicenter prospective study of transoral incisionless fundoplication. *Surg Innov* 2013; **20**: 321-330 [PMID: 22968006 DOI: 10.1177/1553350612459275]
23 **Bell RC**, Mavrelis PG, Barnes WE, Dargis D, Carter BJ, Hoddinott KM, Sewell RW, Trad KS, DaCosta Gill B, Ihde GM. A prospective multicenter registry of patients with chronic gastroesophageal reflux disease receiving transoral incisionless fundoplication. *J Am Coll Surg* 2012; **215**: 794-809 [PMID: 22939637 DOI: 10.1016/j.jamcollsurg.2012.07.014]
24 **Testoni PA**, Testoni S, Mazzoleni G, Vailati C, Passaretti S. Long-term efficacy of transoral incisionless fundoplication with Esophyx (Tif 2.0) and factors affecting outcomes in GERD patients followed for up to 6 years: a prospective single-center study. *Surg Endosc* 2015; **29**: 2770-2780 [PMID: 25480624 DOI: 10.1007/s00464-014-4008-6]
25 **Bell RC**, Fox MA, Barnes WE, Mavrelis PG, Sewell RW, Carter BJ, Ihde GM, Trad KS, Dargis D, Hoddinott KM, Freeman KD, Gunsberger T, Hausmann MG, Gill BD, Wilson E. Univariate and multivariate analyses of preoperative factors influencing symptomatic outcomes of transoral fundoplication. *Surg Endosc* 2014; **28**: 2949-2958 [PMID: 24879134 DOI: 10.1007/s00464-014-3557-z]
26 **Trad KS**, Barnes WE, Simoni G, Shughoury AB, Mavrelis PG, Raza M, Heise JA, Turgeon DG, Fox MA. Transoral incisionless fundoplication effective in eliminating GERD symptoms in partial responders to proton pump inhibitor therapy at 6 months: the TEMPO Randomized Clinical Trial. *Surg Innov* 2015; **22**: 26-40 [PMID: 24756976 DOI: 10.1177/1553350614526788]
27 **Hunter JG**, Kahrilas PJ, Bell RC, Wilson EB, Trad KS, Dolan JP, Perry KA, Oelschlager BK, Soper NJ, Snyder BE, Burch MA, Melvin WS, Reavis KM, Turgeon DG, Hungness ES, Diggs BS. Efficacy of transoral fundoplication vs omeprazole for treatment of regurgitation in a randomized controlled trial. *Gastroenterology* 2015; **148**: 324-333.e5 [PMID: 25448925 DOI: 10.1053/j.gastro.2014.10.009]
28 **Zacherl J**, Roy-Shapira A, Bonavina L, Bapaye A, Kiesslich R, Schoppmann SF, Kessler WR, Selzer DJ, Broderick RC, Lehman GA, Horgan S. Endoscopic anterior fundoplication with the Medigus Ultrasonic Surgical Endostapler (MUSE™) for gastroesophageal reflux disease: 6-month results from a multi-center prospective trial. *Surg Endosc* 2015; **29**: 220-229 [PMID: 25135443 DOI: 10.1007/s00464-014-3731-3]
29 **Roy-Shapira A**, Bapaye A, Date S, Pujari R, Dorwat S. Trans-oral anterior fundoplication: 5-year follow-up of pilot study. *Surg Endosc* 2015; **29**: 3717-3721 [PMID: 25783833]
30 **Kauer WK**, Roy-Shapira A, Watson D, Sonnenschein M, Sonnenschein E, Unger J, Voget M, Stein HJ. Preclinical trial of a modified gastroscope that performs a true anterior fundoplication for the endoluminal treatment of gastroesophageal reflux disease. *Surg Endosc* 2009; **23**: 2728-2731 [PMID: 19357915 DOI: 10.1007/s00464-009-0479-2]
31 **Svoboda P**, Kantorová I, Kozumplík L, Scheer P, Radvan M, Radvanová J, Krass V, Horálek F. Our experience with transoral incisionless plication of gastroesophageal reflux disease: NOTES procedure. *Hepatogastroenterology* 2011; **58**: 1208-1213 [PMID: 21937380 DOI: 10.5754/hge10108]
32 **Fumagalli Romario U**, Barbera R, Repici A, Porta M, Malesci A, Rosati R. Nissen fundoplication after failure of endoluminal fundoplication: short-term results. *J Gastrointest Surg* 2011; **15**: 439-443 [PMID: 21207179 DOI: 10.1007/s11605-010-1409-2]
33 **Furnée EJ**, Broeders JA, Draaisma WA, Schwartz MP, Hazebroek EJ, Smout AJ, van Rijn PJ, Broeders IA. Laparoscopic Nissen fundoplication after failed EsophyX fundoplication. *Br J Surg* 2010; **97**: 1051-1055 [PMID: 20632271 DOI: 10.1002/bjs.7078]
34 **Bell RC**, Hufford RJ, Fearon J, Freeman KD. Revision of failed traditional fundoplication using EsophyX transoral fundoplication. *Surg Endosc* 2013; **27**: 761-767 [PMID: 23052519 DOI: 10.1007/s00464-012-2542-7]
35 **Kim KY**, Kim GH, Kim DU, Wang SG, Lee BJ, Lee JC, Park DY, Song GA. Is ineffective esophageal motility associated with gastropharyngeal reflux disease? *World J Gastroenterol* 2008; **14**: 6030-6035 [PMID: 18932282 DOI: 10.3748/wjg.14.6030]
36 **Nijjar RS**, Watson DI, Jamieson GG, Archer S, Bessell JR, Booth M, Cade R, Cullingford GL, Devitt PG, Fletcher DR, Hurley J, Kiroff G, Martin IJ, Nathanson LK, Windsor JA. Five-year follow-up of a multicenter, double-blind randomized clinical trial of laparoscopic Nissen vs anterior 90 degrees partial fundoplication. *Arch Surg* 2010; **145**: 552-557 [PMID: 20566975 DOI: 10.1001/archsurg.2010.81]
37 **Broeders JA**, Mauritz FA, Ahmed Ali U, Draaisma WA, Ruurda JP, Gooszen HG, Smout AJ, Broeders IA, Hazebroek EJ. Systematic review and meta-analysis of laparoscopic Nissen (posterior total) versus Toupet (posterior partial) fundoplication for gastro-oesophageal reflux disease. *Br J Surg* 2010; **97**: 1318-1330 [PMID: 20641062 DOI: 10.1002/bjs.7174]

P- Reviewer: Dehghani SM, Nagaya M, Teramoto-Matsubara OT
S- Editor: Qi Y **L- Editor**: A **E- Editor**: Wu HL

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i2.190

World J Gastrointest Pharmacol Ther 2016 May 6; 7(2): 190-206
ISSN 2150-5349 (online)

REVIEW

Barrett's esophagus in 2016: From pathophysiology to treatment

Irene Martinucci, Nicola de Bortoli, Salvatore Russo, Lorenzo Bertani, Manuele Furnari, Anna Mokrowiecka, Ewa Malecka-Panas, Vincenzo Savarino, Edoardo Savarino, Santino Marchi

Irene Martinucci, Nicola de Bortoli, Salvatore Russo, Lorenzo Bertani, Santino Marchi, Division of Gastroenterology, University of Pisa, 56124 Pisa, Italy

Manuele Furnari, Vincenzo Savarino, Division of Gastroenterology, University of Genoa, 16100 Genoa, Italy

Anna Mokrowiecka, Ewa Malecka-Panas, Department of Digestive Tract Diseases, Medical University of Lodz, 90-127 Lodz, Poland

Edoardo Savarino, Division of Gastroenterology, University of Padua, 35100 Padua, Italy

Author contributions: Martinucci I and de Bortoli N contributed to this paper by conceiving of and designing the study; Martinucci I, de Bortoli N and Savarino E reviewed and analyzed the literature; Martinucci I, de Bortoli N, Russo S, Bertani L, Furnari M, Mokrowiecka A and Malecka-Panas E drafted the paper; Savarino E, Savarino V and Marchi S performed the critical revision and editing of the manuscript; all authors approved the final version of the study.

Conflict-of-interest statement: The authors declare no potential conflicts of interest and no financial support.

Correspondence to: Irene Martinucci, MD, Division of Gastroenterology, University of Pisa, Via Paradisa 2, 56124 Pisa, Italy. martinucci.irene@gmail.com
Telephone: +39-050-997395
Fax: +39-050-997436

Received: July 30, 2015
Peer-review started: July 31, 2015
First decision: September 16, 2015
Revised: January 20, 2016
Accepted: March 17, 2016
Article in press: March 18, 2016
Published online: May 6, 2016

Abstract

Esophageal complications caused by gastroesophageal reflux disease (GERD) include reflux esophagitis and Barrett's esophagus (BE). BE is a premalignant condition with an increased risk of developing esophageal adenocarcinoma (EAC). The carcinogenic sequence may progress through several steps, from normal esophageal mucosa through BE to EAC. A recent advent of functional esophageal testing (particularly multichannel intraluminal impedance and pH monitoring) has helped to improve our knowledge about GERD pathophysiology, including its complications. Those findings (when properly confirmed) might help to predict BE neoplastic progression. Over the last few decades, the incidence of EAC has continued to rise in Western populations. However, only a minority of BE patients develop EAC, opening the debate regarding the cost-effectiveness of current screening/surveillance strategies. Thus, major efforts in clinical and research practice are focused on new methods for optimal risk assessment that can stratify BE patients at low or high risk of developing EAC, which should improve the cost effectiveness of screening/surveillance programs and consequently significantly affect health-care costs. Furthermore, the area of BE therapeutic management is rapidly evolving. Endoscopic eradication therapies have been shown to be effective, and new therapeutic options for BE and EAC have emerged. The aim of the present review article is to highlight the status of screening/surveillance programs and the current progress of BE therapy. Moreover, we discuss the recent introduction of novel esophageal pathophysiological exams that have improved the knowledge of the mechanisms linking

GERD to BE.

Key words: Gastroesophageal reflux disease; Barrett's esophagus; Esophageal adenocarcinoma; Impedance and pH monitoring; Endoscopy

Core tip: The review highlights the significant progress in the diagnostic and therapeutic management of Barrett's esophagus (BE) thanks to the development of up-and-coming endoscopic technologies. Moreover, we discuss the recent introduction of novel esophageal pathophysiological exams that have improved the knowledge of the mechanisms implicated in the genesis of esophageal mucosal damage, paving the way to the future possibility of predicting BE neoplastic progression. The comparison of endoscopic surveillance and eradication therapy recommendations for BE in currently available guidelines are provided.

Martinucci I, de Bortoli N, Russo S, Bertani L, Furnari M, Mokrowiecka A, Malecka-Panas E, Savarino V, Savarino E, Marchi S. Barrett's esophagus in 2016: From pathophysiology to treatment. *World J Gastrointest Pharmacol Ther* 2016; 7(2): 190-206 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i2/190.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i2.190

INTRODUCTION

Gastroesophageal reflux disease (GERD) is defined as a condition that develops when the reflux of stomach contents causes troublesome symptoms and/or complications[1]. Esophageal complications caused by GERD include reflux esophagitis and Barrett's esophagus (BE), and the latter predisposes patients to esophageal adenocarcinoma (EAC)[1]. BE is a premalignant condition in which the normal stratified squamous epithelium of the distal esophagus is replaced by columnar mucosa with intestinal specialized metaplasia[2].

GERD is a worldwide disease, and evidence suggests an overall increase in its prevalence since 1995[3,4]. As a result, costs related to GERD diagnosis, treatment and surveillance represent a substantial commitment of economic resources[5]. In parallel, over the last few decades, the incidence of EAC has continued to rise in Western populations[6-8]. The totality of evidence supports the idea that the different racial, ethnic and gender distributions of BE may drive the risk of EAC, with incidence rates much higher among male, non-Hispanic whites[7,9]. However, fewer than 10% of GERD patients are likely to progress to a diagnosis of BE at 5 years[10], and only a minority of BE patients develop EAC; the previously estimated risk of 0.5% per year[11-13] was recently lowered to approximately 0.3% per year[14,15]. Furthermore, more than 90% of EAC patients are not known to have BE before diagnosis[16]. In line with these assumptions, the current strategies of BE screening and surveillance programs are debated and show moderate to absent cost-effectiveness[17,18].

The aim of the present review article is to highlight the status of screening/surveillance programs and the current progress of BE therapy. Moreover, we discuss the recent introduction of novel esophageal pathophysiological exams that have improved the knowledge of the mechanisms linking GERD to BE.

ESOPHAGEAL PATHOPHYSIOLOGICAL EXAMS

The overall characteristics and composition of the refluxate, together along with the dysfunction of the anti-reflux barrier, the impairment of mucosal defence, visceral motility and esophageal clearance, represent the complex set of mechanisms that determines GERD manifestation and its complications[19]. To date, it is well known that the refluxate may contain varying concentrations of acid, pepsin, or duodenal contents (*i.e.*, bile acid, pancreatic enzymes) implicated in the development of esophageal mucosal damage[20-22]. In keeping with the spectrum model of GERD, several studies have demonstrated that severity of acid reflux increases from non-erosive reflux disease (NERD) through erosive reflux disease (ERD) up to short (*i.e.*, esophageal intestinal metaplasia up to 3 cm in length, SSBE) and long segments (*i.e.*, esophageal intestinal metaplasia more than 3 cm in length, LSBE) of BE[23-25]. Similarly, the presence of duodenogastroesophageal reflux (DGER), evaluated with a fibreoptic spectrophotometer (Bilitec), increases significantly across the spectrum of GERD from NERD to BE[26-29]. Of note, it has been established that acid and DGER occur simultaneously in the majority of the reflux episodes, and at best, bile reflux may have a synergistic role in producing esophageal damage[26,27].

Over the past decade, the introduction of new technologies with which to study the esophagus from a functional point of view has helped improve our knowledge of GERD pathophysiology. The combination of multichannel intraluminal impedance and pH monitoring (MII-pH) provides a comprehensive characterization of reflux episodes during a 24-h period, detecting both chemical (*i.e.*, acid, weakly acidic or weakly alkaline) and physical properties (*i.e.*, liquid, mixed, gas, proximal extension)[30]. Regarding SSBE, by means of monitoring only pH, the acid exposure time (AET) may be similar to that found in NERD and normal in several patients[31]. Therefore, Frazzoni *et al*[32] assessed reflux parameters *via* a combined MII-pH study in newly diagnosed SSBE, at baseline and during proton pump inhibitor (PPI) therapy. The authors found that MII-pH improved the overall diagnostic yield because the number of reflux episodes was altered in more than one half of patients with normal AET off PPI.

Moreover, 69% of SSBE patients on PPI therapy showed an increased number of total reflux events, the vast majority of which were weakly acidic refluxes[32]. These findings are consistent with other studies in which the number of both acid and weakly acidic reflux episodes was increased in patients with BE[33,34]. In particular, Savarino *et al*[34] highlighted that the greater total exposure of esophageal mucosa to acid and weakly acidic reflux was due to intermittent reflux episodes. Indeed, the authors found a higher frequency of "re-reflux" episodes in BE than in ERD patients[34]. "Re-reflux" episodes (*i.e.*, the occurrence of a further reflux when the basal esophageal pH is already below 4) represent a diagnostic advantage obtained through MII-pH because pH-only monitoring equipment has a lower sampling frequency[35,36]. Moreover, intermittent reflux episodes determining a brief exposure of acid or bile might be more important than continuous exposure concerning the genesis of the overall alterations promoting the progression of BE[37,38]. With regard to the role of weakly acidic refluxes, it is important to realize that in an environment at a pH between 4 and 5.5, pepsins and bile acids can still damage esophageal mucosa[39,40]. Given that the main consequence of PPI therapy is to convert acid refluxes into weakly acidic refluxes without significant changes in the number of total reflux events[41], a regression of intestinal metaplasia with long-term PPI therapy is somewhat doubtful. At last, Bredenoord *et al*[33] found that in patients with BE, only a few reflux episodes reached the proximal esophagus that seems to be more sensitive, likely explaining, at least in part, why these subjects often report fewer symptoms then NERD patients[42,43].

The recent introduction in the clinical and research practice of high-resolution manometry (HRM) has represented a major advance in characterizing esophageal motility abnormalities in GERD patients, with particular regard for dysfunction of the antireflux barrier and impaired esophageal clearance[44,45]. However, at present, the role of HRM in reflux remains restricted to preoperative testing, the identification of possible mechanisms and the exclusion of motility disorders[45]. Of note, several studies have shown that esophageal motility abnormalities are increasingly prevalent with increasing severity of GERD presentation[25,46-49]. In particular, Savarino *et al*[50] evaluated 755 GERD patients through conventional or impedance esophageal manometry and/or MII-pH testing, and they found that ineffective esophageal motility gradually increased from controls and functional heartburn to NERD and from ERD to BE. Likewise, the esophageal clearing function decreased as the severity of mucosal damage increased, with ERD and BE patients having the greatest prevalence of bolus transit abnormalities, which occurred also in cases of normal motility pattern[50].

Finally, a recent study by Frazzoni *et al*[51] assessed that neoplastic progression in SSBE was associated with an impairment of esophageal chemical clearance. Impedance can be used to measure the clearance of a swallowed bolus from the esophagus[52], and a parameter representing esophageal chemical clearance, named the post-reflux swallow-induced peristaltic wave (PSPW) index, can be obtained through MII-pH monitoring[53]. The impairment of chemical clearance represents a crucial mechanism in the pathophysiology of GERD and is not affected by medical or surgical therapy. In fact, the PSPW index has increased the diagnostic yield of MII-pH in GERD patients[54,55]. In this setting, Frazzoni *et al*[51] showed that the PSPW index was lower in SSBE patients with incident dysplasia than in those without it, and a PSPW index cut-off value of 26% was able to discriminate between these two groups of patients. Overall, the authors speculated that predicting neoplastic progression in SSBE based on a low PSPW index might be useful to select those patients deserving a close endoscopic follow-up, thus improving the cost-effectiveness of surveillance programs[51].

DIAGNOSTIC ROLE OF UPPER ENDOSCOPY

To date, the gold standard for the evaluation of BE is high-resolution white-light endoscopy with biopsy sampling performed according to the Seattle protocol[56-59]. The Prague classification represents a reliable and validated endoscopic classification of BE, which records the length of the esophagus involved circumferentially (C) in addition to the maximal length (M) involved at any point[60].

The development of EAC in BE seems to occur through the progression of intestinal metaplasia to low-grade dysplasia (LGD) and high-grade dysplasia (HGD). Thus, the presence of dysplasia represents the most widely used marker of neoplastic progression in BE[61]. High-resolution endoscopes, allowing for a fine definition of the mucosal layer, seem to have high sensitivity for detecting dysplasia and BE-related early neoplasia[62]. Furthermore, a longer inspection time during white-light endoscopy seems to be associated with a higher detection rate of HGD/EAC[63].

Some studies have investigated the detection of intestinal metaplasia with chromoendoscopy. Available data regarding the improvement of methylene blue-targeted biopsy samples, compared with random samples, are conflicting[64-67]. Moreover, methylene blue may damage DNA, so its use is not recommended[68]. The only randomized trial that has evaluated indigo carmine for the detection of dysplasia in BE has not found a higher rate of dysplasia than high-resolution white-light endoscopy[69]. Regarding virtual chromoendoscopy, narrow band imaging (NBI) is the most extensively studied in BE[70]. A meta-analysis of eight studies reported a NBI sensitivity and specificity of 95% and 65%, respectively, for the diagnosis of intestinal metaplasia and of 96% and 94%, respectively, for the diagnosis of HGD[71]. However, the interobserver agreement for the interpretation of NBI images is

moderate, and on a per-patient basis, high-resolution endoscopy alone seems to be sufficient to maximize dysplasia detection[72,73].

Autofluorescence imaging alone has an excessively high false-positive rate of dysplasia detection[74]. Additionally, the use of endoscopic trimodal imaging (*i.e.*, high-resolution endoscopy, autofluorescence imaging and NBI), compared with standard endoscopy with random biopsy sampling, has shown contradictory results[75,76]. Regarding spectroscopy and optical coherence tomography, further studies are warranted to define their usefulness in BE surveillance[77,78]. Randomized crossover studies on the diagnostic yield of acetic acid-enhanced magnification endoscopy for BE intestinal metaplasia have produced contradictory data[79,80]. Using this technique, promising results have been obtained in dysplasia detection[81,82], and it also seems to be more cost-effective than the Seattle protocol in a high-risk population[83]. However, further studies are necessary to ascertain the utility of this technique.

Recently, the use of probe-based confocal laser endomicroscopy combined with high-definition white-light endoscopy significantly improved the ability to detect neoplasia in BE patients compared with high-definition white-light endoscopy[84,85].

Finally, molecular imaging, exploiting fluorescently labelled molecules that bind with a different affinity to dysplastic and non-dysplastic cells, is a promising technique[86,87]. In a recent study, using a novel peptide that binds to areas of HGD and neoplasia, Sturm *et al*[88] reported 75% sensitivity and 97% specificity for neoplasia.

Screening

Because the proportion of EAC patients with a prior diagnosis of BE is low, and given the low incidence of EAC in BE[15,89], performing a screening program for BE with endoscopy in an unselected population is not cost-effective. Currently, most medical societies suggest endoscopic screening for BE in patients with chronic GERD symptoms and multiple risk factors (*i.e.*, 50 years of age or older, white race, male gender, obesity, history of smoking, family history for BE or EAC)[58,59] or in men older than 60 years with reflux symptoms for 10 years[90].

New methods for BE screening are being evaluated with some promising results. Transnasal endoscopy is a well-tolerated method, and it seems to have good accuracy, but further validation is necessary[91]. Moreover, biopsy specimens taken with these endoscopes are small, which could increase sampling bias and hinder the interpretation[92].

Cytosponge is a non-endoscopic esophageal sampling device coupled with immunocytochemistry for trefoil factor 3, a marker of columnar epithelium with intestinal metaplasia[93,94]. In a study involving 504 patients, Kadri *et al*[93] reported a sensitivity and a specificity for the detection of BE of, respectively, 73% and 94%. This test also appears to be more cost-effective for screening than conventional endoscopy[95]. However, the Cytosponge needs further validation, particularly considering the lower sensitivity for SSBE detection.

Recently, a risk-prediction model including multiple demographic and clinical variables (*i.e.*, GERD frequency and duration, age, sex, race, waist-to-hip ratio, Helicobacter pylori status), serum levels of cytokines (IL12p70, IL6, IL8, IL10) and leptin obtained an area under the curve of 0.85, a better result than that achieved by other non-invasive methods[96].

Surveillance

Observational studies have shown that patients with BE receiving an EAC diagnosis during endoscopic surveillance have earlier-stage tumours and higher survival rates than those whose tumours are discovered because of symptoms[97,98]. However, such studies are susceptible to biases that could overestimate the benefits of surveillance. Furthermore, recent studies have reported a lower annual risk of progression from BE to EAC than previously observed (approximately 0.3% per year)[14,15]. The risk of progressing to EAC could also be lower in patients with a persistence of non-dysplastic BE after several surveillance endoscopies[99]. Despite the lack of high-quality evidence, most guidelines recommend surveillance endoscopy every 2-5 years for non-dysplastic BE, as shown in Table 1[57-59,90,100,101]. In cases of an indefinite diagnosis for dysplasia (IND), the risk of progression seems to be only in the first year[102], and it appears higher in patients with multifocal IND[103]. Current guidelines recommend a 6-12 mo interval to repeat, a biopsy (Table 1), and an increased acid suppression in cases of inflammatory infiltration and regenerative changes[57-59,90,100,101]. Because limited data are available, the natural history of LGD in BE is not yet clear. A recent meta-analysis found an annual rate of progression from LGD to EAC of 0.5% but a wide variability across studies[104]. The main issue for LGD diagnosis is a high degree of interobserver variability[105], in part due to the difficulty in differentiating it from reactive changes[106], therefore, a confirmation after an expert histological review is recommended[107]. Immunohistochemistry for p53 overexpression can be particularly useful to improve interobserver agreement for dysplasia detection[106], and it can be recommended as an adjunct to histopathology[58]. In patients with LGD on a single occasion, a repeat endoscopy in 2-12 mo (time interval depending on the society) is recommended, along with a more frequent surveillance if LGD is confirmed (Table 1). There is also evidence that LSBE patients with persistent and multifocal LGD are more likely to progress to EAC[108].

MEDICAL THERAPY

A large retrospective study highlighted how the control of reflux is important in the management of BE,

Table 1 Comparison of endoscopic surveillance recommendations for Barrett's esophagus in currently available guidelines

Guidelines	NDBE	IND	LGD	HDG
BOB CAT[90]	Not recommended[1]	≤ 12 mo	6-12 mo	Not recommended
ACPG[57]	< 3 cm 3-5 yr ≥ 3 cm 2-3 yr	≤ 6 mo	6 mo	Not recommended
BSG[58]	< 3 cm 3-5 yr ≥ 3 cm 2-3 yr	≤ 6 mo	6 mo	Not recommended
ASGE[100]	3-5 yr	No specific time frame	12 mo[2]	3 mo[3]
ACP[101]	3-5 yr	Not recommended	No specific time frame	No specific time frame
AGA[59]	3-5 yr	Not recommended	6-12 mo	3 mo[3]

[1]If undertaken, surveillance should be directed at high-risk groups (*i.e.*, composite risk factors including but not limited to 50 years of age or older, white race, male sex, central obesity, the length of the segment, and the symptom duration, frequency and severity), unless the life expectancy ≤ 5 yr; [2]Six months to confirm LGD; [3]In the absence of eradication therapy. BOB CAT: Benign Barrett's and Cancer Taskforce; ACPG: Australian Clinical Practice Guidelines; BSG: British Society for Gastroenterology; ASGE: American Society for Gastrointestinal Endoscopy; ACP: American College of Physicians; AGA: American Gastroenterological Association; NDBE: Non-dysplastic Barrett's esophagus; IND: Indefinite for dysplasia; LGD: Low-grade dysplasia; HGD: High-grade dysplasia.

showing a significantly lower rate of progression to LGD, HGD, or EAC in patients who had a history of antireflux surgery or PPI use[109]. Moreover, a recent meta-analysis of observational studies showed that PPI therapy was associated with a 71% risk reduction in BE progression with a trend towards a dose-response relationship, considering PPI use for > 2-3 years, protective against EAC or HGD[110]. However, a considerable heterogeneity was observed, and chemopreventive high-quality prospective trials of PPIs in patients with BE are warranted[110,111].

Complete, but not partial, acid suppression by PPIs over 6 mo, as measured by 24-h pH monitoring, decreases markers of epithelial proliferation and increases cell differentiation markers in patients with BE[112]. Similarly, a randomized clinical trial showed that a high-dose esomeprazole promoted a decrease in proliferative markers, concomitantly with a decrease in apoptotic cell death[113]. Overall, PPI therapy seems to be important not only because it reduces the acidity, and therefore the chemical damage, of the refluxate but also because PPIs have anti-inflammatory properties independent of their acid-suppressive effects[114].

A large case-control study by Nguyen *et al*[115] indicated that using PPI, nonsteroidal anti-inflammatory drugs (NSAID)/aspirin, or statin therapy in patients with BE might reduce the risk of developing EAC. Furthermore, an interesting study found that the incubation of isolated cells from mucosal biopsies of BE metaplasia with aspirin and omeprazole together induced a significantly greater reduction in proliferative activity than that induced separately by any of the two drugs, thus suggesting a synergistic effect of the two drugs[116]. To ascertain the efficacy of chemoprevention with PPIs and/or aspirin in BE metaplasia, a large clinical trial (Aspirin Esomeprazole Chemoprevention Trial - AspECT) was planned, the results of which are expected in 2016[117].

Although the exact dose of PPIs and the therapeutic efficacy endpoint are not known, high-dosage PPIs are commonly prescribed in clinical practice. However, the currently available international guidelines are not in a total agreement regarding recommendations for the maintenance treatment with PPIs in patients with BE. The recent international Benign Barrett's and Cancer Taskforce (BOB CAT) consensus group hints at using medical over surgical therapies to prevent BE neoplastic progression[90]. The Australian Clinical Practice Guidelines suggests that only symptomatic patients with BE should be treated with PPI therapy, with the dose titrated to control symptoms[57]. According to the British Society of Gastroenterology, there is not yet sufficient evidence to recommend acid-suppression drugs as chemopreventive agents, even if PPIs have the best clinical profile for symptom management[58]. Moreover, the American Gastroenterological Association (AGA) highlighted that PPI therapy also has effects that, conceivably, might promote the development of cancer in BE (*i.e.*, increasing the serum levels of gastrin, a hormone than can induce proliferation in BE epithelium)[59]. Because the evidence to support potent acid suppression with PPIs as a chemopreventive strategy in BE is largely indirect, the AGA asserts that insufficient data are available to advocate the prescription of PPIs in dosages higher than those necessary to eliminate the symptoms and endoscopic signs of GERD or, for patients without such symptoms and signs, in dosages higher than those suggested as conventional for GERD treatment. Likewise, there are not sufficient data to support the use of esophageal pH monitoring to titrate the PPI dosage to normalize AET in patients with BE[59].

ENDOSCOPIC THERAPY

Over the past decade, evidence has been accumulating on the effectiveness of the endoscopic management in BE treatment. There is generally high level of agreement across various guidelines regarding the management of non-dysplastic BE and BE with HGD or EAC. However, the therapy administered to patients with LGD is often a controversial topic. The changing guidelines for the BE endoscopic management are shown in Table 2.

Management of non-dysplastic and LGD BE

In 2011, AGA proposed using radiofrequency ablation (RFA; with or without endoscopic mucosal resection, EMR) for selected non-dysplastic BE individuals at risk for progression; however, the risk criteria were not fully defined. Then, it was also stated that RFA in LGD leads to reversion to normal-appearing squamous epithelium in > 90% of cases, and ablation should be the

Table 2 Recommendations for endoscopic eradication therapy in Barrett's esophagus

Guidelines	NDBE	LGD	HGD/intramucosal EAC
ACG[118]	Not recommended	Not recommended	Endoscopic ablation or surgical esophagectomy
AGA[59]	RFA (± EMR) for select individuals at risk for progression	RFA is a therapeutic option	Endoscopic therapy with RFA, PDT or EMR EMR in BE dysplasia with a visible mucosal irregularity Before proceeding with esophagectomy, patients with HGD or intramucosal EAC should be referred for evaluation by surgical specialized centres
BAD CAT[120]	-	-	Endoscopic treatment should be preferred over endoscopic surveillance or surgery for the management of most patients with HGD/intramucosal EAC RFA is currently the best available ablation technique for the treatment of flat HGD and for the eradication of residual BE after focal EMR In the HGD endoscopic resection of all visible abnormalities, cap and snare and band ligation with resection are equally effective
ASGE (2012)[100]	Consider endoscopic ablation in select cases	Consider endoscopic resection or ablation	Consider endoscopic resection or RFA ablation. Consider EUS for local staging and lymphadenopathy Consider surgical consultation
BSG[58]	Not recommended	Not routinely recommended	Endoscopic therapy preferred over esophagectomy
ASGE (2013)[123]	-	-	EMR is indicated for nodular BE and T1a EAC and may be used for flat BE with HGD ESD can be used in similar situations but is preferred to EMR for large areas of dysplasia or T1b EAC (*i.e.*, confined to the submucosa) Ablation techniques may be used alone or in combination with mucosal resection techniques
BOB CAT[90]	If the lesion is visible, endoscopic resection for diagnosis is then appropriate ablative therapy		-
	Not recommended	Lower risk: Intense surveillance. Higher risk: Ablative therapy with follow-up	

ACG: American College of Gastroenterology; AGA: American Gastroenterology Association; BAD CAT: Barrett's Dysplasia and Cancer Taskforce; ASGE: American Society for Gastrointestinal Endoscopy; BSG: British Society for Gastroenterology; BOB CAT: Benign Barrett's and Cancer Taskforce; RFA: Radiofrequency ablation; EMR: Endoscopic mucosal resection; PDT: Photodynamic therapy; EUS: Endoscopic ultrasound; ESD: Endoscopic submucosal dissection; BE: Barrett's esophagus; NDBE: Non-dysplastic Barrett's esophagus; LGD: Low-grade dysplasia; HGD: High-grade dysplasia; EAC: Esophageal adenocarcinoma.

therapeutic option in those cases[59]. In the American Society for Gastrointestinal Endoscopy guidelines, endoscopic ablation therapy was suggested as the management option in selected patients with non-dysplastic BE. They also allowed the consideration of endoscopic resection or ablation in all LGD cases[100]. However, according to the British guidelines, the endoscopic treatment was not routinely recommended in non-dysplastic BE or in LGD[58].

Recent management strategies, established by the international BOB CAT consensus, include the following: (1) endoscopic resection/ablation is not recommended in benign BE; and (2) patients with LGD on a single occasion, without higher-risk features, should be managed with endoscopic surveillance continued for 6-12 mo (provided the patient is fit for endoscopy and is not already undergoing therapy)[90]. The absence of dysplasia in two subsequent upper endoscopies identifies a cohort of patients, previously diagnosed with LGD, who are at low risk of neoplastic progression and can keep on routine surveillance. Moreover, the BOB CAT consensus states that BE patients with multifocal LGD and/or with LGD that persists have an increased risk for neoplastic progression than those with focal LGD or with LGD detected on a single endoscopy[90]. The former group should be treated with ablative therapy rather than only followed up with[90].

Management of HGD and early-stage EAC

In HGD, there is a high rate of progression to EAC (6%-19% per year), and endoscopic therapy is a well-established therapy for these cases. All associations recommend endoscopic therapy (with a combination of EMR followed by the ablation of residual BE mucosa) for HGD and intramucosal EAC (Table 2)[58,59,118-120]. Previously, the standard of treatment was esophagectomy due to high cure rates, but it was also characterized by substantial mortality (2%-5%) and morbidity (30%-50%)[121].

In 2013, the European Society for Medical Oncology stated that surgery is the treatment of choice in early EAC (Tis-T1a, N0). However, endoscopic resection is an alternative treatment option for selected patients because similar cure rates in specialized centres have been reported[122]. Similarly to BE with dysplasia, endoscopic therapy for early-stage EAC includes resection and ablation techniques[123]. EMR successfully eradicates 91% to 98% of T1a EAC[123,124], with a

Table 3 Ablation therapy in Barrett's esophagus

Ablation modalities	Description of the technique	Outcome	Ref.
RFA	RFA uses a balloon-based circumferential array of closely spaced electrodes to deliver radiofrequency energy to the esophageal mucosa. With this technique, the mucosa is ablated to the submucosal level. A smaller, endoscope-mounted, radiofrequency catheter ablation device could be used for the focal ablation of metaplasia that could remain after treatment with the circumferential system. A follow-up endoscopy is at 3 mo when any remaining metaplasia is ablated, with a further follow-up endoscopy at 1 yr	A landmark large, multicentre, randomized trial showed that RFA can eliminate HGD, reducing the risk of EAC compared with a sham procedure. Overall, the eradication rates for HGD range from 79% to 90% and from 69% and 97% for NDBE/LGD patients RFA is safer and easier to administer, and it causes fewer major complications, particularly stricture formation, than PDT	[133,145]
APC	APC produces a flow of ionized argon plasma that generates a high-frequency monopolar current to the BE surface under direct vision	Different eradication rates for NDBE and LGD in the short term ranged from 36% to 100% for NDBE and rates of recurrence between 62% and 100% for LGD patients	[133]
PDT	PDT is based on the injection of a light sensitizing drug (*e.g.*, porfimer sodium) into the patient and then the exposure of a portion of the esophagus to light of a specific wavelength, which would lead to dysplasia cell death. Once the photosensitizer is activated by the light, it generates oxygen free radicals that result in cytotoxicity to the mucosal cells	The eradication rates for HGD range from 77% to 100%, and those for NDBE/LGD range from 50%-100% of patients The limitations include the cost of the intravenous agent, the prolonged period (weeks) of photosensitivity following exposure, and an appreciable post-treatment stricture rate	[133]
CRY	CRY is a non-contact method of cryotherapy that involves an endoscopically directed spray of liquid nitrogen at -196 ℃ directly onto the Barrett's mucosa The advantage is a lack of contact with mucosa and hence can be applied to irregularity, which would make the application of contact therapies such as RFA challenging	The rates of complete eradication are approximately 68%-97% for HGD and 57% for NDBE The current literature is inadequate to assess the ability of CRY to achieve sustained reversion of the metaplastic mucosa to normal-appearing squamous epithelium in subjects at any stage of BE. Further longitudinal studies are needed	[133,156]
MPEC	MPEC uses an endoscopic multipolar electrical probe, which is used to control gastrointestinal haemorrhage that applies electrical energy at 50 W so that all BE surfaces are treated	Complete eradication in 65%-100% of NDBE. This technique is very much operator dependent and causes dysphagia as the most common side effect	[133]

RFA: Radiofrequency ablation; APC: Argon plasma coagulation; PDT: Photodynamic therapy; CRY: Cryoablation; MPEC: Multipolar electrocoagulation; EAC: Esophageal adenocarcinoma; BE: Barrett's esophagus; IM: Intestinal metaplasia; NDBE: Non-dysplastic Barrett's esophagus; LGD: Low-grade dysplasia; HGD: High-grade dysplasia.

cancer-free survival similar to and a lower morbidity than surgical resection[125]. The long-term survival of 742 patients with TisN0M0 and T1N0M0 EAC treated with either endoscopic modalities (most commonly EMR) and surgical resection was similar[126]. Zehetner *et al*[127] demonstrated similar survival in patients with HGD and intramucosal EAC treated with endoscopic resection and ablation than surgical resection, with a significantly lower morbidity associated with endoscopic treatment.

Categories of endoscopic BE eradication modalities

Multiple modalities may be employed for the endoscopic eradication of BE. There are two main types of endoscopic therapy: Tissue-acquiring techniques, which include EMR and endoscopic submucosal dissection (ESD), and ablative techniques, which include thermal techniques (RFA, multipolar electrocoagulation, argon plasma coagulation), cryotherapy and photochemical techniques (photodynamic therapy, PDT)[119]. Examples of ablative therapies are shown in Table 3.

The great advantage of both EMR and ESD, compared to ablative therapies, is that specimens for histopathological analysis at the time of treatment can be obtained. The diagnosis of dysplasia and neoplasia in EMR specimens is improved, particularly because of the upstaging of cases previously diagnosed as dysplasia and the assessment of the depth of invasion with the determination of margins of resection[119,128-130], which have crucial implications in the appropriate choice of treatment and outcomes[131]. However, ablation therapies can be applied to larger surface areas and to different resection locations[132].

The AGA guidelines recommended RFA, PDT, cryotherapy, thermal energy application, and EMR in BE eradication in 2011[59,133]. Currently, the most commonly used technologies are RFA and EMR used alone or in combination. In most cases, ablation techniques are used in combination with resection techniques (multimodal therapy), wherein ablation techniques are applied following EMR or ESD that are used to remove macroscopically visible lesions[119].

Endoscopic mucosal resection

EMR is an endoscopic technique useful in the resection of macroscopically visible BE lesions that are less than 2 cm in diameter. To lift the lesion from the muscularis propria, normal saline or dilute epinephrine is first injected into submucosa[119]. EMR can be performed with either EMR-cap or EMR-ligation techniques. The former uses suction to retract the target tissue into a plastic cap that is attached to the endoscope, and a snare is closed around the lesion, followed by electrocautery. The latter uses suction to aspirate the tissue, followed by band deployment, to create a pseudopolyp. Then, a

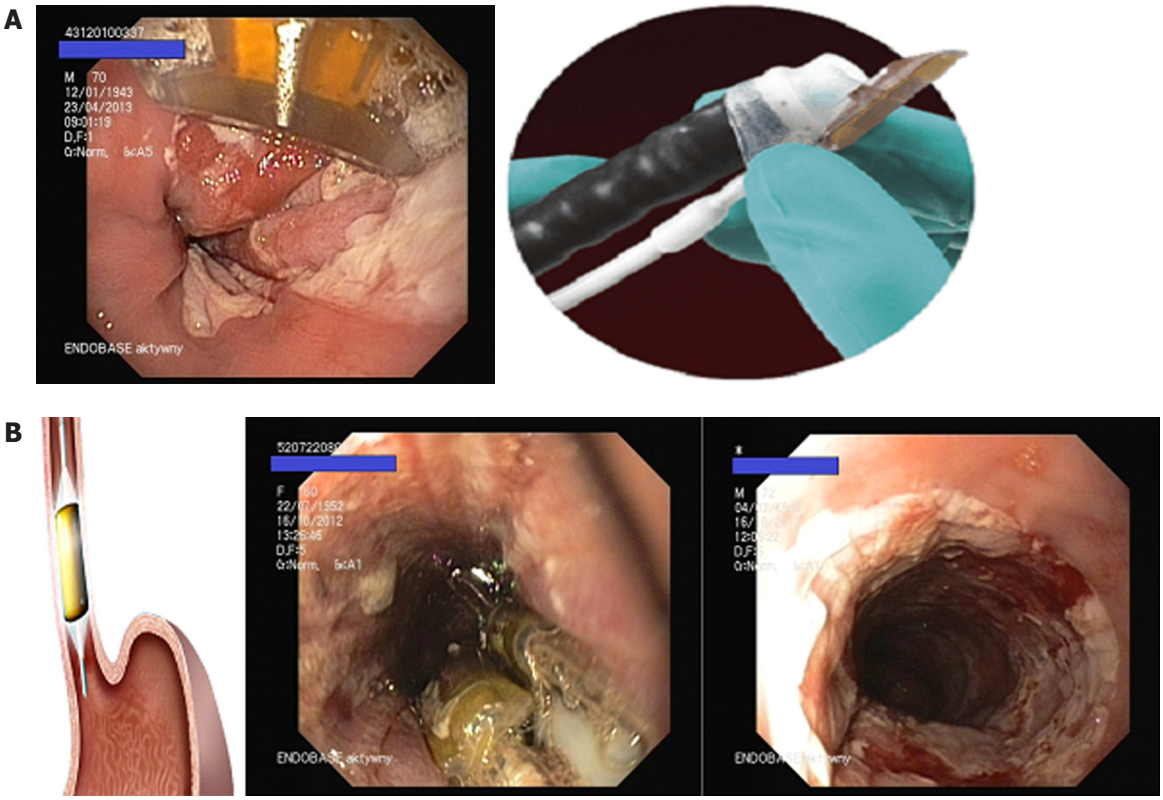

Figure 1 Radiofrequency ablation to treat Barrett's esophagus. A: HALO 90 to treat short segments, islands and tongues of Barrett's esophagus; B: Balloon ablation catheter (HALO 360) intended to treat long-segment circumferential Barrett's esophagus. Material from Department of Digestive Tract Disease, Medical University of Lodz, Poland.

hexagonal snare and electrocautery are used to resect the lesion[119]. These two techniques have shown similar diagnostic accuracy and safety[119,134]. However, with the ability to perform several resections with a single intubation and kit, EMR-ligation technique seems to be faster and less expensive[134].

Complete remission of dysplasia and intestinal metaplasia is achieved in > 80%-90% of patients undergoing EMR with or without concurrent ablative therapy[135,136]. Recently, a large group of patients with BE and T1a EAC treated with endoscopic therapy reported a 96.3% complete response rate. The overall survival rate was 91% at 5 years and 75% at 10 years[137].

Potential complications of EMR are bleeding, perforation, and stricture formation. Delayed bleeding is infrequent, but immediate post-resection bleeding can occur in 10% of patients[138]. Perforation rates are reported to be less than 3%. The extent of mucosa removed by EMR is the risk factor for stricture formations (37% of cases), the majority of which are successfully managed by endoscopic dilation[124,135,139].

ESD

ESD is more likely to achieve an en bloc resection, usually of lesions > 2 cm. First, a lesion is lifted off the muscularis propria with an injected solution; then, dissection in the submucosal plane, using a variety of dissection knives, is performed. ESD in the esophagus is technically more difficult, particularly due to narrow lumen, fibrosis caused by chronic reflux, and the thin wall of the esophagus. Furthermore, esophageal ESD showed frequent complications, such as bleeding, perforation (rates between 2% and 5%), and stricture formation (rates between 5% and 17.2%)[119,140-143]. Prophylactic steroid injection following esophageal ESD has been shown to decrease the risk of stricture formation[144].

Radiofrequency ablation

Radiofrequency ablation (RFA) delivers high-frequency energy to the esophageal mucosa to achieve tissue necrosis. The depth of ablation is between 500 and 1000 μm. There are two systems available: A 3-cm-long balloon ablation catheter (HALO 360) intended to treat circumferential LSBE (Figure 1) and an endoscope-mounted targeted device (HALO 90, HALO 60, HALO ULTRA) to treat SSBE and BE islands and tongues (Figure 1)[132]. The technique involves mucosal ablation under endoscopic guidance followed by the removal of the adhered white coagulum in the ablated area and then by repeat treatment of the same area, all within one endoscopic session (Figure 2). Multiple endoscopic treatments may be required depending on the length of the BE segment and the tissue response. Treatment is usually performed every 2-3 mo[132].

Among patients undergoing RFA, a complete eradication of dysplasia occurred in 90.5% and in 81%

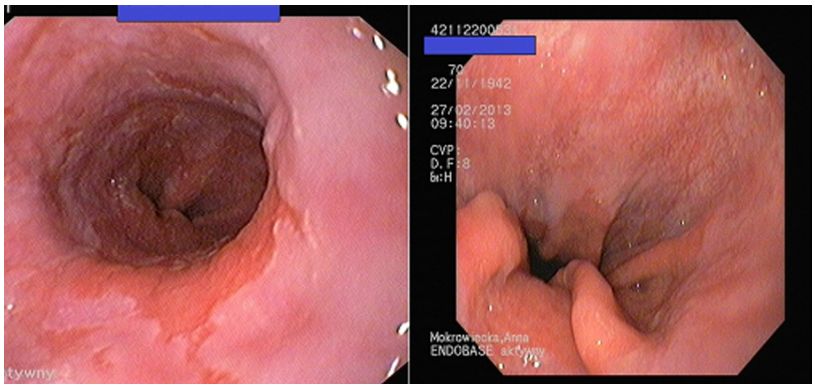

Figure 2 Improvement after radiofrequency ablation of Barrett's esophagus. Material from Department of Digestive Tract Disease, Medical University of Lodz, Poland.

of LGD and HGD patients, respectively, at a 12-mo follow-up. Overall, 77.4% of patients in the ablation group had complete eradication of intestinal metaplasia[145]. Subsequently, RFA therapy provided an acceptable safety profile associated with a low rate of disease progression for up to 3 years[146]. RFA efficacy has been demonstrated in several other studies, with eradication rates of metaplasia and dysplasia ranging from approximately 81%-92.6% and 75%-88.2%, respectively[119,147,148]. Moreover, reductions in rates of progression from LGD and HGD to cancer have been demonstrated in randomized controlled trials with RFA[145,147].

RFA is safe and well tolerated. The most common complications reported include chest pain lasting less than one week, strictures requiring dilation (6%-8%), gastrointestinal haemorrhage (1%), and perforation (less than 1%)[132,145,149].

Incomplete response to ablation is possible, particularly in cases of a longer duration of dysplasia, longer BE segments, a loss of p16 locus or polysomy (detected by FISH) and poor reflux control[119,150-152]. The presence or persistence of intestinal metaplasia under new squamous epithelium is known as "buried metaplasia". Because of its malignant potential, it is important to remember that it could be invisible in endoscopic surveillance and in superficial biopsies. The prevalence of buried metaplasia was 14% after PDT and 0.9% after RFA, but the results could be underestimated[119,153,154]. Similar rates of recurrence have been reported with all modalities of BE endotherapy (RFA, PDT and cryotherapy). Most recurrences can be treated endoscopically if detected early. Thus, post-treatment endoscopic surveillance is needed[119,155,156].

SURGICAL THERAPY

Surgical treatment is indicated particularly in patients who need long-term treatment of GERD (*i.e.*, patients with persistent troublesome symptoms and/or a progression of disease despite adequate PPI therapy)[157,158]. To achieve an increase in the quality of life, proper diagnostic testing should be performed to adequately select patients before surgery[157-159].

Laparoscopic partial and total fundoplications are currently the best available surgical techniques to treat severe GERD[158]. The two major competing procedures are the laparoscopic Nissen fundoplication and the posterior partial Toupet hemifundoplication. Randomized studies have shown a similar outcome at 5 years but a higher rate of side effects (dysphagia, bloating, and flatulence) and a higher reoperation rate in the Nissen group than in the Toupet group[160,161]. In contrast, other studies have reported minor side effects and a lower reoperation rate for the Nissen procedure[162-164]. Because of these controversies, the choice of fundoplication technique should be left to the individual preferences of the surgeon.

Recently, the LOTUS trial showed a comparable rate of symptom control between surgery and escalating doses of PPIs[165]. Surgery should be considered for younger patients, particularly in cases with a high risk of progression with large hiatal hernias, severe reflux symptoms, and a long history of disease to prevent the progression to BE[166,167]. However, there is limited evidence of the effectiveness of antireflux surgery in reducing the extent of BE and the risk of progression to cancer, as well as the regression of BE. Thus, after antireflux surgery, endoscopic surveillance has to be maintained[168-171]. Of note, it has been shown that neoplastic progression after antireflux surgery is due primarily to the subsequent recurrence of reflux[172].

Surgery is still the treatment of choice in early EAC; however, in 2011, AGA stated that most patients with HGD BE (70%-80%) can be successfully treated with endoscopic eradication therapy. Esophagectomy in patients with HGD is an alternative; however, the current data suggest a lower morbidity with ablative therapy[59]. The important issue is the choice of surgical centres specializing in the treatment of foregut cancers and HGD. In 2012, the Barrett's Dysplasia and Cancer Task Force (BAD CAT) consensus group stated that endoscopic treatment is preferred to surgery in most cases of HGD; however, esophagectomy results in a long-term cure. Moreover, there is no strong evidence that fundoplication reverses HGD[120].

For localized EAC without suspected lymph node involvement (T1-2 N0M0), surgery is regarded as a standard treatment. However, the long-term survival does not exceed 25% if regional lymph nodes are involved (pN1-3). Therefore, preoperative treatment can also be justified[122,173]. Preoperative chemoradiotherapy is preferred in EAC for selected patients, particularly in high-risk patients (*i.e.*, those with locally more advanced stages). Even after a complete tumour response to preoperative chemoradiotherapy, operable patients with EAC should proceed to surgery[122].

CONCLUSION

BE is a premalignant condition that affects 1.3%-2% of the adult population[174-176]. Patients with BE have an increased risk of developing EAC through a gradual process, in which metaplastic epithelium without dysplasia evolves to LGD, HGD and eventually EAC. GERD is considered to play a major role in the development of these histologic changes[61,177]. Indeed, GERD symptoms, ERD, and BE have a number of common determinants (*i.e.*, esophagogastric junction dysfunction, impaired esophageal clearance, gastric and duodenal contents of the refluxate), which are implicated in the genesis of esophageal mucosal damage[19]. In keeping with the spectrum model of GERD, the severity of acid reflux, DGER, and esophageal motility abnormalities are increasingly prevalent with the increasing severity of GERD presentation, from NERD, through ERD, and up to BE[25,28]. Over the past decade, the introduction of new technologies (particularly with regard to MII-pH) has increased the overall diagnostic yield of the pathophysiological mechanisms underlying GERD manifestation and its complications (including BE)[32-34,50,51]. In particular, a proper evaluation of impairment of esophageal chemical clearance might help predict BE neoplastic progression[51]. However, future studies are expected to substantiate this finding.

To date, dysplasia is considered the most widely used marker of BE progression to cancer, and generally, its detection warrants intensified surveillance and/or treatment[59]. Considering the large increase in the incidence of EAC[8], effective screening/surveillance programs of BE, coupled with improved therapeutic approaches, represent the hope to reverse this incidence. However, only a minority of BE patients develop EAC, with a current estimated risk of 0.3% per year[14,15]. Moreover, given the large number of subjects with BE, endoscopic examinations represent a substantial commitment of resources[5]. Thus, current strategies of screening and surveillance programs of BE are debated, showing moderate to absent cost-effectiveness[18]. New methods for BE screening are being evaluated with some promising results; however, we still await conclusive data for the best screening approach (*i.e.*, simple, minimally invasive), and additional studies are urgently needed. Clearly, the identification of subgroups of patients at reduced or increased risk for BE development and degeneration would lead to more cost-effective strategies for the prevention of EAC, helping select those patients deserving a close endoscopic follow-up. At present, the management of patients with LGD represents a main issue due to its unpredictable natural history, the lack of cost-effectiveness data regarding the surveillance of LGD and high disagreement between pathologists in LGD diagnosis[100].

The area of BE therapeutic management is rapidly evolving. Unequivocal data on the use of drugs such as PPIs, aspirin or statins in the chemoprevention of BE are lacking. At the moment, there is no doubt regarding the use of PPIs for symptom control[58,59]. Endoscopic eradication therapies have been shown to be effective in patients with BE/EAC, and new therapies have appeared. BE containing HGD and/or early-stage EAC can be treated endoscopically instead of with surgical esophagectomy. Moreover, recent management strategies, including a de-escalation strategy for lower-risk patients and escalation to intervention with follow-up for higher-risk patients, have been established[90,120]. The main objective of endoscopic therapy should be the elimination of all intestinal metaplasia because the recurrence of neoplasia appears to be higher in individuals who do not achieve a full eradication of BE[124].

REFERENCES

1 **Vakil N**, van Zanten SV, Kahrilas P, Dent J, Jones R. The Montreal definition and classification of gastroesophageal reflux disease: a global evidence-based consensus. *Am J Gastroenterol* 2006; **101**: 1900-1920; quiz 1943 [PMID: 16928254 DOI: 10.1111/j.1572-0241.2006.00630.x]

2 **Spechler SJ**. Barrett's esophagus. *Semin Oncol* 1994; **21**: 431-437 [PMID: 8042041]

3 **El-Serag HB**, Sweet S, Winchester CC, Dent J. Update on the epidemiology of gastro-oesophageal reflux disease: a systematic review. *Gut* 2014; **63**: 871-880 [PMID: 23853213 DOI: 10.1136/gutjnl-2012-304269]

4 **Ness-Jensen E**, Lindam A, Lagergren J, Hveem K. Changes in prevalence, incidence and spontaneous loss of gastro-oesophageal reflux symptoms: a prospective population-based cohort study, the HUNT study. *Gut* 2012; **61**: 1390-1397 [PMID: 22190483 DOI: 10.1136/gutjnl-2011-300715]

5 **Peery AF**, Dellon ES, Lund J, Crockett SD, McGowan CE, Bulsiewicz WJ, Gangarosa LM, Thiny MT, Stizenberg K, Morgan DR, Ringel Y, Kim HP, Dibonaventura MD, Carroll CF, Allen JK, Cook SF, Sandler RS, Kappelman MD, Shaheen NJ. Burden of gastrointestinal disease in the United States: 2012 update. *Gastroenterology* 2012; **143**: 1179-1187.e1-3 [PMID: 22885331 DOI: 10.1053/j.gastro.2012.08.002]

6 **Chang CY**, Cook MB, Lee YC, Lin JT, Ando T, Bhatia S, Chow WH, El-Omar EM, Goto H, Li YQ, McColl K, Reddy N, Rhee PL, Sharma P, Sung JJ, Ghoshal U, Wong JY, Wu JC, Zhang J, Ho KY. Current status of Barrett's esophagus research in Asia. *J Gastroenterol Hepatol* 2011; **26**: 240-246 [PMID: 21155883 DOI: 10.1111/j.1440-1746.2010.06529.x]

7 **Cook MB**, Chow WH, Devesa SS. Oesophageal cancer incidence in the United States by race, sex, and histologic type, 1977-2005. *Br J Cancer* 2009; **101**: 855-859 [PMID: 19672254 DOI: 10.1038/sj.bjc.6605246]

8 **Kroep S**, Lansdorp-Vogelaar I, Rubenstein JH, Lemmens VE, van Heijningen EB, Aragonés N, van Ballegooijen M, Inadomi JM. Comparing trends in esophageal adenocarcinoma incidence

and lifestyle factors between the United States, Spain, and the Netherlands. *Am J Gastroenterol* 2014; **109**: 336-343; quiz 335, 344 [PMID: 24343546 DOI: 10.1038/ajg.2013.420]

9 **Corley DA**, Kubo A, Levin TR, Block G, Habel L, Rumore G, Quesenberry C, Buffler P. Race, ethnicity, sex and temporal differences in Barrett's oesophagus diagnosis: a large community-based study, 1994-2006. *Gut* 2009; **58**: 182-188 [PMID: 18978173 DOI: 10.1136/gut.2008.163360]

10 **Malfertheiner P**, Nocon M, Vieth M, Stolte M, Jaspersen D, Koelz HR, Labenz J, Leodolter A, Lind T, Richter K, Willich SN. Evolution of gastro-oesophageal reflux disease over 5 years under routine medical care--the ProGERD study. *Aliment Pharmacol Ther* 2012; **35**: 154-164 [PMID: 22070159 DOI: 10.1111/j.1365-2036.2011.04901.x]

11 **Jankowski JA**, Provenzale D, Moayyedi P. Esophageal adenocarcinoma arising from Barrett's metaplasia has regional variations in the west. *Gastroenterology* 2002; **122**: 588-590 [PMID: 11845805 DOI: 10.1053/gast.2002.31599]

12 **Shaheen NJ**, Crosby MA, Bozymski EM, Sandler RS. Is there publication bias in the reporting of cancer risk in Barrett's esophagus? *Gastroenterology* 2000; **119**: 333-338 [PMID: 10930368 DOI: 10.1053/gast.2000.9302]

13 **Murray L**, Watson P, Johnston B, Sloan J, Mainie IM, Gavin A. Risk of adenocarcinoma in Barrett's oesophagus: population based study. *BMJ* 2003; **327**: 534-535 [PMID: 12958113 DOI: 10.1136/bmj.327.7414.534]

14 **Hvid-Jensen F**, Pedersen L, Drewes AM, Sørensen HT, Funch-Jensen P. Incidence of adenocarcinoma among patients with Barrett's esophagus. *N Engl J Med* 2011; **365**: 1375-1383 [PMID: 21995385 DOI: 10.1056/NEJMoa1103042]

15 **Bhat S**, Coleman HG, Yousef F, Johnston BT, McManus DT, Gavin AT, Murray LJ. Risk of malignant progression in Barrett's esophagus patients: results from a large population-based study. *J Natl Cancer Inst* 2011; **103**: 1049-1057 [PMID: 21680910 DOI: 10.1093/jnci/djr203]

16 **Verbeek RE**, Leenders M, Ten Kate FJ, van Hillegersberg R, Vleggaar FP, van Baal JW, van Oijen MG, Siersema PD. Surveillance of Barrett's esophagus and mortality from esophageal adenocarcinoma: a population-based cohort study. *Am J Gastroenterol* 2014; **109**: 1215-1222 [PMID: 24980881 DOI: 10.1038/ajg.2014.156]

17 **Corley DA**, Mehtani K, Quesenberry C, Zhao W, de Boer J, Weiss NS. Impact of endoscopic surveillance on mortality from Barrett's esophagus-associated esophageal adenocarcinomas. *Gastroenterology* 2013; **145**: 312-319.e1 [PMID: 23673354 DOI: 10.1053/j.gastro.2013.05.004]

18 **Gordon LG**, Mayne GC. Cost-effectiveness of Barrett's oesophagus screening and surveillance. *Best Pract Res Clin Gastroenterol* 2013; **27**: 893-903 [PMID: 24182609 DOI: 10.1016/j.bpg.2013.08.019]

19 **Bredenoord AJ**, Pandolfino JE, Smout AJ. Gastro-oesophageal reflux disease. *Lancet* 2013; **381**: 1933-1942 [PMID: 23477993 DOI: 10.1016/S0140-6736(12)62171-0]

20 **Woodland P**, Sifrim D. The refluxate: The impact of its magnitude, composition and distribution. *Best Pract Res Clin Gastroenterol* 2010; **24**: 861-871 [PMID: 21126699 DOI: 10.1016/j.bpg.2010.09.002]

21 **Lagergren J**, Bergström R, Lindgren A, Nyrén O. Symptomatic gastroesophageal reflux as a risk factor for esophageal adenocarcinoma. *N Engl J Med* 1999; **340**: 825-831 [PMID: 10080844 DOI: 10.1056/NEJM199903183401101]

22 **Koek GH**, Tack J, Sifrim D, Lerut T, Janssens J. The role of acid and duodenal gastroesophageal reflux in symptomatic GERD. *Am J Gastroenterol* 2001; **96**: 2033-2040 [PMID: 11467629 DOI: 10.1111/j.1572-0241.2001.03863.x]

23 **Loughney T**, Maydonovitch CL, Wong RK. Esophageal manometry and ambulatory 24-hour pH monitoring in patients with short and long segment Barrett's esophagus. *Am J Gastroenterol* 1998; **93**: 916-919 [PMID: 9647018 DOI: 10.1111/j.1572-0241.1998.00276.x]

24 **Fass R**, Hell RW, Garewal HS, Martinez P, Pulliam G, Wendel C, Sampliner RE. Correlation of oesophageal acid exposure with Barrett's oesophagus length. *Gut* 2001; **48**: 310-313 [PMID: 11171818 DOI: 10.1136/gut.48.3.310]

25 **Zentilin P**, Conio M, Mele MR, Mansi C, Pandolfo N, Dulbecco P, Gambaro C, Tessieri L, Iiritano E, Bilardi C, Biagini R, Vigneri S, Savarino V. Comparison of the main oesophageal pathophysiological characteristics between short- and long-segment Barrett's oesophagus. *Aliment Pharmacol Ther* 2002; **16**: 893-898 [PMID: 11966497 DOI: 10.1046/j.1365-2036.2002.01237.x]

26 **Champion G**, Richter JE, Vaezi MF, Singh S, Alexander R. Duodenogastroesophageal reflux: relationship to pH and importance in Barrett's esophagus. *Gastroenterology* 1994; **107**: 747-754 [PMID: 8076761 DOI: 10.1016/0016-5085(94)90123-6]

27 **Vaezi MF**, Richter JE. Role of acid and duodenogastroesophageal reflux in gastroesophageal reflux disease. *Gastroenterology* 1996; **111**: 1192-1199 [PMID: 8898632 DOI: 10.1053/gast.1996.v111.pm8898632]

28 **Koek GH**, Sifrim D, Lerut T, Janssens J, Tack J. Multivariate analysis of the association of acid and duodeno-gastro-oesophageal reflux exposure with the presence of oesophagitis, the severity of oesophagitis and Barrett's oesophagus. *Gut* 2008; **57**: 1056-1064 [PMID: 18403496 DOI: 10.1136/gut.2006.119206]

29 **Zaninotto G**, Portale G, Parenti A, Lanza C, Costantini M, Molena D, Ruol A, Battaglia G, Costantino M, Epifani M, Nicoletti L. Role of acid and bile reflux in development of specialised intestinal metaplasia in distal oesophagus. *Dig Liver Dis* 2002; **34**: 251-257 [PMID: 12038808 DOI: 10.1016/S1590-8658(02)80144-X]

30 **Zentilin P**, Dulbecco P, Savarino E, Giannini E, Savarino V. Combined multichannel intraluminal impedance and pH-metry: a novel technique to improve detection of gastro-oesophageal reflux literature review. *Dig Liver Dis* 2004; **36**: 565-569 [PMID: 15460839 DOI: 10.1016/j.dld.2004.03.019]

31 **Frazzoni M**, Manno M, De Micheli E, Savarino V. Pathophysiological characteristics of the various forms of gastro-oesophageal reflux disease. Spectrum disease or distinct phenotypic presentations? *Dig Liver Dis* 2006; **38**: 643-648 [PMID: 16627016 DOI: 10.1016/j.dld.2006.02.020]

32 **Frazzoni M**, Savarino E, Manno M, Melotti G, Mirante VG, Mussetto A, Bertani H, Manta R, Conigliaro R. Reflux patterns in patients with short-segment Barrett's oesophagus: a study using impedance-pH monitoring off and on proton pump inhibitor therapy. *Aliment Pharmacol Ther* 2009; **30**: 508-515 [PMID: 19519732 DOI: 10.1111/j.1365-2036.2009.04063.x]

33 **Bredenoord AJ**, Hemmink GJ, Smout AJ. Relationship between gastro-oesophageal reflux pattern and severity of mucosal damage. *Neurogastroenterol Motil* 2009; **21**: 807-812 [PMID: 19374635 DOI: 10.1111/j.1365-2982.2009.01306.x]

34 **Savarino E**, Zentilin P, Frazzoni M, Cuoco DL, Pohl D, Dulbecco P, Marabotto E, Sammito G, Gemignani L, Tutuian R, Savarino V. Characteristics of gastro-esophageal reflux episodes in Barrett's esophagus, erosive esophagitis and healthy volunteers. *Neurogastroenterol Motil* 2010; **22**: 1061-e280 [PMID: 20557468 DOI: 10.1111/j.1365-2982.2010.01536.x]

35 **Sifrim D**, Castell D, Dent J, Kahrilas PJ. Gastro-oesophageal reflux monitoring: review and consensus report on detection and definitions of acid, non-acid, and gas reflux. *Gut* 2004; **53**: 1024-1031 [PMID: 15194656 DOI: 10.1136/gut.2003.033290]

36 **Shay SS**, Johnson LF, Richter JE. Acid rereflux: a review, emphasizing detection by impedance, manometry, and scintigraphy, and the impact on acid clearing pathophysiology as well as interpreting the pH record. *Dig Dis Sci* 2003; **48**: 1-9 [PMID: 12645783 DOI: 10.1023/A: 1021762310433]

37 **Kaur BS**, Ouatu-Lascar R, Omary MB, Triadafilopoulos G. Bile salts induce or blunt cell proliferation in Barrett's esophagus in an acid-dependent fashion. *Am J Physiol Gastrointest Liver Physiol* 2000; **278**: G1000-G1009 [PMID: 10859231]

38 **Tselepis C**, Morris CD, Wakelin D, Hardy R, Perry I, Luong QT, Harper E, Harrison R, Attwood SE, Jankowski JA. Upregulation of the oncogene c-myc in Barrett's adenocarcinoma: induction of c-myc by acidified bile acid in vitro. *Gut* 2003; **52**: 174-180 [PMID: 12524396 DOI: 10.1136/gut.52.2.174]

39 **Roberts NB**. Review article: human pepsins - their multiplicity, function and role in reflux disease. *Aliment Pharmacol Ther* 2006; **24** Suppl 2: 2-9 [PMID: 16939427 DOI: 10.1111/j.1365-2036.2006.03038.x]

40 **Farré R**, van Malenstein H, De Vos R, Geboes K, Depoortere I, Vanden Berghe P, Fornari F, Blondeau K, Mertens V, Tack J, Sifrim D. Short exposure of oesophageal mucosa to bile acids, both in acidic and weakly acidic conditions, can impair mucosal integrity and provoke dilated intercellular spaces. *Gut* 2008; **57**: 1366-1374 [PMID: 18593808 DOI: 10.1136/gut.2007.141804]

41 **Hemmink GJ**, Bredenoord AJ, Weusten BL, Monkelbaan JF, Timmer R, Smout AJ. Esophageal pH-impedance monitoring in patients with therapy-resistant reflux symptoms: 'on' or 'off' proton pump inhibitor? *Am J Gastroenterol* 2008; **103**: 2446-2453 [PMID: 18684197 DOI: 10.1111/j.1572-0241.2008.02033.x]

42 **Bredenoord AJ**, Weusten BL, Timmer R, Smout AJ. Characteristics of gastroesophageal reflux in symptomatic patients with and without excessive esophageal acid exposure. *Am J Gastroenterol* 2006; **101**: 2470-2475 [PMID: 17090278 DOI: 10.1111/j.1572-0241.2006.00945.x]

43 **Emerenziani S**, Ribolsi M, Sifrim D, Blondeau K, Cicala M. Regional oesophageal sensitivity to acid and weakly acidic reflux in patients with non-erosive reflux disease. *Neurogastroenterol Motil* 2009; **21**: 253-258 [PMID: 19019016 DOI: 10.1111/j.1365-2982.2008.01203.x]

44 **Martinucci I**, de Bortoli N, Giacchino M, Bodini G, Marabotto E, Marchi S, Savarino V, Savarino E. Esophageal motility abnormalities in gastroesophageal reflux disease. *World J Gastrointest Pharmacol Ther* 2014; **5**: 86-96 [PMID: 24868489 DOI: 10.4292/wjgpt.v5.i2.86]

45 **DeVault K**, McMahon BP, Celebi A, Costamagna G, Marchese M, Clarke JO, Hejazi RA, McCallum RW, Savarino V, Zentilin P, Savarino E, Thomson M, Souza RF, Donohoe CL, O'Farrell NJ, Reynolds JV. Defining esophageal landmarks, gastroesophageal reflux disease, and Barrett's esophagus. *Ann N Y Acad Sci* 2013; **1300**: 278-295 [PMID: 24117649 DOI: 10.1111/nyas.12253]

46 **Kahrilas PJ**, Dodds WJ, Hogan WJ, Kern M, Arndorfer RC, Reece A. Esophageal peristaltic dysfunction in peptic esophagitis. *Gastroenterology* 1986; **91**: 897-904 [PMID: 3743966]

47 **Ho KY**, Kang JY. Reflux esophagitis patients in Singapore have motor and acid exposure abnormalities similar to patients in the Western hemisphere. *Am J Gastroenterol* 1999; **94**: 1186-1191 [PMID: 10235190 DOI: 10.1111/j.1572-0241.1999.01063.x]

48 **Diener U**, Patti MG, Molena D, Fisichella PM, Way LW. Esophageal dysmotility and gastroesophageal reflux disease. *J Gastrointest Surg* 2001; **5**: 260-265 [PMID: 11360049]

49 **Wu JC**, Cheung CM, Wong VW, Sung JJ. Distinct clinical characteristics between patients with nonerosive reflux disease and those with reflux esophagitis. *Clin Gastroenterol Hepatol* 2007; **5**: 690-695 [PMID: 17481961 DOI: 10.1016/j.cgh.2007.02.023]

50 **Savarino E**, Gemignani L, Pohl D, Zentilin P, Dulbecco P, Assandri L, Marabotto E, Bonfanti D, Inferrera S, Fazio V, Malesci A, Tutuian R, Savarino V. Oesophageal motility and bolus transit abnormalities increase in parallel with the severity of gastro-oesophageal reflux disease. *Aliment Pharmacol Ther* 2011; **34**: 476-486 [PMID: 21671968 DOI: 10.1111/j.1365-2036.2011.04742.x]

51 **Frazzoni M**, Bertani H, Conigliaro R, Frazzoni L, Losi L, Melotti G. Neoplastic progression in short-segment Barrett's oesophagus is associated with impairment of chemical clearance, but not inadequate acid suppression by proton pump inhibitor therapy. *Aliment Pharmacol Ther* 2014; **40**: 835-842 [PMID: 25087846 DOI: 10.1111/apt.12895]

52 **Conchillo JM**, Smout AJ. Review article: intra-oesophageal impedance monitoring for the assessment of bolus transit and gastro-oesophageal reflux. *Aliment Pharmacol Ther* 2009; **29**: 3-14 [PMID: 18945260 DOI: 10.1111/j.1365-2036.2008.03863.x]

53 **Frazzoni M**, Manta R, Mirante VG, Conigliaro R, Frazzoni L, Melotti G. Esophageal chemical clearance is impaired in gastro-esophageal reflux disease--a 24-h impedance-pH monitoring assessment. *Neurogastroenterol Motil* 2013; **25**: 399-406, e295 [PMID: 23360178 DOI: 10.1111/nmo.12080]

54 **de Bortoli N**, Martinucci I, Savarino E, Tutuian R, Frazzoni M, Piaggi P, Bertani L, Furnari M, Franchi R, Russo S, Bellini M, Savarino V, Marchi S. Association between baseline impedance values and response proton pump inhibitors in patients with heartburn. *Clin Gastroenterol Hepatol* 2015; **13**: 1082-8.e1 [PMID: 25499991 DOI: 10.1016/j.cgh.2014.11.035]

55 **Frazzoni M**, Savarino E, de Bortoli N, Martinucci I, Furnari M, Frazzoni L, Mirante VG, Bertani H, Marchi S, Conigliaro R, Savarino V. Analyses of the Post-reflux Swallow-induced Peristaltic Wave Index and Nocturnal Baseline Impedance Parameters Increase the Diagnostic Yield of Impedance-pH Monitoring of Patients With Reflux Disease. *Clin Gastroenterol Hepatol* 2016; **14**: 40-46 [PMID: 26122764 DOI: 10.1016/j.cgh.2015.06.026]

56 **Levine DS**, Haggitt RC, Blount PL, Rabinovitch PS, Rusch VW, Reid BJ. An endoscopic biopsy protocol can differentiate high-grade dysplasia from early adenocarcinoma in Barrett's esophagus. *Gastroenterology* 1993; **105**: 40-50 [PMID: 8514061]

57 **Whiteman DC**, Appleyard M, Bahin FF, Bobryshev YV, Bourke MJ, Brown I, Chung A, Clouston A, Dickins E, Emery J, Eslick GD, Gordon LG, Grimpen F, Hebbard G, Holliday L, Hourigan LF, Kendall BJ, Lee EY, Levert-Mignon A, Lord RV, Lord SJ, Maule D, Moss A, Norton I, Olver I, Pavey D, Raftopoulos S, Rajendra S, Schoeman M, Singh R, Sitas F, Smithers BM, Taylor AC, Thomas ML, Thomson I, To H, von Dincklage J, Vuletich C, Watson DI, Yusoff IF. Australian clinical practice guidelines for the diagnosis and management of Barrett's esophagus and early esophageal adenocarcinoma. *J Gastroenterol Hepatol* 2015; **30**: 804-820 [PMID: 25612140 DOI: 10.1111/jgh.12913]

58 **Fitzgerald RC**, di Pietro M, Ragunath K, Ang Y, Kang JY, Watson P, Trudgill N, Patel P, Kaye PV, Sanders S, O'Donovan M, Bird-Lieberman E, Bhandari P, Jankowski JA, Attwood S, Parsons SL, Loft D, Lagergren J, Moayyedi P, Lyratzopoulos G, de Caestecker J. British Society of Gastroenterology guidelines on the diagnosis and management of Barrett's oesophagus. *Gut* 2014; **63**: 7-42 [PMID: 24165758 DOI: 10.1136/gutjnl-2013-305372]

59 **Spechler SJ**, Sharma P, Souza RF, Inadomi JM, Shaheen NJ. American Gastroenterological Association medical position statement on the management of Barrett's esophagus. *Gastroenterology* 2011; **140**: 1084-1091 [PMID: 21376940 DOI: 10.1053/j.gastro.2011.01.030]

60 **Sharma P**, Dent J, Armstrong D, Bergman JJ, Gossner L, Hoshihara Y, Jankowski JA, Junghard O, Lundell L, Tytgat GN, Vieth M. The development and validation of an endoscopic grading system for Barrett's esophagus: the Prague C & amp; M criteria. *Gastroenterology* 2006; **131**: 1392-1399 [PMID: 17101315 DOI: 10.1053/j.gastro.2006.08.032]

61 **Hameeteman W**, Tytgat GN, Houthoff HJ, van den Tweel JG. Barrett's esophagus: development of dysplasia and adenocarcinoma. *Gastroenterology* 1989; **96**: 1249-1256 [PMID: 2703113]

62 **Wolfsen HC**, Crook JE, Krishna M, Achem SR, Devault KR, Bouras EP, Loeb DS, Stark ME, Woodward TA, Hemminger LL, Cayer FK, Wallace MB. Prospective, controlled tandem endoscopy study of narrow band imaging for dysplasia detection in Barrett's Esophagus. *Gastroenterology* 2008; **135**: 24-31 [PMID: 18442484 DOI: 10.1053/j.gastro.2008.03.019]

63 **Gupta N**, Gaddam S, Wani SB, Bansal A, Rastogi A, Sharma P. Longer inspection time is associated with increased detection of high-grade dysplasia and esophageal adenocarcinoma in Barrett's esophagus. *Gastrointest Endosc* 2012; **76**: 531-538 [PMID: 22732877 DOI: 10.1016/j.gie.2012.04.470]

64 **Sharma P**, Topalovski M, Mayo MS, Weston AP. Methylene blue chromoendoscopy for detection of short-segment Barrett's esophagus. *Gastrointest Endosc* 2001; **54**: 289-293 [PMID: 11522967]

65 **Canto MI**, Setrakian S, Willis J, Chak A, Petras R, Powe NR, Sivak MV. Methylene blue-directed biopsies improve detection of intestinal metaplasia and dysplasia in Barrett's esophagus. *Gastrointest Endosc* 2000; **51**: 560-568 [PMID: 10805842]

66 **Gossner L**, Pech O, May A, Vieth M, Stolte M, Ell C. Comparison of methylene blue-directed biopsies and four-quadrant biopsies in

the detection of high-grade intraepithelial neoplasia and early cancer in Barrett's oesophagus. *Dig Liver Dis* 2006; **38**: 724-729 [PMID: 16911879 DOI: 10.1016/j.dld.2006.05.025]

67 **Ngamruengphong S**, Sharma VK, Das A. Diagnostic yield of methylene blue chromoendoscopy for detecting specialized intestinal metaplasia and dysplasia in Barrett's esophagus: a meta-analysis. *Gastrointest Endosc* 2009; **69**: 1021-1028 [PMID: 19215918 DOI: 10.1016/j.gie.2008.06.056]

68 **Davies J**, Burke D, Olliver JR, Hardie LJ, Wild CP, Routledge MN. Methylene blue but not indigo carmine causes DNA damage to colonocytes in vitro and in vivo at concentrations used in clinical chromoendoscopy. *Gut* 2007; **56**: 155-156 [PMID: 17172595 DOI: 10.1136/gut.2006.107300]

69 **Kara MA**, Peters FP, Rosmolen WD, Krishnadath KK, ten Kate FJ, Fockens P, Bergman JJ. High-resolution endoscopy plus chromoendoscopy or narrow-band imaging in Barrett's esophagus: a prospective randomized crossover study. *Endoscopy* 2005; **37**: 929-936 [PMID: 16189764 DOI: 10.1055/s-2005-870433]

70 **Sharma P**, Bansal A, Mathur S, Wani S, Cherian R, McGregor D, Higbee A, Hall S, Weston A. The utility of a novel narrow band imaging endoscopy system in patients with Barrett's esophagus. *Gastrointest Endosc* 2006; **64**: 167-175 [PMID: 16860063 DOI: 10.1016/j.gie.2005.10.044]

71 **Mannath J**, Subramanian V, Hawkey CJ, Ragunath K. Narrow band imaging for characterization of high grade dysplasia and specialized intestinal metaplasia in Barrett's esophagus: a meta-analysis. *Endoscopy* 2010; **42**: 351-359 [PMID: 20200809 DOI: 10.1055/s-0029-1243949]

72 **Sharma P**, Hawes RH, Bansal A, Gupta N, Curvers W, Rastogi A, Singh M, Hall M, Mathur SC, Wani SB, Hoffman B, Gaddam S, Fockens P, Bergman JJ. Standard endoscopy with random biopsies versus narrow band imaging targeted biopsies in Barrett's oesophagus: a prospective, international, randomised controlled trial. *Gut* 2013; **62**: 15-21 [PMID: 22315471 DOI: 10.1136/gutjnl-2011-300962]

73 **Curvers WL**, Bohmer CJ, Mallant-Hent RC, Naber AH, Ponsioen CI, Ragunath K, Singh R, Wallace MB, Wolfsen HC, Song LM, Lindeboom R, Fockens P, Bergman JJ. Mucosal morphology in Barrett's esophagus: interobserver agreement and role of narrow band imaging. *Endoscopy* 2008; **40**: 799-805 [PMID: 18828075 DOI: 10.1055/s-2008-1077596]

74 **Kara MA**, Peters FP, Fockens P, ten Kate FJ, Bergman JJ. Endoscopic video-autofluorescence imaging followed by narrow band imaging for detecting early neoplasia in Barrett's esophagus. *Gastrointest Endosc* 2006; **64**: 176-185 [PMID: 16860064 DOI: 10.1016/j.gie.2005.11.050]

75 **Curvers WL**, Alvarez Herrero L, Wallace MB, Wong Kee Song LM, Ragunath K, Wolfsen HC, Prasad GA, Wang KK, Subramanian V, Weusten BL, Ten Kate FJ, Bergman JJ. Endoscopic tri-modal imaging is more effective than standard endoscopy in identifying early-stage neoplasia in Barrett's esophagus. *Gastroenterology* 2010; **139**: 1106-1114 [PMID: 20600033 DOI: 10.1053/j.gastro.2010.06.045]

76 **Curvers WL**, van Vilsteren FG, Baak LC, Böhmer C, Mallant-Hent RC, Naber AH, van Oijen A, Ponsioen CY, Scholten P, Schenk E, Schoon E, Seldenrijk CA, Meijer GA, ten Kate FJ, Bergman JJ. Endoscopic trimodal imaging versus standard video endoscopy for detection of early Barrett's neoplasia: a multicenter, randomized, crossover study in general practice. *Gastrointest Endosc* 2011; **73**: 195-203 [PMID: 21168835 DOI: 10.1016/j.gie.2010.10.014]

77 **Wallace MB**, Perelman LT, Backman V, Crawford JM, Fitzmaurice M, Seiler M, Badizadegan K, Shields SJ, Itzkan I, Dasari RR, Van Dam J, Feld MS. Endoscopic detection of dysplasia in patients with Barrett's esophagus using light-scattering spectroscopy. *Gastroenterology* 2000; **119**: 677-682 [PMID: 10982761 DOI: 10.1053/gast.2000.16511]

78 **Evans JA**, Poneros JM, Bouma BE, Bressner J, Halpern EF, Shishkov M, Lauwers GY, Mino-Kenudson M, Nishioka NS, Tearney GJ. Optical coherence tomography to identify intramucosal carcinoma and high-grade dysplasia in Barrett's esophagus. *Clin Gastroenterol Hepatol* 2006; **4**: 38-43 [PMID: 16431303 DOI: 10.1016/S1542-3565(05)00746-9]

79 **Hoffman A**, Kiesslich R, Bender A, Neurath MF, Nafe B, Herrmann G, Jung M. Acetic acid-guided biopsies after magnifying endoscopy compared with random biopsies in the detection of Barrett's esophagus: a prospective randomized trial with crossover design. *Gastrointest Endosc* 2006; **64**: 1-8 [PMID: 16813794 DOI: 10.1016/j.gie.2005.09.031]

80 **Ferguson DD**, DeVault KR, Krishna M, Loeb DS, Wolfsen HC, Wallace MB. Enhanced magnification-directed biopsies do not increase the detection of intestinal metaplasia in patients with GERD. *Am J Gastroenterol* 2006; **101**: 1611-1616 [PMID: 16863568 DOI: 10.1111/j.1572-0241.2006.00622.x]

81 **Longcroft-Wheaton G**, Duku M, Mead R, Poller D, Bhandari P. Acetic acid spray is an effective tool for the endoscopic detection of neoplasia in patients with Barrett's esophagus. *Clin Gastroenterol Hepatol* 2010; **8**: 843-847 [PMID: 20601133 DOI: 10.1016/j.cgh.2010.06.016]

82 **Pohl J**, Pech O, May A, Manner H, Fissler-Eckhoff A, Ell C. Incidence of macroscopically occult neoplasias in Barrett's esophagus: are random biopsies dispensable in the era of advanced endoscopic imaging? *Am J Gastroenterol* 2010; **105**: 2350-2356 [PMID: 20664531 DOI: 10.1038/ajg.2010.280]

83 **Bhandari P**, Kandaswamy P, Cowlishaw D, Longcroft-Wheaton G. Acetic acid-enhanced chromoendoscopy is more cost-effective than protocol-guided biopsies in a high-risk Barrett's population. *Dis Esophagus* 2012; **25**: 386-392 [PMID: 21981061 DOI: 10.1111/j.1442-2050.2011.01267.x]

84 **Sharma P**, Meining AR, Coron E, Lightdale CJ, Wolfsen HC, Bansal A, Bajbouj M, Galmiche JP, Abrams JA, Rastogi A, Gupta N, Michalek JE, Lauwers GY, Wallace MB. Real-time increased detection of neoplastic tissue in Barrett's esophagus with probe-based confocal laser endomicroscopy: final results of an international multicenter, prospective, randomized, controlled trial. *Gastrointest Endosc* 2011; **74**: 465-472 [PMID: 21741642 DOI: 10.1016/j.gie.2011.04.004]

85 **Canto MI**, Anandasabapathy S, Brugge W, Falk GW, Dunbar KB, Zhang Z, Woods K, Almario JA, Schell U, Goldblum J, Maitra A, Montgomery E, Kiesslich R. In vivo endomicroscopy improves detection of Barrett's esophagus-related neoplasia: a multicenter international randomized controlled trial (with video). *Gastrointest Endosc* 2014; **79**: 211-221 [PMID: 24219822 DOI: 10.1016/j.gie.2013.09.020]

86 **Bird-Lieberman EL**, Neves AA, Lao-Sirieix P, O'Donovan M, Novelli M, Lovat LB, Eng WS, Mahal LK, Brindle KM, Fitzgerald RC. Molecular imaging using fluorescent lectins permits rapid endoscopic identification of dysplasia in Barrett's esophagus. *Nat Med* 2012; **18**: 315-321 [PMID: 22245781 DOI: 10.1038/nm.2616]

87 **Li M**, Anastassiades CP, Joshi B, Komarck CM, Piraka C, Elmunzer BJ, Turgeon DK, Johnson TD, Appelman H, Beer DG, Wang TD. Affinity peptide for targeted detection of dysplasia in Barrett's esophagus. *Gastroenterology* 2010; **139**: 1472-1480 [PMID: 20637198 DOI: 10.1053/j.gastro.2010.07.007]

88 **Sturm MB**, Joshi BP, Lu S, Piraka C, Khondee S, Elmunzer BJ, Kwon RS, Beer DG, Appelman HD, Turgeon DK, Wang TD. Targeted imaging of esophageal neoplasia with a fluorescently labeled peptide: first-in-human results. *Sci Transl Med* 2013; **5**: 184ra61 [PMID: 23658246 DOI: 10.1126/scitranslmed.3004733]

89 **Bhat SK**, McManus DT, Coleman HG, Johnston BT, Cardwell CR, McMenamin U, Bannon F, Hicks B, Kennedy G, Gavin AT, Murray LJ. Oesophageal adenocarcinoma and prior diagnosis of Barrett's oesophagus: a population-based study. *Gut* 2015; **64**: 20-25 [PMID: 24700439 DOI: 10.1136/gutjnl-2013-305506]

90 **Bennett C**, Moayyedi P, Corley DA, DeCaestecker J, Falck-Ytter Y, Falk G, Vakil N, Sanders S, Vieth M, Inadomi J, Aldulaimi D, Ho KY, Odze R, Meltzer SJ, Quigley E, Gittens S, Watson P, Zaninotto G, Iyer PG, Alexandre L, Ang Y, Callaghan J, Harrison R, Singh R, Bhandari P, Bisschops R, Geramizadeh B, Kaye P, Krishnadath S, Fennerty MB, Manner H, Nason KS, Pech O, Konda V, Ragunath K, Rahman I, Romero Y, Sampliner R, Siersema PD, Tack J, Tham TC, Trudgill N, Weinberg DS, Wang J, Wang K, Wong JY, Attwood S, Malfertheiner P, MacDonald D, Barr H, Ferguson MK, Jankowski

J. BOB CAT: A Large-Scale Review and Delphi Consensus for Management of Barrett's Esophagus With No Dysplasia, Indefinite for, or Low-Grade Dysplasia. *Am J Gastroenterol* 2015; **110**: 662-682; quiz 683 [PMID: 25869390 DOI: 10.1038/ajg.2015.55]

91 **Alashkar B**, Faulx AL, Hepner A, Pulice R, Vemana S, Greer KB, Isenberg GA, Falck-Ytter Y, Chak A. Development of a program to train physician extenders to perform transnasal esophagoscopy and screen for Barrett's esophagus. *Clin Gastroenterol Hepatol* 2014; **12**: 785-792 [PMID: 24161352 DOI: 10.1016/j.cgh.2013.10.014]

92 **Shariff MK**, Bird-Lieberman EL, O'Donovan M, Abdullahi Z, Liu X, Blazeby J, Fitzgerald R. Randomized crossover study comparing efficacy of transnasal endoscopy with that of standard endoscopy to detect Barrett's esophagus. *Gastrointest Endosc* 2012; **75**: 954-961 [PMID: 22421496 DOI: 10.1016/j.gie.2012.01.029]

93 **Kadri SR**, Lao-Sirieix P, O'Donovan M, Debiram I, Das M, Blazeby JM, Emery J, Boussioutas A, Morris H, Walter FM, Pharoah P, Hardwick RH, Fitzgerald RC. Acceptability and accuracy of a non-endoscopic screening test for Barrett's oesophagus in primary care: cohort study. *BMJ* 2010; **341**: c4372 [PMID: 20833740 DOI: 10.1136/bmj.c4372]

94 **Lao-Sirieix P**, Boussioutas A, Kadri SR, O'Donovan M, Debiram I, Das M, Harihar L, Fitzgerald RC. Non-endoscopic screening biomarkers for Barrett's oesophagus: from microarray analysis to the clinic. *Gut* 2009; **58**: 1451-1459 [PMID: 19651633 DOI: 10.1136/gut.2009.180281]

95 **Benaglia T**, Sharples LD, Fitzgerald RC, Lyratzopoulos G. Health benefits and cost effectiveness of endoscopic and nonendoscopic cytosponge screening for Barrett's esophagus. *Gastroenterology* 2013; **144**: 62-73.e6 [PMID: 23041329 DOI: 10.1053/j.gastro.2012.09.060]

96 **Thrift AP**, Garcia JM, El-Serag HB. A multibiomarker risk score helps predict risk for Barrett's esophagus. *Clin Gastroenterol Hepatol* 2014; **12**: 1267-1271 [PMID: 24362047 DOI: 10.1016/j.cgh.2013.12.014]

97 **Corley DA**, Levin TR, Habel LA, Weiss NS, Buffler PA. Surveillance and survival in Barrett's adenocarcinomas: a population-based study. *Gastroenterology* 2002; **122**: 633-640 [PMID: 11874995 DOI: 10.1053/gast.2002.31879]

98 **Aldulaimi DM**, Cox M, Nwokolo CU, Loft DE. Barrett's surveillance is worthwhile and detects curable cancers. A prospective cohort study addressing cancer incidence, treatment outcome and survival. *Eur J Gastroenterol Hepatol* 2005; **17**: 943-950 [PMID: 16093872 DOI: 10.1097/00042737-200509000-00010]

99 **Gaddam S**, Singh M, Balasubramanian G, Thota P, Gupta N, Wani S, Higbee AD, Mathur SC, Horwhat JD, Rastogi A, Young PE, Cash BD, Bansal A, Vargo JJ, Falk GW, Lieberman DA, Sampliner RE, Sharma P. Persistence of nondysplastic Barrett's esophagus identifies patients at lower risk for esophageal adenocarcinoma: results from a large multicenter cohort. *Gastroenterology* 2013; **145**: 548-553.e1 [PMID: 23714382 DOI: 10.1053/j.gastro.2013.05.040]

100 **Evans JA**, Early DS, Fukami N, Ben-Menachem T, Chandrasekhara V, Chathadi KV, Decker GA, Fanelli RD, Fisher DA, Foley KQ, Hwang JH, Jain R, Jue TL, Khan KM, Lightdale J, Malpas PM, Maple JT, Pasha SF, Saltzman JR, Sharaf RN, Shergill A, Dominitz JA, Cash BD. The role of endoscopy in Barrett's esophagus and other premalignant conditions of the esophagus. *Gastrointest Endosc* 2012; **76**: 1087-1094 [PMID: 23164510 DOI: 10.1016/j.gie.2012.08.004]

101 **Shaheen NJ**, Weinberg DS, Denberg TD, Chou R, Qaseem A, Shekelle P. Upper endoscopy for gastroesophageal reflux disease: best practice advice from the clinical guidelines committee of the American College of Physicians. *Ann Intern Med* 2012; **157**: 808-816 [PMID: 23208168 DOI: 10.7326/0003-4819-157-11-201212040-00008]

102 **Horvath B**, Singh P, Xie H, Thota PN, Allende DS, Pai RK, Patil DT, Plesec TP, Goldblum JR, Liu X. Risk for esophageal neoplasia in Barrett's esophagus patients with mucosal changes indefinite for dysplasia. *J Gastroenterol Hepatol* 2015; **30**: 262-267 [PMID: 25087917 DOI: 10.1111/jgh.12696]

103 **Younes M**, Lauwers GY, Ertan A, Ergun G, Verm R, Bridges M, Woods K, Meriano F, Schmulen C, Johnson C, Barroso A, Schwartz J, McKechnie J, Lechago J. The significance of "indefinite for dysplasia" grading in Barrett metaplasia. *Arch Pathol Lab Med* 2011; **135**: 430-432 [PMID: 21466357 DOI: 10.1043/2010-0097-OA.1]

104 **Singh S**, Manickam P, Amin AV, Samala N, Schouten LJ, Iyer PG, Desai TK. Incidence of esophageal adenocarcinoma in Barrett's esophagus with low-grade dysplasia: a systematic review and meta-analysis. *Gastrointest Endosc* 2014; **79**: 897-909.e4; quiz 983.e1, 983.e3 [PMID: 24556051 DOI: 10.1016/j.gie.2014.01.009]

105 **Wani S**, Falk GW, Post J, Yerian L, Hall M, Wang A, Gupta N, Gaddam S, Singh M, Singh V, Chuang KY, Boolchand V, Gavini H, Kuczynski J, Sud P, Bansal A, Rastogi A, Mathur SC, Young P, Cash B, Goldblum J, Lieberman DA, Sampliner RE, Sharma P. Risk factors for progression of low-grade dysplasia in patients with Barrett's esophagus. *Gastroenterology* 2011; **141**: 1179-1186, 1186.e1 [PMID: 21723218 DOI: 10.1053/j.gastro.2011.06.055]

106 **Kaye PV**, Haider SA, Ilyas M, James PD, Soomro I, Faisal W, Catton J, Parsons SL, Ragunath K. Barrett's dysplasia and the Vienna classification: reproducibility, prediction of progression and impact of consensus reporting and p53 immunohistochemistry. *Histopathology* 2009; **54**: 699-712 [PMID: 19438745 DOI: 10.1111/j.1365-2559.2009.03288.x]

107 **Duits LC**, Phoa KN, Curvers WL, Ten Kate FJ, Meijer GA, Seldenrijk CA, Offerhaus GJ, Visser M, Meijer SL, Krishnadath KK, Tijssen JG, Mallant-Hent RC, Bergman JJ. Barrett's oesophagus patients with low-grade dysplasia can be accurately risk-stratified after histological review by an expert pathology panel. *Gut* 2015; **64**: 700-706 [PMID: 25034523 DOI: 10.1136/gutjnl-2014-307278]

108 **Abdalla M**, Dhanekula R, Greenspan M, Mobarhan S, Patil A, Jakate S, Giusto D, Silva R, Li H, Melson J. Dysplasia detection rate of confirmatory EGD in nondysplastic Barrett's esophagus. *Dis Esophagus* 2014; **27**: 505-510 [PMID: 23020509 DOI: 10.1111/j.1442-2050.2012.01431.x]

109 **Brown CS**, Lapin B, Wang C, Goldstein JL, Linn JG, Denham W, Haggerty SP, Talamonti MS, Howington JA, Carbray J, Ujiki MB. Reflux control is important in the management of Barrett's Esophagus: results from a retrospective 1,830 patient cohort. *Surg Endosc* 2015; **29**: 3528-3534 [PMID: 25676204 DOI: 10.1007/s00464-015-4103-3]

110 **Singh S**, Garg SK, Singh PP, Iyer PG, El-Serag HB. Acid-suppressive medications and risk of oesophageal adenocarcinoma in patients with Barrett's oesophagus: a systematic review and meta-analysis. *Gut* 2014; **63**: 1229-1237 [PMID: 24221456 DOI: 10.1136/gutjnl-2013-305997]

111 **Islami F**, Kamangar F, Boffetta P. Use of proton pump inhibitors and risk of progression of Barrett's esophagus to neoplastic lesions. *Am J Gastroenterol* 2009; **104**: 2646-2648 [PMID: 19806109 DOI: 10.1038/ajg.2009.369]

112 **Ouatu-Lascar R**, Fitzgerald RC, Triadafilopoulos G. Differentiation and proliferation in Barrett's esophagus and the effects of acid suppression. *Gastroenterology* 1999; **117**: 327-335 [PMID: 10419913]

113 **de Bortoli N**, Martinucci I, Piaggi P, Maltinti S, Bianchi G, Ciancia E, Gambaccini D, Lenzi F, Costa F, Leonardi G, Ricchiuti A, Mumolo MG, Bellini M, Blandizzi C, Marchi S. Randomised clinical trial: twice daily esomeprazole 40 mg vs. pantoprazole 40 mg in Barrett's oesophagus for 1 year. *Aliment Pharmacol Ther* 2011; **33**: 1019-1027 [PMID: 21385192 DOI: 10.1111/j.1365-2036.2011.04616.x]

114 **Kedika RR**, Souza RF, Spechler SJ. Potential anti-inflammatory effects of proton pump inhibitors: a review and discussion of the clinical implications. *Dig Dis Sci* 2009; **54**: 2312-2317 [PMID: 19714466 DOI: 10.1007/s10620-009-0951-9]

115 **Nguyen DM**, Richardson P, El-Serag HB. Medications (NSAIDs, statins, proton pump inhibitors) and the risk of esophageal adenocarcinoma in patients with Barrett's esophagus. *Gastroenterology* 2010; **138**: 2260-2266 [PMID: 20188100 DOI: 10.1053/j.gastro.2010.02.045]

116 **Richter JE**, Penagini R, Tenca A, Pohl D, Dvorak K, Goldman A, Savarino E, Zentilin P, Savarino V, Watson JT, Wong RK, Pace F, Casini V, Peura DA, Herzig SJ, Kamiya T, Pelosini I, Scarpignato C,

Armstrong D, DeVault KR, Bechi P, Taddei A, Freschi G, Ringressi MN, Degli'Innocenti DR, Castiglione F, Masini E, Hunt RH. Barrett's esophagus: proton pump inhibitors and chemoprevention II. *Ann N Y Acad Sci* 2011; **1232**: 114-139 [PMID: 21950810 DOI: 10.1111/j.1749-6632.2011.06048.x]

117 **Das D**, Chilton AP, Jankowski JA. Chemoprevention of oesophageal cancer and the AspECT trial. *Recent Results Cancer Res* 2009; **181**: 161-169 [PMID: 19213566]

118 **Wang KK**, Sampliner RE. Updated guidelines 2008 for the diagnosis, surveillance and therapy of Barrett's esophagus. *Am J Gastroenterol* 2008; **103**: 788-797 [PMID: 18341497 DOI: 10.1111/j.1572-0241.2008.01835.x]

119 **Blevins CH**, Iyer PG. Endoscopic therapy for Barrett's oesophagus. *Best Pract Res Clin Gastroenterol* 2015; **29**: 167-177 [PMID: 25743464 DOI: 10.1016/j.bpg.2014.11.007]

120 **Bennett C**, Vakil N, Bergman J, Harrison R, Odze R, Vieth M, Sanders S, Gay L, Pech O, Longcroft-Wheaton G, Romero Y, Inadomi J, Tack J, Corley DA, Manner H, Green S, Al Dulaimi D, Ali H, Allum B, Anderson M, Curtis H, Falk G, Fennerty MB, Fullarton G, Krishnadath K, Meltzer SJ, Armstrong D, Ganz R, Cengia G, Going JJ, Goldblum J, Gordon C, Grabsch H, Haigh C, Hongo M, Johnston D, Forbes-Young R, Kay E, Kaye P, Lerut T, Lovat LB, Lundell L, Mairs P, Shimoda T, Spechler S, Sontag S, Malfertheiner P, Murray I, Nanji M, Poller D, Ragunath K, Regula J, Cestari R, Shepherd N, Singh R, Stein HJ, Talley NJ, Galmiche JP, Tham TC, Watson P, Yerian L, Rugge M, Rice TW, Hart J, Gittens S, Hewin D, Hochberger J, Kahrilas P, Preston S, Sampliner R, Sharma P, Stuart R, Wang K, Waxman I, Abley C, Loft D, Penman I, Shaheen NJ, Chak A, Davies G, Dunn L, Falck-Ytter Y, Decaestecker J, Bhandari P, Ell C, Griffin SM, Attwood S, Barr H, Allen J, Ferguson MK, Moayyedi P, Jankowski JA. Consensus statements for management of Barrett's dysplasia and early-stage esophageal adenocarcinoma, based on a Delphi process. *Gastroenterology* 2012; **143**: 336-346 [PMID: 22537613 DOI: 10.1053/j.gastro.2012.04.032]

121 **Perry KA**, Enestvedt CK, Pham T, Welker M, Jobe BA, Hunter JG, Sheppard BC. Comparison of laparoscopic inversion esophagectomy and open transhiatal esophagectomy for high-grade dysplasia and stage I esophageal adenocarcinoma. *Arch Surg* 2009; **144**: 679-684 [PMID: 19620549 DOI: 10.1001/archsurg.2009.113]

122 **Stahl M**, Mariette C, Haustermans K, Cervantes A, Arnold D. Oesophageal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2013; **24** Suppl 6: vi51-vi56 [PMID: 24078662 DOI: 10.1093/annonc/mdt342]

123 **Evans JA**, Early DS, Chandraskhara V, Chathadi KV, Fanelli RD, Fisher DA, Foley KQ, Hwang JH, Jue TL, Pasha SF, Sharaf R, Shergill AK, Dominitz JA, Cash BD. The role of endoscopy in the assessment and treatment of esophageal cancer. *Gastrointest Endosc* 2013; **77**: 328-334 [PMID: 23410694 DOI: 10.1016/j.gie.2012.10.001]

124 **Pech O**, Behrens A, May A, Nachbar L, Gossner L, Rabenstein T, Manner H, Guenter E, Huijsmans J, Vieth M, Stolte M, Ell C. Long-term results and risk factor analysis for recurrence after curative endoscopic therapy in 349 patients with high-grade intraepithelial neoplasia and mucosal adenocarcinoma in Barrett's oesophagus. *Gut* 2008; **57**: 1200-1206 [PMID: 18460553 DOI: 10.1136/gut.2007.142539]

125 **Prasad GA**, Wu TT, Wigle DA, Buttar NS, Wongkeesong LM, Dunagan KT, Lutzke LS, Borkenhagen LS, Wang KK. Endoscopic and surgical treatment of mucosal (T1a) esophageal adenocarcinoma in Barrett's esophagus. *Gastroenterology* 2009; **137**: 815-823 [PMID: 19524578 DOI: 10.1053/j.gastro.2009.05.059]

126 **Das A**, Singh V, Fleischer DE, Sharma VK. A comparison of endoscopic treatment and surgery in early esophageal cancer: an analysis of surveillance epidemiology and end results data. *Am J Gastroenterol* 2008; **103**: 1340-1345 [PMID: 18510606 DOI: 10.1111/j.1572-0241.2008.01889.x]

127 **Zehetner J**, DeMeester SR, Hagen JA, Ayazi S, Augustin F, Lipham JC, DeMeester TR. Endoscopic resection and ablation versus esophagectomy for high-grade dysplasia and intramucosal adenocarcinoma. *J Thorac Cardiovasc Surg* 2011; **141**: 39-47 [PMID: 21055772 DOI: 10.1016/j.jtcvs.2010.08.058]

128 **Wani S**, Mathur SC, Curvers WL, Singh V, Alvarez Herrero L, Hall SB, Ulusarac O, Cherian R, McGregor DH, Bansal A, Rastogi A, Ahmed B, Singh M, Gaddam S, Ten Kate FJ, Bergman J, Sharma P. Greater interobserver agreement by endoscopic mucosal resection than biopsy samples in Barrett's dysplasia. *Clin Gastroenterol Hepatol* 2010; **8**: 783-788 [PMID: 20472096 DOI: 10.1016/j.cgh.2010.04.028]

129 **Wani S**, Abrams J, Edmundowicz SA, Gaddam S, Hovis CE, Green D, Gupta N, Higbee A, Bansal A, Rastogi A, Early D, Lightdale CJ, Sharma P. Endoscopic mucosal resection results in change of histologic diagnosis in Barrett's esophagus patients with visible and flat neoplasia: a multicenter cohort study. *Dig Dis Sci* 2013; **58**: 1703-1709 [PMID: 23633158 DOI: 10.1007/s10620-013-2689-7]

130 **Mino-Kenudson M**, Hull MJ, Brown I, Muzikansky A, Srivastava A, Glickman J, Park DY, Zuckerberg L, Misdraji J, Odze RD, Lauwers GY. EMR for Barrett's esophagus-related superficial neoplasms offers better diagnostic reproducibility than mucosal biopsy. *Gastrointest Endosc* 2007; **66**: 660-666; quiz 767, 769 [PMID: 17905005 DOI: 10.1016/j.gie.2007.02.063]

131 **Leggett CL**, Lewis JT, Wu TT, Schleck CD, Zinsmeister AR, Dunagan KT, Lutzke LS, Wang KK, Iyer PG. Clinical and histologic determinants of mortality for patients with Barrett's esophagus-related T1 esophageal adenocarcinoma. *Clin Gastroenterol Hepatol* 2015; **13**: 658-664.e1-3 [PMID: 25151255 DOI: 10.1016/j.cgh.2014.08.016]

132 **Davila ML**, Hofstetter WL. Endoscopic management of Barrett's esophagus with high-grade dysplasia and early-stage esophageal adenocarcinoma. *Thorac Surg Clin* 2013; **23**: 479-489 [PMID: 24199698 DOI: 10.1016/j.thorsurg.2013.07.010]

133 **Spechler SJ**, Sharma P, Souza RF, Inadomi JM, Shaheen NJ. American Gastroenterological Association technical review on the management of Barrett's esophagus. *Gastroenterology* 2011; **140**: e18-52; quiz e13 [PMID: 21376939 DOI: 10.1053/j.gastro.2011.01.031]

134 **Pouw RE**, van Vilsteren FG, Peters FP, Alvarez Herrero L, Ten Kate FJ, Visser M, Schenk BE, Schoon EJ, Peters FT, Houben M, Bisschops R, Weusten BL, Bergman JJ. Randomized trial on endoscopic resection-cap versus multiband mucosectomy for piecemeal endoscopic resection of early Barrett's neoplasia. *Gastrointest Endosc* 2011; **74**: 35-43 [PMID: 21704807 DOI: 10.1016/j.gie.2011.03.1243]

135 **Ell C**, May A, Pech O, Gossner L, Guenter E, Behrens A, Nachbar L, Huijsmans J, Vieth M, Stolte M. Curative endoscopic resection of early esophageal adenocarcinomas (Barrett's cancer). *Gastrointest Endosc* 2007; **65**: 3-10 [PMID: 17185072 DOI: 10.1016/j.gie.2006.04.033]

136 **May A**, Gossner L, Pech O, Fritz A, Günter E, Mayer G, Müller H, Seitz G, Vieth M, Stolte M, Ell C. Local endoscopic therapy for intraepithelial high-grade neoplasia and early adenocarcinoma in Barrett's oesophagus: acute-phase and intermediate results of a new treatment approach. *Eur J Gastroenterol Hepatol* 2002; **14**: 1085-1091 [PMID: 12362099]

137 **Pech O**, May A, Manner H, Behrens A, Pohl J, Weferling M, Hartmann U, Manner N, Huijsmans J, Gossner L, Rabenstein T, Vieth M, Stolte M, Ell C. Long-term efficacy and safety of endoscopic resection for patients with mucosal adenocarcinoma of the esophagus. *Gastroenterology* 2014; **146**: 652-660.e1 [PMID: 24269290 DOI: 10.1053/j.gastro.2013.11.006]

138 **Moss A**, Bourke MJ, Hourigan LF, Gupta S, Williams SJ, Tran K, Swan MP, Hopper AD, Kwan V, Bailey AA. Endoscopic resection for Barrett's high-grade dysplasia and early esophageal adenocarcinoma: an essential staging procedure with long-term therapeutic benefit. *Am J Gastroenterol* 2010; **105**: 1276-1283 [PMID: 20179694 DOI: 10.1038/ajg.2010.1]

139 **Gerke H**, Siddiqui J, Nasr I, Van Handel DM, Jensen CS. Efficacy and safety of EMR to completely remove Barrett's esophagus: experience in 41 patients. *Gastrointest Endosc* 2011; **74**: 761-771 [PMID: 21824611 DOI: 10.1016/j.gie.2011.06.009]

140 **Yoshinaga S**, Gotoda T, Kusano C, Oda I, Nakamura K, Takayanagi

R. Clinical impact of endoscopic submucosal dissection for superficial adenocarcinoma located at the esophagogastric junction. *Gastrointest Endosc* 2008; **67**: 202-209 [PMID: 18226681 DOI: 10.1016/j.gie.2007.09.054]

141 **Teoh AY**, Chiu PW, Yu Ngo DK, Wong SK, Lau JY, Ng EK. Outcomes of endoscopic submucosal dissection versus endoscopic mucosal resection in management of superficial squamous esophageal neoplasms outside Japan. *J Clin Gastroenterol* 2010; **44**: e190-e194 [PMID: 20844363 DOI: 10.1097/MCG.0b013e3181ce52fb]

142 **Repici A**, Hassan C, Carlino A, Pagano N, Zullo A, Rando G, Strangio G, Romeo F, Nicita R, Rosati R, Malesci A. Endoscopic submucosal dissection in patients with early esophageal squamous cell carcinoma: results from a prospective Western series. *Gastrointest Endosc* 2010; **71**: 715-721 [PMID: 20363414 DOI: 10.1016/j.gie.2009.11.020]

143 **Chennat J**, Konda VJ, Ross AS, de Tejada AH, Noffsinger A, Hart J, Lin S, Ferguson MK, Posner MC, Waxman I. Complete Barrett' s eradication endoscopic mucosal resection: an effective treatment modality for high-grade dysplasia and intramucosal carcinoma--an American single-center experience. *Am J Gastroenterol* 2009; **104**: 2684-2692 [PMID: 19690526 DOI: 10.1038/ajg.2009.465]

144 **Hashimoto S**, Kobayashi M, Takeuchi M, Sato Y, Narisawa R, Aoyagi Y. The efficacy of endoscopic triamcinolone injection for the prevention of esophageal stricture after endoscopic submucosal dissection. *Gastrointest Endosc* 2011; **74**: 1389-1393 [PMID: 22136782 DOI: 10.1016/j.gie.2011.07.070]

145 **Shaheen NJ**, Sharma P, Overholt BF, Wolfsen HC, Sampliner RE, Wang KK, Galanko JA, Bronner MP, Goldblum JR, Bennett AE, Jobe BA, Eisen GM, Fennerty MB, Hunter JG, Fleischer DE, Sharma VK, Hawes RH, Hoffman BJ, Rothstein RI, Gordon SR, Mashimo H, Chang KJ, Muthusamy VR, Edmundowicz SA, Spechler SJ, Siddiqui AA, Souza RF, Infantolino A, Falk GW, Kimmey MB, Madanick RD, Chak A, Lightdale CJ. Radiofrequency ablation in Barrett's esophagus with dysplasia. *N Engl J Med* 2009; **360**: 2277-2288 [PMID: 19474425 DOI: 10.1056/NEJMoa0808145]

146 **Shaheen NJ**, Overholt BF, Sampliner RE, Wolfsen HC, Wang KK, Fleischer DE, Sharma VK, Eisen GM, Fennerty MB, Hunter JG, Bronner MP, Goldblum JR, Bennett AE, Mashimo H, Rothstein RI, Gordon SR, Edmundowicz SA, Madanick RD, Peery AF, Muthusamy VR, Chang KJ, Kimmey MB, Spechler SJ, Siddiqui AA, Souza RF, Infantolino A, Dumot JA, Falk GW, Galanko JA, Jobe BA, Hawes RH, Hoffman BJ, Sharma P, Chak A, Lightdale CJ. Durability of radiofrequency ablation in Barrett's esophagus with dysplasia. *Gastroenterology* 2011; **141**: 460-468 [PMID: 21679712 DOI: 10.1053/j.gastro.2011.04.061]

147 **Phoa KN**, van Vilsteren FG, Weusten BL, Bisschops R, Schoon EJ, Ragunath K, Fullarton G, Di Pietro M, Ravi N, Visser M, Offerhaus GJ, Seldenrijk CA, Meijer SL, ten Kate FJ, Tijssen JG, Bergman JJ. Radiofrequency ablation vs endoscopic surveillance for patients with Barrett esophagus and low-grade dysplasia: a randomized clinical trial. *JAMA* 2014; **311**: 1209-1217 [PMID: 24668102 DOI: 10.1001/jama.2014.2511]

148 **Orman ES**, Li N, Shaheen NJ. Efficacy and durability of radiofrequency ablation for Barrett's Esophagus: systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2013; **11**: 1245-1255 [PMID: 23644385 DOI: 10.1016/j.cgh.2013.03.039]

149 **Bulsiewicz WJ**, Kim HP, Dellon ES, Cotton CC, Pasricha S, Madanick RD, Spacek MB, Bream SE, Chen X, Orlando RC, Shaheen NJ. Safety and efficacy of endoscopic mucosal therapy with radiofrequency ablation for patients with neoplastic Barrett's esophagus. *Clin Gastroenterol Hepatol* 2013; **11**: 636-642 [PMID: 23103824 DOI: 10.1016/j.cgh.2012.10.028]

150 **Prasad GA**, Wang KK, Halling KC, Buttar NS, Wongkeesong LM, Zinsmeister AR, Brankley SM, Fritcher EG, Westra WM, Krishnadath KK, Lutzke LS, Borkenhagen LS. Utility of biomarkers in prediction of response to ablative therapy in Barrett's esophagus. *Gastroenterology* 2008; **135**: 370-379 [PMID: 18538141 DOI: 10.1053/j.gastro.2008.04.036]

151 **Krishnan K**, Pandolfino JE, Kahrilas PJ, Keefer L, Boris L, Komanduri S. Increased risk for persistent intestinal metaplasia in patients with Barrett's esophagus and uncontrolled reflux exposure before radiofrequency ablation. *Gastroenterology* 2012; **143**: 576-581 [PMID: 22609385 DOI: 10.1053/j.gastro.2012.05.005]

152 **Timmer MR**, Brankley SM, Gorospe EC, Sun G, Lutzke LS, Iyer PG, Halling KC, Krishnadath KK, Wang KK. Prediction of response to endoscopic therapy of Barrett's dysplasia by using genetic biomarkers. *Gastrointest Endosc* 2014; **80**: 984-991 [PMID: 25085335 DOI: 10.1016/j.gie.2014.06.023]

153 **Gray NA**, Odze RD, Spechler SJ. Buried metaplasia after endoscopic ablation of Barrett's esophagus: a systematic review. *Am J Gastroenterol* 2011; **106**: 1899-1908; quiz 1909 [PMID: 21826111 DOI: 10.1038/ajg.2011.255]

154 **Gupta N**, Mathur SC, Dumot JA, Singh V, Gaddam S, Wani SB, Bansal A, Rastogi A, Goldblum JR, Sharma P. Adequacy of esophageal squamous mucosa specimens obtained during endoscopy: are standard biopsies sufficient for postablation surveillance in Barrett's esophagus? *Gastrointest Endosc* 2012; **75**: 11-18 [PMID: 21907985 DOI: 10.1016/j.gie.2011.06.040]

155 **Dulai PS**, Pohl H, Levenick JM, Gordon SR, MacKenzie TA, Rothstein RI. Radiofrequency ablation for long- and ultralong-segment Barrett's esophagus: a comparative long-term follow-up study. *Gastrointest Endosc* 2013; **77**: 534-541 [PMID: 23290719 DOI: 10.1016/j.gie.2012.10.021]

156 **Gosain S**, Mercer K, Twaddell WS, Uradomo L, Greenwald BD. Liquid nitrogen spray cryotherapy in Barrett's esophagus with high-grade dysplasia: long-term results. *Gastrointest Endosc* 2013; **78**: 260-265 [PMID: 23622979 DOI: 10.1016/j.gie.2013.03.002]

157 **Fuchs KH**, Babic B, Breithaupt W, Dallemagne B, Fingerhut A, Furnee E, Granderath F, Horvath P, Kardos P, Pointner R, Savarino E, Van Herwaarden-Lindeboom M, Zaninotto G. EAES recommendations for the management of gastroesophageal reflux disease. *Surg Endosc* 2014; **28**: 1753-1773 [PMID: 24789125 DOI: 10.1007/s00464-014-3431-z]

158 **Galmiche JP**, Hatlebakk J, Attwood S, Ell C, Fiocca R, Eklund S, Långström G, Lind T, Lundell L. Laparoscopic antireflux surgery vs esomeprazole treatment for chronic GERD: the LOTUS randomized clinical trial. *JAMA* 2011; **305**: 1969-1977 [PMID: 21586712 DOI: 10.1001/jama.2011.626]

159 **Faria R**, Bojke L, Epstein D, Corbacho B, Sculpher M. Cost-effectiveness of laparoscopic fundoplication versus continued medical management for the treatment of gastro-oesophageal reflux disease based on long-term follow-up of the REFLUX trial. *Br J Surg* 2013; **100**: 1205-1213 [PMID: 23775366 DOI: 10.1002/bjs.9190]

160 **Strate U**, Emmermann A, Fibbe C, Layer P, Zornig C. Laparoscopic fundoplication: Nissen versus Toupet two-year outcome of a prospective randomized study of 200 patients regarding preoperative esophageal motility. *Surg Endosc* 2008; **22**: 21-30 [PMID: 18027055 DOI: 10.1007/s00464-007-9546-8]

161 **Zornig C**, Strate U, Fibbe C, Emmermann A, Layer P. Nissen vs Toupet laparoscopic fundoplication. *Surg Endosc* 2002; **16**: 758-766 [PMID: 11997817 DOI: 10.1007/s00464-001-9092-8]

162 **Patti MG**, Robinson T, Galvani C, Gorodner MV, Fisichella PM, Way LW. Total fundoplication is superior to partial fundoplication even when esophageal peristalsis is weak. *J Am Coll Surg* 2004; **198**: 863-869; discussion 869-870 [PMID: 15194064 DOI: 10.1016/j.jamcollsurg.2004.01.029]

163 **Dallemagne B**, Weerts J, Markiewicz S, Dewandre JM, Wahlen C, Monami B, Jehaes C. Clinical results of laparoscopic fundoplication at ten years after surgery. *Surg Endosc* 2006; **20**: 159-165 [PMID: 16333553 DOI: 10.1007/s00464-005-0174-x]

164 **Fein M**, Bueter M, Thalheimer A, Pachmayr V, Heimbucher J, Freys SM, Fuchs KH. Less reflux recurrence following Nissen fundoplication : results of laparoscopic antireflux surgery after 10 years. *Chirurg* 2008; **79**: 759-764 [PMID: 18496658 DOI: 10.1007/s00104-008-1532-8]

165 **Attwood SE**, Lundell L, Hatlebakk JG, Eklund S, Junghard O, Galmiche JP, Ell C, Fiocca R, Lind T. Medical or surgical management of GERD patients with Barrett's esophagus: the LOTUS trial 3-year experience. *J Gastrointest Surg* 2008; **12**: 1646-1654; discussion 1654-1655 [PMID: 18709511 DOI: 10.1007/

s11605-008-0645-1]
166 **Caygill CP**, Watson A, Lao-Sirieix P, Fitzgerald RC. Barrett's oesophagus and adenocarcinoma. *World J Surg Oncol* 2004; **2**: 12 [PMID: 15132744 DOI: 10.1186/1477-7819-2-12]
167 **Avidan B**, Sonnenberg A, Schnell TG, Chejfec G, Metz A, Sontag SJ. Hiatal hernia size, Barrett's length, and severity of acid reflux are all risk factors for esophageal adenocarcinoma. *Am J Gastroenterol* 2002; **97**: 1930-1936 [PMID: 12190156 DOI: 10.1111/j.1572-0241.2002.05902.x]
168 **Rossi M**, Barreca M, de Bortoli N, Renzi C, Santi S, Gennai A, Bellini M, Costa F, Conio M, Marchi S. Efficacy of Nissen fundoplication versus medical therapy in the regression of low-grade dysplasia in patients with Barrett esophagus: a prospective study. *Ann Surg* 2006; **243**: 58-63 [PMID: 16371737]
169 **Zaninotto G**, Parente P, Salvador R, Farinati F, Tieppo C, Passuello N, Zanatta L, Fassan M, Cavallin F, Costantini M, Mescoli C, Battaglia G, Ruol A, Ancona E, Rugge M. Long-term follow-up of Barrett's epithelium: medical versus antireflux surgical therapy. *J Gastrointest Surg* 2012; **16**: 7-14; discussion 14-15 [PMID: 22086718 DOI: 10.1007/s11605-011-1739-8]
170 **Chang EY**, Morris CD, Seltman AK, O'Rourke RW, Chan BK, Hunter JG, Jobe BA. The effect of antireflux surgery on esophageal carcinogenesis in patients with barrett esophagus: a systematic review. *Ann Surg* 2007; **246**: 11-21 [PMID: 17592284 DOI: 10.1097/01.sla.0000261459.10565.e9]
171 **Corey KE**, Schmitz SM, Shaheen NJ. Does a surgical antireflux procedure decrease the incidence of esophageal adenocarcinoma in Barrett's esophagus? A meta-analysis. *Am J Gastroenterol* 2003; **98**: 2390-2394 [PMID: 14638338 DOI: 10.1111/j.1572-0241.2003.08702.x]
172 **Löfdahl HE**, Lu Y, Lagergren P, Lagergren J. Risk factors for esophageal adenocarcinoma after antireflux surgery. *Ann Surg* 2013; **257**: 579-582 [PMID: 23426349 DOI: 10.1097/SLA.0b013e3182888384]
173 **Sjoquist KM**, Burmeister BH, Smithers BM, Zalcberg JR, Simes RJ, Barbour A, Gebski V. Survival after neoadjuvant chemotherapy or chemoradiotherapy for resectable oesophageal carcinoma: an updated meta-analysis. *Lancet Oncol* 2011; **12**: 681-692 [PMID: 21684205 DOI: 10.1016/S1470-2045(11)70142-5]
174 **Ford AC**, Forman D, Reynolds PD, Cooper BT, Moayyedi P. Ethnicity, gender, and socioeconomic status as risk factors for esophagitis and Barrett's esophagus. *Am J Epidemiol* 2005; **162**: 454-460 [PMID: 16076833 DOI: 10.1093/aje/kwi218]
175 **Conio M**, Filiberti R, Blanchi S, Ferraris R, Marchi S, Ravelli P, Lapertosa G, Iaquinto G, Sablich R, Gusmaroli R, Aste H, Giacosa A. Risk factors for Barrett's esophagus: a case-control study. *Int J Cancer* 2002; **97**: 225-229 [PMID: 11774268 DOI: 10.1002/ijc.1583]
176 **Zagari RM**, Fuccio L, Wallander MA, Johansson S, Fiocca R, Casanova S, Farahmand BY, Winchester CC, Roda E, Bazzoli F. Gastro-oesophageal reflux symptoms, oesophagitis and Barrett's oesophagus in the general population: the Loiano-Monghidoro study. *Gut* 2008; **57**: 1354-1359 [PMID: 18424568 DOI: 10.1136/gut.2007.145177]
177 **Conio M**, Blanchi S, Lapertosa G, Ferraris R, Sablich R, Marchi S, D'Onofrio V, Lacchin T, Iaquinto G, Missale G, Ravelli P, Cestari R, Benedetti G, Macrì G, Fiocca R, Munizzi F, Filiberti R. Long-term endoscopic surveillance of patients with Barrett's esophagus. Incidence of dysplasia and adenocarcinoma: a prospective study. *Am J Gastroenterol* 2003; **98**: 1931-1939 [PMID: 14499768 DOI: 10.1111/j.1572-0241.2003.07666.x]

P- Reviewer: Abdel-Salam O, Actis GC **S- Editor**: Kong JX
L- Editor: A **E- Editor**: Wu HL

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i2.207

World J Gastrointest Pharmacol Ther 2016 May 6; 7(2): 207-213
ISSN 2150-5349 (online)

Eosinophilic esophagitis in adults: An update

Monjur Ahmed

Monjur Ahmed, Division of Gastroenterolgy and Hepatology, Department of Internal Medicine, Thomas Jefferson University, Philadelphia, PA 19107, United States

Author contributions: Ahmed M solely contributed to this work.

Conflict-of-interest statement: None to declare.

Correspondence to: Monjur Ahmed, MD, FRCP, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Thomas Jefferson University, 132 South 10th Street, Suite 480, Main Building, Philadelphia, PA 19107, United States. monjur.ahmed@jefferson.edu
Telephone: +1-215-9521493
Fax: +1-215-7551850

Received: November 9, 2015
Peer-review started: November 9, 2015
First decision: December 18, 2015
Revised: January 10, 2016
Accepted: March 14, 2016
Article in press: March 16, 2016
Published online: May 6, 2016

Abstract

Eosinophilic esophagitis is a worldwide chronic allergic disease of the esophagus. In the last decade, there is an epidemic of this entity in the western world. Mostly seen in children and young adults, patients present with dysphagia or food impaction in the emergency room. Characteristic endoscopic findings, esophageal eosinophilia and non-responsiveness to proton pump inhibitors help make the diagnosis. Avoidance of food allergens, administration of steroidal anti-inflammatory medications and dilation of the esophagus are the mainstays of treatment. Investigations are ongoing for mucosal healing and optimum maintenance treatment.

Key words: Eosinophilic esophagitis; Dysphagia; Food bolus impaction; Esophageal eosinophilia; Esophageal stricture

Core tip: While eosinophilic esophagitis is an important differential diagnosis in the field of dysphagia and acute food bolus impaction, the understanding and management of this disease is still in its infancy. It is now considered as the second most common cause of chronic esophagitis. This article focuses on the diagnostic criteria, epidemiology, pathogenesis, pathology, clinical presentation, investigations including endoscopic reference score, current treatment options and future potential agents.

Ahmed M. Eosinophilic esophagitis in adults: An update. *World J Gastrointest Pharmacol Ther* 2016; 7(2): 207-213 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i2/207.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i2.207

INTRODUCTION

Eosinophilic esophagitis (EoE) also known as "asthma of the esophagus" is a chronic immune/antigen mediated disorder of the esophagus affecting both children and adults. It is a clinicopathologic disease characterized clinically by dysphagia and pathologically by esophageal eosinophilia. Diagnosis is made by 3 criteria: (1) symptoms of esophageal dysfunction; (2) presence of $\geqslant$ 15 eosinophils/high power field in at least 1 esophageal biopsy with few exceptions; and (3) eosinophilia limited to the esophagus, with exclusion of other possible causes of esophageal eosinophilia,

including proton pump inhibitors (PPI) responsive esophageal eosinophilia[1].

EPIDEMIOLOGY

The disease is more common in Caucasian population with a male to female ratio of 3:1[2]. Eosinophilic esophagitis has also been seen in African Americans, Asians and Hispanic population. The disease is increasingly being recognized over the last few decades. The prevalence of EoE is currently as high as 50 patients per 100000 population in the United States and Europe[2]. The disease can affect both children and adults. In adults, it mostly affects middle aged men between the age of 30 and 50. Most of the patients with eosinophilic esophagitis have personal history of allergic disorders like bronchial asthma, allergic rhinitis, allergic conjunctivitis or food allergy.

PATHOGENESIS

Exposure of the esophagus to food and aeroallergens in genetically predisposed individuals may initiate the process of eosinophilic esophagitis although the exact mechanism is currently unknown[3]. Foods most commonly implicated in EoE are: Milk, egg, wheat, soy, peanuts, beans, rye and beef. Genome-wide association analysis (GWAS) suggested that CAPN14 at 2p23 locus is upregulated after epithelial exposure to interleukin (IL)-13[4]. Recently, epithelial-derived cytokine thymic stromal lymphopoietin (*TSLP*) gene at 5q22 locus has been identified as a candidate gene in a multicenter GWAS. There is an increased expression of TSLP in patients with EoE. TSLP activates dendritic cells (antigen presenting cells). Food allergen is initially recognized by antigen presenting cells which differentiate CD4 cells into TH1 cells and TH2 cells. TH1 cells secrete interferon-γ and transforming growth factor-β. TH2 cells secrete IL-4, IL-5 and IL-13. There is also single nucleotide polymorphism (SNP) in this TSLP receptor gene in male patients with EoE. This gene is found on the pseudoautosomal region on Xp22.3 and Yp11.3. This finding may explain increased prevalence of EoE in male patients. There is also a suggestion of second hit for the development of EoE. Toll-like receptor-3 (TLR-3) can recognize double-stranded RNA (found in some viruses) and can induce TSLP[5]. IL-5 is responsible for eosinophilic infiltration, growth and survival. Eosinophils secrete various inflammatory cytokines and chemokines including macrophage migration inhibitory factor, tumor necrosis factor, granulocyte-monocyte colony stimulating factors (GMCSF) and toxic granules[6]. Transforming growth factor β1 is a profibrotic molecule and helps in remodeling of the esophagus in EoE. This may explain esophagheal luminal narrowing, stricture formation and dysmotility. Eotaxin-3 is a strong chemotactic agent for esophageal eosinophilia. A single-nucleotide polymorphism in the human *eotaxin-3* gene was associated with disease susceptibility. IL-4 and IL-13 secreted by TH2 can stimulate eotaxin-3. In telomerase-immortalized esophageal squamous cells of EoE patients, IL-4 stimulated eotaxin-3 secretion was blocked by PPI - omeprazole and lansoprazole[7]. The may explain PPI responsiveness of esophageal eosinophilia. Twin and family studies suggest that there is not only increased prevalence of EoE in male sex but also in monozygotic twins and other family member[8].

PATHOLOGY

The major features (Figure 1) include infiltration of numerous eosinophils (usually > 15 per high power field) into the squamous epithelium, layering of eosinophils on the surface layer and eosinophilic microabscess formation (clusters of $\geqslant$ 4 eosinophils). Often necrotic squamous cells are also seen on the surface layer[9]. Minor features include chronic inflammatory infiltrate into the lamina propria with fibrosis of the lamina propria[10], hyperplasia of muscular layers and basal epithelial cells with lengthening of lamina propria papillae, and intercellular edema. One study showed plenty of IgG4-containing plasma cells in the lamina propria[11]. The pathological changes are patchy in distribution, and generally affect the whole length of the esophagus. None of the histologic findings is specific for eosinophilic esophagitis. Esophageal eosinophilia can be found in a variety of disorders including gastroesophageal reflux disease (GERD), proton pump responsive esophageal eosinophilia (PPI-REE), eosinophilic gastroenteritis, hypereosinophilic syndrome, Crohn's disease, connective tissue diseases, drug hypersensitivity, parasitic and fungal infections and achalasia. In clinical practice, the real challenge comes to differentiate EoE from GERD and PPI-REE[12]. Eosinophilic degranulation is seen more profoundly in EoE than in GERD biopsy specimen[13]. In EoE, the eosinophilic inflammation extends beyond mucosa into the submucosa and muscularis propria.

CLINICAL FEATURE

The major symptoms of eosinophilic esophagitis are solid food dysphagia and esophageal food impaction requiring endoscopic removal of food bolus as an emergency case[14]. In one study, EoE was found in 9% of all cases of esophageal food impaction[15]. Commonly, the diagnosis is suspected after a first episode of esophageal food impaction and biopsy showing esophageal eosinophilia. Less commonly, patients present with heartburn and chest pain mimicking gastroesophageal reflux disease. One study found that gender was an important factor in the initial clinical presentation of eosinophilic esophagitis. Men presented with dysphagia and esophageal food impaction more commonly than women. Women presented with heartburn and chest pain more commonly than

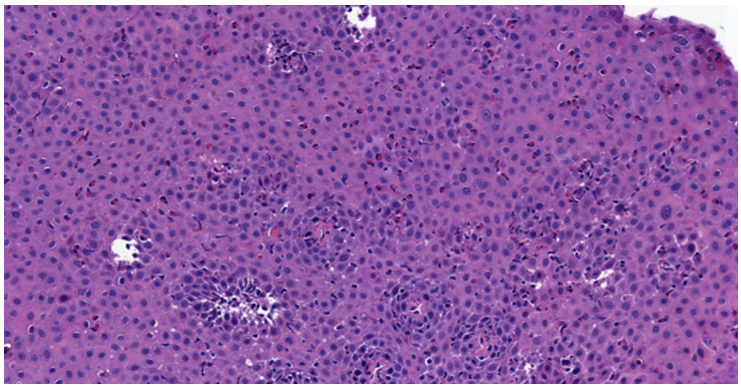

Figure 1 HE staining from the same patient showing many eosinophils, dilated intercellular spaces and basal layer hyperplasia.

men[16]. Diffuse narrowing of the esophageal lumen has been seen in clinical practice as a result of chronic inflammation and fibrosis. Esophageal mucosa is friable and esophageal perforation has been reported during endoscopic esophageal foreign body removal and during esophageal stricture dilation[17]. As aeroallergens play an important role in the pathogenesis, eosinophilic esophagitis was diagnosed more frequently when the environmental pollen counts (grass, trees and weeds) are high; the highest percentage of EoE occurred in the Spring and the lowest percentage in the Winter[18]. Another study showed symptomatic esophageal eosinophilia was diagnosed more frequently in the December/January and May/June periods[19].

INVESTIGATIONS

Lab tests

There is no single Lab test which can support the diagnosis of EoE. Mild peripheral eosinophilia may or may not be present. Peripheral eosinophilia, elevated serum eosinophil-derived neurotoxin and eotaxin-3 (CCL26) may have the potential to act as a biomarker for monitoring EoE[20].

Endsocopy

The esophageal mucosa may look normal in 7% to 10% of cases of EoE[21]. A variety of non-specific features of inflammation can be seen in EoE during endoscopy. The five major endoscopic features of EoE as per EoE endoscopic reference score (EREFS) are edema, rings (Figure 2), exudates, furrows and strictures[22]. Edema is identified by loss of vascular markings and mucosal pallor. Transient concentric rings or trachealization may indicate esophageal longitudinal muscle contraction[23] and fixed rings may indicate fibrous stricture formation due to tissue remodeling. Exudates or white spots or white plaques may mimic candida esophagitis, histologically they are eosinophilic microabscesses. Furrows are vertical lines running parallel to the axis of the esophagus probably due to epithelial edema. Chronic eosinophilic esophagitis may lead to long segment or short segment stricture. Narrow-caliber esophagus due to luminal narrowing of most of the esophagus is infrequently seen in EoE. Crepe paper esophagus occurs due to esophageal mucosal fragility and is recognized by a mucosal tear that occurs during passage of a diagnostic endoscope but neither during endoscope withdrawal nor after esophageal dilation. Although more than one of the above endoscopic findings can be seen in the same patient, none of them is specific for EoE. Recently, esophageal "pull" sign (substantial resistance and mucosal tenting during pulling of the biopsy forcep) was found to be highly specific and responsive to successful therapy in EoE patients[24].

Current recommendation is to take at least 2 to 4 biopsies both proximal and distal halves of esophagus (5 cm above GE junction) and also to take targeted biopsies from abnormal mucosa, *i.e.,* exudates, rings, edema, furrows and strictures. Gastric and duodenal biopsies should also be taken to evaluate eosinophilic gastroenteritis.

BARIUM SWALLOW

Imaging studies are generally not done to diagnose EoE. Barium swallow may show normal esophagus. Sometimes featureless narrow-caliber esophagus, ringed esophagus, and isolated esophageal stricture are seen in EoE. But none is pathognomonic of EoE.

ESOPHAGEAL MANOMETRY

Generally normal peristalsis is seen in EoE. Prolonged esophageal manometry and pH-metry showed ineffective esophageal peristalsis in children with EoE[25]. Twenty-four hours pH study would be normal in EoE unless there is coexistent GERD.

ECHOENDOSCOPY

Echoendoscopy may show hypoechogenesity and thickening of all the layers of the esophageal wall due to inflammation and edema[26].

MANAGEMENT

Firm diagnosis of EoE is essential before offering any treatment. The three most common differential

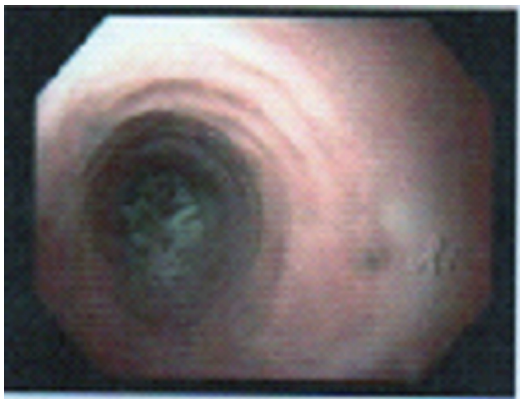

Figure 2 Esophageal rings with food bolus impaction in esophagus.

diagnoses of esophageal eosinophilia include EoE, PPI-REE and GERD. EoE and PPI-REE can be indistinguishable clinically, endoscopically and pathologically. So repeat endoscopy with biopsy after an eight weeks trial of twice daily PPI is essential to differentiate EoE from PPI-REE. Esophageal eosinophilia will persist in EoE. In suspected cases, 24 h pH study should be done to exclude GERD. In EoE, tissue eosinophilia is restricted to the esophagus in contrast to eosinophilic gastroenteritis.

Currently, drugs, diet and dilation are the three main modalities of treatment of EoE.

DRUGS

Topical corticosteroids

Have become the first line medications for the treatment of EoE[27]. Fluticasone metered dose inhaler 880 microgram puffed directly into the mouth without breathing and then dry swallowed twice a day for 6 wk has been found to be effective in reducing symptoms and esophageal eosinophilia[28]. Patients are advised not to take any food or drink or rinse their mouth for half an hour to prevent the medication from washing off the esophageal mucosa. The maximal anti-inflammatory effect is found in proximal esophagus. Oral viscous budesonide (OVB) 1 mg twice a day also decreases dysphagia and esophageal eosinophilia. OVB is easy to swallow, more mucoadherent and is made by mixing aqueous solution of budesonide (1 mg/2 mL) with the sugar substitute sucralose (5 g), chocolate syrup or honey[29]. Both forms of topical corticosteroids are more effective in histologic improvement than symptomatic improvement. Only 1% of the topical steroid is absorbed, so systemic side effects are extremely rare although oral and esophageal candidiasis can occur in up to one third of the time and herpes simplex esophagitis have been reported rarely.

Topical steroid is generally given for 8 wk. If that fails, prolonged or higher doses of topical steroids or systemic steroids or dietary treatment or esophageal dilation should be tried to get symptomatic improvement.

SYSTEMIC STEROIDS

Oral methylprednisolone induced marked clinical and histological improvement in pediatric EoE patients[30]. Because of systemic side effects, this therapy is reserved when other therapeutic interventions fail.

Steroids work by reducing the synthesis of eotaxin-3, IL-5 and GMCSF, and inducing the apoptosis of eosinophils. But recurrence of the EoE occurs after withdrawal of the steroids.

IMMUNOMODULATORS

Azathiopurine and 6-mercaptopurine induced and maintained clinical and histological remission in steroid dependent EoE patients in a case series[31]. They are not currently recommended for routine clinical use in EoE.

MAST CELL STABILIZERS

In a small case series, Cromolyn sodium failed to show any clinical or histologic improvement in EoE patients[32].

LEUKOTRIENE INHIBITORS

Montelukast is an eosinophil stabilizing agent. It improved clinical symptoms in EoE but there was no histological improvement[33].

IL-5 ANTIBODY

Anti-IL-5 antibody has been studied in both pediatric and adult patients with EoE. Mepolizumab significantly reduced esophageal eosinophilia but there was minimum symptomatic improvement[34]. Reslizumab also improved esophageal eosinophilia in EoE but there was no difference in clinical improvement in comparison to placebo[35].

IL-13 ANTIBODY

Anti-IL-13 monoclonal antibody QAX576 was studied in a small number of patients with EoE. There was a significant and sustained decrease of intraepithelial esophageal eosinophil count and a tendency towards clinical improvement[36].

DIET

Dietary therapy is very effective in the management of EoE. It can be used as an initial therapy or when other modalities of treatments fail. Dietary therapy depends on the resources available and can be expensive. As the dietary food allergen is removed, dietary therapy is very effective in inducing and maintaining clinicopathological remission. The three ways of dietary modification include: (1) elemental diet: Amino acid based formula to remove food allergens. This therapy when given for a minimum of 6 wk did both symptomatic and histologic

improvement (95%-98%) in EoE patients[37]. But the amino acid formula is expensive and unpalatable and affects patients' quality of life; and (2) six-food group elimination diet (SFGED): The most common food allergens in EoE include milk, egg, wheat, soy, peanuts/tree nuts and sea food (fish/shellfish). Significant clinical and histological (74%) improvement occurred in EoE patients (children) when they were on this SFGED[38]. Another study showed four-food group elimination diet (FFGED) which includes milk, egg, wheat and legumes, when given for 6 wk, clinicopathological remission occurred in 54% of adult EoE patient[39]. Targeted or tailored elimination diet: This therapy is guided by detection of food allergens by skin prick/patch tests and blood tests. These tests can be not only time consuming but also can give false positive and false negative results. This therapy is offered as per the preference of the patient. Sixty-eight percent of EoE patients had symptomatic improvement on targeted therapy[40].

A dietitian interested in food allergies and EoE should be consulted. An Allergist should also be involved to find out the allergens triggering EoE. Food challenge by introducing one food or food group every 4 to 6 wk should be offered. If the patient is allergic to food, there will be recurrence of symptoms and esophageal eosinophilia[41].

ENDOSCOPIC TREATMENT

Esophageal dilation has definitive role in the management of EoE. It is indicated if the patients do not respond to pharmacological or dietary therapy. It is also very effective in symptomatic esophageal stricture (esophageal diameter < 10 mm), long segment narrowing and narrow caliber esophagus. This modality of treatment improves dysphagia and quality of life but does not reduce esophageal eosinophilia[42]. Either hydrostatic balloon dilation or wire guided bougie dilation can be done. Esophageal diameter should be 15 to 18 mm to relieve dysphagia. Patients may need multiple sessions to achieve this. There is an increased risk of mucosal tear causing post-dilation chest pain for several days[43]. Although initially thought that EoE patients carry higher risk of perforation after esophageal dilation, systematic review did not show any higher risk of perforation (0.1%) in these group of patients[44].

PROGNOSIS

As mentioned earlier, EoE is a chronic inflammatory disease of the esophagus. The inflammation leads to remodeling, fibrosis and stricture. Fortunately, no case of esophageal malignancy has been reported in EoE. Patients are generally diagnosed after several years of their symptoms. Although symptomatic improvement occurs after treatment, recurrence is common after discontinuation of treatment. So maintenance therapy is needed to prevent recurrences. At the present time there is no head to head study to suggest the best maintenance treatment. Continuation of swallowed corticosteroid and/or dietary therapy should be done in all EoE patients particularly in those with history of food impaction, dysphagia, esophageal stricture, and in those with rapid symptomatic and histologic relapse following initial treatment[45].

SUMMARY

EoE has become a common clinical entity in patients with dysphagia and esophageal food impaction. Although the disease is more common in young male patients with allergic disorders, any person can get affected. High degree of suspicion is essential to diagnose this disease. So multiple proximal and distal esophageal biopsies should be taken in EoE suggestive mucosa (EREFS) and even in normal looking mucosa. Other causes of esophageal eosinophilia particularly GERD, PPIREE, eosinophilic gastroenteritis and hypereosinophilic syndrome should be excluded. Patients should be referred to the dietitians interested in food allergies and EoE patients. Patients with endoscopic findings of edema, exudates and furrows should be given topical corticosteroids for 6 to 8 wk. If there is no clinicoopathological improvement, esophageal dilation should be offered. Esophageal dilation followed by topical corticosteroid therapy should be offered to patients with esophageal rings, strictures and narrow caliber lumen. Lowest effective dose of topical corticosteroid should be continued to all EoE patients as maintenance therapy to reduce progression of the disease and relapse.

Macrophage migration inhibitory factor (MIF) is overexpressed in the esophageal mucosa of EoE patients. Recently, in the mice model of EoE, early administration a drug that blocked the action of MIF prevented eosinophilic infiltration in the esophagus. This study can lead to a novel therapy in future if MIF effect can be blocked in EoE patients.

REFERENCES

1 **Park H**. An overview of eosinophilic esophagitis. *Gut Liver* 2014; **8**: 590-597 [PMID: 25368745 DOI: 10.5009/gnl14081]

2 **Hruz P**. Epidemiology of eosinophilic esophagitis. *Dig Dis* 2014; **32**: 40-47 [PMID: 24603379 DOI: 10.1159/000357008]

3 **Raheem M**, Leach ST, Day AS, Lemberg DA. The pathophysiology of eosinophilic esophagitis. *Front Pediatr* 2014; **2**: 41 [PMID: 24910846 DOI: 10.3389/fped.2014.00041]

4 **Kottyan LC**, Davis BP, Sherrill JD, Liu K, Rochman M, Kaufman K, Weirauch MT, Vaughn S, Lazaro S, Rupert AM, Kohram M, Stucke EM, Kemme KA, Magnusen A, He H, Dexheimer P, Chehade M, Wood RA, Pesek RD, Vickery BP, Fleischer DM, Lindbad R, Sampson HA, Mukkada VA, Putnam PE, Abonia JP, Martin LJ, Harley JB, Rothenberg ME. Genome-wide association analysis of eosinophilic esophagitis provides insight into the tissue specificity of this allergic disease. *Nat Genet* 2014; **46**: 895-900 [PMID: 25017104 DOI: 10.1038/ng.3033]

5 **Spergel JM**. New genetic links in eosinophilic esophagitis. *Genome Med* 2010; **2**: 60 [PMID: 20822553 DOI: 10.1186/gm181]

6 **Malhotra N**, Levine J. Eosinophilic esophagitis: an autoimmune esophageal disorder. *Curr Probl Pediatr Adolesc Health Care* 2014;

44: 335-340 [PMID: 25499460 DOI: 10.1016/j.cppeds.2014.10.004]
7 **Zhang X**, Cheng E, Huo X, Yu C, Zhang Q, Pham TH, Wang DH, Spechler SJ, Souza RF. Omeprazole blocks STAT6 binding to the eotaxin-3 promoter in eosinophilic esophagitis cells. *PLoS One* 2012; **7**: e50037 [PMID: 23185525 DOI: 10.1371/journal.pone.0050037]
8 **Alexander ES**, Martin LJ, Collins MH, Kottyan LC, Sucharew H, He H, Mukkada VA, Succop PA, Abonia JP, Foote H, Eby MD, Grotjan TM, Greenler AJ, Dellon ES, Demain JG, Furuta GT, Gurian LE, Harley JB, Hopp RJ, Kagalwalla A, Kaul A, Nadeau KC, Noel RJ, Putnam PE, von Tiehl KF, Rothenberg ME. Twin and family studies reveal strong environmental and weaker genetic cues explaining heritability of eosinophilic esophagitis. *J Allergy Clin Immunol* 2014; **134**: 1084-1092.e1 [PMID: 25258143 DOI: 10.1016/j.jaci.2014.07.021]
9 **Odze RD**. Pathology of eosinophilic esophagitis: what the clinician needs to know. *Am J Gastroenterol* 2009; **104**: 485-490 [PMID: 19174804 DOI: 10.1038/ajg.2008.40]
10 **Philpott H**, Nandurkar S, Thien F, Gibson PR, Royce SG. Eosinophilic esophagitis: a clinicopathological review. *Pharmacol Ther* 2015; **146**: 12-22 [PMID: 25200122 DOI: 10.1016/j.pharmthera.2014.09.001]
11 **Clayton F**, Fang JC, Gleich GJ, Lucendo AJ, Olalla JM, Vinson LA, Lowichik A, Chen X, Emerson L, Cox K, O'Gorman MA, Peterson KA. Eosinophilic esophagitis in adults is associated with IgG4 and not mediated by IgE. *Gastroenterology* 2014; **147**: 602-609 [PMID: 24907494 DOI: 10.1053/j.gastro.2014.05.036]
12 **Dellon ES**. Diagnosis and management of eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2012; **10**: 1066-1078 [PMID: 22728382 DOI: 10.1016/j.cgh.2012.06.003]
13 **Parfitt JR**, Gregor JC, Suskin NG, Jawa HA, Driman DK. Eosinophilic esophagitis in adults: distinguishing features from gastroesophageal reflux disease: a study of 41 patients. *Mod Pathol* 2006; **19**: 90-96 [PMID: 16258505 DOI: 10.1038/modpathol.3800498]
14 **Akyüz U**, Akyüz F, Ozdil K, Altun H, Ağan AF, Ağan A. Food impaction in older age: Think about an eosinophilic esophagitis. *World J Gastrointest Endosc* 2013; **5**: 79-80 [PMID: 23422854 DOI: 10.4253/wjge.v5.i2.79]
15 **Sperry SL**, Crockett SD, Miller CB, Shaheen NJ, Dellon ES. Esophageal foreign-body impactions: epidemiology, time trends, and the impact of the increasing prevalence of eosinophilic esophagitis. *Gastrointest Endosc* 2011; **74**: 985-991 [PMID: 21889135 DOI: 10.1016/j.gie.2011.06.029]
16 **Lynch KL**, Dhalla S, Chedid V, Ravich WJ, Stein EM, Montgomery EA, Bochner BS, Clarke JO. Gender is a determinative factor in the initial clinical presentation of eosinophilic esophagitis. *Dis Esophagus* 2016; **29**: 174-178 [PMID: 25626069 DOI: 10.1111/dote.12307]
17 **Vernon N**, Mohananey D, Ghetmiri E, Ghaffari G. Esophageal rupture as a primary manifestation in eosinophilic esophagitis. *Case Rep Med* 2014; **2014**: 673189 [PMID: 24899902 DOI: 10.1155/2014/673189]
18 **Moawad FJ**, Veerappan GR, Lake JM, Maydonovitch CL, Haymore BR, Kosisky SE, Wong RK. Correlation between eosinophilic oesophagitis and aeroallergens. *Aliment Pharmacol Ther* 2010; **31**: 509-515 [PMID: 19925501 DOI: 10.1111/j.1365-2036.2009.04199.x]
19 **Elias MK**, Kopacova J, Arora AS, Dierkhising RA, Enders FT, Katzka DA, Kryzer LA, Halland M, Smyrk TC, Talley NJ, Alexander JA. The diagnosis of esophageal eosinophilia is not increased in the summer months. *Dysphagia* 2015; **30**: 67-73 [PMID: 25288197 DOI: 10.1007/s00455-014-9574-1]
20 **Konikoff MR**, Blanchard C, Kirby C, Buckmeier BK, Cohen MB, Heubi JE, Putnam PE, Rothenberg ME. Potential of blood eosinophils, eosinophil-derived neurotoxin, and eotaxin-3 as biomarkers of eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2006; **4**: 1328-1336 [DOI: 10.1016/j.cgh.2006.08.013]
21 **Bondar RL**, Stein F, Kassam MS, Dunphy PT, Bennett BS, Johnston KW. Cerebral blood flow velocities by transcranial Doppler during parabolic flight. *J Clin Pharmacol* 1991; **31**: 915-919 [PMID: 1761720 DOI: 10.1111/j.1572-0241.2007.01396.x]
22 **Hirano I**. Role of advanced diagnostics for eosinophilic esophagitis. *Dig Dis* 2014; **32**: 78-83 [PMID: 24603385 DOI: 10.1159/000357014]
23 **Nurko S**, Furuta GT. Eosinophilic esophagitis. Available from: URL: http//www.nature.com/gimo/contents/pt1/full/gimo49.html
24 **Dellon ES**, Gebhart JH, Higgins LL, Hathorn KE, Woosley JT, Shaheen NJ. The esophageal biopsy "pull" sign: a highly specific and treatment-responsive endoscopic finding in eosinophilic esophagitis (with video). *Gastrointest Endosc* 2016; **83**: 92-100 [PMID: 26142556 DOI: 10.1016/j.gie.2015.05.046]
25 **Nurko S**, Rosen R, Furuta GT. Esophageal dysmotility in children with eosinophilic esophagitis: a study using prolonged esophageal manometry. *Am J Gastroenterol* 2009; **104**: 3050-3057 [PMID: 19755968 DOI: 10.1038/ajg.2009.543]
26 **Fox VL**, Nurko S, Teitelbaum JE, Badizadegan K, Furuta GT. High-resolution EUS in children with eosinophilic "allergic" esophagitis. *Gastrointest Endosc* 2003; **57**: 30-36 [PMID: 12518127 DOI: 10.1067/mge.2003.33]
27 **Carr S**, Watson W. Eosinophilic esophagitis. *Allergy Asthma Clin Immunol* 2011; **7** (Suppl 1): S8 [DOI: 10.1186/1710-1492-7-S1-S8]
28 **Remedios M**, Campbell C, Jones DM, Kerlin P. Eosinophilic esophagitis in adults: clinical, endoscopic, histologic findings, and response to treatment with fluticasone propionate. *Gastrointest Endosc* 2006; **63**: 3-12 [PMID: 16377308 DOI: 10.1016/j.gie.2005.07.049]
29 **Straumann A**, Conus S, Degen L, Felder S, Kummer M, Engel H, Bussmann C, Beglinger C, Schoepfer A, Simon HU. Budesonide is effective in adolescent and adult patients with active eosinophilic esophagitis. *Gastroenterology* 2010; **139**: 1526-1537, 1537.e1 [PMID: 20682320 DOI: 10.1053/j.gastro.2010.07.048]
30 **Liacouras CA**, Wenner WJ, Brown K, Ruchelli E. Primary eosinophilic esophagitis in children: successful treatment with oral corticosteroids. *J Pediatr Gastroenterol Nutr* 1998; **26**: 380-385 [PMID: 9552132 DOI: 10.1097/00005176-199804000-00004]
31 **Netzer P**, Gschossmann JM, Straumann A, Sendensky A, Weimann R, Schoepfer AM. Corticosteroid-dependent eosinophilic oesophagitis: azathioprine and 6-mercaptopurine can induce and maintain long-term remission. *Eur J Gastroenterol Hepatol* 2007; **19**: 865-869 [PMID: 17873610 DOI: 10.1097/MEG.0b013e32825a6ab4]
32 **Liacouras CA**, Spergel JM, Ruchelli E, Verma R, Mascarenhas M, Semeao E. Eosinophilic Esophagitis: A 10-Year Experience in 381 Children. *Clin Gastroenterol Hepatol* 2005; **3**: 1198-1206 [DOI: 10.1016/S1542-3565(05)00885-2]
33 **Attwood SE**, Lewis CJ, Bronder CS, Morris CD, Armstrong GR, Whittam J. Eosinophilic oesophagitis: a novel treatment using Montelukast. *Gut* 2003; **52**: 181-185 [PMID: 12524397 DOI: 10.1136/gut.52.2.181]
34 **Straumann A**, Conus S, Grzonka P, Kita H, Kephart G, Bussmann C, Beglinger C, Smith DA, Patel J, Byrne M, Simon HU. Anti-interleukin-5 antibody treatment (mepolizumab) in active eosinophilic oesophagitis: a randomised, placebo-controlled, double-blind trial. *Gut* 2010; **59**: 21-30 [PMID: 19828470 DOI: 10.1136/gut.2009.178558]
35 **Spergel JM**, Rothenberg ME, Collins MH, Furuta GT, Markowitz JE, Fuchs G, O'Gorman MA, Abonia JP, Young J, Henkel T, Wilkins HJ, Liacouras CA. Reslizumab in children and adolescents with eosinophilic esophagitis: results of a double-blind, randomized, placebo-controlled trial. *J Allergy Clin Immunol* 2012; **129**: 456-463, 463.e1-3 [PMID: 22206777 DOI: 10.1016/j.jaci.2011.11.044]
36 **Rothenberg ME**, Wen T, Greenberg A, Alpan O, Enav B, Hirano I, Nadeau K, Kaiser S, Peters T, Perez A, Jones I, Arm JP, Strieter RM, Sabo R, Gunawardena KA. Intravenous anti-IL-13 mAb QAX576 for the treatment of eosinophilic esophagitis. *J Allergy Clin Immunol* 2015; **135**: 500-507 [PMID: 25226850 DOI: 10.1016/j.jaci.2014.07.049]
37 **Kelly KJ**, Lazenby AJ, Rowe PC, Yardley JH, Perman JA, Sampson HA. Eosinophilic esophagitis attributed to gastroesophageal reflux: improvement with an amino acid-based formula. *Gastroenterology* 1995; **109**: 1503-1512 [PMID: 7557132 DOI: 10.1016/0016-5085(95)90637-1]

38 **Kagalwalla AF**, Sentongo TA, Ritz S, Hess T, Nelson SP, Emerick KM, Melin-Aldana H, Li BU. Effect of six-food elimination diet on clinical and histologic outcomes in eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2006; **4**: 1097-1102 [PMID: 16860614 DOI: 10.1016/j.cgh.2006.05.026]

39 **Molina-Infante J**, Arias A, Barrio J, Rodríguez-Sánchez J, Sanchez-Cazalilla M, Lucendo AJ. Four-food group elimination diet for adult eosinophilic esophagitis: A prospective multicenter study. *J Allergy Clin Immunol* 2014; **134**: 1093-1099.e1 [PMID: 25174868 DOI: 10.1016/j.jaci.2014.07.023]

40 **Wolf WA**, Jerath MR, Sperry SL, Shaheen NJ, Dellon ES. Dietary elimination therapy is an effective option for adults with eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2014; **12**: 1272-1279 [PMID: 24440337 DOI: 10.1016/j.cgh.2013.12.034]

41 **Gonsalves N**, Yang GY, Doerfler B, Ritz S, Ditto AM, Hirano I. Elimination diet effectively treats eosinophilic esophagitis in adults; food reintroduction identifies causative factors. *Gastroenterology* 2012; **142**: 1451-1459.e1; quiz e14- e15 [PMID: 22391333 DOI: 10.1053/j.gastro.2012.03.001]

42 **Schoepfer AM**, Gonsalves N, Bussmann C, Conus S, Simon HU, Straumann A, Hirano I. Esophageal dilation in eosinophilic esophagitis: effectiveness, safety, and impact on the underlying inflammation. *Am J Gastroenterol* 2010; **105**: 1062-1070 [PMID: 19935783 DOI: 10.1038/ajg.2009.657]

43 **Bohm ME**, Richter JE. Review article: oesophageal dilation in adults with eosinophilic oesophagitis. *Aliment Pharmacol Ther* 2011; **33**: 748-757 [PMID: 21320137 DOI: 10.1111/j.1365-2036.2011.04593.x]

44 **Jacobs JW**, Spechler SJ. A systematic review of the risk of perforation during esophageal dilation for patients with eosinophilic esophagitis. *Dig Dis Sci* 2010; **55**: 1512-1515 [PMID: 20238250 DOI: 10.1007/s10620-010-1165-x]

45 **Dellon ES**, Gonsalves N, Hirano I, Furuta GT, Liacouras CA, Katzka DA. ACG clinical guideline: Evidenced based approach to the diagnosis and management of esophageal eosinophilia and eosinophilic esophagitis (EoE). *Am J Gastroenterol* 2013; **108**: 679-692; quiz 693 [PMID: 23567357 DOI: 10.1038/ajg.2013.71]

P- Reviewer: Fabozzi M, Peng SY, Zhu X **S- Editor**: Qi Y
L- Editor: A **E- Editor**: Wu HL

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i2.214

World J Gastrointest Pharmacol Ther 2016 May 6; 7(2): 214-216
ISSN 2150-5349 (online)

MINIREVIEWS

Minimally invasive surgery for inflammatory bowel disease: Current perspectives

Badri Shrestha

Badri Shrestha, Sheffield Teaching Hospitals NHS Trust, Sheffield S5 7AU, United Kingdom

Author contributions: Shrestha B performed the planning and writing of the paper.

Conflict-of-interest statement: There is no conflict of interest associated with the author contributing in this manuscript.

Correspondence to: Badri Shrestha, MD, FRCS, FACS, Consultant Surgeon, Sheffield Teaching Hospitals NHS Trust, Herries Road, Sheffield S5 7AU, United Kingdom. shresthabm@doctors.net.uk
Telephone: +44-114-2434343
Fax: +44-114-2714604

Received: December 29, 2015
Peer-review started: January 1, 2016
First decision: February 2, 2016
Revised: February 17, 2016
Accepted: March 24, 2016
Article in press: March 25, 2016
Published online: May 6, 2016

Abstract

The surgical management of complicated and recurrent inflammatory bowel disease (IBD), has remained a challenge. Minimally invasive surgery (MIS), in the form of laparoscopic resections, single port approach and robotic-assisted dissections in the management of IBD, have been examined in several prospective studies. All of them have shown advantages over open surgery in terms of reduction of physical trauma of surgery, recovery time, better cosmetic outcomes and shorter hospitalization. However, it is important to appreciate that not all patients with IBD are suitable for MIS, so a combination of both open and MIS should be adopted to achieve optimum outcomes. A review on this subject performed by Neumann *et al* in this issue of *World Journal of Gastrointestinal Pharmacology and Therapeutics* have provided evidence in support of the contemporary practice of MIS in the management of IBD and the accompanying commentary further critically evaluates their application in clinical practice.

Key words: Minimally invasive surgery; Ulcerative colitis; Crohn's disease; Laparoscopy; Robotic-assisted surgery

Core tip: The advantages conferred by minimally invasive surgery (MIS) in the management inflammatory bowel disease (IBD) are well established. Currently available evidences support the application of MIS in the management of IBD, although the decision to adopt MIS, open surgery or combination of both, has to be made on case-by-case basis, based upon the understanding of the advantages and disadvantages of individual technique, available resources and local expertise.

Shrestha B. Minimally invasive surgery for inflammatory bowel disease: Current perspectives. *World J Gastrointest Pharmacol Ther* 2016; 7(2): 214-216 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i2/214.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i2.214

INTRODUCTION

Neumann *et al*[1] have performed a review of the MIS

in the management of inflammatory bowel disease (IBD), which is a rapidly expanding field relevant to the practicing surgeons in the colorectal specialty. Surgical management of both Crohn's disease (CD) and ulcerative colitis (UC), has remained challenging because of associated complications, such as abscesses, fistula and strictures. The complex anatomy resulting from previous open operations, which is particularly present in recurrent CD, and the immunosuppressed state of the patients resulting from medical treatment further compounds the management[2].

ADVANTAGES OF MINIMALLY INVASIVE SURGERY IN IBD

It is a common practice to adopt conservative surgical resection procedures in IBD, which necessitates repeated surgery for recurrent disease, particularly in young group of patients. Repeated open surgery leading to intra-abdominal adhesions, scarred abdominal wall contributes to significant morbidity and risk to future surgery. Better cosmetic outcomes and significant reduction in the length of hospital stay and recovery time have been achieved with the application of minimally invasive surgery (MIS) compared with open approach[3]. Since the beginning of the millennium, MIS has gained popularity and has become the gold standard as a safe surgical strategy in primary and complicated cases of CD as well as proctocolectomies and restorative proctocolectomies for patients with UC, both in paediatric and adult patients, and in elective and emergency settings[4-7].

CURRENT EVIDENCES

There is lack of randomised trials comparing laparoscopic colorectal surgery (LCS) with open surgery for the management of IBD. Majority of the published studies are either case control or cohort studies, which have shown reduced hospital stay, comparable or fewer complications but with an increased operating time associated with LCS. Emergency LCS can be safely undertaken providing there is appropriate patient selection, the surgeon is adequately experienced and there are sufficient resources to allow for potentially more complex operations[8,9].

Although available evidence suggest laparoscopic approach for recurrent IBD is safe with comparable outcomes to open surgery, there are still controversies on the application of laparoscopic approach for recurrent CD due to prolonged operating time and higher incidence of open conversion[10]. Staged procedures combining LCS and open surgery is indicated where reconstructions are required for continence, which does reduce the morbidity significantly. Hand-assisted laparoscopic surgery does reduce the operating time, has reduced conversion rates and reduces the hazards of prolonged anaesthesia, hence should be in the armamentarium of surgeons[11]. Single port laparoscopic surgery (SPLS) is an attractive modification, although more data is required to prove its superiority over other techniques[12].

Limited data is available on the robotic - assisted dissection of rectum for IBD. However, there is a favourable trend in the use of robotic - assisted surgery for treating colorectal IBD, where the operating time and cost are influenced by the learning curve of the surgeon[13]. Technical developments have introduced extraction of the specimen through natural orifices and transanal MIS, which avoid extra incision and scar on the anterior abdominal wall. However, there is no robust data available on their application in the treatment of IBD[14].

CONCLUSION

The conclusions made by Neumann *et al*[1] is pertinent as the application of MIS in the treatment of IBD has expanded significantly and become standard practice due to its advantages over open surgery. However, it is important to appreciate the fact that not all cases of IBD are suitable for LCS, particularly complicated and recurrent CD and UC, where combination of open and LCS should be considered for achieving best outcomes. The efficacy of SPLS and robotic-assisted surgery still remains to be assessed in prospective studies.

REFERENCES

1 **Neumann PA**, Rijcken E. Minimally invasive surgery for inflammatory bowel disease: Review of current developments and future perspectives. *World J Gastrointest Pathophysiol* 2016; 7: 217-226

2 **Watanabe M**. Current status of and outlook for the latest minimally invasive surgery for colorectal diseases. *Nihon Geka Gakkai Zasshi* 2015; **116**: 297-301 [PMID: 26630735]

3 **Sica GS**, Biancone L. Surgery for inflammatory bowel disease in the era of laparoscopy. *World J Gastroenterol* 2013; **19**: 2445-2448 [PMID: 23674844 DOI: 10.3748/wjg.v19.i16.2445]

4 **Dunker MS**, Bemelman WA, Slors JF, van Hogezand RA, Ringers J, Gouma DJ. Laparoscopic-assisted vs open colectomy for severe acute colitis in patients with inflammatory bowel disease (IBD): a retrospective study in 42 patients. *Surg Endosc* 2000; **14**: 911-914 [PMID: 11080402]

5 **Holder-Murray J**, Marsicovetere P, Holubar SD. Minimally invasive surgery for inflammatory bowel disease. *Inflamm Bowel Dis* 2015; **21**: 1443-1458 [PMID: 25989341 DOI: 10.1097/MIB.0000000000000316]

6 **Pini-Prato A**, Faticato MG, Barabino A, Arrigo S, Gandullia P, Mazzola C, Disma N, Montobbio G, Mattioli G. Minimally invasive surgery for paediatric inflammatory bowel disease: Personal experience and literature review. *World J Gastroenterol* 2015; **21**: 11312-11320 [PMID: 26525138 DOI: 10.3748/wjg.v21.i40.11312]

7 **Mahida JB**, Asti L, Deans KJ, Minneci PC, Nwomeh BC. Laparoscopic bowel resection for pediatric inflammatory bowel disease. *J Surg Res* 2015; **199**: 130-136 [PMID: 25935468 DOI: 10.1016/j.jss.2015.04.009]

8 **Chand M**, Siddiqui MR, Gupta A, Rasheed S, Tekkis P, Parvaiz A, Mirnezami AH, Qureshi T. Systematic review of emergent laparoscopic colorectal surgery for benign and malignant disease. *World J Gastroenterol* 2014; **20**: 16956-16963 [PMID: 25493008 DOI: 10.3748/wjg.v20.i45.16956]

9 **Horváth G**, Simonka Z, Lázár G. Comparison of the results of

laparotomy and laparoscopic surgery in patients with Crohn's disease. *Orv Hetil* 2014; **155**: 24-29 [PMID: 24379093 DOI: 10.1556/OH.2014.29794]

10 **Goyer P**, Alves A, Bretagnol F, Bouhnik Y, Valleur P, Panis Y. Impact of complex Crohn's disease on the outcome of laparoscopic ileocecal resection: a comparative clinical study in 124 patients. *Dis Colon Rectum* 2009; **52**: 205-210 [PMID: 19279413 DOI: 10.1007/DCR.0b013e31819c9c08]

11 **Jadlowiec CC**, Mannion EM, Thielman MJ, Bartus CM, Johnson KH, Sardella WV, Vignati PV, Cohen JL. Evolution of technique in performance of minimally invasive colectomies. *Dis Colon Rectum* 2014; **57**: 1090-1097 [PMID: 25101605 DOI: 10.1097/DCR.0000000000000178]

12 **Keshava A**, Young CJ, Richardson GL, De-Loyde K. A historical comparison of single incision and conventional multiport laparoscopic right hemicolectomy. *Colorectal Dis* 2013; **15**: e618-e622 [PMID: 23937552 DOI: 10.1111/codi.12380]

13 **Byrn JC**, Hrabe JE, Charlton ME. An initial experience with 85 consecutive robotic-assisted rectal dissections: improved operating times and lower costs with experience. *Surg Endosc* 2014; **28**: 3101-3107 [PMID: 24928229 DOI: 10.1007/s00464-014-3591-x]

14 **Lacy AM**, Saavedra-Perez D, Bravo R, Adelsdorfer C, Aceituno M, Balust J. Minilaparoscopy-assisted natural orifice total colectomy: technical report of a minilaparoscopy-assisted transrectal resection. *Surg Endosc* 2012; **26**: 2080-2085 [PMID: 22258297 DOI: 10.1007/s00464-011-2117-z]

P- Reviewer: Jonaitis L, Munoz M, Naito Y **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Wu HL

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i2.217

World J Gastrointest Pharmacol Ther 2016 May 6; 7(2): 217-226
ISSN 2150-5349 (online)

MINIREVIEWS

Minimally invasive surgery for inflammatory bowel disease: Review of current developments and future perspectives

Philipp-Alexander Neumann, Emile Rijcken

Philipp-Alexander Neumann, Emile Rijcken, Department of General and Visceral Surgery, University Clinic of Muenster, 48149 Muenster, Germany

Author contributions: Neumann PA and Rijcken E solely contributed to this paper.

Conflict-of-interest statement: None.

Correspondence to: Dr. Emile Rijcken, MD, Department of General and Visceral Surgery, University Clinic of Muenster, Albert-Schweitzer Campus 1, 48149 Muenster, Germany. rijcken@uni-muenster.de
Telephone: +49-251-8356301
Fax: +49-251-8356414

Received: December 13, 2015
Peer-review started: December 14, 2015
First decision: January 18, 2016
Revised: February 9, 2016
Accepted: March 7, 2016
Article in press: March 9, 2016
Published online: May 6, 2016

Abstract

Patients with inflammatory bowel disease (IBD) comprise a population of patients that have a high likelihood of both surgical treatment at a young age and repetitive operative interventions. Therefore surgical procedures need to aim at minimizing operative trauma with best postoperative recovery. Minimally invasive techniques have been one of the major advancements in surgery in the last decades and are nowadays almost routinely performed in colorectal resections irrespective of underlying disease. However due to special disease related characteristics such as bowel stenosis, interenteric fistula, abscesses, malnutrition, repetitive surgeries, or immunosuppressive medications, patients with IBD represent a special cohort with specific needs for surgery. This review summarizes current evidence of minimally invasive surgery for patients with Crohn's disease or ulcerative colitis and gives an outlook on the future perspective of technical advances in this highly moving field with its latest developments in single port surgery, robotics and trans-anal techniques.

Key words: Inflammatory bowel disease; Minimally invasive surgery; Laparoscopy; Colorectal; Robotic

Core tip: Laparoscopic techniques have been applied to a wide variety of surgical procedures for inflammatory bowel disease (IBD). Beside the feasibility and safety, numerous short time advantages for laparoscopic techniques such as reduced trauma, reduction of morbidity, and reduced hospital stays have been well documented for IBD patients as well. Newly emerging minimally invasive techniques such as single port laparoscopic surgery, robotic surgery or transanal techniques will further expand the field of IBD surgery.

Neumann PA, Rijcken E. Minimally invasive surgery for inflammatory bowel disease: Review of current developments and future perspectives. *World J Gastrointest Pharmacol Ther* 2016; 7(2): 217-226 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i2/217.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i2.217

INTRODUCTION

Medical treatment is still considered the first line approach for patients with inflammatory bowel disease (IBD)[1]. However despite novel therapeutic strategies, up to 80% of patients with Crohn's disease and 30% of patients with ulcerative colitis still require surgery during their course of disease[2,3]. The vast majority of patients will be operated at a fairly young age[4]. Furthermore, substantial numbers of patients especially with Crohn's disease might require repetitive surgery due to complications or disease recurrence. Therefore development of surgical techniques to minimize operative trauma has been of great interest. In fact minimally invasive surgery has been one of the recent major advances in surgery in the last decades[5].

In the following, current evidence for minimally invasive surgery for IBD will be discussed followed by an outline of possible future advances in this moving field.

Current status of minimally invasive surgery for IBD

For Crohn's disease as well as ulcerative colitis, development of minimally invasive techniques has evolved with more caution than in other colorectal diseases. This was mostly due to special disease related characteristics that are associated with IBD such as bowel inflammation with obliteration of surgical layers, complicated anatomy following multiple operations, potentially fistulizing disease or difficulties in anastomotic healing. But also the patient's impaired physical condition due to chronic inflammation, bowel obstruction, malnutrition, anemia, hypoalbuminemia, or the need for immunosuppressive medication such as steroids, azathioprine, or anti-TNF-α agents; doubts have been raised whether minimally invasive surgery is suitable for patients suffering from IBD. However, during the last decades, nearly all primary surgical procedures for IBD, varying from stricturoplasties, segmental resections, or proctocolectomies, were reported to having been performed safely in a laparoscopic fashion, even with substantial advantages as compared to conventional approaches in large clinical series and trials. Today minimally invasive surgery is broadly accepted as a safe surgical strategy in primary and complicated cases of Crohn's disease as well as restorative proctocolectomies for patients with ulcerative colitis[6-8]. This is also reflected by incorporation of laparoscopic approaches into several clinical guidelines by national and international medical societies on IBD[9,10]. Although most of the possible advantages of minimally invasive approaches like shorter hospital stay, less wound infections, and reduced pain are true for both entities of IBD, special clinical characteristics of the diseases require separate discussion (for an overview of the cited literature for the respective technical advancements see also Table 1).

Laparoscopic surgery for Crohn's disease

With ileocolic involvement being the most common disease pattern, ileocolic resections for refractory stricturing disease represent the main surgical treatment for patients with Crohn's disease. A large body of literature has been published comparing laparoscopic to open ileocolic resections with 2 randomized trials and 3 meta-analyzes representing the highest level of evidence[11-15]. Altogether the trials have shown comparable results with a trend towards faster recovery of bowel function, shorter hospital stay and fewer complications in the laparoscopic groups. However none of these reached statistical significance in the randomized trials, which might mostly be due to a comparably low number of included inpatients. Recently the patients of both randomized trials have been followed up for long term results and the studies have been published consecutively[16,17]. Altogether data suggested no differences between the different approaches in terms of recurrence of disease or long term morbidity, again being limited by a small number of included patients.

For recurrent disease following initial surgical resection acquisition of data is more complex due to complicated anatomy and the inhomogeneous presentation of disease. However, recently a growing amount of literature has been published on outcomes following laparoscopic and open approaches for recurrent disease[18,19]. Data has shown that a laparoscopic approach for recurrent disease seems safe with comparable outcomes to open surgery. However, whether a laparoscopic approach for recurrent disease irrespective of the initial approach is advantageous is still in debate. Most specifically it seems not clear yet, whether in case of recurrence the possible advantages of laparoscopic surgery can be maintained following midline laparotomy of the initial operation[20]. Another subject of debate is the feasibility of laparoscopic surgery for cases with disease related complications such as penetrating disease. Recently in a prospective study, Goyer *et al*[21] compared outcomes following laparoscopic or open surgery for ileocolic resection for penetrating or recurrent disease in 54 patients to non-complicated primary Crohn's disease in 70 cases. Overall penetrating disease was associated with a higher likelihood of conversions, higher number of diverting stoma being performed, and longer operative times compared to primary non-penetrating disease. However no difference was observed whether an open or laparoscopic approach was chosen. Recently in a nationwide data analysis in the United States, Lesperance *et al*[22] investigated the use and outcomes of laparoscopic surgery for patients with Crohn's disease. All patients from the Nationwide inpatient Sample database that have been operated between 2000 and 2004 were analyzed. A total number of 389911 patients received treatment because of Crohn's disease and 12% (n = 49609) required surgical treatment. Independent predictors for patients being operated laparoscopically were: Age below 35 years, female gender, admission to a teaching hospital, ileocecal disease and lower disease stage. Compared to open operations minimally invasive operations were associated with lower percentage

Table 1 Minimally Invasive techniques for inflammatory bowel diseases

Technical development		Indications[1]	Performed procedures	Advantages	Ref.
Laparoscopy/ laparoscopically assisted	UC	Elective surgery[1] Refractory disease[1] Malignancy	Proctocolectomy + IPAA	Reduced trauma Similar recovery Similar LOS Similar morbidity	[28,29,32,33]
		Urgent surgery[1] High immuno-suppression[1]	Subtotal colectomy	Reduced trauma Faster recovery Reduced LOS	[5,23-27,31]
	CD	Primary disease[1] Recurrent disease Complicated disease Stenosing disease[1] Penetrating disease Fistulizing disease	Ileo-/colonic resection	Reduced trauma Faster recovery Reduced LOS Less morbidity	[11-22]
HALS	UC	Elective surgery[1] Refractory disease[1] Malignancy Complex cases Learning curve of lap	Proctocolectomy + IPAA Subtotal colectomy	Reduced trauma Similar recovery Similar LOS Similar morbidity Reduced operative time compared to lap Possibly less conversions to open resections compared to lap	[30,34-37]
	CD	Fistulizing disease Elective surgery[1] Refractory disease[1] Malignancy Complex cases	Ileo-/colonic resection	Reduced trauma Similar recovery Similar LOS Similar morbidity Reduced operative time compared to lap Possibly less conversions to open resections compared to lap	[37]
SPLS	UC	(Elective refractory disease)	Proctocolectomy + IPAA[2]	Fewer number of incisions Comparable morbidity[2]	[41-43]
	CD	Elective[1] Stenosing disease[1] Recurrent disease[2] Disease related complications[2]	Ileo-/colonic resection	Shorter hospital stay compared to lap Reduced pain compared to lap Similar morbidity compared to lap	[38-40]
NOTES/NOSE	UC	-	Proctectomy	-	[54-57,59]
	CD	Perianal fistulizing disease Stenosing disease	Proctectomy Colectomy Ileocolic resections	Reduction of needed incisions for specimen removal	
Trans-anal minimally invasive surgery	UC	Elective surgery[1] Refractory disease[1] Malignancy	Transanal proctectomy for IPAA	Transanal removal of colon with performance of anastomosis	[59]
	CD	Perianal fistulizing disease Supraanal stenosis	Transanal completion proctectomy	Transanal removal of colon with performance of anastomosis/ perineal closure	[52,53,58]
Robotic surgery	UC/CD	Elective surgery[1] Refractory disease[1] Malignancy	Completion proctectomy following laparoscopic colectomy	Comparable postoperative morbitidy	[45-49]

[1]Indicates main indications for the respective procedure; lap = multitrocar laparoscopic surgery; [2]Indicates only limited evidence available. UC: Ulcerative colitis; CD: Crohn's disease; IPAA: Ileal pouch-anal anastomosis; HALS: Hand assisted laparoscopic surgery; SPLS: Single port laparoscopic surgery; NOSE: Natural orifice specimen extraction; TAMIS: Trans-anal minimally invasive surgery; LOS: Length of stay.

of complications, shorter length of stay, and reduced mortality. Open operations were more likely performed for patients in fistulizing disease and when an ostomy was necessary[22]. This indicates that there is still a need for conventional surgery in patients with Crohn's disease, especially for those with complicated disease and repetitive surgery. Patient selection is highly valuable for the surgeon's decision whether to perform an open or laparoscopic approach. However, for patients with primary stricturing ileocolic disease, a laparoscopic approach seems to be the method of choice today.

Laparoscopic surgery for ulcerative colitis

For ulcerative colitis the main indications for surgery are a refractory course of disease, risk of malignant transformation and emergency indications for severe colitis refractory to medical treatment. In case of emergency indications a subtotal colectomy without primary anastomoses is recommended[1]. The rationale behind this is to remove most of the diseased colon

while minimizing the risk of surgical complications such as anastomotic leakage. Whether a laparoscopic or open approach should be selected for subtotal colectomy in the emergency setting has been investigated by multiple studies[23-27]. Most of these show similar results for laparoscopic or open resection, some showing favorable results for laparoscopy concerning postoperative morbidity, return of bowel function and length of stay. On the down part laparoscopic surgery is associated with longer operative times. Taken together laparoscopy seems to be safe for cases of medical refractory severe colitis; however no study has yet shown feasibility of laparoscopic approaches for complications such as perforation or toxic megacolon[5]. Therefore "emergency surgery" should be interpreted as "urgent surgery" for refractory disease in studies as mentioned above, while critical bleeding or free perforations with four quadrant peritonitis still seem to be a domain of conventional surgery in ulcerative colitis.

In case of elective surgery for medically refractory disease, performance of an ileal J-pouch with ileal pouch anal anastomosis (IPAA) following proctocolectomy has become the method of choice. In the majority of cases the operation is performed as a two staged procedure with proctocolectomy and pouch formation under protection of a diverting loop-ileostomy in the first setting and stoma reversal in the second. With laparoscopy being more routinely performed today, literature about comparison of open to laparoscopic approaches has increased[28-30]. Results so far show similar outcomes in terms of intra-operative blood loss, postoperative morbidity, time to bowel function and length of hospital stay. In most studies different approaches of laparoscopic assisted operations have been compared to open resections. Mostly for laparoscopy a laparoscopic assisted approach is employed with addition of a Pfannenstiel-incision for specimen removal and pouch formation. Limitations of comparative studies so far might have been a potential selection bias within the different groups. Therefore Gu *et al*[31] have recently analyzed their outcomes of laparoscopic and open total colectomy, adjusted for possible confounders. They report that after statistical adjustments for covariates such as age, comorbidities, ASA score and others, patients with the laparoscopic approach still had favorable outcomes in terms of postoperative recovery. In a recent meta-analysis published by Singh *et al*[32], the authors investigated operative outcomes following laparoscopic *vs* open restorative proctocolectomy with functional results as primary outcome measures and intraoperative details, short term outcomes as well as adverse events as secondary end points. A total of 27 studies with 2428 patients were analyzed. Laparoscopic operations were performed in 45.1% of the operations and were associated with a shorter length of stay, less wound infections and reduced intraoperative blood loss. There were no differences in terms of pouch failure with a tendency of better pouch function following minimally invasive operations. Another prospective randomized controlled single center trial was performed by a German group in 2013 (LapConPouch-Trial)[33]. Blood loss was used as the primary endpoint, unfortunately the trial had to be stopped due to insufficient patient recruitment. However the results were published after a total of 21 patients were included in each group (laparoscopic *vs* open restorative proctocolectomy) with statistical analysis being performed exploratively. In their mixed population of patients with ulcerative colitis and familial adenomatous polyposis no differences in terms of blood loss were found. No differences were noted in secondary outcomes such as length of hospital stay, postoperative pain, bowel function and quality of life (QOL). The different technical strategies of laparoscopic or laparoscopic assisted restorative proctocolectomy respectively will be discussed below.

TECHNICAL DEVELOPMENTS AND FUTURE PERSPECTIVES

Hand assisted laparoscopic surgery

The idea of combining the benefits of minimally invasive surgery with the possibility of tactile feedback and manual assistance while performing complex colorectal resections has led to the development of the hand assisted laparoscopic approach. One of the first descriptions of the technique for colonic resections has been published by Bemelman *et al*[34] in 1996. They reported their initial experience in using a "pneumo-sleeve" to provide abdominal access of the surgeons hand while preserving pneumoperitoneum. For a limited number of five patients with mostly diverticular disease, they have shown feasibility of the approach that enables tactile feedback and hand assistance during laparoscopic resections. In 2004 Nakajima *et al*[35] published their experience of hand assisted laparoscopic surgery (HALS) colectomies compared to classic laparoscopic approaches. A total of 12 HALS resections (5 total proctocolectomies, 7 total abdominal colectomies) have been compared to 11 laparoscopic resections. Most significantly, total operative time could be reduced by almost 1 h using the HALS-technique. Total blood loss and length of incisions were similar in both groups since in the majority of the laparoscopic cases an additional Pfannenstiel-incision was used for specimen retrieval and usage of stapling devices. The authors concluded that even in centers with a high level of laparoscopic experience employment of HALS-ports can lead to further improvement of postoperative outcomes. Maartense *et al*[30] have compared HALS to open restorative proctocolectomy in a prospective randomized trial. A total number of 60 patients have been included. The authors have evaluated postoperative QOL as the primary end-point of the study and operating time, blood loss, conversion rates, morbidity, morphine requirement, mortality and costs as secondary end points. Operating time in the laparoscopic groups was significantly longer than in the

open group (214 min compared to 133 min, $P = 0.001$). In the minimally invasive group 5 patients had major complications with anastomotic leakages of IPAA in 2 patients compared to 4 patients of the open group. In the laparoscopic group revision surgery was performed laparoscopically while in the open group relaparotomy was performed. In the postoperative period no differences between the groups were recorded in terms of recovery, pain, morphine requirement and return to normal diet. Most interestingly, with regard to the primary endpoint, no differences in development of QOL measures were noted. In both groups QOL significantly dropped during the first 2 wk following surgery. This was irrespective of the type of surgery. Patients of both groups returned to baseline level after 4 wk and 3 mo after surgery patients had better QOL scores than before surgery. Altogether the study could not show any measurable advantage of the minimally invasive approach. The authors argue that probably the impact of the operation on the QOL outweighed the possible advantages of smaller incisions. However it has to be noted that in their analysis the main difference was only the colectomy part of the operation since the proctectomy was performed *via* a Pfannenstiel incision in the minimally invasive group and a midline incision in the open group. This might also explain the missing differences in terms of pain medication.

A Cochrane analysis from the Netherlands that included data from 11 trials with a total number of 607 patients of whom 253 (41%) have been operated using a laparoscopic approach showed no significant differences in terms of the main postoperative outcomes[36]. There was no significant difference concerning morbidity or mortality between the groups. Although until publication of the study only 1 randomized controlled trial has been performed and could be included in the analysis, over all outcomes of the studies show increasing safety of laparoscopic approaches for restorative proctocolectomies. With more and more evidence on the safety of laparoscopic resections and with advancing learning curves in minimally invasive colorectal surgery, many surgeons leave hand-assisted procedures in favor of full laparoscopic procedures, putting hand-assisted surgery in the background. In an interesting retrospective study Jadlowiec *et al*[37] investigated the technical evolution of laparoscopic colorectal resection within their tertiary center. Minimally invasive procedures for IBD showed steady growth with a higher number of pure laparoscopic operations, a decrease in the amount of HALS-procedures and a plateau in open resections. Altogether irrespective of the indication, although decreasing in total numbers, HALS-operations were still chosen for more complex cases - especially with possible advantages in case of present fistulizing disease. As the authors point out, HALS surgery has still a role as a learning instrument in the acquisition of surgical skills prior to performance of pure laparoscopic resections. With respect to conversion rates the possibility to perform HALS complimentary to laparoscopic resections might result in less conversions to open resections.

Single port laparoscopic surgery

Single port laparoscopic surgery (SPLS) was developed to further reduce the operative trauma through reduction of needed incisions to only one. Usually a paraumbilical or transumbilical incision is used for insertion of a single port for introduction of the camera as well as all working instruments.

Our group has recently reported our experience with single incision surgery for elective ileocolic resections in Crohn's disease[38]. In a match pair analysis 20 single incision ileocolic resections for stricturing ileocolic Crohn's disease were compared to 20 multi-trocar resections for the same indication. Altogether the results between the two approaches were comparable in terms of postoperative morbidity, postoperative pain and conversion rates (SPLS group 5%, laparoscopy group 10%). Another study that compared postoperative outcomes of single-trocar *vs* multi-trocar ileocecal resections was published in 2013[39]. Twenty one patients who had ileocolic resections using a single port approach were matched and compared to patients with ileocolic resections in a multitrocar approach. Matching criteria comprised BMI, length of diseased bowel resected and the presence of fistulizing disease. Comparison of the 21 single port patients had little but significantly shorter length of hospital stay and less morphine use on postoperative day 1. All other outcome measures such as postoperative pain and complications were similar in both groups. Taken the results of the 2 studies together, in this well-defined and elective setting of surgery for primary ileocolic disease, single port surgery was fully comparable to the multi-trocar approach, however long term results are still under investigation. Furthermore only a few studies have analyzed outcomes following single port surgery for complicated or recurrent Crohn's disease. To analyze the feasibility of single port laparoscopy in patients with complicated or recurrent disease, an Irish group has recently investigated their patients that presented either with urgent interventions ($n = 15$), prior abdominal interventions ($n = 8$), obstruction ($n = 7$), intraabdominal mass ($n = 6$), fistulizing disease ($n = 6$) or abscess ($n = 4$). For all indications the operation was initiated using a single port approach[40]. For introduction of the instruments a surgical glove port was used. In most cases ileocolonic resections were performed. Conversion rate was 15% and associated with the complexity of clinical presentation. Overall the authors conclude that even in patients with disease related complications or recurrent disease initial single port laparoscopy can be used with acceptable morbidity and conversion rates. Especially long term evaluation of the cosmetic results will be most interesting since reduction in incision length and avoidance of additional incisions is the biggest difference between the techniques, with claimed benefits in postoperative pain, adhesions, and cosmetic results for the single port

technique.

For ulcerative colitis, Geisler *et al*[41] reported their initial experience with single port proctocolectomy and ileal-pouch-anal-anastomosis. Although limited by a fairly low number of 5 patients with ulcerative colitis as well as familial adenomatous polyposis, they have shown technical feasibility of the method. In the following years SPLS proctocolectomy has also been investigated by other groups[42,43]. Gash *et al*[42] described a series of patients where restorative proctocolectomy was performed using a SPLS trocar through the existing or planed ileostomy site. In their analysis they reported no complications associated with the approach with good function of the pouch following ileostomy reversal. Consecutively Bulian *et al*[43] described the case of a patient who had previous subtotal colectomy and ileostomy for restorative proctocolectomy as a three staged approach. SPLS was used for formation of the pouch through the ostomy site and the rectal stump was resected and closed extra-abdominally. Pouch formation was performed outside the abdomen and the pouch-anal anastomosis was performed using the SPLS trocar without further incisions.

Altogether SPLS seems to be feasible and safe for elective colorectal resections for IBD. Nevertheless, the SPLS technique is elaborate and needs getting used to even for experienced laparoscopic surgeons. Therefore there is doubt if SPLS will get adapted by most surgeons in future. Moreover long term data is still missing and evidence is needed, whether the approach is suitable for different indications of complicated Crohn's disease. Additionally, yet no true advantages other than fewer numbers of incisions have been reported and it is still open whether these differences have a significant impact on the QOL of the patients.

Robotic surgery for IBD

Ongoing innovation in the field of robotic surgery and its progressing use in different surgical disciplines starting with urology and gynecology has now let to its increasing use in colorectal surgery. Pigazzi *et al*[44] published their initial experience with low rectal resections for rectal cancer using the da vinci robotic system. Literature published so far has shown feasibility of the robotic approach for performance of proctectomy in patients with rectal cancer. In 2012 Miller *et al*[45] published their short term results of robotic *vs* laparoscopic surgery for patients with IBD. In a case-matched study design they analyzed 17 robotic proctectomies following laparoscopic total abdominal colectomy. There were no conversions to open surgery and the results were comparable between the 2 groups. However at the beginning of the study the authors reported longer operation times, slower postoperative recovery and longer length of stay in the robotic group, but these differences equalized during the study period. Postoperative mortality, especially anastomotic leakage did not differ between the 2 groups. The study is certainly limited by a low number of patients and a retrospective design; however it has demonstrated a possible combination of laparoscopic and robotic surgery for proctocolectomy in patients with IBD. In the same year McLemore *et al*[46] published their initial results of a case series about robotic-assisted laparoscopic two-staged restorative proctectomy for toxic ulcerative colitis. In three cases with toxic ulcerative colitis that had previously undergone laparoscopic colectomy a robotic-assisted completion proctectomy with ileal-J-Pouch anastomosis was performed. These preliminary results have added to the combined experience of a robotic-assisted approach for completion proctectomy and pouch formation following laparoscopic colectomy. Possible advantages of robotic surgery are mostly expected in rectal resections because of the limited space in the lower rectum. Here usage of robotic assisted operations is believed to bring advantages in terms of nerve preserving operations with possibly better oncologic outcomes. However, robotic colorectal operations have also been used in locations other than the rectum. In a single case presentation Tou *et al*[47] have recently shown technical feasibility of robotic assisted performance of strictureplasty for refractory stenosis of the terminal ileum. Following laparoscopic exploration of the abdomen, the robot was successfully used for the incision as well as performance of a two layered anastomosis. Additionally Juo *et al*[48] have published their experience with robotic single incision colorectal resections for different indications. They reported on 31 right hemicolectomies, 20 sigmoid colectomies, 5 left hemicolectomies, 2 low anterior resections and 1 total colectomy. Although only 1 patient with IBD was included, the study has certainly shown technical feasibility of robotic assisted surgery in colorectal operations of different extensions. Especially the conversion rate to open procedures was comparably low (6.8%). Postoperative complications occurred in 27.1% of the cases. Five of those were classified severe complications, three moderate, and seven mild complications[48]. With more and more experience in colorectal robotic surgery, development to total robotic operations with performance of intraabdominal anastomoses has gained more attention. In a case series Lujan *et al*[49] published their experience with intracorporeal anastomoses following right hemicolectomy for a mixed cohort of indications. In their 58 operations, 52 anastomoses were performed intra-corporally with a complication rate of 19% and only 1 anastomotic leakage. Although an additional incision for extraction of the specimen was used, the study reports feasibility of the approach for different indications. However, long term data will be necessary to estimate the true impact of robotic assisted colorectal surgery for IBD. While robotic approaches in the low pelvis appear to be reasonable, robotic techniques in more than one abdominal quadrant as required for example for proctocolectomy for ulcerative colitis seem to be very elaborate with doubts concerning medical and economic benefits so far.

Natural orifice specimen extraction techniques for IBD

Avoidance of further incisions to extract the resected specimen is a further step towards fully laparoscopic operations. Without the need to remove the resected bowel through the abdominal wall, additional incisions such as a Pfannenstiel incision can be avoided. Not only for colon resections in patients with ulcerative colitis but also for ileocolic resections and colectomies in patients with Crohn's disease this method of specimen extraction is feasible[50,51]. Especially for patients with colectomy or proctectomy, extraction of the specimen through the rectum has been described in different studies[52,53]. For ileocolic resections, Eshuis *et al*[51] have reported that the specimen can be extracted by an intraoperative endoscopist before suturing of the anastomosis. In case of performance of ostomies, the specimen can also be extracted through the planned ostomy site. Limitations of these emerging techniques are technical difficulty, potentially longer operating times and possible difficulties with extraction of large specimen.

Transperineal completion proctectomy

Performance of completion proctectomy is considered the last option for refractory perianal fistulizing disease with rectal involvement. In the literature around 10%-20% of the patients with Crohn's disease will eventually require proctectomy[54,55]. As an alternative to a low Hartman procedure, where an anterior rectal resection is completed by stapling of the rectum at the dentate line, an interspincteric resection procedure has recently been described as the method of choice for completion proctectomy in patients with Crohn's disease[56]. The procedure can be performed *via* a transperineal approach and usage of ultrasonic dissection of the rectum. An advantage of the technique compared to a low Hartman situation is complete resection of rectal mucosa and thereby reduction of Crohn's associated symptoms. Furthermore, as the authors point out, close dissection of the rectum is preservation of the rectal mesentery with only a small residual cavity in the lesser pelvis. For patients with ulcerative colitis completion proctectomy is generally performed following subtotal colectomy with performance of terminal ileostomy without the possibility of reconstructive surgery. In these cases the remaining rectal stump is a potential source of residual inflammation and associated morbidity. Liyanage *et al*[57] have introduced an alternative approach for performance of completion proctectomy other than abdomino-perineal resection. Using an endoscopic microsurgery TEM-equipment the authors performed perineal proctectomy following abdominal subtotal colectomy for ulcerative colitis. Twelve patients have been included in their preliminary study. The operation was initiated by an intersphincteric dissection following insertion of the proctoscope and performance of close rectal dissection. The specimen was then extracted perineally and the external sphincter was closed using an absorbable suture. In four patients there was delayed healing in the perineal wound with no associated morbidity. Altogether the authors have illustrated that in their limited number of patients, employment of TEM instruments for performance of trans-anal intersphincteric dissection and completion proctectomy is feasible with acceptable outcomes.

Transanal reconstructive rectal surgery

One of the latest technical advances in colorectal surgery is the further development of trans-anal minimally invasive surgery. The main principle of transanal surgical approaches has already been developed decades ago and now has gained a revival in attention. The method has mostly been employed for local excisions of rectal neoplasia but lately has also been successfully expanded for treatment of other distal colorectal disease[58]. Basic principle of the technique is the idea of easy transanal entrance to the mesorectal plane for rectal excisions such as in total mesorectal excision (TME) in case of rectal cancer. For the transanal introduction of the instruments different port systems have been used, altogether they represent the base for the so called transanal minimally invasive surgery (TAMIS)-platform for the use of regular laparoscopic instruments. The technique has mostly been investigated for performance of TME with the idea of a nerve sparing approach in rectal cancer. In most cases TAMIS is combined with abdominal laparoscopic surgery for performance of proximal mobilization of the colon and rectum prior to transanal resection and performance of anastomosis. The latter is an important point which distinguishes transanal minimally invasive surgery from transanal completion proctectomy. With performance of TAMIS the operation is aimed at performing a primary anastomosis following transanal resection. TAMIS has not been performed for patients with Crohn's disease, however one could imagine possible indications for TAMIS resections for example in case of low rectal stenosis or high supra-anal fistulae. Just recently Tasende *et al*[59] have published their short term outcomes in a prospective case series of patients with ulcerative colitis that underwent proctocolectomy and J-pouch formation in a three step procedure using a combined laparoscopic/natural orifice specimen extraction (NOSE) approach with transanal minimally invasive completion proctectomy. Initial subtotal colectomy with terminal ileostomy was performed laparoscopically in a NOSE approach with the colon being removed transrectally. In the second operation ileostomy removal and pouch formation was performed and followed by transanal proctectomy and performance of anastomosis. Ultimately in the third operation reversal of loop ileostomy was performed. A total number of 16 patients were included with mean operative times of 162.2 min (SD 40.5) for the first step and 170 min (SD 50.1) for the second step. Three months after ileostomy reversal patients had a mean 24 h defecation frequency of 5.5 (SD 1.7), which is comparable to results published in the literature. The majority of patients (75%) could retain stools for more than 30 min indicating sufficient function of the anal sphincter.

Altogether the results open a promising possibility for further development of the technique.

CONCLUSION

Taken together, published data so far has shown feasibility of laparoscopic approaches for primary, recurrent and complicated cases of IBD. Interpretation of the data is still limited by a small number of randomized trials with low numbers of patients being enrolled. Especially for complicated cases of penetrating disease careful selection of patients together with a high level of laparoscopic expertise seems to be the main influencing factor for good short and long term outcomes. Studies investigating the best population that would benefit most by laparoscopic approaches are still missing. However today, in specialized centers primary resections such as ileocecal resection for Crohn's disease or restorative proctocolectomy for ulcerative colitis are almost routinely performed at least with laparoscopic assistance[5].

REFERENCES

1 **Travis SP**, Stange EF, Lémann M, Oresland T, Bemelman WA, Chowers Y, Colombel JF, D'Haens G, Ghosh S, Marteau P, Kruis W, Mortensen NJ, Penninckx F, Gassull M. European evidence-based Consensus on the management of ulcerative colitis: Current management. *J Crohns Colitis* 2008; **2**: 24-62 [PMID: 21172195 DOI: 10.1016/j.crohns.2007.11.002]

2 **Filippi J**, Allen PB, Hébuterne X, Peyrin-Biroulet L. Does anti-TNF therapy reduce the requirement for surgery in ulcerative colitis? A systematic review. *Curr Drug Targets* 2011; **12**: 1440-1447 [PMID: 21466486]

3 **Targownik LE**, Singh H, Nugent Z, Bernstein CN. The epidemiology of colectomy in ulcerative colitis: results from a population-based cohort. *Am J Gastroenterol* 2012; **107**: 1228-1235 [PMID: 22613902 DOI: 10.1038/ajg.2012.127]

4 **Baumgart DC**, Sandborn WJ. Inflammatory bowel disease: clinical aspects and established and evolving therapies. *Lancet* 2007; **369**: 1641-1657 [PMID: 17499606 DOI: 10.1016/S0140-6736(07)60751-X]

5 **Maggiori L**, Panis Y. Surgical management of IBD--from an open to a laparoscopic approach. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 297-306 [PMID: 23419288 DOI: 10.1038/nrgastro.2013.30]

6 **Neumann PA**, Rijcken EJ, Bruewer M. Current status of laparoscopic surgery for patients with Crohn's disease. *Int J Colorectal Dis* 2013; **28**: 599-610 [PMID: 23588872 DOI: 10.1007/s00384-013-1684-y]

7 **Buskens CJ**, Sahami S, Tanis PJ, Bemelman WA. The potential benefits and disadvantages of laparoscopic surgery for ulcerative colitis: A review of current evidence. *Best Pract Res Clin Gastroenterol* 2014; **28**: 19-27 [PMID: 24485252 DOI: 10.1016/j.bpg.2013.11.007]

8 **Yamamoto T**, Watanabe T. Surgery for luminal Crohn's disease. *World J Gastroenterol* 2014; **20**: 78-90 [PMID: 24415860 DOI: 10.3748/wjg.v20.i1.78]

9 **Dignass A**, Lindsay JO, Sturm A, Windsor A, Colombel JF, Allez M, D'Haens G, D'Hoore A, Mantzaris G, Novacek G, Oresland T, Reinisch W, Sans M, Stange E, Vermeire S, Travis S, Van Assche G. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 2: current management. *J Crohns Colitis* 2012; **6**: 991-1030 [PMID: 23040451 DOI: 10.1016/j.crohns.2012.09.002]

10 **Kornbluth A**, Sachar DB. Ulcerative colitis practice guidelines in adults: American College Of Gastroenterology, Practice Parameters Committee. *Am J Gastroenterol* 2010; **105**: 501-523; quiz 524 [PMID: 20068560 DOI: 10.1038/ajg.2009.727]

11 **Maartense S**, Dunker MS, Slors JF, Cuesta MA, Pierik EG, Gouma DJ, Hommes DW, Sprangers MA, Bemelman WA. Laparoscopic-assisted versus open ileocolic resection for Crohn's disease: a randomized trial. *Ann Surg* 2006; **243**: 143-149; discussion 150-153 [PMID: 16432345 DOI: 10.1097/01.sla.0000197318.37459.ec]

12 **Milsom JW**, Hammerhofer KA, Böhm B, Marcello P, Elson P, Fazio VW. Prospective, randomized trial comparing laparoscopic vs. conventional surgery for refractory ileocolic Crohn's disease. *Dis Colon Rectum* 2001; **44**: 1-8; discussion 8-9 [PMID: 11805557]

13 **Tan JJ**, Tjandra JJ. Laparoscopic surgery for Crohn's disease: a meta-analysis. *Dis Colon Rectum* 2007; **50**: 576-585 [PMID: 17380366 DOI: 10.1007/s10350-006-0855-0]

14 **Rosman AS**, Melis M, Fichera A. Metaanalysis of trials comparing laparoscopic and open surgery for Crohn's disease. *Surg Endosc* 2005; **19**: 1549-1555 [PMID: 16235128 DOI: 10.1007/s00464-005-0114-9]

15 **Tilney HS**, Constantinides VA, Heriot AG, Nicolaou M, Athanasiou T, Ziprin P, Darzi AW, Tekkis PP. Comparison of laparoscopic and open ileocecal resection for Crohn's disease: a metaanalysis. *Surg Endosc* 2006; **20**: 1036-1044 [PMID: 16715212 DOI: 10.1007/s00464-005-0500-3]

16 **Eshuis EJ**, Slors JF, Stokkers PC, Sprangers MA, Ubbink DT, Cuesta MA, Pierik EG, Bemelman WA. Long-term outcomes following laparoscopically assisted versus open ileocolic resection for Crohn's disease. *Br J Surg* 2010; **97**: 563-568 [PMID: 20175126 DOI: 10.1002/bjs.6918]

17 **Stocchi L**, Milsom JW, Fazio VW. Long-term outcomes of laparoscopic versus open ileocolic resection for Crohn's disease: follow-up of a prospective randomized trial. *Surgery* 2008; **144**: 622-627; discussion 627-628 [PMID: 18847647 DOI: 10.1016/j.surg.2008.06.016]

18 **Brouquet A**, Bretagnol F, Soprani A, Valleur P, Bouhnik Y, Panis Y. A laparoscopic approach to iterative ileocolonic resection for the recurrence of Crohn's disease. *Surg Endosc* 2010; **24**: 879-887 [PMID: 19730944 DOI: 10.1007/s00464-009-0682-1]

19 **Hasegawa H**, Watanabe M, Nishibori H, Okabayashi K, Hibi T, Kitajima M. Laparoscopic surgery for recurrent Crohn's disease. *Br J Surg* 2003; **90**: 970-973 [PMID: 12905550 DOI: 10.1002/bjs.4136]

20 **Aytac E**, Stocchi L, Remzi FH, Kiran RP. Is laparoscopic surgery for recurrent Crohn's disease beneficial in patients with previous primary resection through midline laparotomy? A case-matched study. *Surg Endosc* 2012; **26**: 3552-3556 [PMID: 22648125 DOI: 10.1007/s00464-012-2361-x]

21 **Goyer P**, Alves A, Bretagnol F, Bouhnik Y, Valleur P, Panis Y. Impact of complex Crohn's disease on the outcome of laparoscopic ileocecal resection: a comparative clinical study in 124 patients. *Dis Colon Rectum* 2009; **52**: 205-210 [PMID: 19279413 DOI: 10.1007/DCR.0b013e31819c9c08]

22 **Lesperance K**, Martin MJ, Lehmann R, Brounts L, Steele SR. National trends and outcomes for the surgical therapy of ileocolonic Crohn's disease: a population-based analysis of laparoscopic vs. open approaches. *J Gastrointest Surg* 2009; **13**: 1251-1259 [PMID: 19301075 DOI: 10.1007/s11605-009-0853-3]

23 **Chung TP**, Fleshman JW, Birnbaum EH, Hunt SR, Dietz DW, Read TE, Mutch MG. Laparoscopic vs. open total abdominal colectomy for severe colitis: impact on recovery and subsequent completion restorative proctectomy. *Dis Colon Rectum* 2009; **52**: 4-10 [PMID: 19273949 DOI: 10.1007/DCR.0b013e3181975701]

24 **Marceau C**, Alves A, Ouaissi M, Bouhnik Y, Valleur P, Panis Y. Laparoscopic subtotal colectomy for acute or severe colitis complicating inflammatory bowel disease: a case-matched study in 88 patients. *Surgery* 2007; **141**: 640-644 [PMID: 17462464 DOI: 10.1016/j.surg.2006.12.012]

25 **Telem DA**, Vine AJ, Swain G, Divino CM, Salky B, Greenstein AJ, Harris M, Katz LB. Laparoscopic subtotal colectomy for medically refractory ulcerative colitis: the time has come. *Surg Endosc* 2010; **24**: 1616-1620 [PMID: 20204417 DOI: 10.1007/s00464-009-0819-2]

26 **Ouaïssi M**, Alves A, Bouhnik Y, Valleur P, Panis Y. Three-step ileal

pouch-anal anastomosis under total laparoscopic approach for acute or severe colitis complicating inflammatory bowel disease. *J Am Coll Surg* 2006; **202**: 637-642 [PMID: 16571435 DOI: 10.1016/j.jamcollsurg.2005.12.016]

27 **Ouaïssi M**, Lefevre JH, Bretagnol F, Alves A, Valleur P, Panis Y. Laparoscopic 3-step restorative proctocolectomy: comparative study with open approach in 45 patients. *Surg Laparosc Endosc Percutan Tech* 2008; **18**: 357-362 [PMID: 18716534 DOI: 10.1097/SLE.0b013e3181772d75]

28 **El-Gazzaz GS**, Kiran RP, Remzi FH, Hull TL, Geisler DP. Outcomes for case-matched laparoscopically assisted versus open restorative proctocolectomy. *Br J Surg* 2009; **96**: 522-526 [PMID: 19358179 DOI: 10.1002/bjs.6578]

29 **Fichera A**, Silvestri MT, Hurst RD, Rubin MA, Michelassi F. Laparoscopic restorative proctocolectomy with ileal pouch anal anastomosis: a comparative observational study on long-term functional results. *J Gastrointest Surg* 2009; **13**: 526-532 [PMID: 19015927 DOI: 10.1007/s11605-008-0755-9]

30 **Maartense S**, Dunker MS, Slors JF, Cuesta MA, Gouma DJ, van Deventer SJ, van Bodegraven AA, Bemelman WA. Hand-assisted laparoscopic versus open restorative proctocolectomy with ileal pouch anal anastomosis: a randomized trial. *Ann Surg* 2004; **240**: 984-991; discussion 991-992 [PMID: 15570204]

31 **Gu J**, Stocchi L, Remzi FH, Kiran RP. Total abdominal colectomy for severe ulcerative colitis: does the laparoscopic approach really have benefit? *Surg Endosc* 2014; **28**: 617-625 [PMID: 24196546 DOI: 10.1007/s00464-013-3218-7]

32 **Singh P**, Bhangu A, Nicholls RJ, Tekkis P. A systematic review and meta-analysis of laparoscopic vs open restorative proctocolectomy. *Colorectal Dis* 2013; **15**: e340-e351 [PMID: 23560590 DOI: 10.1111/codi.12231]

33 **Schiessling S**, Leowardi C, Kienle P, Antolovic D, Knebel P, Bruckner T, Kadmon M, Seiler CM, Büchler MW, Diener MK, Ulrich A. Laparoscopic versus conventional ileoanal pouch procedure in patients undergoing elective restorative proctocolectomy (LapConPouch Trial)-a randomized controlled trial. *Langenbecks Arch Surg* 2013; **398**: 807-816 [PMID: 23686277 DOI: 10.1007/s00423-013-1088-z]

34 **Bemelman WA**, Ringers J, Meijer DW, de Wit CW, Bannenberg JJ. Laparoscopic-assisted colectomy with the dexterity pneumo sleeve. *Dis Colon Rectum* 1996; **39**: S59-S61 [PMID: 8831548]

35 **Nakajima K**, Lee SW, Cocilovo C, Foglia C, Kim K, Sonoda T, Milsom JW. Hand-assisted laparoscopic colorectal surgery using GelPort. *Surg Endosc* 2004; **18**: 102-105 [PMID: 12958676 DOI: 10.1007/s00464-002-8648-6]

36 **Ahmed Ali U**, Keus F, Heikens JT, Bemelman WA, Berdah SV, Gooszen HG, van Laarhoven CJ. Open versus laparoscopic (assisted) ileo pouch anal anastomosis for ulcerative colitis and familial adenomatous polyposis. *Cochrane Database Syst Rev* 2009; **(1)**: CD006267 [PMID: 19160273 DOI: 10.1002/14651858.CD006267.pub2]

37 **Jadlowiec CC**, Mannion EM, Thielman MJ, Bartus CM, Johnson KH, Sardella WV, Vignati PV, Cohen JL. Evolution of technique in performance of minimally invasive colectomies. *Dis Colon Rectum* 2014; **57**: 1090-1097 [PMID: 25101605 DOI: 10.1097/DCR.0000000000000178]

38 **Rijcken E**, Mennigen R, Argyris I, Senninger N, Bruewer M. Single-incision laparoscopic surgery for ileocolic resection in Crohn's disease. *Dis Colon Rectum* 2012; **55**: 140-146 [PMID: 22228156 DOI: 10.1097/DCR.0b013e31823d0e0d]

39 **Gardenbroek TJ**, Verlaan T, Tanis PJ, Ponsioen CY, D'Haens GR, Buskens CJ, Bemelman WA. Single-port versus multiport laparoscopic ileocecal resection for Crohn's disease. *J Crohns Colitis* 2013; **7**: e443-e448 [PMID: 23507422 DOI: 10.1016/j.crohns.2013.02.015]

40 **Moftah M**, Nazour F, Cunningham M, Cahill RA. Single port laparoscopic surgery for patients with complex and recurrent Crohn's disease. *J Crohns Colitis* 2014; **8**: 1055-1061 [PMID: 24589026 DOI: 10.1016/j.crohns.2014.02.003]

41 **Geisler DP**, Condon ET, Remzi FH. Single incision laparoscopic total proctocolectomy with ileopouch anal anastomosis. *Colorectal Dis* 2010; **12**: 941-943 [PMID: 19895601 DOI: 10.1111/j.1463-1318.2009.02115.x]

42 **Gash KJ**, Goede AC, Kaldowski B, Vestweber B, Dixon AR. Single incision laparoscopic (SILS) restorative proctocolectomy with ileal pouch-anal anastomosis. *Surg Endosc* 2011; **25**: 3877-3880 [PMID: 21761270 DOI: 10.1007/s00464-011-1814-y]

43 **Bulian DR**, Knuth J, Krakamp B, Heiss MM. Restorative restproctectomy as single-port surgery through the ostomy site in a three-stage procedure. *Surg Endosc* 2012; **26**: 3688-3690 [PMID: 22710655 DOI: 10.1007/s00464-012-2394-1]

44 **Pigazzi A**, Ellenhorn JD, Ballantyne GH, Paz IB. Robotic-assisted laparoscopic low anterior resection with total mesorectal excision for rectal cancer. *Surg Endosc* 2006; **20**: 1521-1525 [PMID: 16897284 DOI: 10.1007/s00464-005-0855-5]

45 **Miller AT**, Berian JR, Rubin M, Hurst RD, Fichera A, Umanskiy K. Robotic-assisted proctectomy for inflammatory bowel disease: a case-matched comparison of laparoscopic and robotic technique. *J Gastrointest Surg* 2012; **16**: 587-594 [PMID: 21964583 DOI: 10.1007/s11605-011-1692-6]

46 **McLemore EC**, Cullen J, Horgan S, Talamini MA, Ramamoorthy S. Robotic-assisted laparoscopic stage II restorative proctectomy for toxic ulcerative colitis. *Int J Med Robot* 2012; **8**: 178-183 [PMID: 22113993 DOI: 10.1002/rcs.445]

47 **Tou S**, Pavesi E, Nasser A, Mazirka P, Bergamaschi R. Robotic-assisted strictureplasty for Crohn's disease. *Tech Coloproctol* 2015; **19**: 253-254 [PMID: 25567362 DOI: 10.1007/s10151-014-1260-7]

48 **Juo YY**, Agarwal S, Luka S, Satey S, Obias V. Single-Incision Robotic Colectomy (SIRC) case series: initial experience at a single center. *Surg Endosc* 2015; **29**: 1976-1981 [PMID: 25303915 DOI: 10.1007/s00464-014-3896-9]

49 **Lujan HJ**, Molano A, Burgos A, Rivera B, Plasencia G. Robotic right colectomy with intracorporeal anastomosis: experience with 52 consecutive cases. *J Laparoendosc Adv Surg Tech A* 2015; **25**: 117-122 [PMID: 25622223 DOI: 10.1089/lap.2014.0199]

50 **Gardenbroek TJ**, Eshuis EJ, van Acker GJ, Tanis PJ, Bemelman WA. Alternative specimen extraction techniques after laparoscopic emergency colectomy in inflammatory bowel disease. *Surg Endosc* 2012; **26**: 408-412 [PMID: 21909858 DOI: 10.1007/s00464-011-1888-6]

51 **Eshuis EJ**, Voermans RP, Stokkers PC, van Berge Henegouwen MI, Fockens P, Bemelman WA. Laparoscopic resection with transcolonic specimen extraction for ileocaecal Crohn's disease. *Br J Surg* 2010; **97**: 569-574 [PMID: 20155789 DOI: 10.1002/bjs.6932]

52 **Larson DW**, Dozois E, Sandborn WJ, Cima R. Total laparoscopic proctocolectomy with Brooke ileostomy: a novel incisionless surgical treatment for patients with ulcerative colitis. *Surg Endosc* 2005; **19**: 1284-1287 [PMID: 16132322 DOI: 10.1007/s00464-004-8245-y]

53 **Wexner SD**. Restorative proctectomy with colon pouch-anal anastomosis by laparoscopic transanal pull-through: an available option for low rectal cancer? *Surg Endosc* 2007; **21**: 1679 [PMID: 17593441 DOI: 10.1007/s00464-007-9438-y]

54 **Basu A**, Wexner SD. Perianal Crohn's Disease. *Curr Treat Options Gastroenterol* 2002; **5**: 197-206 [PMID: 12003714]

55 **Williamson PR**, Hellinger MD, Larach SW, Ferrara A. Twenty-year review of the surgical management of perianal Crohn's disease. *Dis Colon Rectum* 1995; **38**: 389-392 [PMID: 7720446]

56 **Gardenbroek TJ**, Tanis PJ, Buskens CJ, Bemelman WA. Surgery for Crohn's disease: new developments. *Dig Surg* 2012; **29**: 275-280 [PMID: 22922840 DOI: 10.1159/000341567]

57 **Liyanage C**, Ramwell A, Harris GJ, Levy BF, Simson JN. Transanal endoscopic microsurgery: a new technique for completion proctectomy. *Colorectal Dis* 2013; **15**: e542-e547 [PMID: 24011233 DOI: 10.1111/codi.12316]

58 **Atallah S**, Albert M, Debeche-Adams T, Larach S. Transanal minimally invasive surgery (TAMIS): applications beyond local

excision. *Tech Coloproctol* 2013; **17**: 239-243 [PMID: 23207713 DOI: 10.1007/s10151-012-0945-z]

59 **Tasende MM**, Delgado S, Jimenez M, Del Gobbo GD, Fernández-Hevia M, DeLacy B, Balust J, Lacy AM. Minimal invasive surgery: NOSE and NOTES in ulcerative colitis. *Surg Endosc* 2015; **29**: 3313-3318 [PMID: 25669637 DOI: 10.1007/s00464-015-4087-z]

P- Reviewer: Shrestha BM, Soria F, Yan XQ **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Wu HL

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i2.227

World J Gastrointest Pharmacol Ther 2016 May 6; 7(2): 227-234
ISSN 2150-5349 (online)

MINIREVIEWS

Update on management of Barrett's esophagus

Fernando Macías-García, J Enrique Domínguez-Muñoz

Fernando Macías-García, J Enrique Domínguez-Muñoz, Department of Gastroenterology and Hepatology, University Hospital of Santiago de Compostela, 15706 Santiago de Compostela, Spain

Fernando Macías-García, J Enrique Domínguez-Muñoz, Foundation for Research in Digestive Diseases (FIENAD), University Hospital of Santiago de Compostela, 15706 Santiago de Compostela, Spain

Author contributions: Macías-García F wrote the paper; Domínguez-Muñoz JE supervision and final approval of the article.

Conflict-of-interest statement: Authors declare no conflict of interests for this article.

Correspondence to: Fernando Macías-García, MD, Department of Gastroenterology and Hepatology, University Hospital of Santiago de Compostela, Choupana s/n, 15706 Santiago de Compostela, Spain. fmacgar@yahoo.es
Telephone: +34-98-1951364
Fax: +34-98-1951365

Received: April 25, 2015
Peer-review started: April 27, 2015
First decision: September 8, 2015
Revised: December 15, 2015
Accepted: February 14, 2016
Article in press: February 16, 2016
Published online: May 6, 2016

Abstract

Barrett's esophagus (BE) is a common condition that develops as a consequence of gastroesophageal reflux disease. The significance of Barrett's metaplasia is that predisposes to cancer development. This article provides a current evidence-based review for the management of BE and related early neoplasia. Controversial issues that impact the management of patients with BE, including definition, screening, clinical aspects, diagnosis, surveillance, and management of dysplasia and early cancer have been assessed.

Key words: Barrett's esophagus; Barrett metaplasia; Esophageal adenocarcinoma; Gastroesophageal reflux disease; Radiofrequency ablation

Core tip: Barrett's esophagus (BE) is a common condition that predisposes to cancer development. This article provides a current evidence-based review for controversial issues that impact the management of patients with BE, including clinical aspects, diagnosis, surveillance, and management of dysplasia and early cancer.

Macías-García F, Domínguez-Muñoz JE. Update on management of Barrett's esophagus. *World J Gastrointest Pharmacol Ther* 2016; 7(2): 227-234 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i2/227.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i2.227

INTRODUCTION

Since Barrett's esophagus (BE) was first described in 1950[1], the definition of this condition has been modified on several occasions. Presently, it is defined as the condition in which any extent of metaplastic columnar epithelium that predisposes to cancer development replaces the stratified squamous epithelium that normally lines the distal esophagus[2]. Since

only intestinal metaplasia (with globet cells) clearly predisposes to malignancy, its presence is required for the diagnosis. Nevertheless, some scientific societies consider that the presence of cardiac mucosa (with mucus-secreting columnar cells without goblet cells) are also diagnostic of BE[3]. However, because the risk for malignancy of cardia-type epithelium remains unclear, it is not generally recommended to use the term "Barrett's esophagus" in that context[2,4].

EPIDEMIOLOGY AND CLINICAL ISSUES

BE is a common condition, with an estimated prevalence in the general adult population between 2% and 7%[5,6], and an incidence rate varying between 23.1 and 32.7 per 100000 person-year[5,7,8]. It is observed in 4% of patients undergoing an upper gastrointestinal endoscopy, and in 9% of men over 50 years[9].

Risk factors for BE include the presence of severe and longstanding gastroesophageal reflux, for which the biliary-pancreatic content seems to have a significant role[10], as well as advanced age, male sex, white race, obesity, and tobacco use. Conversely, factors that might protect against BE include the use of NSAIDs, Helicobacter pylori, and high consumption of fruits and vegetables[11].

Although BE is an asymptomatic disease, it is the most important known risk factor for the development of esophageal adenocarcinoma (EA), a tumor that has increased its incidence six-fold over the last four decades in Western countries, becoming the fastest growing cause of cancer mortality[12]. For BE patients, with a probability of 0.5% per year[13], the risk of developing EA is between 40 and 50 higher than for the general population[14-16].

The malignant degeneration cascade is thought to occur from nondysplastic intestinal metaplasia, to low-grade (LGD) and then high-grade dysplasia (HGD), and eventually EA[2,17]. The rate of progression from LGD to either HGD or EA ranges from 0.5% to 13.4% per patient per year[18]. The annual risk of progression from HGD to EA is 10% (ranges between 6% and 19%)[19,20].

SCREENIG FOR BE

Although this practice is not supported by high-quality evidence, screening for BE can be suggested in patients with chronic gastroesophageal reflux disease (GERD) symptoms who have at least one additional risk factor for EA. The risk factors include: 50 years or older, male sex, white race, hiatal hernia, elevated body-mass index, intrabdominal body-fat distribution, or tobacco use[21-24].

ENDOSCOPIC DIAGNOSIS

The gastroesophageal junction (GEJ) is not anatomically well defined, but it is accepted as the proximal limit of the gastric folds under partial insufflation. The squamocolumnar junction is bounded by the pale pink squamous mucosa of the esophagus, which contrasts with the red columnar gastric mucosa. The diagnosis of BE requires that the columnar epithelium extends above the GEJ, and the presence of columnar metaplasia confirmed in the esophageal biopsy[21].

BE has been divided in short (< 3 cm) or long-segment (≥ 3 cm), depending on the length of the metaplastic epithelium[25], but it is not clear that this classification can be clinical helpful, since there is no definitive evidence that the extent of the metaplastic segment increases the risk of cancer[26]. Prague's classification is a more recent system for describing BE endoscopically that evaluates both the circumferential extent (C) and the maximum extent (M) of Barrett's metaplasia[27]. However, since no endoscopic technique allows to either differentiate the intestinal metaplasia from gastric metaplasia or recognize the presence of dysplasia, a biopsy specimen is always required for diagnosis.

SURVEILLANCE IN BE

Although there is no data from randomized controlled trials, surveillance is generally recommended because it has been correlated in published studies with earlier stage diagnosis and improved survival from cancer[3]. However, surveillance strategies are limited by the low incidence of cancer in patients with BE, and by the various difficulties in the interpretation of the presence of dysplasia (because of random sample collection, possibility of false negatives in the evaluation of the biopsies, or high variability for the interpretation of dysplasia). Nevertheless, most clinical guidelines[2,3,28] recommend endoscopic surveillance in patients with BE. The goal is the early detection of LGD and of its progression to HGD or early stage cancer (lymph node involvement varies between 0% and 2%, respectively). However, no long-term trials have been performed to definitively answer the question of whether endoscopic surveillance really reduces cancer incidence or mortality.

A high-resolution endoscopy is strongly recommended for the accurate evaluation of BE. A 4-quadrant biopsy sampling should be performed every 2 cm or every 1 cm (if known or suspected dysplasia). Additionally, specific biopsies of any suspicious lesions should be submitted separately.

Advanced imaging techniques (such as chromoendoscopy or electronic chromoendoscopy, narrow band imaging, confocal laser endomicroscopy or magnification) are not superior to standard white light endoscopy and, therefore, are not recommended for routine use. However, these technologies may be helpful to adequately address biopsies if dysplasia is suspected[2,29].

When no dysplasia is detected after 2 consecutive endoscopies within 6-12 mo, the usual recommendation is to repeat the test after 3-5 years. When indeterminate-grade dysplasia is detected, it is recommended

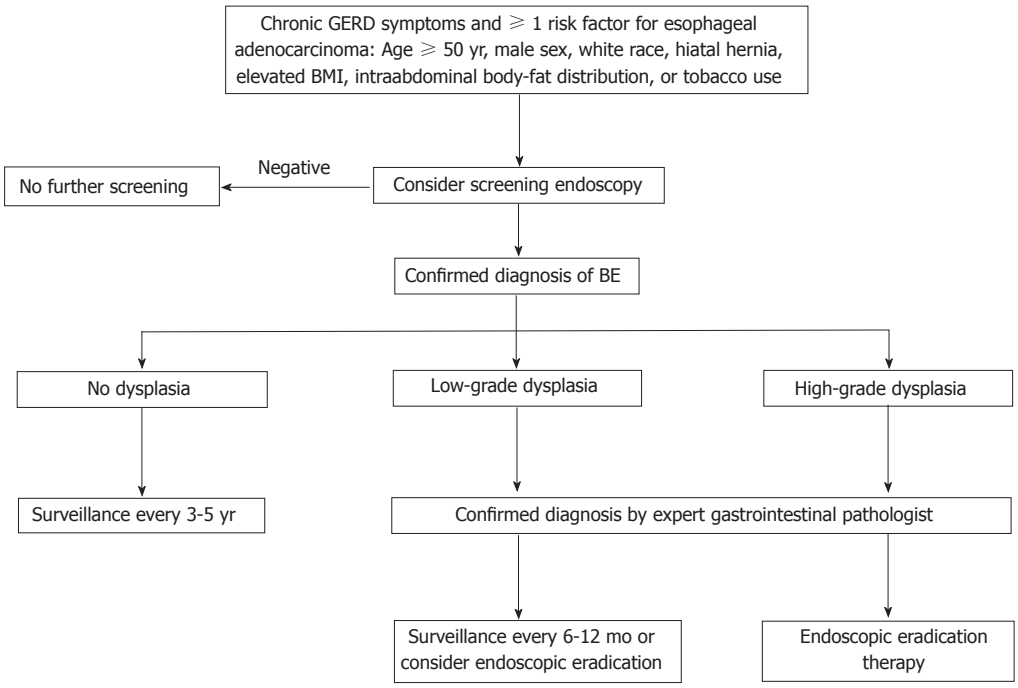

Figure 1 Algorithm for the screening, surveillance and management of Barrett's esophagus. GERD: Gastroesophageal reflux disease; BMI: Body mass index. BE: Barrett's esophagus.

to increase the antisecretory treatment to heal the esophageal inflammation and then repeat the biopsy after 6 mo. When LGD is detected, the recommendation is to perform an endoscopic control after 6-12 mo, and then an annual endoscopy until the absence of dysplasia is confirmed in two consecutive annual controls; alternatively, endoscopic eradication therapy can also be considered. When HGD is detected, endoscopic eradication therapy is strongly advised (consider surveillance every 3 mo only in selected cases). An algorithm for the screening, surveillance and management of BE is shown in Figure 1.

MANAGEMENT OF DYSPLASIA AND EARLY CANCER

HGD and intramucosal EA

Traditionally, esophagectomy has been recommended for patients with BE and either HGD or early EA, but the high morbidity and mortality of this technique, alongside with the development of new endoscopic techniques, are modifying this approach. Even in high-volume centers, the mortality rate of esophagectomy for HGD or early EA ranges from 0% to 4%[30]. Actually, surgery should be reserved for those patients with infiltration of the submucosal layer, and/or low grade or lack of response to endoscopic treatment.

The goal of endoscopic eradication therapy for patients with BE is to permanently eradicate all intestinal metaplasia and achieve a complete reversion to squamous epithelium[2,31]. Several studies have shown that HGD/T1m neoplasms can be eradicated in up 80%-100% cases, as well as the BE with intestinal metaplasia can be removed of in > 75% of cases[20,32-36]. Moreover, a significantly higher rate of progression to cancer has been shown in the endoscopic surveillance group comparing with the ablative treatment group (after initial endoscopic mucosal resection (EMR) where appropriate)[20,37]. Therefore, instead of surveillance, endoscopic eradication therapy with radiofrequency ablation (RFA), photodynamic therapy (PDT), or EMR is recommended for treatment of patients with confirmed HGD or intramucosal adenocarcinoma (T1a) within BE[2]. Major complications of these techniques include strictures, hemorrhage and perforation. Minor complications include temporary chest pain, fever and odynophagia.

Survival after endoscopic resection is similar to that expected after surgical treatment, but with less morbidity[32-34]. Therefore, since HGD is not associated with metastatic nodal spread when the existence of a deeper invasion has been excluded by EMR, endoscopic treatment is preferred over surgery in most patients with BE and HGD[32,33,38-40]. But, on the other hand, endoscopic therapies are associated with a higher rate of recurrence of the HGD[32-34,38,41], although it can usually be treated endoscopically[32-34,42]. Because of that, surgical resection should be reserved until the endoscopic treatment fails[32-34].

RFA is effective transforming an esophagus with pathological cells into an esophagus with a normal mucosa, without genetic abnormalities that may become premalignant[43]. A recent systematic review[44] suggests that success rates are higher with RFA, with a sustained disappearance of the HGD in up to 90% of patients[20,23,35,43,45]. RFA ablation is a safe, long-

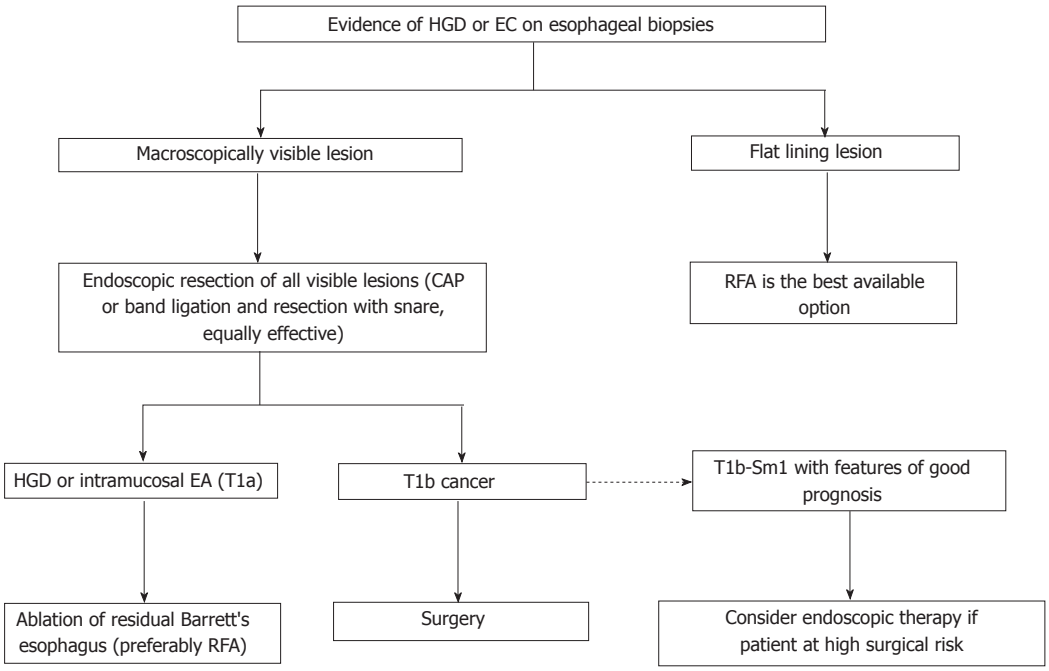

Figure 2 Management of high-grade dysplasia and early cancer in Barrett's esophagus. RFA: Radiofrequency ablation; BE: Barrett's esophagus; HGD: High-grade dysplasia; EC: Early cancer; EA: Esophageal adenocarcinoma.

lasting therapy (up to 5 years) that is associated with a significant reduction in the relative risk for neoplastic progression[20,35,46-48]. This technique is usually performed in various sessions to completely eradicate the metaplasia. The most common adverse event is the stenosis (up to 5% of patients)[49], but the rate of severe side effects of RFA is lower than with other ablative techniques[2]. Compared to other options, such as surgical treatment, photodynamic therapy, or follow up, RFA ablation is the most cost-effective strategy in patients with HGD[50].

In cases of HGD on a visible mucosal lesion, EMR is needed for an adequate diagnosis and depth staging[51] that can lead to a significant change in the management[52-55], since if an EA is found in the EMR sample, the risk of malignant adenopathy is related to the depth of invasion[56,57]. In this sense, the cap and snare technique with submucosal injection, and the band ligation technique without submucosal injection are considered to be equally effective[3]. Ideally, EMR should be applied in less than two thirds of the circumference of the esophagus to avoid strictures[29]. If a stenosis appears, it can be usually treated with endoscopic dilatation[58-60].

Endoscopic ablation of residual BE is currently recommended after completion of EMR of all visible HGD/T1a lesions. Several case series have reported recurrence of neoplasia if any residual BE is left untreated (11% to 30%, with a mean follow-up of 3 years)[32,58], and ablation of the residual BE is associated with a lower recurrence[20,36,40,41,61,62]. Consequently, RFA is currently the best available technique for the treatment of flat HGD and for eradicating residual BE after EMR[3,29,63].

Among alternative ablative techniques, PDT has been effectively used to ablate HGD, reducing the risk of progression to cancer compared with surveillance alone[64]. However, adverse events associated with this technology are common (development of esophageal stricture, 36% after PDT *vs* 6% after RFA) and may be severe[22,42]. Moreover, HGD can even persist in up to 33%-50% of the patients[65,66]. Long follow-up controlled studies comparing PDT with surgical resection and the other endoscopic therapies are needed to adequately assess this technique. Cryotherapy has not been assessed in randomized controlled trials, and it is not currently indicated as an alternative endoscopic eradication therapy. Small randomized controlled trials using argon plasma coagulation have reported anecdotal high-success rates[67].

In the case of an early EA extending into the submucosal layer, surgery should be considered as the best option[29], since in T1a context the rate of lymph node involvement is extremely low (< 3%) but the risk increases up to 20%-25% when the submucosal layer is affected. However, in selected T1b-Sm1 cases (invasion limited to the superficial layer of the submucosa), and with low-risk histopathologic features (invasion < 500 μm; G1-G2 grade, no lympho-vascular invasion), endoscopic therapy could be an option instead of esophagectomy (especially in high surgical risk patients)[68,69]. Endoscopic ultrasound evaluation of visible lymph nodes is advised in this setting.

The algorithm for the management of BE with HGD or early cancer is shown in Figure 2.

LGD

Up to 25%-40% of BE patients will be diagnosed with

LGD during follow-up[70]. Most guidelines recommend endoscopic surveillance (every 6-12 mo) to rule out dysplastic progression. However, there are several doubts related to the evolution of the LGD. In some cases LGD may progress to HGD or EA, but it can also remain stable or even disappear in subsequent controls. Still, a significant progression rate from LGD to HGD or EA (13.4% per person-year) has been recently reported[18], suggesting that the endoscopic treatment in this population may also be justified.

The impact of RFA on the risk of neoplastic progression in BE patients with LGD is not clear, but RFA leads to reversion to normal-appearing squamous epithelium in > 90% of LGD cases[2]. A recent randomized controlled study[71] including 136 BE patients with confirmed LGD (68 patients undergoing RFA ± EMR *vs* 68 patients followed endoscopically) showed that RFA was associated with a significant reduction on the risk of neoplastic progression at 3 years follow-up: 26.5% in the follow-up group *vs* 1.5% in the ablative treatment group (95%CI: 14.1% to 35.9%; $P < 0.001$). This result corresponds to an NNT of 4. Full eradication of dysplasia and intestinal metaplasia were persistently achieved in most patients of the ablative group. Therefore, the authors conclude that ablative therapy should also be considered for patients with a confirmed LGD.

BE without dysplasia

Endoscopic eradication therapy could not yet be recommended in patients with BE without dysplasia, because the low risk of progression to EA (0.1% to 0.3% per year)[14,72-74] and the side effects potentially associated with the endoscopy therapy (10%-15%).

FOLLOW-UP AFTER ERADICATION

After endoscopic or surgery eradication of HGD, endoscopic follow-up is mandatory[75,76]. An evidence-based strategy for surveillance after subtotal esophagectomy is to perform endoscopy at 2, 5, and 10 years after surgery, and every 2-year once BE has been detected[29]. The follow-up interval for the endoscopic ablative therapy is still unclear.

CHEMOPREVENTION AND SYMPTOMATIC CONTROL IN BE

GERD therapy is clearly indicated in the presence of GERD symptoms and/or reflux esophagitis. Although chemoprevention with acid-suppressing drugs can not yet be recommended, some observational studies have found an association between anti-reflux therapy and a lower rate of progression to EA, even in patients without GERD symptoms[77]. These results indirectly suggest a cancer-protective role for proton pump inhibitors (PPIs) in BE, and are strong enough to warrant conventional-dose PPI treatment for patients who have no symptoms or endoscopic signs of GERD[11]. However, acid- suppressing therapies, specifically PPIs, have not proven to reduce risk of progression to dysplasia or cancer[2,3]. PPIs are also used to prevent acid reflux and allow for reepithelialization by squamous epithelium after EMR or ablation.

The risk of EA among patients treated with antireflux surgery, and among those who received medical treatment with PPIs is similar[78]. Thus, antireflux surgery does not protect against cancer, and its indications in BE patients are the same as in GERD patients.

There is currently no definitive evidence to advise the use of aspirin or other chemopreventive agents in BE patients. The use of aspirin is only recommended in BE patients with cardiovascular risk factors (for which aspirin therapy is indicated) because the benefit-risk balance is clearly favorable only in this situation.

REFERENCES

1 **Barrett NR**. Chronic peptic ulcer of the oesophagus and "oesophagitis". *Br J Surg* 1950; **38**: 175-182 [PMID: 14791960 DOI: 10.1002/bjs.18003815005]

2 **Spechler SJ**, Sharma P, Souza RF, Inadomi JM, Shaheen NJ. American Gastroenterological Association medical position statement on the management of Barrett's esophagus. *Gastroenterology* 2011; **140**: 1084-1091 [PMID: 21376940 DOI: 10.1053/j.gastro.2011.01.030]

3 **Fitzgerald RC**, di Pietro M, Ragunath K, Ang Y, Kang JY, Watson P, Trudgill N, Patel P, Kaye PV, Sanders S, O'Donovan M, Bird-Lieberman E, Bhandari P, Jankowski JA, Attwood S, Parsons SL, Loft D, Lagergren J, Moayyedi P, Lyratzopoulos G, de Caestecker J. British Society of Gastroenterology guidelines on the diagnosis and management of Barrett's oesophagus. *Gut* 2014; **63**: 7-42 [PMID: 24165758 DOI: 10.1136/gutjnl-2013-305372]

4 **Riddell RH**, Odze RD. Definition of Barrett's esophagus: time for a rethink--is intestinal metaplasia dead? *Am J Gastroenterol* 2009; **104**: 2588-2594 [PMID: 19623166 DOI: 10.1038/ajg.2009.390]

5 **Ronkainen J**, Aro P, Storskrubb T, Johansson SE, Lind T, Bolling-Sternevald E, Vieth M, Stolte M, Talley NJ, Agréus L. Prevalence of Barrett's esophagus in the general population: an endoscopic study. *Gastroenterology* 2005; **129**: 1825-1831 [PMID: 16344051 DOI: 10.1053/j.gastro.2005.08.053]

6 **Rex DK**, Cummings OW, Shaw M, Cumings MD, Wong RK, Vasudeva RS, Dunne D, Rahmani EY, Helper DJ. Screening for Barrett's esophagus in colonoscopy patients with and without heartburn. *Gastroenterology* 2003; **125**: 1670-1677 [PMID: 14724819 DOI: 10.1053/j.gastro.2003.09.030]

7 **Hayeck TJ**, Kong CY, Spechler SJ, Gazelle GS, Hur C. The prevalence of Barrett's esophagus in the US: estimates from a simulation model confirmed by SEER data. *Dis Esophagus* 2010; **23**: 451-457 [PMID: 20353441 DOI: 10.1111/j.1442-2050.2010.01054.x]

8 **van Soest EM**, Dieleman JP, Siersema PD, Sturkenboom MC, Kuipers EJ. Increasing incidence of Barrett's oesophagus in the general population. *Gut* 2005; **54**: 1062-1066 [PMID: 15857935 DOI: 10.1136/gut.2004.063685]

9 **Ford AC**, Forman D, Reynolds PD, Cooper BT, Moayyedi P. Ethnicity, gender, and socioeconomic status as risk factors for esophagitis and Barrett's esophagus. *Am J Epidemiol* 2005; **162**: 454-460 [PMID: 16076833 DOI: 10.1093/aje/kwi218]

10 **Huo X**, Juergens S, Zhang X, Rezaei D, Yu C, Strauch ED, Wang JY, Cheng E, Meyer F, Wang DH, Zhang Q, Spechler SJ, Souza RF. Deoxycholic acid causes DNA damage while inducing apoptotic resistance through NF-κB activation in benign Barrett's epithelial cells. *Am J Physiol Gastrointest Liver Physiol* 2011; **301**: G278-G286 [PMID: 21636532 DOI: 10.1152/ajpgi.00092.2011]

11 **Spechler SJ**, Souza RF. Barrett's esophagus. *N Engl J Med* 2014; **371**: 836-845 [PMID: 25162890 DOI: 10.1056/NEJMra1314704]
12 **National Cancer Institute**. Fast stats: esophagus cancer. Available from: URL: http://seer.cancer.gov/faststats/selections.php
13 **Spechler SJ**. Barrett's esophagus: Clinical issues. *Gastrointest Endosc Clin N Am* 2011; **21**: 1-7 [PMID: 21112493 DOI: 10.1016/j.giec.2010.09.012]
14 **Jung KW**, Talley NJ, Romero Y, Katzka DA, Schleck CD, Zinsmeister AR, Dunagan KT, Lutzke LS, Wu TT, Wang KK, Frederickson M, Geno DM, Locke GR, Prasad GA. Epidemiology and natural history of intestinal metaplasia of the gastroesophageal junction and Barrett's esophagus: a population-based study. *Am J Gastroenterol* 2011; **106**: 1447-1455; quiz 1456 [PMID: 21483461 DOI: 10.1038/ajg.2011.130]
15 **Hvid-Jensen F**, Pedersen L, Drewes AM, Sørensen HT, Funch-Jensen P. Incidence of adenocarcinoma among patients with Barrett's esophagus. *N Engl J Med* 2011; **365**: 1375-1383 [PMID: 21995385 DOI: 10.1056/NEJMoa1103042]
16 **Solaymani-Dodaran M**, Logan RF, West J, Card T, Coupland C. Risk of oesophageal cancer in Barrett's oesophagus and gastro-oesophageal reflux. *Gut* 2004; **53**: 1070-1074 [PMID: 15247170 DOI: 10.1136/gut.2003.028076]
17 **Shaheen NJ**, Richter JE. Barrett's oesophagus. *Lancet* 2009; **373**: 850-861 [PMID: 19269522 DOI: 10.1016/S0140-6736(09)60487-6]
18 **Curvers WL**, ten Kate FJ, Krishnadath KK, Visser M, Elzer B, Baak LC, Bohmer C, Mallant-Hent RC, van Oijen A, Naber AH, Scholten P, Busch OR, Blaauwgeers HG, Meijer GA, Bergman JJ. Low-grade dysplasia in Barrett's esophagus: overdiagnosed and underestimated. *Am J Gastroenterol* 2010; **105**: 1523-1530 [PMID: 20461069 DOI: 10.1038/ajg.2010.171]
19 **Rastogi A**, Puli S, El-Serag HB, Bansal A, Wani S, Sharma P. Incidence of esophageal adenocarcinoma in patients with Barrett's esophagus and high-grade dysplasia: a meta-analysis. *Gastrointest Endosc* 2008; **67**: 394-398 [PMID: 18045592 DOI: 10.1016/j.gie.2007.07.019]
20 **Shaheen NJ**, Sharma P, Overholt BF, Wolfsen HC, Sampliner RE, Wang KK, Galanko JA, Bronner MP, Goldblum JR, Bennett AE, Jobe BA, Eisen GM, Fennerty MB, Hunter JG, Fleischer DE, Sharma VK, Hawes RH, Hoffman BJ, Rothstein RI, Gordon SR, Mashimo H, Chang KJ, Muthusamy VR, Edmundowicz SA, Spechler SJ, Siddiqui AA, Souza RF, Infantolino A, Falk GW, Kimmey MB, Madanick RD, Chak A, Lightdale CJ. Radiofrequency ablation in Barrett's esophagus with dysplasia. *N Engl J Med* 2009; **360**: 2277-2288 [PMID: 19474425 DOI: 10.1056/NEJMoa0808145]
21 **Spechler SJ**. Barrett esophagus and risk of esophageal cancer: a clinical review. *JAMA* 2013; **310**: 627-636 [PMID: 23942681 DOI: 10.1001/jama.2013.226450]
22 **Spechler SJ**, Sharma P, Souza RF, Inadomi JM, Shaheen NJ. American Gastroenterological Association technical review on the management of Barrett's esophagus. *Gastroenterology* 2011; **140**: e18-52; quiz e13 [PMID: 21376939 DOI: 10.1053/j.gastro.2011.01.031]
23 **Evans JA**, Early DS, Fukami N, Ben-Menachem T, Chandrasekhara V, Chathadi KV, Decker GA, Fanelli RD, Fisher DA, Foley KQ, Hwang JH, Jain R, Jue TL, Khan KM, Lightdale J, Malpas PM, Maple JT, Pasha SF, Saltzman JR, Sharaf RN, Shergill A, Dominitz JA, Cash BD. The role of endoscopy in Barrett's esophagus and other premalignant conditions of the esophagus. *Gastrointest Endosc* 2012; **76**: 1087-1094 [PMID: 23164510 DOI: 10.1016/j.gie.2012.08.004]
24 **Shaheen NJ**, Weinberg DS, Denberg TD, Chou R, Qaseem A, Shekelle P. Upper endoscopy for gastroesophageal reflux disease: best practice advice from the clinical guidelines committee of the American College of Physicians. *Ann Intern Med* 2012; **157**: 808-816 [PMID: 23208168 DOI: 10.7326/0003-4819-157-11-201212040-00008]
25 **Sharma P**, Morales TG, Sampliner RE. Short segment Barrett' s esophagus--the need for standardization of the definition and of endoscopic criteria. *Am J Gastroenterol* 1998; **93**: 1033-1036 [PMID: 9672325 DOI: 10.1111/j.1572-0241.1998.00324.x]
26 **Rudolph RE**, Vaughan TL, Storer BE, Haggitt RC, Rabinovitch PS, Levine DS, Reid BJ. Effect of segment length on risk for neoplastic progression in patients with Barrett esophagus. *Ann Intern Med* 2000; **132**: 612-620 [PMID: 10766679 DOI: 10.7326/0003-4819-132-8-200004180-00003]
27 **Sharma P**, Dent J, Armstrong D, Bergman JJ, Gossner L, Hoshihara Y, Jankowski JA, Junghard O, Lundell L, Tytgat GN, Vieth M. The development and validation of an endoscopic grading system for Barrett's esophagus: the Prague C & amp; M criteria. *Gastroenterology* 2006; **131**: 1392-1399 [PMID: 17101315 DOI: 10.1053/j.gastro.2006.08.032]
28 **Wang KK**, Sampliner RE. Updated guidelines 2008 for the diagnosis, surveillance and therapy of Barrett's esophagus. *Am J Gastroenterol* 2008; **103**: 788-797 [PMID: 18341497 DOI: 10.1111/j.1572-0241.2008.01835.x]
29 **Bennett C**, Vakil N, Bergman J, Harrison R, Odze R, Vieth M, Sanders S, Gay L, Pech O, Longcroft-Wheaton G, Romero Y, Inadomi J, Tack J, Corley DA, Manner H, Green S, Al Dulaimi D, Ali H, Allum B, Anderson M, Curtis H, Falk G, Fennerty MB, Fullarton G, Krishnadath K, Meltzer SJ, Armstrong D, Ganz R, Cengia G, Going JJ, Goldblum J, Gordon C, Grabsch H, Haigh C, Hongo M, Johnston D, Forbes-Young R, Kay E, Kaye P, Lerut T, Lovat LB, Lundell L, Mairs P, Shimoda T, Spechler S, Sontag S, Malfertheiner P, Murray I, Nanji M, Poller D, Ragunath K, Regula J, Cestari R, Shepherd N, Singh R, Stein HJ, Talley NJ, Galmiche JP, Tham TC, Watson P, Yerian L, Rugge M, Rice TW, Hart J, Gittens S, Hewin D, Hochberger J, Kahrilas P, Preston S, Sampliner R, Sharma P, Stuart R, Wang K, Waxman I, Abley C, Loft D, Penman I, Shaheen NJ, Chak A, Davies G, Dunn L, Falck-Ytter Y, Decaestecker J, Bhandari P, Ell C, Griffin SM, Attwood S, Barr H, Allen J, Ferguson MK, Moayyedi P, Jankowski JA. Consensus statements for management of Barrett's dysplasia and early-stage esophageal adenocarcinoma, based on a Delphi process. *Gastroenterology* 2012; **143**: 336-346 [PMID: 22537613 DOI: 10.1053/j.gastro.2012.04.032]
30 **Tseng EE**, Wu TT, Yeo CJ, Heitmiller RF. Barrett's esophagus with high grade dysplasia: surgical results and long-term outcome--an update. *J Gastrointest Surg* 2003; **7**: 164-170; discussion 170-171 [PMID: 12600440 DOI: 10.1016/S1091-255X(02)00153-1]
31 **Dunbar KB**. Endoscopic eradication therapy for mucosal neoplasia in Barrett's esophagus. *Curr Opin Gastroenterol* 2013; **29**: 446-453 [PMID: 23703368 DOI: 10.1097/MOG.0b013e3283622848]
32 **Peters FP**, Kara MA, Rosmolen WD, Aalders MC, Ten Kate FJ, Bultje BC, Krishnadath KK, Fockens P, van Lanschot JJ, van Deventer SJ, Bergman JJ. Endoscopic treatment of high-grade dysplasia and early stage cancer in Barrett's esophagus. *Gastrointest Endosc* 2005; **61**: 506-514 [PMID: 15812401 DOI: 10.1016/S0016-5107(05)00063-5]
33 **Ell C**, May A, Pech O, Gossner L, Guenter E, Behrens A, Nachbar L, Huijsmans J, Vieth M, Stolte M. Curative endoscopic resection of early esophageal adenocarcinomas (Barrett's cancer). *Gastrointest Endosc* 2007; **65**: 3-10 [PMID: 17185072 DOI: 10.1016/j.gie.2006.04.033]
34 **Pech O**, Behrens A, May A, Nachbar L, Gossner L, Rabenstein T, Manner H, Guenter E, Huijsmans J, Vieth M, Stolte M, Ell C. Long-term results and risk factor analysis for recurrence after curative endoscopic therapy in 349 patients with high-grade intraepithelial neoplasia and mucosal adenocarcinoma in Barrett's oesophagus. *Gut* 2008; **57**: 1200-1206 [PMID: 18460553 DOI: 10.1136/gut.2007.142539]
35 **Pouw RE**, Wirths K, Eisendrath P, Sondermeijer CM, Ten Kate FJ, Fockens P, Devière J, Neuhaus H, Bergman JJ. Efficacy of radiofrequency ablation combined with endoscopic resection for barrett's esophagus with early neoplasia. *Clin Gastroenterol Hepatol* 2010; **8**: 23-29 [PMID: 19602454 DOI: 10.1016/j.cgh.2009.07.003]
36 **Pouw RE**, Seewald S, Gondrie JJ, Deprez PH, Piessevaux H, Pohl H, Rösch T, Soehendra N, Bergman JJ. Stepwise radical endoscopic resection for eradication of Barrett's oesophagus with early neoplasia in a cohort of 169 patients. *Gut* 2010; **59**: 1169-1177

[PMID: 20525701 DOI: 10.1136/gut.2010.210229]
37 **Overholt BF**, Lightdale CJ, Wang KK, Canto MI, Burdick S, Haggitt RC, Bronner MP, Taylor SL, Grace MG, Depot M. Photodynamic therapy with porfimer sodium for ablation of high-grade dysplasia in Barrett's esophagus: international, partially blinded, randomized phase III trial. *Gastrointest Endosc* 2005; **62**: 488-498 [PMID: 16185958 DOI: 10.1016/j.gie.2005.06.047]
38 **Pohl H**, Sonnenberg A, Strobel S, Eckardt A, Rösch T. Endoscopic versus surgical therapy for early cancer in Barrett's esophagus: a decision analysis. *Gastrointest Endosc* 2009; **70**: 623-631 [PMID: 19394011 DOI: 10.1016/j.gie.2008.11.047]
39 **Westerterp M**, Koppert LB, Buskens CJ, Tilanus HW, ten Kate FJ, Bergman JJ, Siersema PD, van Dekken H, van Lanschot JJ. Outcome of surgical treatment for early adenocarcinoma of the esophagus or gastro-esophageal junction. *Virchows Arch* 2005; **446**: 497-504 [PMID: 15838647 DOI: 10.1007/s00428-005-1243-1]
40 **Vieth M**, Ell C, Gossner L, May A, Stolte M. Histological analysis of endoscopic resection specimens from 326 patients with Barrett' s esophagus and early neoplasia. *Endoscopy* 2004; **36**: 776-781 [PMID: 15326572 DOI: 10.1055/s-2004-825802]
41 **Prasad GA**, Wu TT, Wigle DA, Buttar NS, Wongkeesong LM, Dunagan KT, Lutzke LS, Borkenhagen LS, Wang KK. Endoscopic and surgical treatment of mucosal (T1a) esophageal adenocarcinoma in Barrett's esophagus. *Gastroenterology* 2009; **137**: 815-823 [PMID: 19524578 DOI: 10.1053/j.gastro.2009.05.059]
42 **Badreddine RJ**, Prasad GA, Wang KK, Song LM, Buttar NS, Dunagan KT, Lutzke LS, Borkenhagen LS. Prevalence and predictors of recurrent neoplasia after ablation of Barrett's esophagus. *Gastrointest Endosc* 2010; **71**: 697-703 [PMID: 19959164 DOI: 10.1016/j.gie.2009.08.031]
43 **Pouw RE**, Gondrie JJ, Rygiel AM, Sondermeijer CM, ten Kate FJ, Odze RD, Vieth M, Krishnadath KK, Bergman JJ. Properties of the neosquamous epithelium after radiofrequency ablation of Barrett' s esophagus containing neoplasia. *Am J Gastroenterol* 2009; **104**: 1366-1373 [PMID: 19491850 DOI: 10.1038/ajg.2009.88]
44 **Semlitsch T**, Jeitler K, Schoefl R, Horvath K, Pignitter N, Harnoncourt F, Siebenhofer A. A systematic review of the evidence for radiofrequency ablation for Barrett's esophagus. *Surg Endosc* 2010; **24**: 2935-2943 [PMID: 20464420 DOI: 10.1007/s00464-010-1087-x]
45 **Beaumont H**, Gondrie JJ, McMahon BP, Pouw RE, Gregersen H, Bergman JJ, Boeckxstaens GE. Stepwise radiofrequency ablation of Barrett's esophagus preserves esophageal inner diameter, compliance, and motility. *Endoscopy* 2009; **41**: 2-8 [PMID: 19160152 DOI: 10.1055/s-0028-1103451]
46 **Phoa KN**, Pouw RE, van Vilsteren FG, Sondermeijer CM, Ten Kate FJ, Visser M, Meijer SL, van Berge Henegouwen MI, Weusten BL, Schoon EJ, Mallant-Hent RC, Bergman JJ. Remission of Barrett' s esophagus with early neoplasia 5 years after radiofrequency ablation with endoscopic resection: a Netherlands cohort study. *Gastroenterology* 2013; **145**: 96-104 [PMID: 23542068 DOI: 10.1053/j.gastro.2013.03.046]
47 **van Vilsteren FG**, Pouw RE, Seewald S, Alvarez Herrero L, Sondermeijer CM, Visser M, Ten Kate FJ, Yu Kim Teng KC, Soehendra N, Rösch T, Weusten BL, Bergman JJ. Stepwise radical endoscopic resection versus radiofrequency ablation for Barrett's oesophagus with high-grade dysplasia or early cancer: a multicentre randomised trial. *Gut* 2011; **60**: 765-773 [PMID: 21209124 DOI: 10.1136/gut.2010.229310]
48 **Fleischer DE**, Overholt BF, Sharma VK, Reymunde A, Kimmey MB, Chuttani R, Chang KJ, Muthasamy R, Lightdale CJ, Santiago N, Pleskow DK, Dean PJ, Wang KK. Endoscopic radiofrequency ablation for Barrett's esophagus: 5-year outcomes from a prospective multicenter trial. *Endoscopy* 2010; **42**: 781-789 [PMID: 20857372 DOI: 10.1055/s-0030-1255779]
49 **Orman ES**, Li N, Shaheen NJ. Efficacy and durability of radiofrequency ablation for Barrett's Esophagus: systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2013; **11**: 1245-1255 [PMID: 23644385 DOI: 10.1016/j.cgh.2013.03.039]
50 **Inadomi JM**, Somsouk M, Madanick RD, Thomas JP, Shaheen NJ. A cost-utility analysis of ablative therapy for Barrett's esophagus. *Gastroenterology* 2009; **136**: 2101-2114.e1-6 [PMID: 19272389 DOI: 10.1053/j.gastro.2009.02.062]
51 **Larghi A**, Lightdale CJ, Memeo L, Bhagat G, Okpara N, Rotterdam H. EUS followed by EMR for staging of high-grade dysplasia and early cancer in Barrett's esophagus. *Gastrointest Endosc* 2005; **62**: 16-23 [PMID: 15990814 DOI: 10.1016/S0016-5107(05)00319-6]
52 **Peters FP**, Brakenhoff KP, Curvers WL, Rosmolen WD, Fockens P, ten Kate FJ, Krishnadath KK, Bergman JJ. Histologic evaluation of resection specimens obtained at 293 endoscopic resections in Barrett's esophagus. *Gastrointest Endosc* 2008; **67**: 604-609 [PMID: 18155214 DOI: 10.1016/j.gie.2007.08.039]
53 **Hull MJ**, Mino-Kenudson M, Nishioka NS, Ban S, Sepehr A, Puricelli W, Nakatsuka L, Ota S, Shimizu M, Brugge WR, Lauwers GY. Endoscopic mucosal resection: an improved diagnostic procedure for early gastroesophageal epithelial neoplasms. *Am J Surg Pathol* 2006; **30**: 114-118 [PMID: 16330950 DOI: 10.1097/01.pas.0000180438.56528.a0]
54 **Mino-Kenudson M**, Hull MJ, Brown I, Muzikansky A, Srivastava A, Glickman J, Park DY, Zuckerberg L, Misdraji J, Odze RD, Lauwers GY. EMR for Barrett's esophagus-related superficial neoplasms offers better diagnostic reproducibility than mucosal biopsy. *Gastrointest Endosc* 2007; **66**: 660-66; quiz 767, 769 [PMID: 17905005 DOI: 10.1016/j.gie.2007.02.063]
55 **Moss A**, Bourke MJ, Hourigan LF, Gupta S, Williams SJ, Tran K, Swan MP, Hopper AD, Kwan V, Bailey AA. Endoscopic resection for Barrett's high-grade dysplasia and early esophageal adenocarcinoma: an essential staging procedure with long-term therapeutic benefit. *Am J Gastroenterol* 2010; **105**: 1276-1283 [PMID: 20179694 DOI: 10.1038/ajg.2010.1]
56 **Stein HJ**, Feith M, Bruecher BL, Naehrig J, Sarbia M, Siewert JR. Early esophageal cancer: pattern of lymphatic spread and prognostic factors for long-term survival after surgical resection. *Ann Surg* 2005; **242**: 566-573; discussion 573-575 [PMID: 16192817 DOI: 10.1097/01.sla.0000184211.75970.85]
57 **Bollschweiler E**, Baldus SE, Schröder W, Prenzel K, Gutschow C, Schneider PM, Hölscher AH. High rate of lymph-node metastasis in submucosal esophageal squamous-cell carcinomas and adenocarcinomas. *Endoscopy* 2006; **38**: 149-156 [PMID: 16479422 DOI: 10.1055/s-2006-924993]
58 **Lopes CV**, Hela M, Pesenti C, Bories E, Caillol F, Monges G, Giovannini M. Circumferential endoscopic resection of Barrett' s esophagus with high-grade dysplasia or early adenocarcinoma. *Surg Endosc* 2007; **21**: 820-824 [PMID: 17294308 DOI: 10.1007/s00464-006-9187-3]
59 **Giovannini M**, Bories E, Pesenti C, Moutardier V, Monges G, Danisi C, Lelong B, Delpero JR. Circumferential endoscopic mucosal resection in Barrett's esophagus with high-grade intraepithelial neoplasia or mucosal cancer. Preliminary results in 21 patients. *Endoscopy* 2004; **36**: 782-787 [PMID: 15326573 DOI: 10.1055/s-2004-825813]
60 **Seewald S**, Akaraviputh T, Seitz U, Brand B, Groth S, Mendoza G, He X, Thonke F, Stolte M, Schroeder S, Soehendra N. Circumferential EMR and complete removal of Barrett's epithelium: a new approach to management of Barrett's esophagus containing high-grade intraepithelial neoplasia and intramucosal carcinoma. *Gastrointest Endosc* 2003; **57**: 854-859 [PMID: 12776032 DOI: 10.1016/S0016-5107(03)70020-0]
61 **Gondrie JJ**, Pouw RE, Sondermeijer CM, Peters FP, Curvers WL, Rosmolen WD, Krishnadath KK, Ten Kate F, Fockens P, Bergman JJ. Stepwise circumferential and focal ablation of Barrett's esophagus with high-grade dysplasia: results of the first prospective series of 11 patients. *Endoscopy* 2008; **40**: 359-369 [PMID: 18494131 DOI: 10.1055/s-2007-995567]
62 **Gondrie JJ**, Pouw RE, Sondermeijer CM, Peters FP, Curvers WL, Rosmolen WD, Ten Kate F, Fockens P, Bergman JJ. Effective treatment of early Barrett's neoplasia with stepwise circumferential and focal ablation using the HALO system. *Endoscopy* 2008; **40**: 370-379 [PMID: 18494132 DOI: 10.1055/s-2007-995589]
63 **Rees JR**, Lao-Sirieix P, Wong A, Fitzgerald RC. Treatment for

Barrett's oesophagus. *Cochrane Database Syst Rev* 2010; **(1)**: CD004060 [PMID: 20091557 DOI: 10.1002/14651858.CD004060.pub2]

64 **Fayter D**, Corbett M, Heirs M, Fox D, Eastwood A. A systematic review of photodynamic therapy in the treatment of pre-cancerous skin conditions, Barrett's oesophagus and cancers of the biliary tract, brain, head and neck, lung, oesophagus and skin. *Health Technol Assess* 2010; **14**: 1-288 [PMID: 20663420 DOI: 10.3310/hta14370]

65 **Overholt BF**, Wang KK, Burdick JS, Lightdale CJ, Kimmey M, Nava HR, Sivak MV, Nishioka N, Barr H, Marcon N, Pedrosa M, Bronner MP, Grace M, Depot M. Five-year efficacy and safety of photodynamic therapy with Photofrin in Barrett's high-grade dysplasia. *Gastrointest Endosc* 2007; **66**: 460-468 [PMID: 17643436 DOI: 10.1016/j.gie.2006.12.037]

66 **Hage M**, Siersema PD, van Dekken H, Steyerberg EW, Haringsma J, van de Vrie W, Grool TE, van Veen RL, Sterenborg HJ, Kuipers EJ. 5-aminolevulinic acid photodynamic therapy versus argon plasma coagulation for ablation of Barrett's oesophagus: a randomised trial. *Gut* 2004; **53**: 785-790 [PMID: 15138203 DOI: 10.1136/gut.2003.028860]

67 **Li YM**, Li L, Yu CH, Liu YS, Xu CF. A systematic review and meta-analysis of the treatment for Barrett's esophagus. *Dig Dis Sci* 2008; **53**: 2837-2846 [PMID: 18427992 DOI: 10.1007/s10620-008-0257-3]

68 **Gotoda T**, Yanagisawa A, Sasako M, Ono H, Nakanishi Y, Shimoda T, Kato Y. Incidence of lymph node metastasis from early gastric cancer: estimation with a large number of cases at two large centers. *Gastric Cancer* 2000; **3**: 219-225 [PMID: 11984739 DOI: 10.1007/PL00011720]

69 **Manner H**, May A, Pech O, Gossner L, Rabenstein T, Günter E, Vieth M, Stolte M, Ell C. Early Barrett's carcinoma with "low-risk" submucosal invasion: long-term results of endoscopic resection with a curative intent. *Am J Gastroenterol* 2008; **103**: 2589-2597 [PMID: 18785950 DOI: 10.1111/j.1572-0241.2008.02083.x]

70 **Vieth M**. Low-grade dysplasia in Barrett's esophagus - an innocent bystander? Contra. *Endoscopy* 2007; **39**: 647-649 [PMID: 17611921 DOI: 10.1055/s-2007-966638]

71 **Phoa KN**, van Vilsteren FG, Weusten BL, Bisschops R, Schoon EJ, Ragunath K, Fullarton G, Di Pietro M, Ravi N, Visser M, Offerhaus GJ, Seldenrijk CA, Meijer SL, ten Kate FJ, Tijssen JG, Bergman JJ. Radiofrequency ablation vs endoscopic surveillance for patients with Barrett esophagus and low-grade dysplasia: a randomized clinical trial. *JAMA* 2014; **311**: 1209-1217 [PMID: 24668102 DOI: 10.1001/jama.2014.2511]

72 **Desai TK**, Krishnan K, Samala N, Singh J, Cluley J, Perla S, Howden CW. The incidence of oesophageal adenocarcinoma in non-dysplastic Barrett's oesophagus: a meta-analysis. *Gut* 2012; **61**: 970-976 [PMID: 21997553 DOI: 10.1136/gutjnl-2011-300730]

73 **Wani S**, Falk G, Hall M, Gaddam S, Wang A, Gupta N, Singh M, Singh V, Chuang KY, Boolchand V, Gavini H, Kuczynski J, Sud P, Reddymasu S, Bansal A, Rastogi A, Mathur SC, Young P, Cash B, Lieberman DA, Sampliner RE, Sharma P. Patients with nondysplastic Barrett's esophagus have low risks for developing dysplasia or esophageal adenocarcinoma. *Clin Gastroenterol Hepatol* 2011; **9**: 220-227; quiz e226 [PMID: 21115133 DOI: 10.1016/j.cgh.2010.11.008]

74 **Bhat S**, Coleman HG, Yousef F, Johnston BT, McManus DT, Gavin AT, Murray LJ. Risk of malignant progression in Barrett's esophagus patients: results from a large population-based study. *J Natl Cancer Inst* 2011; **103**: 1049-1057 [PMID: 21680910 DOI: 10.1093/jnci/djr203]

75 **Hamilton SR**, Yardley JH. Regnerative of cardiac type mucosa and acquisition of Barrett mucosa after esophagogastrostomy. *Gastroenterology* 1977; **72**: 669-675 [PMID: 838221 DOI: 10.1016/S0016-5085(77)80152-2]

76 **Dresner SM**, Griffin SM, Wayman J, Bennett MK, Hayes N, Raimes SA. Human model of duodenogastro-oesophageal reflux in the development of Barrett's metaplasia. *Br J Surg* 2003; **90**: 1120-1128 [PMID: 12945080 DOI: 10.1002/bjs.4169]

77 **Kastelein F**, Spaander MC, Steyerberg EW, Biermann K, Valkhoff VE, Kuipers EJ, Bruno MJ. Proton pump inhibitors reduce the risk of neoplastic progression in patients with Barrett's esophagus. *Clin Gastroenterol Hepatol* 2013; **11**: 382-388 [PMID: 23200977 DOI: 10.1016/j.cgh.2012.11.014]

78 **Spechler SJ**. Does Barrett's esophagus regress after surgery (or proton pump inhibitors)? *Dig Dis* 2014; **32**: 156-163 [PMID: 24603402 DOI: 10.1159/000357184]

P- Reviewer: Nagahara H **S- Editor**: Kong JX **L- Editor**: A
E- Editor: Wu HL

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i2.235

World J Gastrointest Pharmacol Ther 2016 May 6; 7(2): 235-241
ISSN 2150-5349 (online)

MINIREVIEWS

Predictive factors for a severe clinical course in ulcerative colitis: Results from population-based studies

Magnus Hofrenning Wanderås, Bjørn A Moum, Marte Lie Høivik, Øistein Hovde

Magnus Hofrenning Wanderås, Department of Medicine, Innlandet Hospital Trust, 2819 Gjøvik, Norway

Magnus Hofrenning Wanderås, Faculty of Medicine, University of Oslo, 0450 Oslo, Norway

Bjørn A Moum, Marte Lie Høivik, Department of Gastroenterology, Oslo University Hospital, 0424 Oslo, Norway

Bjørn A Moum, Øistein Hovde, Institute of Clinical Medicine, University of Oslo, 0424 Oslo, Norway

Øistein Hovde, Department of Gastroenterology, Innlandet Hospital Trust, 2819 Gjøvik, Norway

Author contributions: Wanderås MH and Hovde Ø contributed equally to the design of the review; Wanderås MH was responsible for drafting the article; Hovde Ø was responsible for revising the article thoroughly and for critical intellectual content; Moum BA and Høivik ML made essential contributions in the early phase, and both provided important feedback in the final stage of drafting; all authors approved the final version.

Conflict-of-interest statement: There is no conflict of interest associated with any of the authors who contributed to this manuscript.

Correspondence to: Øistein Hovde, Associate Professor, Department of Gastroenterology, Innlandet Hospital Trust, Kyrre Greppsgt. 11, 2819 Gjøvik, Norway. oistein.hovde@sykehuset-innlandet.no
Telephone: +47-450-02658
Fax: +47-611-57439

Received: June 30, 2015
Peer-review started: July 4, 2015
First decision: July 17, 2015
Revised: January 26, 2016
Accepted: March 9, 2016
Article in press: March 14, 2016
Published online: May 6, 2016

Abstract

Ulcerative colitis (UC) is characterized by chronic inflammation of the large bowel in genetically susceptible individuals exposed to environmental risk factors. The disease course can be difficult to predict, with symptoms ranging from mild to severe. There is no generally accepted definition of severe UC, and no single outcome is sufficient to classify a disease course as severe. There are several outcomes indicating a severe disease course, including progression of the disease's extension, a high relapse rate, the development of acute severe colitis, colectomy, the occurrence of colorectal cancer and UC-related mortality. When evaluating a patient's prognosis, it is helpful to do so in relation to these outcomes. Using these outcomes also makes it easier to isolate factors predictive of severe disease. The aims of this article are to evaluate different disease outcomes and to present predictive factors for these outcomes.

Key words: Ulcerative colitis; Disease course; Prognosis; Severity; Colectomy; Relapse; Acute severe colitis; Cancer; Mortality

Core tip: The disease course of ulcerative colitis (UC) can be difficult to predict. There is no generally accepted definition of severe UC. There are several outcomes indicating a severe disease course, including progression of the disease extension, a high relapse

rate, the development of acute severe colitis, colectomy, the occurrence of colorectal cancer and UC-related mortality. Using these outcomes is helpful when determining patient prognosis and also makes it easier to isolate predictive factors for severe disease. The aim of this article is to evaluate different disease outcomes and to present predictive factors for these outcomes.

Wanderås MH, Moum BA, Høivik ML, Hovde Ø. Predictive factors for a severe clinical course in ulcerative colitis: Results from population-based studies. *World J Gastrointest Pharmacol Ther* 2016; 7(2): 235-241 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i2/235.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i2.235

INTRODUCTION

Ulcerative colitis (UC) is a chronic relapsing inflammatory disease of the colon. Risk factors may be both genetical and environmental[1]. Disease onset usually occurs in young adults, and the symptoms range from mild to severe. The course of the disease is unpredictable, and with the prospect of a life-long disease, it is crucial for patients and physicians to have the most precise information possible regarding disease course and treatment options. The natural progress of the disease, the medical treatment including the benefits and side effects, and the possibility of having surgery at a young age are among the issues specialists are faced with.

There is no generally accepted definition of severe UC. However, there are several outcomes that indicate a severe disease course, including progression of the disease's extension, a high relapse rate, the development of acute severe colitis (ASC), colectomy, the occurrence of colorectal cancer (CRC) and UC-related mortality[2]. When evaluating a patient's prognosis, it can be helpful to do so in relation to these disease outcomes. This approach can simplify the process of determining predictive factors for severe disease as factors for each outcome can be identified separately.

The aims of the present article are firstly to describe the different outcomes used to determine whether a course of UC is severe or not, and secondly to present predictive factors for the different disease outcomes.

Study methods

This overview includes population-based studies published between 1993 and 2015 in the English language. We performed electronic searches in the PubMed, Cochrane, and Medline databases with the following key words: "ulcerative colitis" and "inflammatory bowel diseases", combined with free text searches for "diagnosis", "population based", "clinical", "course", "prognosis", "surgery", "colectomy", "relapse", "recurrence", "progression", "disease extension", "acute severe colitis", "complications", "cancer", "colo-rectal cancer", and "mortality".

WHAT CONSTITUTES SEVERE UC?

As of today, there is no generally accepted definition of what constitutes a severe clinical course of UC. The disease course varies markedly between patients, and the decision whether to classify a certain disease phenotype as severe can be based on several possible disease outcomes, each of which depicts ways that severe UC can behave. One example is whether a course of quiescent disease for several years abruptly ending in an episode of acute severe colitis and colectomy is more or less severe than a course characterized by frequent flares barely manageable by medication. Both of these disease courses could at some point be described as severe.

In the following, six of the most commonly seen disease outcomes are evaluated, and predictive factors for these outcomes are presented.

Progression of disease extension

UC always affects the rectum and extends proximally. However, how far proximally it extends at the time of diagnosis varies greatly, from involving the rectum alone (proctitis) to involving the whole colon (pancolitis)[3]. Furthermore, it is not uncommon that the distribution of the disease may change. In a one-year follow-up study, Moum *et al*[4] found that in 399 patients diagnosed with UC, 66% had changes in colonic involvement from the time of diagnosis to follow-up, 14% had extended proximally, 22% had regressed, and 30% showed normalization at colonoscopy after initial medical treatment.

In a population-based follow-up cohort of 423 cases, the Inflammatory Bowel South East Norway (IBSEN) study identified 288 patients with disease extension distal to the splenic flexure at time of diagnosis. Sixty-one of these patients (21.2%) experienced progression to extensive colitis; 39 of the patients (13.5%) experienced progression within the first five years, and 22 of the patients (7.6%) experienced progression during the subsequent five years[5]. Additionally, 39 of 140 patients (28%) initially diagnosed with proctitis had extended to left-sided colitis. A review from 2012 found similar numbers, stating that 25%-50% of patients with distal colitis experienced progression to more extensive disease over time[3].

Progression of disease extension indicates a poor prognosis. Etchevers *et al*[6] compared patients with stable distal UC (disease limited to rectum and sigmoid colon) with patients having disease progression from distal to extensive (disease with involvement of at least the descending colon). In the group with progression of disease distribution, there was a significantly higher prevalence of extra-intestinal manifestations, steroid-refractory disease course, requirement for immunosuppressive and - modulating medications (including thiopurines, cyclosporine and infliximab) and surgery

than in the group with stable distal UC. However, these differences were not found when comparing stable extensive disease with those having disease progression from distal to extensive. A South Korean study[7] found a higher prevalence of chronic disease activity (> 6 mo), relapse, and hospitalization due to UC relapse in patients with disease progression than in patients with stable disease distribution.

Several factors predictive of disease progression have been identified. Factors at time of diagnosis include a higher Mayo score and the use of corticosteroids. During follow-up, disease progression has been significantly associated with chronic disease activity after diagnosis, as well as hospitalization and disease relapse[7]. Preexisting independent factors predictive of disease progression include younger age at diagnosis and the presence of primary sclerosing cholangitis (PSC)[5,6].

Relapse rate

A relapse is defined as an increase in UC-related symptoms requiring consultation with a physician and leading to changes in medication or surgery[8,9]. The course of UC is usually characterized by relapses that alternate with periods of remission. The severity of relapses varies from mild increases in symptoms to life-threatening colitis requiring surgery[10].

Relapse rates have been evaluated in several studies. One year after a diagnosis of UC, 50% of patients in the IBSEN study had registered one or more relapse[11]. Five years after diagnosis, 78% had registered at least one relapse[9], and the 10-year cumulative relapse rate was 83%[5]. This number is higher than reported in a European population-based cohort study[8], where a 10-year cumulative rate of 67% for the first relapse after diagnosis was established. A Danish study[12] reported a cumulative risk of first relapse after diagnosis of 51%, 75% and 79% at one, five and seven years, respectively, whereas a study from the Netherlands[13] found an overall relapse rate of 85% after 10 years of disease.

Factors predictive of a higher relapse rate have been identified in several studies. The 5-year follow-up IBSEN study[9] found that relapse was more frequent in young patients (mean age 38.5 in patients with relapse *vs* 46.0 years in patients without relapse), and at the 10-year follow-up[5], they found that patients older than 50 years of age had a reduced risk compared to patients younger than 30 years of age. The study also found that relapse was more frequent in female patients. Additionally, during the last five years of the 10-year follow-up, a smaller proportion of patients who initially had an ESR > 30 mm experienced relapse than those with initial ESR < 30 mm (30% *vs* 50%; $P < 0.001$).

The results from a large population-based inception cohort[8] support age as a predictive factor, with a higher total number of relapses in patients younger than 20 years of age than in patients older than 30 years of age. The study also found that the total number of relapses was higher in never-smokers than in current smokers. Furthermore, patients who experienced their first relapse < 1 year after diagnosis experienced an increased number of relapses in the subsequent years compared with those with their first relapse between 1-2 years after diagnosis and > 2 years after diagnosis.

Acute severe colitis

The development of acute severe colitis is considered a medical emergency and is potentially life-threatening. Diagnosis of this condition is often based on Truelove and Witts' severity index: bloody stool frequency ⩾ 6 per day, plus at least one of the following: Pulse > 90/min, temperature > 37.8 ℃, Hb < 10.5 g/dL, or ESR > 30 mm/h[14-16]. Modifications of these criteria can be applied, such as using CRP > 10 instead of ESR elevation[17].

Two recent studies indicate that ASC affects approximately 25% of UC patients[17,18]. Data from the United Kingdom show that ASC is the presenting feature leading to hospitalization and diagnosis in 10%-20% of patients[17]. An Oxford-based cohort consisting of 750 UC patients also found that ASC occurred in approximately 25% of the patients (186/750); in these patients, ASC occurred as a presenting feature or occurred within one year of diagnosis, at a cumulative total of 54%. In 18% of the patients, ASC occurred for the first time 1-5 years after diagnosis, and it occurred after five years in 28% of the patients[14].

The first-line medical treatment for ASC is intravenous corticosteroids[17]. If the patient does not respond to this ("steroid-refractory colitis"), second-line therapy with infliximab or cyclosporine is considered. The results on the efficacy of these treatments are not conclusive[15,17-19], but the risk of in-hospital mortality is no higher for patients treated with second-line medical therapy than for those undergoing surgery[17]. The proportion of patients with ASC undergoing surgery varies from 17% to 40%[14,17,20]. A trend toward fewer surgical interventions for ASC was noted from 2008 to 2010, with 34% *vs* 25% operated in the respective years[17]. Delayed surgery for ASC patients not responding to medical rescue therapy might increase the risk of postoperative complications[16].

Mortality in ASC patients is relatively low compared to that of the background population. A Canadian study[20] found a total mortality rate among 1991 ASC patients of 1%. However, among those who underwent surgery, the mortality rate was approximately 3.8%. A Danish cohort that included UC patients undergoing acute or elective colectomy between 1996 and 2010, with a 30-d follow-up, found a mortality rate of 5.2% among those who underwent emergency surgery[21]. United Kingdom data also suggest a total mortality rate among ASC patients close to 1%. However, this rate increases to close to 3% among those with steroid-refractory colitis[17].

In the literature, predictive factors for ASC are

scarce. However, in an abstract, Cesarini *et al*[22] presented a prognostic index for the development of ASC within 3 years to be applied at the time of diagnosis. The three factors included in this index were extensive disease, CRP > 10 mg/L and Hb < 12.1 g/dL (women)/< 13.8 g/dL (men). The index was tested on three cohorts in England and Sweden, and of the patients who scored 3/3 at diagnosis, 8/11 (73%), 18/18 (100%) and 13/14 (93%) subsequently developed ASC. Another study supports disease extension being predictive of ASC, with a significantly higher number of patients with extensive disease in the ASC cohort than in the non-ASC cohort (30% *vs* 11%)[14]. The same was seen with regard to patients whose disease had progressed from proctitis and left-sided colitis to extensive colitis (81% *vs* 21%).

Colectomy

Colectomy indications can be categorized into those for acute surgery and those for elective surgery[23]. An emergency colectomy is performed when a hospitalized colitis patient develops life-threatening complications unresponsive to medical treatment. Elective colectomy is most frequently performed due to either refractory disease, intolerance to medical treatment or colonic neoplasia.

Colectomy rates for UC have varied between cohorts and across time[24]. In 1994, a Danish study reported that 25% of UC patients underwent colectomy within the first ten years of diagnosis[25]. The reason for this high rate may have been a tendency of specialists to choose colectomy after the second relapse over conservative medical treatment.

Newer assessments have shown a 10-year cumulative colectomy rate of approximately 10%[5,26]. Hoie *et al*[27] found a 10-year cumulative colectomy rate of 8.7% and a significant difference between southern and northern European centers (3.9% *vs* 10.4%). In the province of Manitoba, Canada, the 10-year colectomy rate decreased significantly over time from 12.7% (1987-1991) to 9.3% (1997-2001)[26]. Thus, a trend can be seen toward a lower colectomy rate in UC patients. This is supported in a review from 2013; in the review 10-year surgery rates as high as 35% were reported before 1990, and rates declining to < 10% were reported after 1990[28]. This observation is also supported by Kaplan *et al*[24], who reported a significant decrease in colectomy rates in UC patients between 1997 and 2009. However, the study showed that the rate of emergency colectomies remained stable.

In a population-based surveillance cohort identifying 666 UC patients who underwent surgery, a total of 27% of the patients had a postoperative complication, whereas postoperative mortality occurred at 1.5%. The main independent predictors of complications were advanced age, comorbidity and emergency surgery[29]. Although mortality related to severe attacks of UC has substantially decreased to less than 1% in past decades, a delay in surgery can increase the risk of postoperative complications and mortality[16,20,29]. Sixty percent of patients treated with emergency colectomy experienced some sort of complication during follow-up[16].

In a systematic review from 2014, Dias *et al*[30] identified clinical predictors of colectomy in patients with UC. They found a reduced colectomy risk for female patients and for smokers, whereas a higher risk was noted for patients with extensive disease, for patients who took corticosteroids at least once and for patients who were hospitalized.

Cancer development

UC may be complicated by the development of CRC[31]. Inflammatory bowel disease (IBD)-associated CRC (IBD-CRC) affects patients at a younger age than sporadic CRC. The prognoses for sporadic CRC and IBD-CRC are similar, with a 5-year survival of approximately 50%[32].

A Danish study reporting CRC risk in a nationwide cohort of 47374 patients with IBD over a 30-year period found that the relative risk (RR) of developing CRC in UC patients was 1.07 (95%CI: 0.95-1.21), which means that the risk for CRC in UC patients was comparable to that in the general population[33]. The overall RR for CRC in UC patients decreased from 1.34 (95%CI: 1.13-1.58) in 1979-1988 to 0.57 (95%CI: 0.41-0.80) in 1999-2008.

A meta-analysis to determine CRC risk in UC patients showed that UC increases the risk of CRC 2.4-fold, which represents a total CRC occurrence of 1.6% during the first 14 years of follow-up[34]. The authors concluded that Eaden *et al*[35] overestimated the long-term CRC risk among UC patients in reporting a cumulative incidence of CRC of 2% at 10 years and 8% at 20 years of follow-up for any patient with UC. In this meta-analysis[34], restricted to unselected patients in population-based cohorts and including sporadic CRC cases, the numbers were only 0.4% and 1.1%–5.3%, respectively. The authors conclude that a UC diagnosis no longer seems to be associated with increased CRC risk, although subgroups of patients remain at increased risk. A recent systematic review supports this observation[36]. The decreasing risk of CRC might result from improvements in therapy.

However, a Finnish study found a higher cancer incidence in male IBD patients, with UC patients at increased risk for developing CRC and biliary tract cancer[37]. In addition to this finding, a study from 2009 found a significantly increased risk of CRC development in IBD patients with concomitant PSC[38]. The 10- and 20-year risk rates were 14% and 31%, respectively, compared to 2% and 2% in PSC patients without IBD.

The association between IBD and cancer in general was evaluated in the fifteen-year follow-up of the European Collaborative Study Group of Inflammatory Bowel Disease[39]. The total cancer prevalence was 9.1%, with most patients having a single extra-intestinal neoplasm. In Northern centers there were more

intestinal cancers, whereas in southern centers there were more extra-intestinal cancers. In this IBD cohort, the frequency of observed cancers was not different from that expected in the background population.

Regarding predictive factors for the development of CRC in UC patients, the aforementioned results indicate that it is debated whether a correlation exists between UC and a higher than normal risk of developing CRC. Thus, UC-specific factors predictive of CRC may not exist. However, a Finnish study found that male gender increased the risk of developing CRC in the context of UC, and concomitant PSC also increased CRC risk.

Mortality

Early studies on the prognosis in IBD showed significant reductions in survival, whereas reports from the last two decades have been more optimistic[40-43]. In an updated examination of mortality in the Copenhagen cohort (median follow-up 19 years), no significant increase in overall mortality in UC was reported. The IBSEN study reported on mortality in an inception cohort gathered between 1990 and 1994 and followed the patients for 10 years[5]. No elevated mortality was found, either overall or in subgroups of patients. These data have now been extended up to 20 years, still showing no overall increased mortality[44]. In a population-based registry of 1254 Finnish adult IBD patients accrued between 1986 and 2007, the standard mortality rate (SMR) was 0.90 (95%CI: 0.77-1.06)[45]. Similar data were seen in a large multicenter study in Europe following patients for 10 and 15 years, with no increase in overall mortality[39,46].

In the modern era of managing patients with UC, mortality rates seem to have decreased. A recent meta-analysis comprising 22 studies did not detect an increased risk of death in UC patients compared to the background population. The pooled SMR from the 10 population-based inception studies was 1.1 (95%CI: 0.9-1.2)[47].

Colectomy reduces mortality in UC by removing the risk of dysplasia/CRC and the inflammatory burden of the disease. However, peri- and postoperative mortality might lead to an increased total mortality risk. In the Danish IBD registry spanning from 1996 to 2010, 50% of patients admitted as emergency cases underwent colectomy[21]. In UC patients undergoing emergency surgery, the 30-d mortality rate was 5.2% compared with 0.9% among elective cases. Low hospital total colectomy volume, comorbidity and advanced age were associated with increased 30-d mortality in UC patients undergoing emergency surgery.

UC occurs more frequently in nonsmokers; therefore, smoking-related mortality is decreased[48]. However, one study concluded that in UC patients, mortality from respiratory disorders (smoking-related diseases, asthma, pulmonary embolism, and pneumonia) was significantly increased[49]. Hematological malignancy mortality rates from leukemia and non-Hodgkin's lymphoma were not increased.

The overall mortality in UC has been declining, and today it is no higher than that in the background population. Hence, it is difficult to identify predictive factors related to the disease course that increase mortality. However, trends leading to an increased mortality risk in subgroups of UC patients are seen.

SUMMARY OF SEVERE UC AND ITS PREDICTIVE FACTORS

No generally accepted definition of severe UC exists. However, several disease outcomes can indicate a more severe disease course.

Progression of disease extension is a poor prognostic factor. Predictive factors include higher Mayo score, higher endoscopic score, corticosteroid use, younger age at diagnosis and presence of PSC at time of diagnosis.

Relapse of disease is frequently seen, with 70%-80% of UC patients experiencing at least one relapse. Predictive factors for a higher relapse rate include younger age, female gender, initial ESR < 30 mm, no smoking and early relapse (within 1 year after diagnosis).

Acute severe colitis is a potentially life-threatening condition occurring in approximately 25% of patients with a total mortality of 1%, increasing to 3% in those who undergo surgery. Predictive factors are scarce, but they include extensive disease, CRP > 10 mg/L and Hb < 12.1/13.8 (women/men).

Colectomy, whether an emergency procedure or elective, is performed when conservative treatments have not succeeded at inducing remission. Reduced colectomy risk was found in female patients and smoking patients, whereas higher risk was found in patients with extensive disease, corticosteroid use and hospitalization.

Neither CRC nor mortality has consistently been shown to be increased among UC patients. However, male UC patients and patients with PSC are at an increased risk of developing CRC. Additionally, trends leading to increased mortality risk in subgroups of UC patients are seen.

REFERENCES

1 **Khor B**, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. *Nature* 2011; **474**: 307-317 [PMID: 21677747 DOI: 10.1038/nature10209]

2 **Yarur AJ**, Strobel SG, Deshpande AR, Abreu MT. Predictors of aggressive inflammatory bowel disease. *Gastroenterol Hepatol* (N Y) 2011; **7**: 652-659 [PMID: 22298958]

3 **Ordás I**, Eckmann L, Talamini M, Baumgart DC, Sandborn WJ. Ulcerative colitis. *Lancet* 2012; **380**: 1606-1619 [PMID: 22914296 DOI: 10.1016/s0140-6736(12)60150-0]

4 **Moum B**, Ekbom A, Vatn MH, Elgjo K. Change in the extent of colonoscopic and histological involvement in ulcerative colitis over time. *Am J Gastroenterol* 1999; **94**: 1564-1569 [PMID: 10364026 DOI: 10.1111/j.1572-0241.1999.01145.x]

5 **Solberg IC**, Lygren I, Jahnsen J, Aadland E, Høie O, Cvancarova M, Bernklev T, Henriksen M, Sauar J, Vatn MH, Moum B. Clinical

course during the first 10 years of ulcerative colitis: results from a population-based inception cohort (IBSEN Study). *Scand J Gastroenterol* 2009; **44**: 431-440 [PMID: 19101844 DOI: 10.1080/00365520802600961]
6 **Etchevers MJ**, Aceituno M, García-Bosch O, Ordás I, Sans M, Ricart E, Panés J. Risk factors and characteristics of extent progression in ulcerative colitis. *Inflamm Bowel Dis* 2009; **15**: 1320-1325 [PMID: 19235909 DOI: 10.1002/ibd.20897]
7 **Kim B**, Park SJ, Hong SP, Kim TI, Kim WH, Cheon JH. Proximal disease extension and related predicting factors in ulcerative proctitis. *Scand J Gastroenterol* 2014; **49**: 177-183 [PMID: 24325564 DOI: 10.3109/00365521.2013.867360]
8 **Höie O**, Wolters F, Riis L, Aamodt G, Solberg C, Bernklev T, Odes S, Mouzas IA, Beltrami M, Langholz E, Stockbrügger R, Vatn M, Moum B. Ulcerative colitis: patient characteristics may predict 10-yr disease recurrence in a European-wide population-based cohort. *Am J Gastroenterol* 2007; **102**: 1692-1701 [PMID: 17555460 DOI: 10.1111/j.1572-0241.2007.01265.x]
9 **Henriksen M**, Jahnsen J, Lygren I, Sauar J, Kjellevold Ø, Schulz T, Vatn MH, Moum B. Ulcerative colitis and clinical course: results of a 5-year population-based follow-up study (the IBSEN study). *Inflamm Bowel Dis* 2006; **12**: 543-550 [PMID: 16804390 DOI: 10.1097/01.MIB.0000225339.91484.fc]
10 **Cosnes J**, Gower-Rousseau C, Seksik P, Cortot A. Epidemiology and natural history of inflammatory bowel diseases. *Gastroenterology* 2011; **140**: 1785-1794 [PMID: 21530745 DOI: 10.1053/j.gastro.2011.01.055]
11 **Moum B**, Vatn MH, Ekbom A, Aadland E, Fausa O, Lygren I, Sauar J, Schulz T, Stray N. Incidence of ulcerative colitis and indeterminate colitis in four counties of southeastern Norway, 1990-93. A prospective population-based study. The Inflammatory Bowel South-Eastern Norway (IBSEN) Study Group of Gastroenterologists. *Scand J Gastroenterol* 1996; **31**: 362-366 [PMID: 8726304]
12 **Vester-Andersen MK**, Vind I, Prosberg MV, Bengtsson BG, Blixt T, Munkholm P, Andersson M, Jess T, Bendtsen F. Hospitalisation, surgical and medical recurrence rates in inflammatory bowel disease 2003-2011—a Danish population-based cohort study. *J Crohns Colitis* 2014; **8**: 1675-1683 [PMID: 25154681 DOI: 10.1016/j.crohns.2014.07.010]
13 **Romberg-Camps MJ**, Dagnelie PC, Kester AD, Hesselink-van de Kruijs MA, Cilissen M, Engels LG, Van Deursen C, Hameeteman WH, Wolters FL, Russel MG, Stockbrügger RW. Influence of phenotype at diagnosis and of other potential prognostic factors on the course of inflammatory bowel disease. *Am J Gastroenterol* 2009; **104**: 371-383 [PMID: 19174787 DOI: 10.1038/ajg.2008.38]
14 **Dinesen LC**, Walsh AJ, Protic MN, Heap G, Cummings F, Warren BF, George B, Mortensen NJ, Travis SP. The pattern and outcome of acute severe colitis. *J Crohns Colitis* 2010; **4**: 431-437 [PMID: 21122540 DOI: 10.1016/j.crohns.2010.02.001]
15 **Croft A**, Walsh A, Doecke J, Cooley R, Howlett M, Radford-Smith G. Outcomes of salvage therapy for steroid-refractory acute severe ulcerative colitis: ciclosporin vs. infliximab. *Aliment Pharmacol Ther* 2013; **38**: 294-302 [PMID: 23786158 DOI: 10.1111/apt.12375]
16 **Randall J**, Singh B, Warren BF, Travis SP, Mortensen NJ, George BD. Delayed surgery for acute severe colitis is associated with increased risk of postoperative complications. *Br J Surg* 2010; **97**: 404-409 [PMID: 20101648 DOI: 10.1002/bjs.6874]
17 **Lynch RW**, Lowe D, Protheroe A, Driscoll R, Rhodes JM, Arnott ID. Outcomes of rescue therapy in acute severe ulcerative colitis: data from the United Kingdom inflammatory bowel disease audit. *Aliment Pharmacol Ther* 2013; **38**: 935-945 [PMID: 24004000 DOI: 10.1111/apt.12473]
18 **Chang KH**, Burke JP, Coffey JC. Infliximab versus cyclosporine as rescue therapy in acute severe steroid-refractory ulcerative colitis: a systematic review and meta-analysis. *Int J Colorectal Dis* 2013; **28**: 287-293 [PMID: 23114475 DOI: 10.1007/s00384-012-1602-8]
19 **Narula N**, Fine M, Colombel JF, Marshall JK, Reinisch W. Systematic Review: Sequential Rescue Therapy in Severe Ulcerative Colitis: Do the Benefits Outweigh the Risks? *Inflamm Bowel Dis* 2015; **21**: 1683-1694 [PMID: 25839775 DOI: 10.1097/mib.0000000000000350]
20 **Turner D**, Walsh CM, Steinhart AH, Griffiths AM. Response to corticosteroids in severe ulcerative colitis: a systematic review of the literature and a meta-regression. *Clin Gastroenterol Hepatol* 2007; **5**: 103-110 [PMID: 17142106 DOI: 10.1016/j.cgh.2006.09.033]
21 **Tøttrup A**, Erichsen R, Sværke C, Laurberg S, Srensen HT. Thirty-day mortality after elective and emergency total colectomy in Danish patients with inflammatory bowel disease: a population-based nationwide cohort study. *BMJ Open* 2012; **2**: e000823 [PMID: 22492386 DOI: 10.1136/bmjopen-2012-000823]
22 **Cesarini M**, Collins G, Ronnblom A, Santos A, Sjoberg D, Parkes M, Keshav S, Travis S. Predicting the risk of acute severe colitis (ASC) at diagnosis of Ulcerative Colitis (UC): external validation. *J Crohns Colitis* 2015; **9** Suppl 1: S117-S118 [DOI: 10.1093/ecco-jcc/jju027.197]
23 **Monstad I**, Hovde O, Solberg IC, A Moum B. Clinical course and prognosis in ulcerative colitis: results from population-based and observational studies. *Ann Gastroenterol* 2014; **27**: 95-104 [PMID: 24733679]
24 **Kaplan GG**, Seow CH, Ghosh S, Molodecky N, Rezaie A, Moran GW, Proulx MC, Hubbard J, MacLean A, Buie D, Panaccione R. Decreasing colectomy rates for ulcerative colitis: a population-based time trend study. *Am J Gastroenterol* 2012; **107**: 1879-1887 [PMID: 23165448 DOI: 10.1038/ajg.2012.333]
25 **Langholz E**, Munkholm P, Davidsen M, Binder V. Course of ulcerative colitis: analysis of changes in disease activity over years. *Gastroenterology* 1994; **107**: 3-11 [PMID: 8020674]
26 **Targownik LE**, Singh H, Nugent Z, Bernstein CN. The epidemiology of colectomy in ulcerative colitis: results from a population-based cohort. *Am J Gastroenterol* 2012; **107**: 1228-1235 [PMID: 22613902 DOI: 10.1038/ajg.2012.127]
27 **Hoie O**, Wolters FL, Riis L, Bernklev T, Aamodt G, Clofent J, Tsianos E, Beltrami M, Odes S, Munkholm P, Vatn M, Stockbrügger RW, Moum B. Low colectomy rates in ulcerative colitis in an unselected European cohort followed for 10 years. *Gastroenterology* 2007; **132**: 507-515 [PMID: 17258717 DOI: 10.1053/j.gastro.2006.11.015]
28 **Bernstein CN**, Ng SC, Lakatos PL, Moum B, Loftus EV. A review of mortality and surgery in ulcerative colitis: milestones of the seriousness of the disease. *Inflamm Bowel Dis* 2013; **19**: 2001-2010 [PMID: 23624887 DOI: 10.1097/MIB.0b013e318281f3bb]
29 **de Silva S**, Ma C, Proulx MC, Crespin M, Kaplan BS, Hubbard J, Prusinkiewicz M, Fong A, Panaccione R, Ghosh S, Beck PL, Maclean A, Buie D, Kaplan GG. Postoperative complications and mortality following colectomy for ulcerative colitis. *Clin Gastroenterol Hepatol* 2011; **9**: 972-980 [PMID: 21806954 DOI: 10.1016/j.cgh.2011.07.016]
30 **Dias CC**, Rodrigues PP, da Costa-Pereira A, Magro F. Clinical predictors of colectomy in patients with ulcerative colitis: systematic review and meta-analysis of cohort studies. *J Crohns Colitis* 2015; **9**: 156-163 [PMID: 25518058 DOI: 10.1093/ecco-jcc/jju016]
31 **Ullman TA**, Itzkowitz SH. Intestinal inflammation and cancer. *Gastroenterology* 2011; **140**: 1807-1816 [PMID: 21530747 DOI: 10.1053/j.gastro.2011.01.057]
32 **Dyson JK**, Rutter MD. Colorectal cancer in inflammatory bowel disease: what is the real magnitude of the risk? *World J Gastroenterol* 2012; **18**: 3839-3848 [PMID: 22876036 DOI: 10.3748/wjg.v18.i29.3839]
33 **Jess T**, Simonsen J, Jørgensen KT, Pedersen BV, Nielsen NM, Frisch M. Decreasing risk of colorectal cancer in patients with inflammatory bowel disease over 30 years. *Gastroenterology* 2012; **143**: 375-381.e1; quiz e13-14 [PMID: 22522090 DOI: 10.1053/j.gastro.2012.04.016]
34 **Jess T**, Rungoe C, Peyrin-Biroulet L. Risk of colorectal cancer in patients with ulcerative colitis: a meta-analysis of population-based cohort studies. *Clin Gastroenterol Hepatol* 2012; **10**: 639-645 [PMID: 22289873 DOI: 10.1016/j.cgh.2012.01.010]
35 **Eaden JA**, Abrams KR, Mayberry JF. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. *Gut* 2001; **48**: 526-535 [PMID:

11247898]
36 **Castaño-Milla C**, Chaparro M, Gisbert JP. Systematic review with meta-analysis: the declining risk of colorectal cancer in ulcerative colitis. *Aliment Pharmacol Ther* 2014; **39**: 645-659 [PMID: 24612141 DOI: 10.1111/apt.12651]
37 **Jussila A**, Virta LJ, Pukkala E, Färkkilä MA. Malignancies in patients with inflammatory bowel disease: a nationwide register study in Finland. *Scand J Gastroenterol* 2013; **48**: 1405-1413 [PMID: 24131389 DOI: 10.3109/00365521.2013.846402]
38 **Claessen MM**, Vleggaar FP, Tytgat KM, Siersema PD, van Buuren HR. High lifetime risk of cancer in primary sclerosing cholangitis. *J Hepatol* 2009; **50**: 158-164 [PMID: 19012991 DOI: 10.1016/j.jhep.2008.08.013]
39 **Katsanos KH**, Tatsioni A, Pedersen N, Shuhaibar M, Ramirez VH, Politi P, Rombrechts E, Pierik M, Clofent J, Beltrami M, Bodini P, Freitas J, Mouzas I, Fornaciari G, Moum B, Lakatos PL, Vermeire S, Langholz E, Odes S, Morain CO, Stockbrügger R, Munkholm P, Tsianos EV. Cancer in inflammatory bowel disease 15 years after diagnosis in a population-based European Collaborative follow-up study. *J Crohns Colitis* 2011; **5**: 430-442 [PMID: 21939917 DOI: 10.1016/j.crohns.2011.04.013]
40 **Farrokhyar F**, Swarbrick ET, Grace RH, Hellier MD, Gent AE, Irvine EJ. Low mortality in ulcerative colitis and Crohn's disease in three regional centers in England. *Am J Gastroenterol* 2001; **96**: 501-507 [PMID: 11232697]
41 **Ekbom A**, Helmick CG, Zack M, Holmberg L, Adami HO. Survival and causes of death in patients with inflammatory bowel disease: a population-based study. *Gastroenterology* 1992; **103**: 954-960 [PMID: 1499945]
42 **Probert CS**, Jayanthi V, Wicks AC, Mayberry JF. Mortality in patients with ulcerative colitis in Leicestershire, 1972-1989. An epidemiological study. *Dig Dis Sci* 1993; **38**: 538-541 [PMID: 8444086]
43 **Jess T**, Frisch M, Simonsen J. Trends in overall and cause-specific mortality among patients with inflammatory bowel disease from 1982 to 2010. *Clin Gastroenterol Hepatol* 2013; **11**: 43-48 [PMID: 23022699 DOI: 10.1016/j.cgh.2012.09.026]
44 **Hovde Ø**, Småstuen MC, Høivik ML, Bernklev T, Huppertz-Hauss G, Høie O, Jahnsen J, Stray N, Henriksen M, Solberg IC, Moum BA. Mortality and Causes of Death in Ulcerative Colitis: Results from 20 Years of Follow-up in the IBSEN Study. *Inflamm Bowel Dis* 2016; **22**: 141-145 [PMID: 26355464 DOI: 10.1097/MIB.0000000000000582]
45 **Manninen P**, Karvonen AL, Huhtala H, Rasmussen M, Salo M, Mustaniemi L, Pirttiniemi I, Collin P. Mortality in ulcerative colitis and Crohn's disease. A population-based study in Finland. *J Crohns Colitis* 2012; **6**: 524-528 [PMID: 22398058 DOI: 10.1016/j.crohns.2011.10.009]
46 **Höie O**, Schouten LJ, Wolters FL, Solberg IC, Riis L, Mouzas IA, Politi P, Odes S, Langholz E, Vatn M, Stockbrügger RW, Moum B. Ulcerative colitis: no rise in mortality in a European-wide population based cohort 10 years after diagnosis. *Gut* 2007; **56**: 497-503 [PMID: 17028127]
47 **Selinger CP**, Leong RW. Mortality from inflammatory bowel diseases. *Inflamm Bowel Dis* 2012; **18**: 1566-1572 [PMID: 22275300 DOI: 10.1002/ibd.22871]
48 **Lunney PC**, Leong RW. Review article: Ulcerative colitis, smoking and nicotine therapy. *Aliment Pharmacol Ther* 2012; **36**: 997-1008 [PMID: 23072629 DOI: 10.1111/apt.12086]
49 **Bastida G**, Beltrán B. Ulcerative colitis in smokers, non-smokers and ex-smokers. *World J Gastroenterol* 2011; **17**: 2740-2747 [PMID: 21734782 DOI: 10.3748/wjg.v17.i22.2740]

P- Reviewer: Pierce ES **S- Editor**: Gong XM **L- Editor**: A
E- Editor: Wu HL

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i2.242

World J Gastrointest Pharmacol Ther 2016 May 6; 7(2): 242-253
ISSN 2150-5349 (online)

ORIGINAL ARTICLE

Basic Study

Oral tolerance is inducible during active dextran sulfate sodium-induced colitis

Satoshi Ino, Chikara Kohda, Kosuke Takeshima, Hiroki Ishikawa, Tomoko Norose, Toshiko Yamochi, Masafumi Takimoto, Hiroshi Takahashi, Kazuo Tanaka

Satoshi Ino, Chikara Kohda, Kosuke Takeshima, Hiroki Ishikawa, Kazuo Tanaka, Department of Microbiology and Immunology, School of Medicine, Showa University, Tokyo 142-8555, Japan

Tomoko Norose, Toshiko Yamochi, Masafumi Takimoto, Department of Pathology and Laboratory Medicine, School of Medicine, Showa University, Tokyo 142-8555, Japan

Hiroshi Takahashi, Division of Gastroenterology, Department of Internal Medicine, Showa University, Yokohama 227-8501, Japan

Author contributions: Ino S, Kohda C and Takeshima K performed the majority of experiments and analyzed the data; Norose T, Yamochi T and Takimoto M contributed pathological analysis; Kohda C, Ishikawa H, Takahashi H and Tanaka K designed and coordinated the research; Ino S and Kohda C wrote the paper.

Institutional review board statement: All procedures involving experimental animals were approved by the Institutional Animal Care and Use Committee of Showa University (Permit Number: 04127) and complied with the Guide for the Care and Use of Laboratory Animals (7th and 8th edition, ILAR-NRC). In our facility, the Animal Care and Use Committee of Showa University also functions as an Institutional Review Board for animal experiments.

Institutional animal care and use committee statement: All procedures involving experimental animals were approved by the Institutional Animal Care and Use Committee of Showa University (Permit Number: 04127) and complied with the Guide for the Care and Use of Laboratory Animals (7th and 8th edition, ILAR-NRC).

Conflict-of-interest statement: To the best of our knowledge, no conflict of interest exists.

Data sharing statement: A technical appendix, statistical code, and dataset are available from the corresponding author at kohda@med.showa-u.ac.jp. All participants provided informed consent for data sharing.

Correspondence to: Chikara Kohda, PhD, Assistant Professor, Department of Microbiology and Immunology, School of Medicine, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan. kohda@med.showa-u.ac.jp
Telephone: +81-3-37848131
Fax: +81-3-37843069

Received: November 27, 2015
Peer-review started: November 28, 2015
First decision: December 24, 2015
Revised: January 20, 2016
Accepted: February 16, 2016
Article in press: February 17, 2016
Published online: May 6, 2016

Abstract

AIM: To investigate whether oral tolerance is inducible during the active phase of dextran sulfate sodium (DSS)-induced colitis.

METHODS: Colitis was induced in 6- to 8-wk-old female BALB/c mice by the administration of 2% DSS. To induce oral tolerance, mice that received water with DSS [DSS (+)] and mice that received autoclaved water [DSS (-)] were intragastrically (i.g.) administered ovalbumin (OVA) as a tolerogen before systemic challenge with OVA. Following this, serum levels of OVA-specific IgE antibodies were measured. In mice with active

colitis, $CD4^+CD25^+Foxp3^+$ cell and B10 cell frequencies were evaluated using flow cytometry. Cytokine mRNA expression profiles were evaluated by reverse transcription real-time polymerase chain reaction.

RESULTS: Regardless of the presence of DSS colitis, OVA-specific immunoglobulin E concentrations were significantly reduced in mice that were i.g. administered OVA compared to mice that were i.g. administered PBS [DSS (+): 4.4 (4.2-9.5) ng/mL *vs* 83.9 (66.1-123.2) ng/mL, $P < 0.01$; DSS (-): 27.7 (0.1-54.5) ng/mL *vs* 116.5 (80.6-213.6) ng/mL, $P < 0.01$]. These results demonstrated that oral tolerance was induced in both the presence and absence of colitis. In the spleen and mesenteric lymph nodes (MLN), the frequencies of $CD4^+CD25^+Foxp3^+$ cells and B10 cells, both of which are associated with oral tolerance, did not significantly change. In the spleen, interferon-γ mRNA expression significantly decreased in mice with colitis [DSS (+): 0.42 (0.31-0.53) *vs* DSS (-): 1.00 (0.84-1.39), $P < 0.01$]. The expression levels of other cytokines did not significantly change.

CONCLUSION: Oral tolerance is inducible during active DSS colitis. The stability of regulatory cell populations in the spleen and MLN in colitis might correlate with these results.

Key words: Cytokine; Dextran sulfate sodium colitis; Oral tolerance; Regulatory T cell; Regulatory B cell

Core tip: Our study is the first to demonstrate that oral tolerance is inducible during the active phase of dextran sulfate sodium (DSS)-induced colitis. Lymphocytic infiltration into the large intestine mucosa associated with epithelial defects did not influence oral tolerance. In DSS colitis, the frequencies of $CD4^+CD25^+Foxp3^+$ T cells and B10 cells in the spleen and mesenteric lymph nodes remained stable. This stability might have led to the induction of oral tolerance in DSS colitis. Accordingly, if an appropriate antigen is chosen, then oral immunotherapy may be applicable for the treatment of ulcerative colitis.

Ino S, Kohda C, Takeshima K, Ishikawa H, Norose T, Yamochi T, Takimoto M, Takahashi H, Tanaka K. Oral tolerance is inducible during active dextran sulfate sodium-induced colitis. *World J Gastrointest Pharmacol Ther* 2016; 7(2): 242-253 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i2/242.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i2.242

INTRODUCTION

Inflammatory bowel disease (IBD) includes Crohn's disease (CD) and ulcerative colitis (UC). The precise cause of IBD remains unknown. IBD is a multifactorial disease resulting from excessive immune responses to various environmental factors and is associated with genetic background. As a result of these excessive immune responses, T helper cell type (Th)0 cells differentiate into Th1, Th17 or Th2 cells in response to interleukin (IL)-12/IL-18, IL-6/tissue growth factor (TGF)-β or IL-4, respectively[1].

Regulatory T cells (Tregs) have been shown to suppress conventional T cells through multiple mechanisms, including the generation of immunosuppressive cytokines, such as TGF-β and IL-10, and *via* direct contact with effector T cells or antigen-presenting cells[2]. Decreases in the anti-inflammatory activity of Tregs may therefore be equal in importance to the enhancement of effector mechanisms in contributing to IBD pathogenesis[1].

Regulatory B cells (Bregs) are functionally characterized by their capacity to produce IL-10, a potent inhibitory cytokine. These cells have been designated as B10 cells because their ability to downregulate immune responses and inflammatory disease is attributable to IL-10. The absence of B10 cells exacerbates disease symptoms in mouse models[3]. Breg dysfunction has been reported to influence the pathogeneses of various autoimmune and allergic diseases. In addition to autoimmune and allergic diseases, intestinal inflammation is also regulated by Breg functions, a relationship that has been confirmed by several studies using mouse models of colitis[4]. Additionally, in humans, the depletion of B cells using anti-CD20 (rituximab) for various disorders has been reported to either exacerbate colitis or result in spontaneous colitis[5,6].

Oral tolerance is a phenomenon in which systemic immunity is suppressed relative to orally administered antigens. Treg involvement has been demonstrated as a mechanism for the induction of oral tolerance. Tregs are naturally produced in the thymus (nTreg) and are also induced in peripheral tissues (pTreg). Induced, antigen-specific Tregs can then circulate and establish systemic tolerance to their corresponding antigens. This phenomenon largely contributes to the induction of oral tolerance[7]. In recent years, Bregs have also been indicated to be involved in oral tolerance[8]. Consistent with the regulatory role of B cells, B cell-deficient mice are defective in developing oral tolerance[9].

The administration of dextran sulfate sodium (DSS) can induce colitis in animal models. This induced colitis is similar in appearance to human UC both clinically and histologically[10,11]. Several reports have evaluated Treg dynamics during active DSS colitis tolerance[12,13]; however, few reports have assessed Breg dynamics during active DSS colitis.

Although oral immunotherapy has been applied for various immune disorders, this treatment modality is considered ineffective for IBD because IBD patients have dysfunctional oral tolerance[14,15]. Although the effectiveness of oral immunotherapy for CD patients was recently reported[16,17], there are currently few reports regarding oral immunotherapy for UC patients.

In this study, we utilized a DSS colitis model to explore the potential use of oral immunotherapy during the active phase of UC. The oral administration of colon extracted protein (CEP) prior to the onset of DSS colitis has been shown to induce immune tolerance, downregulate the inflammatory immune response and alleviate DSS-induced colitis[18,19]. However, to the best of our knowledge, no report thus far has evaluated the effectiveness of the oral administration of CEP after the onset of DSS colitis, during the active phase of the disease.

The purpose of this study was to investigate whether oral tolerance is inducible during the active phase of DSS colitis. Additionally, we determined how cytokine levels and regulatory cell populations change in colitis in the mesenteric lymph nodes (MLN) and the spleen and how these changes influence the induction of oral tolerance. Furthermore, we explored the potential use of oral immunotherapy during the active phase of UC.

MATERIALS AND METHODS

Mice

Specific pathogen-free (SPF) BALB/c mice were purchased from Charles River Laboratories Japan (Yokohama, Kanagawa, Japan). All experiments were performed using 6- to 8-wk-old female mice. The protocol used in the current study was designed to minimize pain and discomfort to the animals and was approved by the Institutional Animal Care and Use Committee of Showa University. The mice had access to water and food *ad libitum* and were housed in SPF conditions with alternating light-dark cycles for one week prior to experimentation. Intragastric gavage was performed using straight gavage needles appropriate for each animal's size. All animals were euthanized using CO_2 prior to tissue collection.

Induction of colitis

An overview of the experimental setup is provided in Figure 1A and B. DSS administration was performed as previously described with slight modification[11,20]. DSS colitis was induced by the administration of 2% DSS with a molecular weight ranging between 36 and 50 kDa (MP Biomedicals, Solon, OH, United States) *ad libitum* from day 1 through day 11. As a control, a subset of mice was provided with autoclaved water for the entire study period. All mice were clinically evaluated based on body weight and a scoring system comprising evaluations of stool consistency and fecal blood, as described previously[20].

Histological analysis of DSS-induced colitis

On day 8, the colons of the mice were removed and fixed in 10% buffered formalin and then embedded in paraffin, sliced into sections, and stained with hematoxylin and eosin. The stained sections were examined by two pathologists for evidence of colitis using a previously described histological scoring method[21].

Oral tolerance induction and immunization

To induce oral tolerance, the mice that received 2% DSS from day 1 through day 11 and the mice that received autoclaved water were intragastrically (i.g.) administered either 5 mg/d ovalbumin (OVA) or PBS as a control for 4 consecutive days from day 8 through day 11. To induce systemic antibody (Ab) production in response to OVA antigen, the mice were intraperitoneally (i.p.) injected with 1 μg of OVA antigen plus 0.1 mg of aluminum hydroxide (alum) (Thermo Scientific, Rockford, IL, United States) on days 14, 28, 42, and 56. Following this, blood samples were collected on day 63 to measure serum anti-OVA-specific IgE Ab concentrations, as described previously[22].

ELISA analysis to measure serum anti-OVA-specific IgE Ab concentrations

Serum OVA-specific IgE concentrations were measured by ELISA. Briefly, 50 μg/mL of OVA was dissolved in 0.1 mol/L sodium carbonate buffer (pH 9.5) and incubated with serum samples in a 96-well immunoplate at 4 ℃ overnight. The samples were treated with protein-free blocking buffer T20 (PBS) (Thermo Scientific) to inhibit nonspecific binding. After washing, the serum samples or OVA-specific IgE antibody (Ab) standards (Acris Antibodies, San Diego, CA, United States) and biotin-conjugated anti-mouse IgE Abs (Southern Biotech, Birmingham, AL, United States) were then plated in the wells and incubated for 1 h. All wells were sequentially incubated with HRP-conjugated streptavidin (eBioscience, San Diego, CA, United States). OVA-specific IgE Ab was detected using a TMB Microwell Peroxidase Substrate System (KPL, Gaithersburg, MD, United States) and measured at an absorbance of 450 nm after the addition of H_2SO_4.

Flow cytometric analysis to detect changes in $CD4^+Foxp3^+$ cell and B10 cell frequencies

Single-cell suspensions were prepared from the MLN and spleen on day 14. The following antibodies were used in this study: Anti-CD16/CD32 Ab as an Fc-blocker; FITC-conjugated anti-CD4 Ab; BV421-conjugated anti-CD25 Ab; Alexa Fluor-conjugated anti-Foxp3 Ab (BD Biosciences, San Diego, CA, United States); BV650-conjugated anti-CD3 Ab; FITC-conjugated anti-CD19 Ab; BV510-conjugated anti-CD5 Ab; PE-conjugated anti-CD1d Ab; and PE/Cy7-conjugated anti-IL-10 Ab (BioLegend, San Diego, CA, United States). Dead cells were detected using a Zombie Red Fixable Viability Kit (BioLegend) according to the manufacturer's recommended protocol. Following the staining of surface antigens, intracellular Foxp3 or IL-10 staining was performed using a Transcription Factor Buffer set (BD Biosciences) according to the manufacturer's recommended protocol. All cells were

analyzed using a LSRFortessa flow cytometer (BD Biosciences).

B cell stimulation for the analysis of B10 cells

Analysis of intracellular IL-10 was performed using flow cytometry as previously described[23]. Briefly, isolated spleen cells were resuspended (2×10^6/mL) in complete medium (RPMI 1640) (Wako Pure Chemical Industries, Osaka, Japan) containing 10% FBS with 5×10^{-5} mol/L 2-mercaptoethanol, 10 μg/mL lipopolysaccharide (LPS), 50 ng/mL phorbol 12-myristate 13-acetate (PMA), 500 ng/mL ionomycin (Sigma-Aldrich, St. Louis, MO, United States) and 1 μg/mL Brefeldin A (BioLegend) for 5 h in 24-well flat-bottom plates.

Reverse transcription real-time polymerase chain reaction analysis to detect cytokine mRNA expression

Total RNA was extracted from whole MLN and spleen cells on day 14 using an RNeasy Mini Kit (Qiagen, Tokyo, Japan). Reverse transcription was performed using a QuantiTect Reverse Transcription Kit (Qiagen). Real-time polymerase chain reaction (PCR) was performed using a LightCycler 480 Ⅱ system (Roche Diagnostics, Mannheim, Germany) with LightCycler 480 Probes Master (Roche Diagnostics). The following PCR primers were used: TaqMan® Gene Expression Assays for mouse IL-4 (Assay ID: Mm00445259_m1), *IL-6* (Assay ID: Mm00446190_m1), *IL-10* (Assay ID: Mm 00439614_m1), interferon-γ (IFN-γ) (Assay ID: Mm01168134_m1), tumor necrosis factor (TNF)-α (Assay ID: Mm00443258_m1), and *GAPDH* (Assay ID: Mm99999915_g1) (Applied Biosystems, Foster City, CA, United States). All values were normalized against the expression of the housekeeping gene *GAPDH*.

Statistical analysis

Statistical analyses were performed using the Mann-Whitney *U* test, and a *P* value < 0.05 was considered to indicate a statistically significant difference.

RESULTS

Establishment of experimental colitis

DSS is widely used to induce intestinal inflammation. From day 8 to day 16, the mice that received water containing 2% DSS [DSS (+) mice] had significantly lower body weights than the mice that received autoclaved water [DSS (-) mice] (Figure 1B, $P < 0.05$ to $P < 0.001$). With the progression of colitis, the DSS (+) mice exhibited diarrhea and visible fecal blood. On day 12, the disease activity index (DAI) of the DSS (+) mice was significantly higher than that of the DSS (-) mice (Figure 1C, $P < 0.01$). On day 8, the average colon length of the DSS (+) mice was shorter than that of the DSS (-) mice (Figure 1D and E, $P < 0.01$). Histologically, on day 8, DSS colitis was characterized by epithelial defects, submucosal edema (F1) and inflammatory cell infiltration (F2) (Figure 1F). The histological scores for the DSS (+) mice were significantly higher than those of the DSS (-) mice (Figure 1G, $P < 0.05$).

Induction of oral tolerance in an experimental colitis model

DSS (+) mice and DSS (-) mice were i.g. administered 5 mg/d OVA or PBS, respectively, for 4 consecutive days before undergoing an i.p. administered challenge with 1 μg OVA plus 0.1 mg of alum every two weeks for a total of four times. Serum samples were collected from the mice 1 wk after each challenge, and OVA-specific IgE concentrations were measured. Regardless of the presence of DSS colitis, the mice that were i.g. administered OVA had significantly lower OVA-specific IgE concentrations than the mice i.g. administered PBS [DSS (+): 4.4 (4.2-9.5) ng/mL *vs* 83.9 (66.1-123.2) ng/mL, $P < 0.01$; DSS (-): 27.7 (0.1-54.5) ng/mL *vs* 116.5 (80.6-213.6) ng/mL, $P < 0.01$] (Figure 2). These results demonstrated that oral tolerance was inducible with or without colitis.

Determination of Treg frequency in the spleen and MLN using flow cytometric analysis

As indicated above, oral tolerance was inducible with or without colitis. We hypothesized that MLN and spleen populations of $CD4^+CD25^+Foxp3^+$ cells, which are involved in oral tolerance, remained stable regardless of the presence of colitis, although the colitis mice did exhibit epithelial defects and inflammatory cell infiltration into the colonic mucosa. In the spleen, the frequency of $CD4^+CD25^+$ cells among $CD4^+$ T cells and the frequency of $CD4^+Foxp3^+$ cells among $CD4^+$ T cells were 5.6% (5.35%-5.75%) for the control mice and 6.6% (5.4%-6.6%) for the DSS (+) mice (Figure 3B) and 8.0% (7.0%-8.6%) for the control mice and 7.9% (7.2%-8.5%) for the DSS (+) mice (Figure 3C), respectively. The $CD4^+Foxp3^+$ cells included both $CD4^+CD25^+Foxp3^+$ T cell and $CD4^+CD25^-Foxp3^+$ T cell populations. In the spleen, the frequencies of $CD4^+CD25^+Foxp3^+$ cells and $CD4^+CD25^-Foxp3^+$ cells among $CD4^+$ T cells were 5.0% (4.4%-5.2%) for the control mice and 4.5% (4.5%-5.1%) for the DSS (+) mice (Figure 3D) and 3.0% (2.6%-3.4%) for the control mice and 3.4% (2.7%-3.4%) for the DSS (+) mice (Figure 3E), respectively. In the MLN, the frequencies of $CD4^+$ $CD25^+$ T cells among $CD4^+$ T cells and $CD4^+Foxp3^+$ T cells among $CD4^+$ T cells were 7.4% (6.8%-7.9%) for the control mice and 7.1% (4.9%-16.6%) for the DSS (+) mice (Figure 3G) and 9.4% (9.1%-9.8%) for the control mice and 12.2% (8.3%-20.4%) for the DSS (+) mice (Figure 3H), respectively. In the MLN, the frequencies of $CD4^+CD25^+Foxp3^+$ cells and $CD4^+CD25^-Foxp3^+$ cells among $CD4^+$ T cells were 7.0% (6.6%-7.2%) for the control mice and 6.5% (4.5%-13.6%) for the DSS (+) mice (Figure 3I) and 2.6% (2.2%-2.8%) for the control mice and 5.7% (3.5%-7.1%) for the DSS (+) mice (Figure 3J), respectively. These findings demonstrate that $CD4^+CD25^+$ cell frequency among $CD4^+$ T cells in the spleen tended to increase in colitis, while the

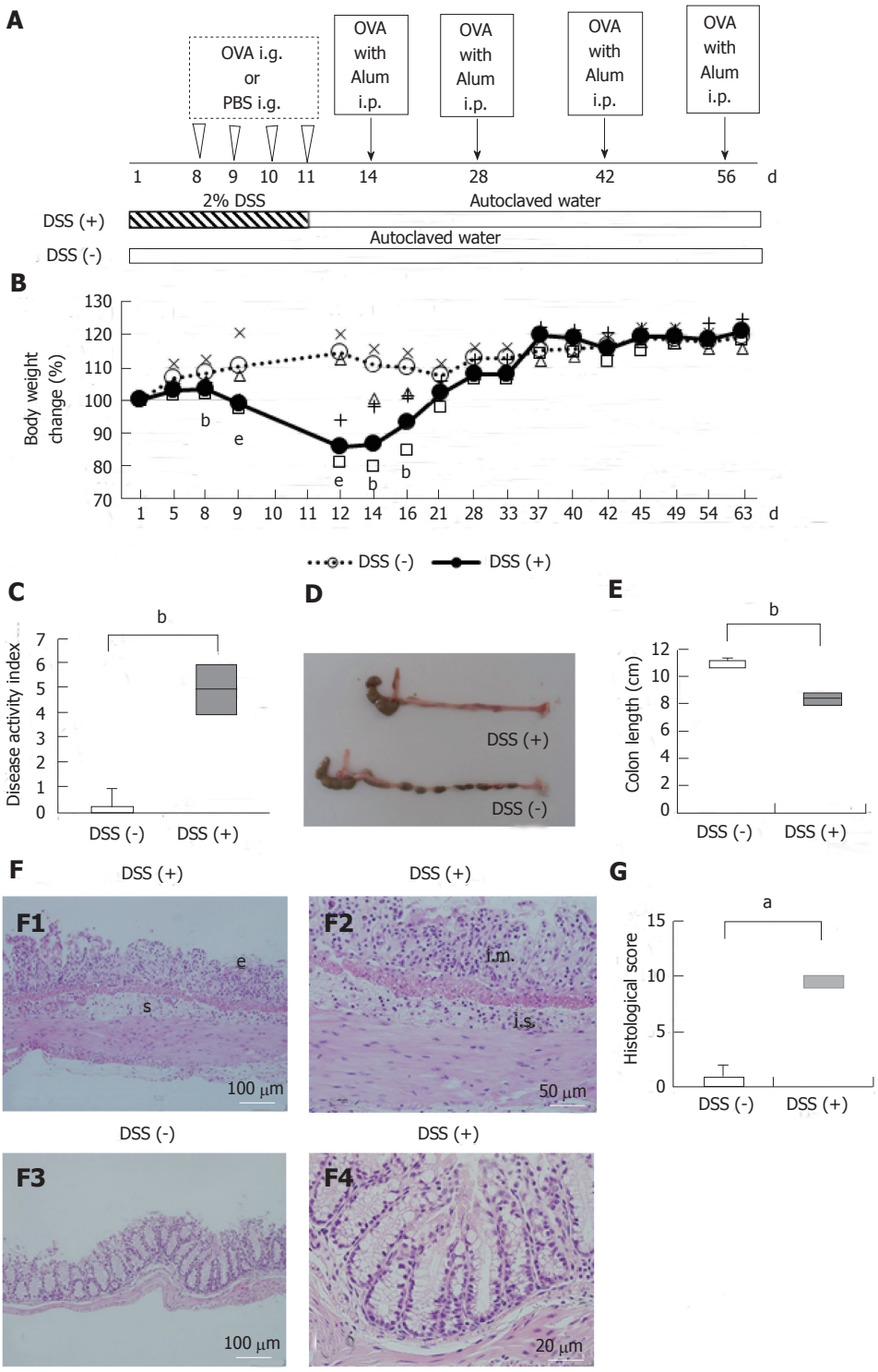

Figure 1 Administration of dextran sulfate sodium to induce colitis. A: Experimental design for the induction of oral tolerance and dextran sulfate sodium (DSS) colitis; B: Percent changes in the body weights of mice that received 2% DSS [DSS (+) mice] and mice that received autoclaved water (control mice). The body weights of the DSS (+) mice (median: Closed circles, first quartile: Plus, third quartile: Open box) were significantly lower than those of the control mice (median: Open circles, first quartile: X-mark, third quartile: Open triangle) from day 8 to day 16; C: Disease activity index (DAI) for the DSS (+) mice and the control mice on day 12. The DAI of the DSS (+) mice (closed bar) was significantly increased compared to that of the control mice (open bar); D: A representative image of colons collected from the DSS (+) mice and the control mice on day 8. The median colon length in the DSS (+) mice was shorter than that in the control mice; E: Colon lengths for the DSS (+) mice and the control mice on day 8. On day 8, the median colon length for the DSS (+) mice (closed bar) was significantly shorter than that for the control mice (open bar); F: Representative hematoxylin and eosin staining of colon sections. On day 8, the colons were removed, fixed in 10% buffered formalin, embedded in paraffin, cut into sections, and stained with hematoxylin and eosin. The DSS (+) mice showed pathological changes characterized by epithelial defects, submucosal edema (F1) and inflammatory cell infiltration in the mucosal layer and submucosal layer (F2). The control mice showed minimal chronic inflammatory cells in the lamina propria with regularly spaced crypts and did not show epithelial defects or submucosal edema (F3, F4); G: Histological scores for the DSS (+) mice and the control mice on day 8. The DSS (+) mice (closed bar) had significantly higher histological scores than the control mice (open bar). The data are shown as the median and interquartile range of three to six mice per group. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{e}P < 0.001$. e: Epithelial; s: Submucosal edema; i.m.: Inflammatory cell infiltration in the mucosal layer; i.s.: Inflammatory cell infiltration in the submucosal layer.

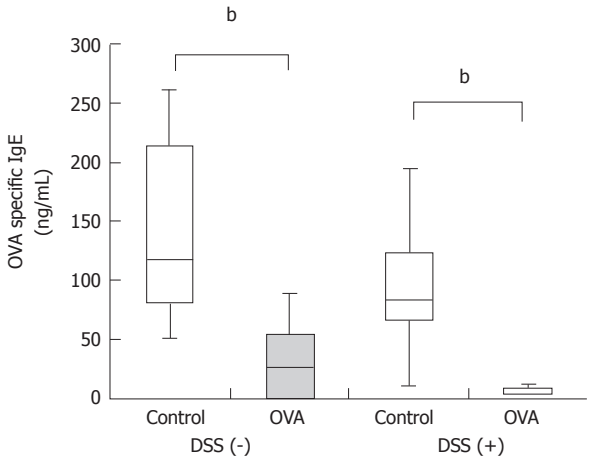

Figure 2 Ovalbumin-specific IgE concentrations in the sera of mice with and without dextran sulfate sodium colitis. On day 63, serum OVA-specific immunoglobulin E (IgE) concentrations were measured by ELISA. The mice that were i.g. administered OVA exhibited significantly lower OVA-specific IgE concentrations (closed bar) than the control mice (open bar) both in the presence and absence of DSS colitis. All results represent at least two independent experiments with four to six mice in each group. The data are shown as the median and interquartile range. $^{b}P < 0.01$. OVA: Ovalbumin; DSS: Dextran sulfate sodium; ELISA: Enzyme-linked immuno sorbent assay; i.g.: Intragastrically.

frequencies of $CD4^+CD25^+Foxp3^+$ cells and $CD4^+CD25^-Foxp3^+$ cells among $CD4^+$ T cells did not change. Our findings additionally revealed that $CD4^+CD25^+Foxp3^+$ cell frequency among $CD4^+$ T cells in the MLN did not change during colitis, while $CD4^+CD25^-Foxp3^+$ Treg frequency increased significantly (Figure 3J, $P < 0.05$). Inflammatory cell infiltration into the colonic mucosa did not influence the stability of $CD4^+CD25^+Foxp3^+$ T cell populations in the spleen, although $CD4^+$ T cells in the spleen were activated during colitis. Moreover, inflammatory cell infiltration did not influence the stability of $CD4^+CD25^+Foxp3^+$ T cell populations in the MLN; however, inflammatory cell infiltration increased the population of $CD4^+CD25^-Foxp3^+$ cells in the MLN. These results suggest that the stability of $CD4^+CD25^+Foxp3^+$ T cell populations in the spleen and MLN may play a role in oral tolerance induction in DSS colitis, and elevated numbers of $CD4^+CD25^-Foxp3^+$ Tregs in the MLN may help sustain homeostasis during colitis.

Measurement of Breg frequency in the spleen using flow cytometric analysis

B10 cells are associated with the induction of oral tolerance[8]. Consistent with the regulatory role of B cells, B cell-deficient mice are defective in the ability to develop oral tolerance[9]. We hypothesized that B10 cell populations, similarly to $CD4^+CD25^+Foxp3^+$ cell populations, remain stable during colitis. Therefore, we examined whether any differences existed in $CD19^+IL\text{-}10^+$ cell populations in the spleens of mice with and without DSS colitis. The frequencies of $CD19^+IL\text{-}10^+$ cells among $CD19^+$ cells and $CD19^+CD5^+CD1d^{Hi}$ cells among $CD19^+$ cells, as well as $CD19^+CD5^+CD1d^{Hi}$ $IL\text{-}10^+$ cells among $CD19^+CD5^+CD1d^{Hi}$ cells were 0.8% (0.7%-1.2%) for the control mice and 1.4% (0.9%-1.9%) for the DSS (+) mice (Figure 4B), 1.5% (1.2%-2.5%) for the control mice and 2.4% (1.3%-2.8%) for the DSS (+) mice (Figure 4C), and 7.6% (6.4%-8.4%) for the control mice and 11.3% (6.9%-14.5%) for the DSS (+) mice (Figure 4D), respectively. The frequencies of $CD19^+IL\text{-}10^+$ cells in the spleens of the DSS colitis mice were comparable to those in the spleens of the control mice. $CD19^+CD5^+CD1d^{Hi}$ cell frequency among $CD19^+$ cells did not change during DSS colitis. However, $IL\text{-}10^+$ cell frequency among $CD19^+CD5^+CD1d^{Hi}$ cells tended to increase in the spleens of the DSS colitis mice relative to the control mice. These results suggest that DSS colitis may act either directly or indirectly to promote IL-10 production within $CD19^+CD5^+CD1d^{Hi}$ cells from the spleen.

Reverse transcription real-time PCR evaluation of cytokine levels in the spleen and MLN during colitis

As indicated above, $CD4^+CD25^+Foxp3^+$ Treg and B10 cell populations did not decrease during DSS colitis. We next investigated how the levels of cytokines, which influence the function and differentiation of Tregs and B10 cells, change during colitis. In the spleens of the mice with colitis, IFN-γ mRNA expression was significantly lower than that in the mice without colitis ($P < 0.01$) (Figure 5A). However, IFN-γ expression in the MLN were comparable between mice with and without colitis (Figure 5B). Additionally, there were no significant differences in IL-4, IL-6, IL-10 or TNF-α mRNA expression in the spleens or MLNs of mice with and without colitis (Figure 5).

DISCUSSION

In the current study, we revealed that oral tolerance is inducible during the active phase of DSS colitis. We hypothesized that the MLN and spleen, both of which are involved in oral tolerance induction, maintained stability during colitis. We also investigated the manners in which cytokine levels and regulatory cell populations, such as those for $Foxp3^+$ T cells and B10 cells, change during colitis.

Histologically, DSS colitis was characterized by epithelial defects and inflammatory cell infiltration. In previous studies, DSS colitis has been shown to exhibit a Th1-predominant profile[24] or a Th1-Th17-predominant profile within the colonic mucosa[25]. During the acute phase of DSS colitis, no differences were found in Foxp3 mRNA expression in colonic tissues from DSS colitis mice and normal mice; however, during the chronic phase, *Foxp3* mRNA expression increased[12]. In the current study, we revealed that $CD4^+CD25^+$ cell frequency among $CD4^+$ T cells in the spleen tended to increase during colitis, while $CD4^+Foxp3^+$ cell frequency among $CD4^+$ T cells did not change. CD25 is known as an activation marker. These results suggest that DSS colitis

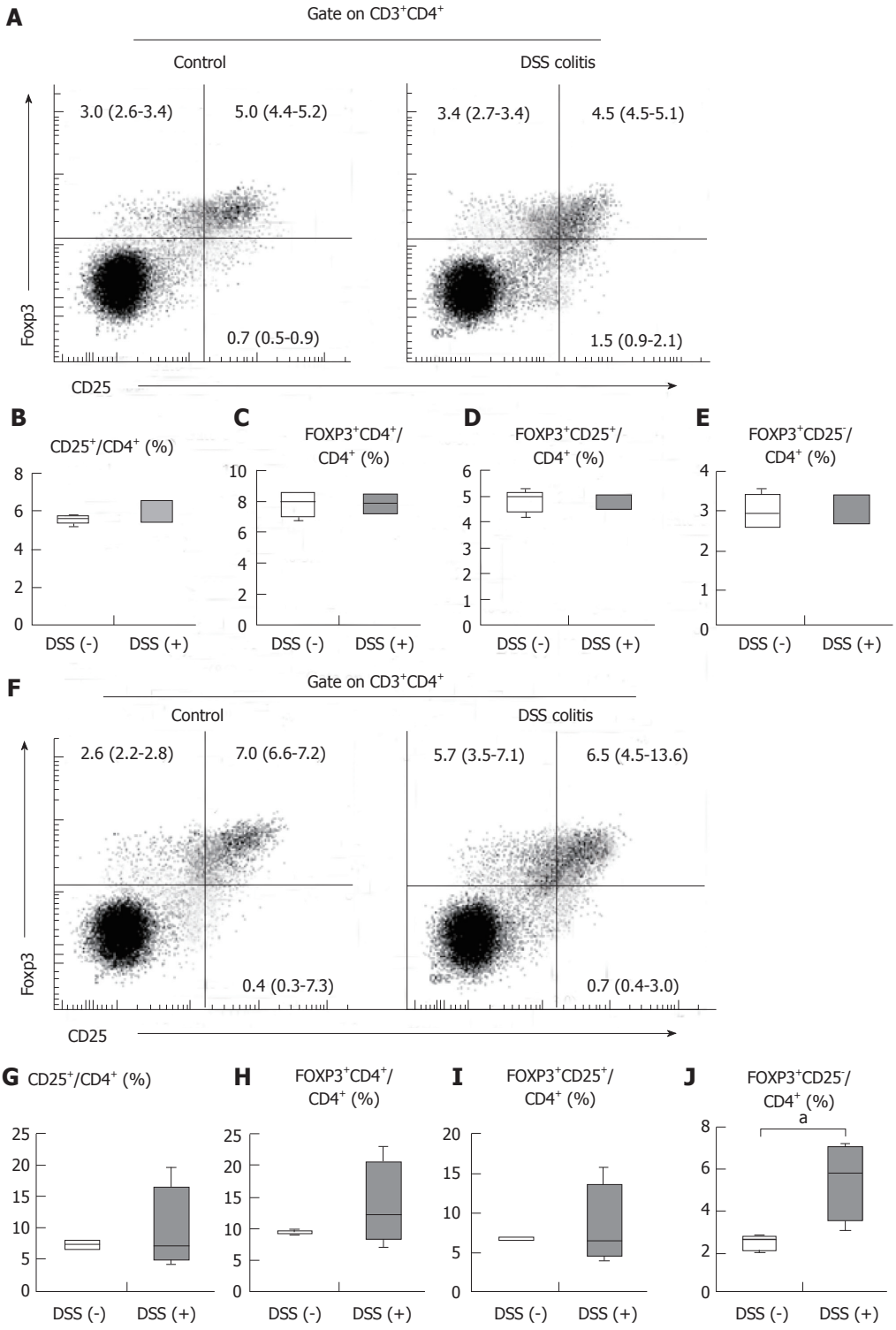

Figure 3 Regulatory T cell frequencies in the spleen and mesenteric lymph nodes as determined by flow cytometry. A: Analysis of CD25 and Foxp3 expression in the spleen. Representative data are shown for control mice (left panel) and DSS colitis mice (right panel); B: $CD4^+CD25^+$ cell frequencies in the spleens of DSS colitis mice and control mice. $CD4^+CD25^+$ cell frequency tended to increase in the spleens of DSS colitis mice (closed bar) compared to those of control mice (open bar); C: $CD4^+Foxp3^+$ cell frequencies in the spleens of DSS colitis mice and control mice. $CD4^+Foxp3^+$ cell frequency was comparable in the spleens of DSS colitis mice (closed bar) and control mice (open bar); D: $CD4^+CD25^+Foxp3^+$ cell frequencies in the spleens of DSS colitis mice and control mice. $CD4^+CD25^+Foxp3^+$ cell frequency was comparable in the spleens of DSS colitis mice (closed bar) and control mice (open bar); E: $CD4^+CD25^-Foxp3^+$ cell frequencies in the spleens of DSS colitis mice and control mice. $CD4^+CD25^-Foxp3^+$ cell frequency was comparable in the spleens of DSS colitis mice (closed bar) and control mice (open bar); F: Analysis of CD25 and Foxp3 expression in the MLN. Representative data are shown for control mice (left panel) and DSS colitis mice (right panel); G: $CD4^+CD25^+$ cell frequencies in the MLNs of DSS colitis and control mice. $CD4^+CD25^+$ cell frequency was comparable in the MLNs of DSS colitis mice (closed bar) and control mice (open bar); H: $CD4^+Foxp3^+$ cell frequencies in the MLNs of DSS colitis mice and control mice. $CD4^+Foxp3^+$ cell frequency tended to increase in the MLNs of DSS colitis mice (closed bar) compared with control mice (open bar); I: $CD4^+CD25^+Foxp3^+$ cell frequencies in the MLNs of DSS colitis mice and control mice. $CD4^+CD25^+Foxp3^+$ cell frequency was comparable in the MLNs of DSS colitis mice (closed bar) and control mice (open bar); J: $CD4^+CD25^-Foxp3^+$ cell frequencies in the MLNs of DSS colitis mice and control mice. $CD4^+CD25^-Foxp3^+$ cell frequency was significantly higher in the MLNs of DSS colitis mice (closed bar) compared with control mice (open bar). All results represent at least two independent experiments with four to six mice in each group. The data are shown as the median and interquartile range. $^aP < 0.05$. MLN: Mesenteric lymph nodes; DSS: Dextran sulfate sodium.

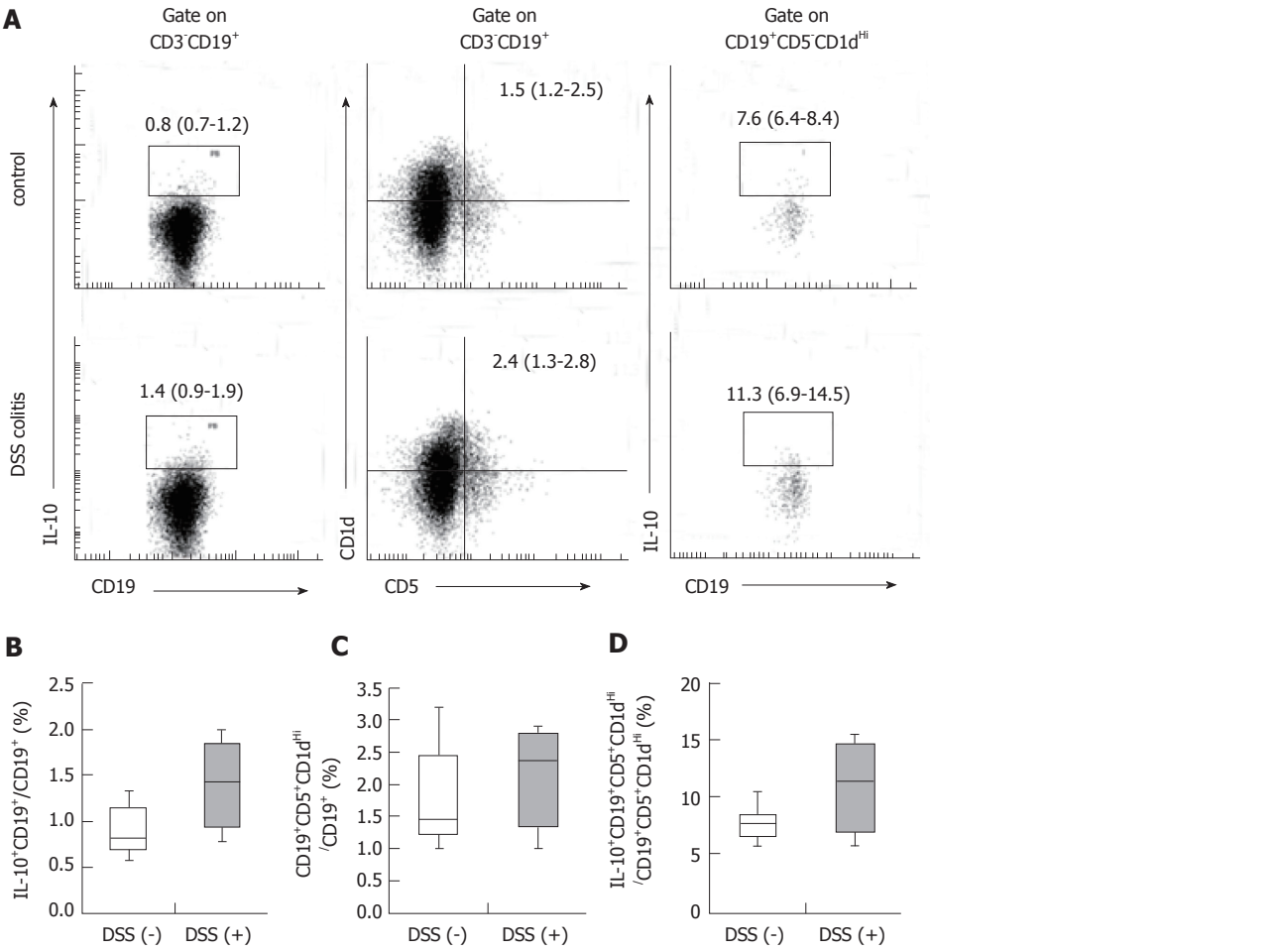

Figure 4 Regulatory B cell frequency in the spleen as determined by flow cytometry. A: Analysis of $CD19^+IL\text{-}10^+$ cell populations (left panel), $CD19^+CD5^+CD1d^{Hi}$ cell populations (center panel) and $CD19^+CD5^+CD1d^{Hi}IL\text{-}10^+$ cell populations (right panel) in the spleen based on flow cytometry. Representative data are shown for control mice (top panel) and DSS colitis mice (bottom panel); B: $CD19^+IL^-10^+$ cell frequencies among $CD19^+$ cells in the spleens of DSS colitis mice and control mice. $CD19^+IL\text{-}10^+$ cell frequency among $CD19^+$ cells was comparable in the spleens of DSS mice (closed bar) and control mice (open bar); C: $CD19^+CD5^+CD1d^{Hi}$ cell frequencies among $CD19^+$ cells in the spleens of DSS colitis mice and control mice. $CD19^+CD5^+CD1d^{Hi}$ cell frequency among $CD19^+$ cells was comparable in the spleens of DSS mice (closed bar) and control mice (open bar); D: $IL\text{-}10^+$ cell frequencies among $CD19^+CD5^+CD1d^{Hi}$ cell populations in the spleens of DSS colitis mice and control mice. $IL\text{-}10^+$ cell frequency among the $CD19^+CD5^+CD1d^{Hi}$ cell population tended to increase in the spleens of the DSS mice (closed bar) compared with those of the control mice (open bar). All results represent at least two independent experiments with four to six mice in each group. The data are shown as the median and interquartile range. DSS: Dextran sulfate sodium; IL: Interleukin.

activated $CD4^+$ cells in the spleen while sustaining the stability of $CD4^+Foxp3^+$ cell populations in the spleen. We further revealed that $CD4^+CD25^+$ cell frequency among $CD4^+$ T cells in the MLN did not change during colitis, while $CD4^+Foxp3^+$ cell frequency among $CD4^+$ T cells tended to increase. In previous studies, $CD4^+Foxp3^+$ Treg frequencies in the MLNs and spleens of mice with colitis were lower than those in mice without colitis[13]. The target organs and mouse species assessed, as well as the concentrations of DSS, evaluation timing and evaluation methodology used may explain the differences in these results.

$CD4^+Foxp3^+$ T cells include $CD4^+CD25^+Foxp3^+$ cell and $CD4^+CD25^-Foxp3^+$ cell populations. The function of $CD4^+CD25^-Foxp3^+$ Tregs remains unclear. One previous study indicated that $CD4^+CD25^-FoxP3^+$ Tregs act similarly to conventional Tregs to a certain extent[26]; however, another study demonstrated that $CD4^+CD25^-Foxp3^+$ Tregs differ from $CD4^+CD25^+$ Tregs both phenotypically and functionally[27,28]. We revealed that mice with and without colitis had comparable frequencies of $CD4^+CD25^+Foxp3^+$ Tregs within the MLN, while $CD4^+CD25^-Foxp3^+$ Treg frequency significantly increased during colitis. $CD4^+CD25^-FoxP3^+$ Tregs may retain a suppressive function in an inflammatory environment[28]. Taken together, the above data indicate that $CD4^+CD25^-Foxp3^+$ Tregs may play roles in maintaining homeostasis in the MLN and in inducing oral tolerance during DSS colitis.

Recently, Bregs have been shown to play an important role in oral tolerance in addition to Tregs. Allergen-specific, IL-10-producing B cells are involved in the development of tolerance to food allergens[8]. The proportion of IL-10-producing B cells following antigen stimulation was shown to decrease in an allergy group, whereas it increased in a tolerant group[29]. We hypothesized that, similarly to $CD4^+CD25^+Foxp3^+$ cell populations, B10 cell populations remain stable

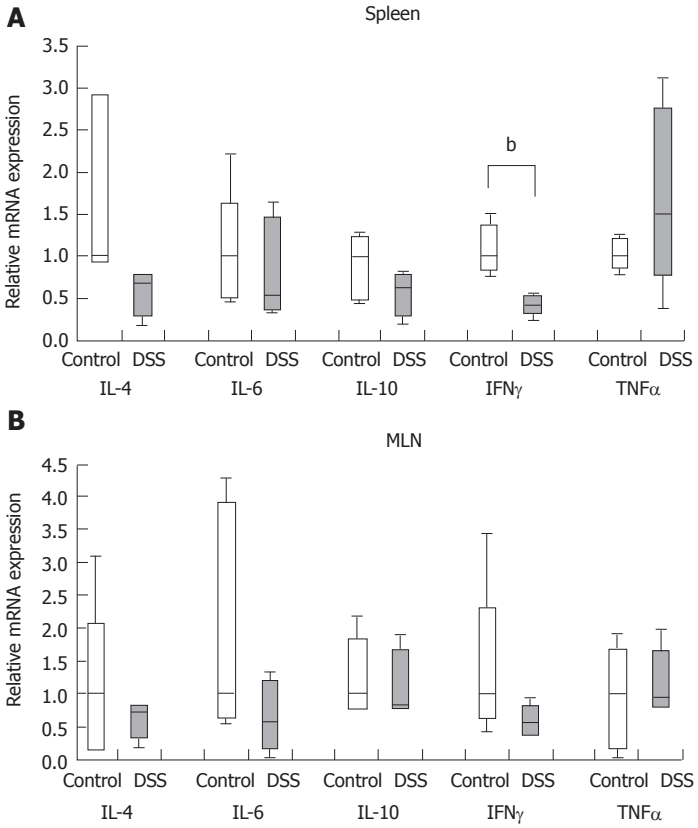

Figure 5 Evaluation of cytokine mRNA expression in the spleen and mesenteric lymph nodes during colitis. A: Evaluation of cytokine mRNA expression in the spleen during colitis. IFN-γ mRNA expression was significantly lower in the spleens of mice with colitis compared to those of mice without colitis. There were no significant differences in IL-4, IL-6, IL-10 or TNF-α mRNA expression; B: Evaluation of cytokine mRNA expression in the MLN in mice with colitis. In the MLN, there was no significant difference in IL-4, IL-6, IL-10, IFN-γ, or TNF-α expression between mice with and without colitis. Expression values were normalized to the expression of the housekeeping gene *GAPDH*. All results represent at least two independent experiments with four to six mice in each group. The data are shown as the median and interquartile range. $^{b}P < 0.01$. MLN: Mesenteric lymph nodes; DSS: Dextran sulfate sodium; TNF: Tumor necrosis factor; IFN: Interferon; IL: Interleukin.

during colitis. The frequencies of $CD19^{+}IL\text{-}10^{+}$ cells in the spleens of DSS colitis mice were comparable to those in the spleens of control mice. $CD19^{+}CD5^{+}CD1d^{Hi}$ is the predominant source of IL-10 production[30]. $CD19^{+}CD5^{+}CD1d^{Hi}$ cell frequency among $CD19^{+}$ cells did not change during DSS colitis; however, $CD19^{+}CD5^{+}CD1d^{Hi}IL\text{-}10^{+}$ cell frequency within the $CD19^{+}CD5^{+}CD1d^{Hi}$ cell population tended to increase. These results suggest that DSS colitis may act either directly or indirectly to promote IL-10 production from $CD19^{+}CD5^{+}CD1d^{Hi}$ cells in the spleen. B10 cells have also been shown to inhibit intestinal injury in DSS colitis mice[31]. A previous study showed that B10 cell populations did not decrease in the spleen during DSS colitis, similar to the present results[32]. Both the current study and the referenced study suggest that DSS colitis does not decrease B10 cell frequency, inhibit B cell IL-10 production, or inhibit B10 cell functions associated with oral tolerance. However, there were limitations associated with our analysis of B10 cells. Whole cells from the spleen were stimulated with LPS, PMA and ionomycin. Thus, it is not possible to exclude the effects of cells other than B cells on Bregs.

Cytokines can influence Treg function[33] and Breg differentiation[34,35]. As indicated above, the frequencies of $CD4^{+}CD25^{+}Foxp3^{+}$ Tregs and B10 cells did not decrease during DSS colitis. We therefore investigated how cytokines, which influence the functions of Tregs and B10 cells, change during colitis.

IFN-γ appears to play an important role in food allergen tolerance induction. Specific oral immunotherapy using IFN-γ may induce tolerance induction in both IgE-mediated[36,37] and non-IgE-mediated food allergies[37]. IFN-γ can both promote and subvert Treg suppressive activity in various settings, and the balance between these opposing functions likely depends on contextual factors, such as the timing and extent of the expression[33]. IFN-γ induces murine $CD5^{+}$ B1 cells to adopt a macrophage-like morphology. Macrophage-like B1 cells express high levels of CD5[38]. Moreover, IFN-γ induces allergen-specific B10 responses and promotes tolerogenic function[29,34]. In previous studies, during the acute phase of DSS colitis, colonic Th cells have been shown to exhibit a Th1 profile, rather than a Th2 or Th17 profile[24]. Moreover, DSS colitis leads to a Th1-Th17 response during its active phase[25]. In the current study, we revealed that IFN-γ mRNA expression was reduced in the spleens of mice with DSS, while its expression did not change in the MLN. This change in IFN-γ mRNA expression in the spleen did not influence oral tolerance, whereas the stability of IFN-γ expression in the MLN may have influenced the induction of oral tolerance.

IL-4 inhibits Treg function[33]. IL-4 receptor signaling has been shown to impair the capacity of Tregs to suppress mast cell activation and expansion, which in turn drives Th2-cell reprogramming of Tregs[39]. Moreover, IL-4 inhibits mouse $CD5^{+}$ B1 cells from adopting a macrophage-like morphology[38]. In previous studies, DSS colitis mice have not exhibited increased IL-4 production from colonic T cells[24]. Similarly, in the current study, we revealed that DSS colitis mice did not exhibit increased IL-4 mRNA expression in either the spleen or MLN. This stability of IL-4 mRNA expression

in the spleen and MLN may influence oral tolerance induction.

IL-6 subverts Treg cell function[33] and is essential for the differentiation of IL-10-producing B cells. Bregs are induced by the gut microbiota; this induction is driven by IL-6 production[35]. During both the acute and chronic phases of DSS colitis, serum IL-6 concentrations increase[25]. In the current study, we revealed that IL-6 mRNA expression was stable in the spleens and MLNs of mice with DSS colitis. This stability may influence oral tolerance induction.

IL-10 signaling is required to maintain Treg and Breg functions. IL-10 exhibits anti-inflammatory effects in part through its regulation of Treg stability and function both under steady-state conditions and during inflammation[33]. Autocrine stimulation of IL-10 is critical toward enriching IL-10 production in $CD40^{Hi}CD5^{+}$ Bregs both *in vitro* and *in vivo*[40]. In DSS colitis, serum IL-10 levels have been shown to remain stable during the acute phase, whereas these levels increase during the chronic phase[25]. In the current study, we revealed that IL-10 mRNA expression was stable in the spleens and MLNs of mice with DSS colitis. This stability may influence oral tolerance induction.

TNF-α can both promote and subvert Treg cell function[34]. In DSS colitis, serum TNF-α concentrations increase during the acute phase but remain stable during the chronic phase[25]. We revealed that TNF-α mRNA expression was stable in the spleens and MLNs of mice with DSS colitis. This stability may influence oral tolerance induction.

In our cytokine analysis, IFN-γ expression decreased in the spleens of mice with DSS colitis, whereas IFN-γ expression in the MLN and IL-4, IL-6, IL-10 and TNF-α expression in both the spleen and the MLN remained stable. The cytokine profiles associated with DSS colitis may help to maintain the function and differentiation of Tregs and Bregs, which in turn are associated with oral tolerance. However, it should be noted that we only evaluated cytokine mRNA expression and not cytokine production.

Oral immunotherapy has been utilized for various immune disorders. However, oral immunotherapy is considered only poorly effective for IBD because IBD patients have dysfunctional oral tolerance[14,15]. One mechanism underlying this dysfunction is small intestinal permeability. In an IL-10 knock out model, increasing small intestinal permeability was shown to prevent the development of oral tolerance[41]. Several studies have also shown that a defect in intestinal epithelial permeability may be involved in the pathogenesis of IBD. Supporting this concept, other studies have shown that increased intestinal permeability precedes the onset of colitis in experimental animal models of IBD[41-43]. Conversely, IBD family members with no clinical symptoms exhibit dysfunctional oral tolerance, although small intestinal permeability is within the normal range. Thus, other genetic backgrounds are likely involved in dysfunctional oral tolerance[44]. In previous reports, the absence of functional inducible nitric oxide synthase (iNOS) enhanced the efficacy of oral tolerance[45]. Conversely, nitric oxide (NO) and iNOS production were increased both in colonic tissues collected from IBD patients and in a DSS-induced colitis model[46,47]. These data suggest that dysfunctional oral tolerance in IBD patients might be due to NO induction in addition to inflammation.

Although the effectiveness of oral immunotherapy for CD patients has recently been reported[16,17], there are few reports regarding the use of oral immunotherapy for UC patients. DSS-induced colitis serves as an experimental animal model of UC[10,11]. In the present study, we used this DSS colitis model to explore the potential use of oral immunotherapy as a treatment during the active phase of UC. Oral administration of CEP prior to the onset DSS colitis has been shown to induce immune tolerance, downregulate the inflammatory immune response and alleviate DSS-induced colitis[18,19]. However, no reports have evaluated oral tolerance following the oral administration of CEP after DSS colitis has developed.

To the best of our knowledge, the current study is the first to demonstrate that oral tolerance is inducible during the active phase of DSS colitis. Lymphocytic infiltration into the large intestine mucosa associated with epithelial defects did not influence oral tolerance. In addition to that used here, there are many other mouse models of IBD available for use. Further research evaluating oral tolerance in these models is warranted prior to clinical translation. Our study suggests that the choice of an appropriate antigen will enhance the effectiveness of oral immunotherapy for the treatment of UC.

COMMENTS

Background

Oral immunotherapy is considered only poorly effective for inflammatory bowel disease (IBD) because IBD patients have dysfunctional oral tolerance. Although the effectiveness of oral immunotherapy for Crohn's disease patients has recently been reported, there are few reports regarding the use of oral immunotherapy for ulcerative colitis (UC) patients. Dextran sulfate sodium (DSS) colitis serves as an animal model of UC. Oral administration of colon extract protein (CEP) prior to the onset of DSS colitis has been shown to alleviate colitis; however, the effectiveness of oral administration of CEP after the onset of DSS colitis has not been evaluated.

Research frontiers

The purpose of this study was to investigate whether oral tolerance is inducible during the active phase of DSS colitis. Additionally, The authors determined how cytokine levels and regulatory cell populations, such as those of $Foxp3^{+}$ T cells and B10 cells, which are associated with oral tolerance, change in colitis in the mesenteric lymph nodes and the spleen.

Innovations and breakthroughs

This study is the first to demonstrate that oral tolerance is inducible during the active phase of DSS colitis. Lymphocytic infiltration into the large intestine mucosa associated with epithelial defects did not influence oral tolerance. The frequency of $CD4^{+}CD25^{+}Foxp3^{+}$ cells and B10 cells, which are associated with oral tolerance, did not change significantly. In the spleen, IFN-γ mRNA expression decreased in mice with colitis, but the expression levels of other

cytokines did not significantly change. This stability in regulatory cell populations and the observed cytokine profiles might influence oral tolerance induction during DSS colitis.

Applications

This study suggests that if an appropriate antigen is chosen, then oral immunotherapy may be applicable for the treatment of UC.

Terminology

Oral tolerance is a phenomenon in which systemic immunity is suppressed following the oral administration of antigens. Oral immunotherapy has been applied for various immune disorders.

Peer-review

The manuscript by Ino *et al* is an interesting study and first to demonstrate that oral tolerance is inducible in the active phase of DSS colitis. In addition the authors tried to make a link between oral tolerance and the numbers of Treg and B10 cells.

REFERENCES

1 **Geremia A**, Biancheri P, Allan P, Corazza GR, Di Sabatino A. Innate and adaptive immunity in inflammatory bowel disease. *Autoimmun Rev* 2014; **13**: 3-10 [PMID: 23774107 DOI: 10.1016/j.autrev.2013.06.004]

2 **Vignali DA**, Collison LW, Workman CJ. How regulatory T cells work. *Nat Rev Immunol* 2008; **8**: 523-532 [PMID: 18566595 DOI: 10.1038/nri2343]

3 **Tedder TF**. B10 cells: a functionally defined regulatory B cell subset. *J Immunol* 2015; **194**: 1395-1401 [PMID: 25663677 DOI: 10.4049/jimmunol.1401329]

4 **Oka A**, Ishihara S, Mishima Y, Tada Y, Kusunoki R, Fukuba N, Yuki T, Kawashima K, Matsumoto S, Kinoshita Y. Role of regulatory B cells in chronic intestinal inflammation: association with pathogenesis of Crohn's disease. *Inflamm Bowel Dis* 2014; **20**: 315-328 [PMID: 24390063 DOI: 10.1097/01.MIB.0000437983.14544.d5]

5 **El Fassi D**, Nielsen CH, Kjeldsen J, Clemmensen O, Hegedüs L. Ulcerative colitis following B lymphocyte depletion with rituximab in a patient with Graves' disease. *Gut* 2008; **57**: 714-715 [PMID: 18408106 DOI: 10.1136/gut.2007.138305]

6 **Ardelean DS**, Gonska T, Wires S, Cutz E, Griffiths A, Harvey E, Tse SM, Benseler SM. Severe ulcerative colitis after rituximab therapy. *Pediatrics* 2010; **126**: e243-e246 [PMID: 20566611 DOI: 10.1542/peds.2009-3395]

7 **Adel-Patient K**, Wavrin S, Bernard H, Meziti N, Ah-Leung S, Wal JM. Oral tolerance and Treg cells are induced in BALB/c mice after gavage with bovine β-lactoglobulin. *Allergy* 2011; **66**: 1312-1321 [PMID: 21615416 DOI: 10.1111/j.1398-9995.2011.02653.x]

8 **Noh J**, Lee JH, Noh G, Bang SY, Kim HS, Choi WS, Cho S, Lee SS. Characterisation of allergen-specific responses of IL-10-producing regulatory B cells (Br1) in Cow Milk Allergy. *Cell Immunol* 2010; **264**: 143-149 [PMID: 20646682 DOI: 10.1016/j.cellimm.2010.05.013]

9 **Gonnella PA**, Waldner HP, Weiner HL. B cell-deficient (mu MT) mice have alterations in the cytokine microenvironment of the gut-associated lymphoid tissue (GALT) and a defect in the low dose mechanism of oral tolerance. *J Immunol* 2001; **166**: 4456-4464 [PMID: 11254701 DOI: 10.4049/jimmunol.166.7.4456]

10 **Cooper HS**, Murthy SN, Shah RS, Sedergran DJ. Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Lab Invest* 1993; **69**: 238-249 [PMID: 8350599]

11 **Okayasu I**, Hatakeyama S, Yamada M, Ohkusa T, Inagaki Y, Nakaya R. A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. *Gastroenterology* 1990; **98**: 694-702 [PMID: 1688816]

12 **Bento AF**, Leite DF, Marcon R, Claudino RF, Dutra RC, Cola M, Martini AC, Calixto JB. Evaluation of chemical mediators and cellular response during acute and chronic gut inflammatory response induced by dextran sodium sulfate in mice. *Biochem Pharmacol* 2012; **84**: 1459-1469 [PMID: 23000912 DOI: 10.1016/j.bcp.2012.09.007]

13 **Chen J**, Xie L, Toyama S, Hünig T, Takahara S, Li XK, Zhong L. The effects of Foxp3-expressing regulatory T cells expanded with CD28 superagonist antibody in DSS-induced mice colitis. *Int Immunopharmacol* 2011; **11**: 610-617 [PMID: 21163250 DOI: 10.1016/j.intimp.2010.11.034]

14 **Kraus TA**, Toy L, Chan L, Childs J, Mayer L. Failure to induce oral tolerance to a soluble protein in patients with inflammatory bowel disease. *Gastroenterology* 2004; **126**: 1771-1778 [PMID: 15188172 DOI: 10.1053/j.gastro.2004.03.076]

15 **Kraus TA**, Cheifetz A, Toy L, Meddings JB, Mayer L. Evidence for a genetic defect in oral tolerance induction in inflammatory bowel disease. *Inflamm Bowel Dis* 2006; **12**: 82-88; discussion 81 [PMID: 16432371 DOI: 10.1097/01.MIB.0000200343.61707.52]

16 **Margalit M**, Israeli E, Shibolet O, Zigmond E, Klein A, Hemed N, Donegan JJ, Rabbani E, Goldin E, Ilan Y. A double-blind clinical trial for treatment of Crohn's disease by oral administration of Alequel, a mixture of autologous colon-extracted proteins: a patient-tailored approach. *Am J Gastroenterol* 2006; **101**: 561-568 [PMID: 16542292 DOI: 10.1111/j.1572-0241.2006.00441.x]

17 **Israeli E**, Zigmond E, Lalazar G, Klein A, Hemed N, Goldin E, Ilan Y. Oral mixture of autologous colon-extracted proteins for the Crohn's disease: A double-blind trial. *World J Gastroenterol* 2015; **21**: 5685-5694 [PMID: 25987796 DOI: 10.3748/wjg.v21.i18.5685]

18 **Ye Y**, Yue M, Jin X, Chen S, Li Y. The effect of oral tolerance on the roles of small intestinal intraepithelial lymphocytes in murine colitis induced by dextran sodium sulfate. *Int J Colorectal Dis* 2012; **27**: 583-593 [PMID: 22246420 DOI: 10.1007/s00384-011-1354-x]

19 **Yue M**, Shen Z, Yu CH, Ye H, Ye YF, Li YM. Effects of appendectomy and oral tolerance on dextran sulfate sodium colitis. *World J Gastroenterol* 2011; **17**: 2437-2445 [PMID: 21633645 DOI: 10.3748/wjg.v17.i19.2437]

20 **Wirtz S**, Neufert C, Weigmann B, Neurath MF. Chemically induced mouse models of intestinal inflammation. *Nat Protoc* 2007; **2**: 541-546 [PMID: 17406617 DOI: 10.1038/nprot.2007.41]

21 **Dieleman LA**, Ridwan BU, Tennyson GS, Beagley KW, Bucy RP, Elson CO. Dextran sulfate sodium-induced colitis occurs in severe combined immunodeficient mice. *Gastroenterology* 1994; **107**: 1643-1652 [PMID: 7958674]

22 **Ishikawa H**, Tanaka K, Maeda Y, Aiba Y, Hata A, Tsuji NM, Koga Y, Matsumoto T. Effect of intestinal microbiota on the induction of regulatory CD25+ CD4+ T cells. *Clin Exp Immunol* 2008; **153**: 127-135 [PMID: 18460018 DOI: 10.1111/j.1365-2249.2008.03668.x]

23 **Hong C**, Gao XM. Purification and immunophenotypic characterization of murine B10 B cells. *Methods Mol Biol* 2014; **1190**: 35-44 [PMID: 25015271 DOI: 10.1007/978-1-4939-1161-5_3]

24 **Kim YS**, Lee MH, Ju AS, Rhee KJ. Th17 responses are not induced in dextran sodium sulfate model of acute colitis. *Immune Netw* 2011; **11**: 416-419 [PMID: 22346784 DOI: 10.4110/in.2011.11.6.416]

25 **Alex P**, Zachos NC, Nguyen T, Gonzales L, Chen TE, Conklin LS, Centola M, Li X. Distinct cytokine patterns identified from multiplex profiles of murine DSS and TNBS-induced colitis. *Inflamm Bowel Dis* 2009; **15**: 341-352 [PMID: 18942757 DOI: 10.1002/ibd.20753]

26 **Bonelli M**, Savitskaya A, Steiner CW, Rath E, Smolen JS, Scheinecker C. Phenotypic and functional analysis of CD4+ CD25- Foxp3+ T cells in patients with systemic lupus erythematosus. *J Immunol* 2009; **182**: 1689-1695 [PMID: 19155519 DOI: 10.4049/jimmunol.182.3.1689]

27 **Iikuni N**, Lourenço EV, Hahn BH, La Cava A. Cutting edge: Regulatory T cells directly suppress B cells in systemic lupus erythematosus. *J Immunol* 2009; **183**: 1518-1522 [PMID: 19570829 DOI: 10.4049/jimmunol.0901163]

28 **Tang Y**, Peng LP, Qin GX, Sun JT, Xu LJ, Jiang YF. CD4(+)CD25(-)Foxp3(+) T cells play a role in tuberculous hydrothorax rather than malignant hydrothorax. *J Transl Med* 2015; **13**: 268 [PMID: 26283421 DOI: 10.1186/s12967-015-0618-6]

29 **Lee SJ**, Noh G, Lee JH. In Vitro Induction of Allergen-Specific

Interleukin-10-Producing Regulatory B Cell Responses by Interferon-γ in Non-Immunoglobulin E-Mediated Milk Allergy. *Allergy Asthma Immunol Res* 2013; **5**: 48-54 [PMID: 23277878 DOI: 10.4168/aair.2013.5.1.48]

30 **Yanaba K**, Bouaziz JD, Matsushita T, Magro CM, St Clair EW, Tedder TF. B-lymphocyte contributions to human autoimmune disease. *Immunol Rev* 2008; **223**: 284-299 [PMID: 18613843 DOI: 10.1111/j.1600-065X.2008.00646.x]

31 **Yanaba K**, Yoshizaki A, Asano Y, Kadono T, Tedder TF, Sato S. IL-10-producing regulatory B10 cells inhibit intestinal injury in a mouse model. *Am J Pathol* 2011; **178**: 735-743 [PMID: 21281806 DOI: 10.1016/j.ajpath.2010.10.022]

32 **Wang L**, Ray A, Jiang X, Wang JY, Basu S, Liu X, Qian T, He R, Dittel BN, Chu Y. T regulatory cells and B cells cooperate to form a regulatory loop that maintains gut homeostasis and suppresses dextran sulfate sodium-induced colitis. *Mucosal Immunol* 2015; **8**: 1297-1312 [PMID: 25807185 DOI: 10.1038/mi.2015.20]

33 **Smigiel KS**, Srivastava S, Stolley JM, Campbell DJ. Regulatory T-cell homeostasis: steady-state maintenance and modulation during inflammation. *Immunol Rev* 2014; **259**: 40-59 [PMID: 24712458 DOI: 10.1111/imr.12170]

34 **Noh J**, Noh G, Lee SJ, Lee JH, Kim A, Kim HS, Choi WS. Tolerogenic effects of interferon-gamma with induction of allergen-specific interleukin-10-producing regulatory B cell (Br1) changes in non-IgE-mediated food allergy. *Cell Immunol* 2012; **273**: 140-149 [PMID: 22336594 DOI: 10.1016/j.cellimm.2011.12.006]

35 **Rosser EC**, Oleinika K, Tonon S, Doyle R, Bosma A, Carter NA, Harris KA, Jones SA, Klein N, Mauri C. Regulatory B cells are induced by gut microbiota-driven interleukin-1β and interleukin-6 production. *Nat Med* 2014; **20**: 1334-1339 [PMID: 25326801 DOI: 10.1038/nm.3680]

36 **Noh G**, Lee SS. A pilot study of interferon-gamma-induced specific oral tolerance induction (ISOTI) for immunoglobulin E-mediated anaphylactic food allergy. *J Interferon Cytokine Res* 2009; **29**: 667-675 [PMID: 19642905 DOI: 10.1089/jir.2009.0001]

37 **Lee JH**, Noh G, Noh J, Lee S, Choi WS, Kim HS, Lee K, Choi S, Jin H, Cho S, Lee S. Clinical characteristics of oral tolerance induction of IgE-mediated and non-IgE-mediated food allergy using interferon gamma. *Allergy Asthma Proc* 2010; **31**: e39-e47 [PMID: 20819314 DOI: 10.2500/aap.2010.31.3345]

38 **Koide N**, Sugiyama T, Mori I, Mu MM, Hamano T, Yoshida T, Yokochi T. Change of mouse CD5(+) B1 cells to a macrophage-like morphology induced by gamma interferon and inhibited by interleukin-4. *Clin Diagn Lab Immunol* 2002; **9**: 1169-1174 [PMID: 12414746 DOI: 10.1128/cdli.9.6.1169-1174.2002]

39 **Noval Rivas M**, Burton OT, Wise P, Charbonnier LM, Georgiev P, Oettgen HC, Rachid R, Chatila TA. Regulatory T cell reprogramming toward a Th2-cell-like lineage impairs oral tolerance and promotes food allergy. *Immunity* 2015; **42**: 512-523 [PMID: 25769611 DOI: 10.1016/j.immuni.2015.02.004]

40 **Kim HS**, Lee JH, Han HD, Kim AR, Nam ST, Kim HW, Park YH, Lee D, Lee MB, Park YM, Kim HS, Kim YM, You JC, Choi WS. Autocrine stimulation of IL-10 is critical to the enrichment of IL-10-producing CD40(hi)CD5(+) regulatory B cells in vitro and in vivo. *BMB Rep* 2015; **48**: 54-59 [PMID: 25341924 DOI: 10.5483/BMBRep.2015.48.1.213]

41 **Arrieta MC**, Madsen KL, Field CJ, Meddings JB. Increasing small intestinal permeability worsens colitis in the IL-10-/- mouse and prevents the induction of oral tolerance to ovalbumin. *Inflamm Bowel Dis* 2015; **21**: 8-18 [PMID: 25517593 DOI: 10.1097/MIB.0000000000000253]

42 **Madsen KL**, Malfair D, Gray D, Doyle JS, Jewell LD, Fedorak RN. Interleukin-10 gene-deficient mice develop a primary intestinal permeability defect in response to enteric microflora. *Inflamm Bowel Dis* 1999; **5**: 262-270 [PMID: 10579119 DOI: 10.1002/ibd.3780050405]

43 **Resta-Lenert S**, Smitham J, Barrett KE. Epithelial dysfunction associated with the development of colitis in conventionally housed mdr1a-/- mice. *Am J Physiol Gastrointest Liver Physiol* 2005; **289**: G153-G162 [PMID: 15774938 DOI: 10.1152/ajpgi.00395.2004]

44 **Kraus TA**, Toy L, Chan L, Childs J, Cheifetz A, Mayer L. Failure to induce oral tolerance in Crohn's and ulcerative colitis patients: possible genetic risk. *Ann N Y Acad Sci* 2004; **1029**: 225-238 [PMID: 15681761 DOI: 10.1196/annals.1309.054]

45 **Kahn DA**, Archer DC, Kelly CJ. Absence of functional inducible NO synthase enhances the efficacy of tolerance induced by high dose antigen feeding. *J Immunol* 2000; **165**: 6116-6122 [PMID: 11086044 DOI: 10.4049/jimmunol.165.11.6116]

46 **Martín MC**, Martinez A, Mendoza JL, Taxonera C, Díaz-Rubio M, Fernández-Arquero M, de la Concha EG, Urcelay E. Influence of the inducible nitric oxide synthase gene (NOS2A) on inflammatory bowel disease susceptibility. *Immunogenetics* 2007; **59**: 833-837 [PMID: 17955236 DOI: 10.1007/s00251-007-0255-1]

47 **Beck PL**, Xavier R, Wong J, Ezedi I, Mashimo H, Mizoguchi A, Mizoguchi E, Bhan AK, Podolsky DK. Paradoxical roles of different nitric oxide synthase isoforms in colonic injury. *Am J Physiol Gastrointest Liver Physiol* 2004; **286**: G137-G147 [PMID: 14665440 DOI: 10.1152/ajpgi.00309.2003]

P- Reviewer: Hokama A, Trifan A, Velin D **S- Editor**: Gong ZM
L- Editor: A **E- Editor**: Wu HL

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i2.254

World J Gastrointest Pharmacol Ther 2016 May 6; 7(2): 254-260
ISSN 2150-5349 (online)

ORIGINAL ARTICLE

Retrospective Study

Clinical utility of quantitative multi-antibody Polycheck immunoassays in the diagnosis of coeliac disease

Ewa Konopka, Maciej Grzywnowicz, Beata Oralewska, Joanna Cielecka-Kuszyk, Ilona Trojanowska, Bożena Cukrowska

Ewa Konopka, Joanna Cielecka-Kuszyk, Ilona Trojanowska, Bożena Cukrowska, Department of Pathology, the Children's Memorial Health Institute, 04-730 Warsaw, Poland

Maciej Grzywnowicz, Department of Experimental Hematooncology, Medical University of Lublin, 20-059 Lublin, Poland

Beata Oralewska, Department of Gastroenerology, Hepatology, Nutritional Disorders and Pediatrics, the Children's Memorial Health Institute, 04-730 Warsaw, Poland

Author contributions: Konopka E and Grzywnowicz M contributed equally; all authors contributed to this manuscript.

Supported by S135/2013 and 229/14 grants from the Children's Memorial Health Institute.

Institutional review board statement: The study was reviewed and approved by the Children's Memorial Health Institute Bioethical Committee Review Board.

Informed consent statement: All patient involved in the study obtained informed consent statement from the principal investigator.

Conflict-of-interest statement: Grzywnowicz M is an employee of the EMMA Paweł Majsiak company. For the remaining authors, none are declared.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at b.cukrowska@czd.pl. Informed consent for data sharing was not obtained. The presented data are anonymized and there is no risk identification.

Correspondence to: Bożena Cukrowska, Professor, Department of Pathology, the Children's Memorial Health Institute, Aleja Dzieci Polskich 20, 04-730 Warsaw, Poland. b.cukrowska@czd.pl
Telephone: +48-228-151091
Fax: +48-228-15197

Received: April 10, 2015
Peer-review started: April 11, 2015
First decision: August 1, 2015
Revised: October 13, 2015
Accepted: January 21, 2016
Article in press: January 22, 2016
Published online: May 6, 2016

Abstract

AIM: To evaluate the clinical utility of multi-antibody strategies in the diagnosis of coeliac disease (CD), the new quantitative Polycheck immunoassays were analysed.

METHODS: Polycheck Celiac Panels (PCPs) are immunoenzyme screening assays for the quantitative measurement of coeliac-specific immunoglobulin class G (IgG) or class A (IgA) in serum. Lines of relevant antigens are coated together with five IgG or IgA standard lines used for the standard curve as positive control. PCP IgA consists of human recombinant human tissue transglutaminase (tTG) and deamidated gliadin peptides (DGP) as targets to detect IgA antibodies. PCP IgG consists of tTG, DGP and IF (intrinsic factor) antigens to detect antibodies in IgG class. PCPs were performed on 50 CD patients, including 6 cases with selective IgA deficiency, and 50 non-coeliac controls. CD diagnosis was performed according to the ESPGHAN recommendations: The presence of specific anti-tTG-

IgA or anti-DGP-IgG (in the case of IgA deficiency) antibodies, typical histopathological changes in duodenal mucosa described in Marsh-Oberhüber classification as at least grade 2. The diagnosis of the majority of the control subjects was functional gastrointestinal disorders. The PCP results were compared with reference EliA Celikey.

RESULTS: The usage of PCPs led to the correct identification of all CD patients. In our study, PCPs showed 100% agreement with the histopathological results. PCP IgA test showed a 98% concordance and correlated positively (R = 0.651, *P* = 0.0014) with EliA Celikey test. The highest specificity and positive predictive value (both 100%) were observed for the detection of Polycheck anti-tTG-IgA antibodies. The highest sensitivity and negative predictive value (both 100%) were achieved by Polycheck anti-DGP-IgG antibody detection. The best performance (98% sensitivity and negative predictive value, 100% specificity and positive predictive value, diagnostic accuracy - AU ROC 99%) was observed for the strategy of using both PCP IgA and IgG and determining positive outcomes of the test with two or more coeliac-specific antibodies detected. The majority of coeliac patients had multiple antibodies. All four antibodies were detected in 7 (14%) cases, 19 children (38%) were positive for three antibodies and 23 (46%) were positive for two antibodies.

CONCLUSION: The present study showed that detection of coeliac-specific antibodies with multi-antibody PCPs is effective and efficacious in the diagnosis of CD.

Key words: Coeliac disease; Tissue transglutaminase; Deamidated gliadin peptides; Multi-antibody tests; Polycheck celiac panels

Core tip: Detection of coeliac-specific antibodies has become a useful tool in the diagnostics of coeliac disease. Different serology test combinations have been found to improve diagnosis in comparison to a single antibody test. Recently, multi-antibody strategy has been implemented in immunoassays. In this study we have found that multi-parametric quantitative Polycheck immunoassay is reliable in reference to intestinal biopsy results and measurements of anti-tissue transglutaminase-IgA by a reference method. The best overall clinical performance was obtained by a combination of both IgA and IgG panels, with two and more positively detected antibodies, to determine the outcome.

Konopka E, Grzywnowicz M, Oralewska B, Cielecka-Kuszyk J, Trojanowska I, Cukrowska B. Clinical utility of quantitative multi-antibody Polycheck immunoassays in the diagnosis of coeliac disease. *World J Gastrointest Pharmacol Ther* 2016; 7(2): 254-260 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i2/254.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i2.254

INTRODUCTION

Coeliac disease (CD) is a chronic immune-based systemic disorder caused by intolerance to dietary gluten in individuals with genetic predisposition. Gluten is a storage protein in wheat, barley, and rye, which triggers an inflammatory state in the small intestine, leading to the induction of the cytotoxic intra-epithelial lymphocytes, reduction of villus height, hyperplastic cryptae and finally to complete villus atrophy. CD is characterised by the presence of specific antibodies, including specific ones against a disease inducing factor: Deamidated gliadin peptides (DGP), as well as autoantibodies against tissue transglutaminase 2 (tTG).

Assessing the levels of serum antibodies that were applied in the diagnosis of CD for over 40 years, starting from the determination of anti-gliadin and anti-reticulin antibody levels[1]. The discovery that a major target of autoantibodies in CD is tTG, which is related to deamidation of gliadin peptides by this enzyme, allowed to better understand the pathogenic pathway of events leading to CD development[2,3]. In recent years, the usage of native gliadin as the target of serology diagnostics of CD was withdraw from the CD routine diagnosis due to inferior performance compared to a highly specific and sensitive anti-tTG tests[4]. Deamidation of gliadin peptides enhances their immunogenicity, which leads to a higher specificity and sensitivity of tests for anti-DGP-IgA and -IgG antibodies than native gliadin tests[4,5]. Studies on the performance of anti-DGP-IgG tests in the diagnosis of CD showed that it is comparable with anti-tTG-IgA tests[6,7]. In contrast to anti-tTG-IgA, anti-DGP-IgG tests are not affected by the presence of hemolysis in a tested serum sample[8] and are effective in the detection of CD in patients with selective IgA deficiency[4,9].

The European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) published, in 2012[10], clinical guidelines including algorithms of CD diagnosis with the crucial role of serology tests. According to ESPGHAN recommendations, the initial approach to patients with suspected CD includes serological screening for anti-tTG-IgA and measurement of a total IgA level to exclude selective IgA deficiency, *i.e.*, immunodeficiency which occurs in CD patients, with a relevance of 2%-8%[10]. The initial usage of anti-tTG-IgA tests is based on its both high sensitivity and specificity values[7]. In the case of confirmed selective IgA deficiency, anti-tTG-IgG, anti-DGP-IgG or anti-endomysial IgG tests are recommended to detect CD[10]. An alternative approach, especially recommended for CD screening in at risk groups, consists of direct testing for

Table 1 Characteristics of the patients

	Coeliac disease	Non-coeliac disease
No. of patients	50	50
Females	28 (56%)	29 (58%)
Males	22 (44%)	21 (42%)
Mean age in years	8.7 ± 4.7 (2.5-17.5)[2]	11.6 ± 4.8 (3-17.5)
Histopathological results[1]		
Marsh 0	0	50 (100%)
Marsh II	6 (12%)	0
Marsh III (a-c)	44 (88%)	0

[1]Biopsy results were classified according to the Marsh-Oberhüber classification[11]; [2]In the coeliac disease patients study group were 3 children ⩾ 2 years old.

anti-tTG-IgA and anti-DGP-IgG, which allows to omit total IgA testing and reduces the number of tests that are needed to be performed[10].

The methods of detection of CD-specific antibodies have been based so far on various immunoenzymatic assays allowing individual measurements of one antibody type per blood sample. Recently, Polycheck Celiac Panels (PCPs) have been introduced as a new diagnostic option in CD. PCPs represent a unique approach in measurement of coeliac-specific antibodies, by combining the detection of multiple antibodies in a single blood sample with a quantitative standard curve based immunoassay on the nitrocellulose membrane. ESPGHAN guidelines recommend a validation for every antibody test being used for CD diagnosis, by comparing the results of a novel test with results obtained from histopathological examination of small intestine specimens, and another reference serology test with high specificity and sensitivity[10]. The aim of this study was the assessment of the sensitivity, specificity and clinical utility of multi-antibody Polycheck IgA and IgG immunoassays in the diagnosis of CD in reference to histology results and the detection of anti-tTG-IgA antibodies by a reference method.

MATERIALS AND METHODS

Study design

This is a retrospective study, which was designed to investigate the sensitivity and specificity of Polycheck Celiac IgA and IgG (Biocheck, GmbH, Muenster, Germany) quantitative, multi-parametric immunoassays in the diagnosis of CD. According to ESPGHAN recommendations, test results were validated with reference methods: Intestinal biopsy and EliA Celikey IgA method for anti-tTG-IgA detection, along with another serological test with known high sensitivity and specificity (Thermo Scientific, Phadia GmbH, Freiburg, Germany). Test results were validated in a group of children with biopsy-proven CD and a control group of non-CD children.

Patients

This study enrolled 50 paediatric patients with CD and 50 non-coeliac age and sex matched control children, treated in the Children's Memorial Health Institute, Warsaw, Poland, between January 2013 and September 2014. All patients underwent intestinal biopsy during endoscopy, with the histological examination of the small intestine specimens classified according Marsh-Oberhüber scale[11]. Serum samples collected from all children and stored at -20 ℃ were used for antibody detection. CD diagnosis was performed according to the ESPGHAN recommendations: The presence of specific anti-tTG-IgA or anti-DGP-IgG (in the case of IgA deficiency) antibodies, typical histopathological changes in duodenal mucosa described in Marsh-Oberhüber classification as at least grade 2. Out of 50 children 46 were observed for at least one year after introduction of gluten free diet, and in all but one improvement of clinical syndromes and systematic decrease in specific CD antibodies were noticed. The child without serological and clinical improvement did not comply with dietary recommendations. The patients's characteristics are presented in Table 1. Out of 50 CD patients, the selective IgA deficiency was detected in 6 children (12%). The diagnosis of the majority of the control subjects was functional gastrointestinal disorders. Two control cases were classified as inflammatory bowel disease. Written, informed consent was obtained from all patients with respect to the use of their blood for scientific purposes.

Detection of antibody by single-antibody immunoassay

The fluoroimmunoassay Elia Celikey IgA, and for patients with IgA deficiency EliA Gliadin DP IgG kits (Thermo Scientific, Phadia GmbH, Freiburg, Germany) were used for the detection of anti-tTG-IgA and anti-DPG-IgG antibodies. Single, well-based immunoassays were performed according to the manufacturer's protocols using an automated Thermo Scientific Phadia 100 system (Freiburg, Germany). The antibody level > 10 U/mL was considered positive.

PCPs

PCPs are immunoassays designed as nitrocellulose membrane strips with different antigens placed in individual lines. Polycheck Panel IgA consists of human recombinant tTG and DGP antigen lines as targets to detect IgA antibodies. Polycheck Panel IgG consists of human recombinant tTG, DGP and IF (intrinsic factor) antigens to detect antibodies in IgG class. Antibody detection by Polycheck Panels were performed according to the manufacture's protocol. Briefly, patients sera, diluted at 1:100, were incubated for 45 min at room temperature. In the next step, anti-human-IgG or -IgA monoclonal detection antibodies were added for 30 min. Finally, the substrates (5'bromo-4'chloro-3' indolylphosphate/4' nitro-bluetetrazolium; BCIP/NBT) were added for 20 min, and the colour intensity of the specific lines corresponding to antibody concentration was scanned and the result was calculated according to the calibrator curve present in each cassette. For

Table 2 Statistical sensitivity and specificity of Polycheck Celiac IgA and IgG tests for single antibody and for selected multi-antibody combinations

Specific coeliac antibody	Sensitivity %	Specificity %	PPV %	NPV %	AU ROC	LR+[2]	LR-
Single antibody positivity							
Anti-tTG-IgA[1]	97.7%	100.0%	100.0%	98.0%	98.9%	-	0.023
Anti-tTG-IgG	48.0%	98.0%	96.0%	65.3%	73.0%	24.000	0.531
Anti-DPG-IgA[1]	34.1%	98.0%	93.8%	62.8%	68.1%	17.045	0.673
Anti-DPG-IgG	100.0%	96.0%	96.2%	100.0%	98.0%	25.000	0.000
Combination of two or more positive antibodies							
Anti-tTG-IgA/-IgG + anti-DGP-IgG/-IgA	98.0%	100.0%	100.0%	98.0%	99.0%	-	0.020

[1]Calculations after excluding patients with selective IgA deficiency; [2]For strategies, where specificity of a test/combination was 100%, the likehood ratio for a positive result could not be calculated. PPV: Positive predictive value; NPV: Negative predictive value; AU ROC: Area under a receiving operator characteristic curve; LR+: Likehood ratio for a positive result; LR-: Likehood ratio for a negative result.

the quantification of antibody concentrations, Biocheck Imaging Software (BIS) was used. The antibody concentration > 0.8 kU/L was considered as positive. The assays have an equivocal range defined, 0.3-0.8 kU/L. For statistical purposes, equivocal results were considered as negative in the analysis.

Statistical analysis

The diagnostic performance of Polycheck serological tests was determined by calculating the sensitivity, specificity, positive and negative predictive values (PPVs and NPVs), areas under the receiving operator characteristic curves (AU ROC) and likehood ratios (LR). Data were analysed using Statistica 10 software (StatSoft, Poland). Correlations of results were computed with the Spearman rank correlation coefficient.

RESULTS

Sensitivity and specificity of antibody tests on panels

We analysed for the each antibody test: Sensitivity, specificity, PPV, NPV, AU ROC, and likehood ratios for positive (LR+) and negative (LR-) results. Statistical performance of antibody tests is presented in Table 2.

The highest specificity and PPV (both 100%) were observed for the detection of anti-tTG-IgA antibodies. The highest sensitivity and NPV (both 100%) were calculated for anti-DGP-IgG antibodies detection. Anti-tTG-IgG and anti-DGP-IgA were specific (both 98% of specificity); however, they presented low sensitivity (48% and 30% respectively). Diagnostic accuracy determined by the AU ROC curve value for anti-DGP-IgG was 98%, for anti-tTG-IgA 93%, for anti-tTG-IgG 73% and for anti-DGP-IgA 64%.

Considering clinical value of tests, LR+ was significant for all detected antibodies detected antibodies. Due to 100% specificity, LR+ for anti-tTG-IgA was incalculable, however, indicating the highest value of performing a diagnostic test.

Two panels combination

Since PCPs were designed as multi-antibody assays, we verified the statistical value of them in the combination of two panels. Detection of anti-tTG (IgA and IgG) and anti-DGP (IgA and IgG) in the combination of both PCPs when two or more antibodies were positive showed the best statistical performance among the analysed tests (individual or in combination). The 98% of sensitivity and NPV, and 100% of specificity and PPV resulted in excellent diagnostic accuracy (AU ROC 99%). The value for LR+ was incalculable due to 100% specificity, which pointed the best reliability of the positive result. For the negative result LR- was 0.02. Values for the combinations are summarised in Table 2.

Validation against the reference

Polycheck tTG-IgA results showed significant correlation with EliA Celikey IgA results: R = 0.651, P = 0.0014 (n = 21). Both assays matched anti-tTG-IgA positive results in 43 out of 44 cases (98% agreement). One of the children, who had positive anti-tTG-IgA antibodies measured with the EliA Celikey IgA kit, was negative for the Polycheck IgA immunoassay, but the level of anti-tTG-IgA was 0.48, which is a borderline value for PCPs. Simultaneously, this child had the single positive result for anti-DGP-IgG antibody, determined by Polycheck IgG. All children with biopsy-proven CD have positive antibodies detected with PCPs (100% agreement with the histopathological results).

Antibody profiles in the study group

The majority of coeliac patients had multiple antibodies detected. Selective IgA deficiency has been described for 6 out of 50 CD patients. All four antibodies were detected in 7 (14%) cases, 19 children (38%) were positive for three antibodies and 23 (46%) were positive for two antibodies. Only the patients with a normal IgA level had single positive anti-DGP-IgG; however, anti-tTG-IgA was borderline in these cases. All CD patients had positive anti-DGP-IgG antibodies. All but one CD patients with normal IgA level had positive anti-tTG-IgA. All 6 children with selective IgA deficiency had both positive anti-DGP-IgG and anti-tTG-IgG. In a non-coeliac control group, 4 children had single positive results, but none had multiple antibodies detected. Summary of obtained antibody profiles for both CD and

non-CD controls are presented in Table 3.

DISCUSSION

The traditional golden standard in the diagnosis of CD is the intestinal biopsy. However, morphological changes of intestinal mucosa are not CD-specific and they might be caused by other pathological conditions[12]. The biopsy is an invasive procedure, and the histopathological results are strongly dependent on the experience of the pathologist[13,14]. Therefore, usage of serology tests to detect coeliac-specific antibodies has been increasing simultaneously with the improvement of the methodology, *i.e*., replacing non-human tTG with a human recombinant tTG and introduction of DGP.

This study was retrospective with preselected patients with biopsy proven CD and with positive single anti-tTG-IgA or anti-DPG-IgG antibodies, which were used as references to validate the performance of any new immunoassays. PCPs detect CD specific multi-antibodies and follow ESPGHAN recommendations stating that novel anti-tTG and anti-DGP tests should produce quantitative, numerical values, expressed in arbitrary units[10]. Obtained results show that PCPs fulfil ESPGHAN requirements by achieving 98% agreement with the reference Elia Celikey IgA test and 100% agreement with biopsy results when using Polycheck Celiac IgG and IgA tests alone, or in combination. There was one discrepancy in the anti-tTG-IgA testing, and this was where a CD child had negative anti-tTG-IgA results with the Polycheck IgA, but positive with the EliA Celikey IgA test. However, the result, classified as negative, has fitted the equivocal range, and this patient had the positive anti-DGP-IgG antibodies. Therefore, the child would have been correctly diagnosed as coeliac-positive with combination of both IgA and IgG PCPs.

Recently, a limited number of strategies using different combinations of tests detecting simultaneously more than the single coeliac-specific antibody were developed in an attempt to achieve better clinical performance, and to define applicative approaches, allowing the omission of a biopsy during CD diagnosis[15-17]. PCPs are the multi-antibody detecting system, therefore, their performance could be considered in several ways. Each panel measures either IgA or IgG specific coeliac-antibodies from a single serum sample, which is a novel approach, not utilising any combinations of separate tests, *e.g.*, immunoenzymatic tests[4]. Our study has shown that the detection of anti-tTG-IgA and anti-DGP-IgG has the best performance in the diagnosis of CD, and that anti-DGP-IgG has a comparable diagnostic value as anti-tTG-IgA. This result is concordant with earlier studies[5,6,16,17]. The diagnostic accuracy of anti-tTG-IgG was better than anti-DGP-IgA, which is concordant with previously made meta-analysis[7]. Considering the overall characteristics, the best performance was observed for the combination of two PCPs (4 antibodies: Anti-tTG-IgA/-IgG and anti-DGP-IgA/-IgG) with double or more positive tests required to determine a positive results. This strategy showed excellent 98% sensitivity and NPV, 100% specificity and PPV, with overall diagnostic accuracy of 99%. Our results were comparable with previous studies, showing that a combination of more than one antibody test creates the better CD diagnostic opportunity than single antibody testing[15-17]. Multi-antibody testing might lead to the lower sensitivity in exchange for higher specificity[4,16,17]; however, in this study, the multi-antibody strategy achieved still higher sensitivity (98%), which is only slightly reduced sensitivity when compared to the anti-DGP-IgG test. Multiple strategies with any positive antibody used in determination of positivity were expected to have a lower specificity and higher sensitivity than any single antibody test[4], which was observed in this study as well. Our results indicate that the most beneficial for CD diagnosis is multi-antibody testing with two and more positive antibodies.

The IgA deficiency is a CD associated condition more common among CD patients than in the general population, therefore, we found it to be suitable to include IgA deficient patients in the characterised study group. The prevalence of selective IgA deficiency was 12% in our CD patients. The observed high sensitivity of anti-tTg-IgA (97.7%) decreased to 86% after including IgA deficient patients (data not shown). The significant advantage of the presented multi-antibody combination was the highest diagnostic accuracy without discriminating on normal and IgA deficient patients. Performing simultaneous multi- antibody detection in both IgA and IgG classes might provide one-step, time-saving diagnosis of CD, independent from selective IgA deficiency.

In this study, we have calculated the likehood ratios for CD in the studied population. The LR for the positive results is the ratio of the probability of a coeliac-positive patient acquiring a positive test result to the probability that non-coeliac patients acquires positive test results, while LR for the negative result describes the opposite situation. It was pointed that LRs are useful in the clinical interpretation of CD antibody tests, since they are independent from the prevalence of CD[17]. We found that the highest LR was observed for double positive tests strategies; however, the precise value could not be calculated because of 100% of PPV in those combinations. The lowest LR for negative results was obtained for double negative tests strategies with the IgG panel alone or quadruple negative tests in combination. Similarly, Vermeersch *et al*[17], observed the highest LR for the positive result in CD testing for the strategy with double positive outcomes for coeliac antibodies, and the lowest LR for the negative result for double negative tests. Sugai *et al*[16] described the comparable test results for LR for positive results; however, for CD exclusion, the lower LR was observed for triple negative than for double negative results.

ESPGHAN guidelines[10] allow, under certain circumstances, to omit a biopsy as a confirmatory procedure and to base it on the serology results and genetic background

Table 3 Antibody profile in celiac disease patients and non-celiac disease controls

Anti-tTG-IgA	Anti-tTG-IgG	Anti-DGP-IgA	Anti-DGP-IgG	CD patients *n* = 50	Non-CD controls *n* = 50	Total *n* = 100	Classification in combination (anti-tTG-IgA/IgG + anti-DGP-IgG/IgA)
+	+	+	+	7 (14%)	0	7	49 positives
+	+	-	+	11 (22%)	0	11	
+	-	+	+	8 (16%)	0	8	
+	-	-	+	17 (34%)	0	17	
-	+	-	+	6[1] (12%)	0	6	
-	-	-	+	1 (2%)	2 (4%)	3	5 not classified
-	-	+	-	0	1 (2%)	1	
-	+	-	-	0	1 (2%)	1	
-	-	-	-	0	46 (92%)	46	46 negatives

[1]Patients with confirmed selective IgA deficiency. +: Antibody present; -: Antibody absent; Using the combination of four antibodies (anti-tTG-IgA/IgG + anti-DGP-IgG/IgA) classified 49 children as CD positive, 46 as CD negative, and 5 were neither classified as CD positive nor negative with further verification needed. CD: Celiac disease; DGP: Deamidated gliadin peptides; tTG: Tissue transglutaminase.

in CD diagnosis. Recently, several studies have evaluated the performances of different combinations of antibody tests and their utility in avoiding intestinal biopsies[15-17]. It was found that multi-antibody screening in CD could be successfully used to increase the overall performance of serology diagnostics[15-17]. Our aim was to assess how quantitative, screening Polycheck immunoassays would perform in the diagnosis of childhood CD, since that approach might became more popular in practice. In this study, we have shown that the significant majority of CD children (49/50) have more than one positive coeliac-specific antibody, and that 46 out 50 children with no CD have been recorded as negative for all four antibodies (Table 3), and the best diagnostic accuracy was achieved with the combination of two or more positive antibodies. With defining an outcome as positive with two or more detected antibodies, and as negative with all four non-detected antibodies, it could allow to avoid 98% of intestinal biopsies with no missed CD cases in the analysed study group. Sugai *et al*[16], showed that combinations of two different assays would allow the avoidance of 92%-98.7% of all intestinal biopsies in the high-risk group, and 92.1%-99% in the low-risk group (3-5 missed CD cases), which is compatible with our findings. It is worth emphasising that no CD case was missed with the combination presented in this study. Bürgin-Wolff *et al*[15], with usage of three assays (anti-tTG-IgA + anti-DGP-IgG/-IgA) would avoid 78% of biopsies; however, IgA-deficient cases were excluded in that study. The combination presented in this study could be used regardless of IgA deficiency. The limitation of this study is the preselection of the study group; therefore, future validations of the presented strategy are required.

In conclusion, we found Polycheck Celiac IgA and IgG panels to be reliable immunoassays in CD diagnostics. Both panels presented very good clinical performance, which was even better in the combination with double or more detected antibodies as positive results, meeting criteria set by the ESPGHAN guidelines[10]. They showed 100% agreement with biopsy results and provide numerical, quantitative results. The detection of IgG coeliac antibodies by PCPs, especially with the well-defined performance of anti-DPG-IgG, determined high clinical utility in the group of patients including cases with selective IgA deficiency. The combination of both panels is reliable and effective in CD diagnosis, regardless of any selective IgA deficiency.

COMMENTS

Background

Serological testing of coeliac specific antibodies: Anti-tissue transglutaminase (tTG) and deamidated gliadin peptide [deamidated gliadin peptides (DGP) immunoglobulin A (IgA) and immunoglobulin G (IgG) antibodies] has become increasingly important for the diagnosis of coeliac disease (CD), starting to rival the biopsy results. The discovery that a major target of autoantibodies in CD is tTG, which is related to deamidation of gliadin peptides by this enzyme, allowed to understand better the pathogenic pathway of events leading to CD development. Introduction of the human tissue transglutaminase into diagnostic methods was the major breakthrough in the diagnosis of CD. In recent years, the usage of native gliadin as the target of serology diagnostics of CD was withdraw from the CD routine diagnosis due to inferior performance compared to a highly specific and sensitive tests anti-tTG IgA and anti-deamidated gliadin IgG. It was found that anti-DGP of the IgG class might be useful for the identification of coeliac disease in children under 2-3 years of age and in patients with IgA deficiency. The European Society for Paediatric Gastroenterology, Hepatology, and Nutrition published in 2012 clinical guidelines pointed that the serology diagnostic of coeliac disease is crucial in determination of following diagnostic steps. It also allows to omit the biopsy under certain conditions based only on coeliac specific antibodies and genetic background. Finally, it points an alternative approach, especially recommended for CD screening in at risk groups, consisting direct testing for anti-tTG-IgA and anti-DGP-IgG, what allows to omit total IgA testing and reduces a number of tests needed to perform.

Research frontiers

Since accuracy of coeliac-specific serological tests have improved and multi-antibody approaches have occurred, the effectives of such strategies is the focus of up-to-date studies, often as potential alternative to biopsy results. Additionally, the evaluation of clinical use of new markers such as anti-DGP is the important part of the research field.

Innovations and breakthroughs

The aim of this study was the evaluation of the clinical utility of new multi-antibody quantitative Polycheck Celiac Panels immunoassays in the diagnosis of CD, since several strategies detecting coeliac-specific antibodies are being recently investigated. The study has shown concordant with earlier studies results that the detection of anti-tTG-IgA and anti-DGP-IgG has the best performance in the diagnosis of CD, and that anti-DGP-IgG has a comparable diagnostic value as

anti-tTG-IgA. In study, the best performance was observed for the combination of two polycheck celiac panels (4 antibodies: anti-tTG-IgA/-IgG and anti-DGP-IgA/-IgG) with double or more positive tests required to determine a positive results. This strategy showed excellent 98% sensitivity and NPV, 100% specificity and PPV, with overall diagnostic accuracy of 99%. These results were comparable with previous studies, showing that a combination of more than one antibody test creates the better CD diagnostic opportunity than single antibody testing.

Applications

The use of multi-antibody strategy, like use of polycheck celiac panels, which consists coeliac-antibodies in both IgG and IgG classes, could be beneficial in patients with coexistent to CD selective IgA deficiency. In contrary to cascade approach, multiple serological tests might increase the sensitivity of the diagnostic strategy and lead to faster diagnosis, since serology several markers are tested simultaneously.

Terminology

DGP: Gliadin peptides deamidated by tissue transglutaminase 2 enzyme, which leads to great increase of their immunogenicity; Multi-parametric, multi-antibody serology tests: The tests which allows to measure more than one coealiac-specific antibody from one serum sample in a single measurement test; Quantitative test: Test which produces the quantitative results with exact concentration of antibodies being measured.

Peer-review

In this study the authors have explored the utility of quantitative multi-antibody Polycheck immunoassays in the diagnosis of celiac disease.

REFERENCES

1 **Seah PP**, Fry L, Rossiter MA, Hoffbrand AV, Holborow EJ. Anti-reticulin antibodies in childhood coeliac disease. *Lancet* 1971; **2**: 681-682 [PMID: 4105712 DOI: 10.1016/S0140-6736(71)92248-3]

2 **Dieterich W**, Ehnis T, Bauer M, Donner P, Volta U, Riecken EO, Schuppan D. Identification of tissue transglutaminase as the autoantigen of celiac disease. *Nat Med* 1997; **3**: 797-801 [PMID: 9212111 DOI: 10.1038/nm0797-797]

3 **Jabri B**, Sollid LM. Tissue-mediated control of immunopathology in coeliac disease. *Nat Rev Immunol* 2009; **9**: 858-870 [PMID: 19935805 DOI: 10.1038/nri2670]

4 **Rashtak S**, Ettore MW, Homburger HA, Murray JA. Comparative usefulness of deamidated gliadin antibodies in the diagnosis of celiac disease. *Clin Gastroenterol Hepatol* 2008; **6**: 426-432; quiz 370 [PMID: 18304884 DOI: 10.1016/j.cgh.2007.12.030]

5 **Prause C**, Ritter M, Probst C, Daehnrich C, Schlumberger W, Komorowski L, Lieske R, Richter T, Hauer AC, Stern M, Uhlig HH, Laass MW, Zimmer KP, Mothes T. Antibodies against deamidated gliadin as new and accurate biomarkers of childhood coeliac disease. *J Pediatr Gastroenterol Nutr* 2009; **49**: 52-58 [PMID: 19465869 DOI: 10.1097/MPG.0b013e318195dae3]

6 **Sugai E**, Vázquez H, Nachman F, Moreno ML, Mazure R, Smecuol E, Niveloni S, Cabanne A, Kogan Z, Gómez JC, Mauriño E, Bai JC. Accuracy of testing for antibodies to synthetic gliadin-related peptides in celiac disease. *Clin Gastroenterol Hepatol* 2006; **4**: 1112-1117 [PMID: 16860613 DOI: 10.1016/j.cgh.2006.05.004]

7 **Lewis NR**, Scott BB. Meta-analysis: deamidated gliadin peptide antibody and tissue transglutaminase antibody compared as screening tests for coeliac disease. *Aliment Pharmacol Ther* 2010; **31**: 73-81 [PMID: 19664074 DOI: 10.1111/j.1365-2036.2009.04110.x]

8 **Arguelles-Grande C**, Norman GL, Bhagat G, Green PH. Hemolysis interferes with the detection of anti-tissue transglutaminase antibodies in celiac disease. *Clin Chem* 2010; **56**: 1034-1036 [PMID: 20224046 DOI: 10.1373/clinchem.2009.141242]

9 **Villalta D**, Tonutti E, Prause C, Koletzko S, Uhlig HH, Vermeersch P, Bossuyt X, Stern M, Laass MW, Ellis JH, Ciclitira PJ, Richter T, Daehnrich C, Schlumberger W, Mothes T. IgG antibodies against deamidated gliadin peptides for diagnosis of celiac disease in patients with IgA deficiency. *Clin Chem* 2010; **56**: 464-468 [PMID: 20022984 DOI: 10.1373/clinchem.2009.128132]

10 **Husby S**, Koletzko S, Korponay-Szabó IR, Mearin ML, Phillips A, Shamir R, Troncone R, Giersiepen K, Branski D, Catassi C, Lelgeman M, Mäki M, Ribes-Koninckx C, Ventura A, Zimmer KP. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. *J Pediatr Gastroenterol Nutr* 2012; **54**: 136-160 [PMID: 22197856 DOI: 10.1097/MPG.0b013e31821a23d0]

11 **Oberhuber G**, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. *Eur J Gastroenterol Hepatol* 1999; **11**: 1185-1194 [PMID: 10524652 DOI: 10.1097/00042737-199910000-00019]

12 **Husby S**, Murray JA. Diagnosing coeliac disease and the potential for serological markers. *Nat Rev Gastroenterol Hepatol* 2014; **11**: 655-663 [PMID: 25266110 DOI: 10.1038/nrgastro.2014.162]

13 **Collin P**, Kaukinen K, Vogelsang H, Korponay-Szabó I, Sommer R, Schreier E, Volta U, Granito A, Veronesi L, Mascart F, Ocmant A, Ivarsson A, Lagerqvist C, Bürgin-Wolff A, Hadziselimovic F, Furlano RI, Sidler MA, Mulder CJ, Goerres MS, Mearin ML, Ninaber MK, Gudmand-Høyer E, Fabiani E, Catassi C, Tidlund H, Alainentalo L, Mäki M. Antiendomysial and antihuman recombinant tissue transglutaminase antibodies in the diagnosis of coeliac disease: a biopsy-proven European multicentre study. *Eur J Gastroenterol Hepatol* 2005; **17**: 85-91 [PMID: 15647647 DOI: 10.1097/00042737-200501000-00017]

14 **Mubarak A**, Nikkels P, Houwen R, Ten Kate F. Reproducibility of the histological diagnosis of celiac disease. *Scand J Gastroenterol* 2011; **46**: 1065-1073 [PMID: 21668407 DOI: 10.3109/00365521.2011.589471]

15 **Bürgin-Wolff A**, Mauro B, Faruk H. Intestinal biopsy is not always required to diagnose celiac disease: a retrospective analysis of combined antibody tests. *BMC Gastroenterol* 2013; **13**: 19 [PMID: 23343249 DOI: 10.1186/1471-230X-13-19]

16 **Sugai E**, Moreno ML, Hwang HJ, Cabanne A, Crivelli A, Nachman F, Vázquez H, Niveloni S, Argonz J, Mazure R, La Motta G, Caniggia ME, Smecuol E, Chopita N, Gómez JC, Mauriño E, Bai JC. Celiac disease serology in patients with different pretest probabilities: is biopsy avoidable? *World J Gastroenterol* 2010; **16**: 3144-3152 [PMID: 20593499 DOI: 10.3748/wjg.v16.i25.3144]

17 **Vermeersch P**, Geboes K, Mariën G, Hoffman I, Hiele M, Bossuyt X. Serological diagnosis of celiac disease: comparative analysis of different strategies. *Clin Chim Acta* 2012; **413**: 1761-1767 [PMID: 22771970 DOI: 10.1016/j.cca.2012.06.024]

P- Reviewer: Makharia GK **S- Editor**: Ji FF **L- Editor**: A **E- Editor**: Wu HL

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i2.261

World J Gastrointest Pharmacol Ther 2016 May 6; 7(2): 261-267
ISSN 2150-5349 (online)

ORIGINAL ARTICLE

Retrospective Study

Factors associated with visceral fat accumulation in the general population in Okinawa, Japan

Shingo Arakaki, Tatsuji Maeshiro, Akira Hokama, Kunikazu Hoshino, Shuichi Maruwaka, Miwa Higashiarakawa, Gretchen Parrott, Tetsuo Hirata, Kozen Kinjo, Jiro Fujita

Shingo Arakaki, Tatsuji Maeshiro, Kunikazu Hoshino, Shuichi Maruwaka, Miwa Higashiarakawa, Gretchen Parrott, Tetsuo Hirata, Jiro Fujita, Department of Infectious, Respiratory, and Digestive Medicine, University of the Ryukyus, Nishihara, Okinawa 903-0215, Japan

Akira Hokama, Department of Endoscopy, University of the Ryukyus, Nishihara, Okinawa 903-0215, Japan

Kozen Kinjo, Okinawa Health Promotion Foundation, Haebaru, Okinawa 903-0215, Japan

Author contributions: Arakaki S designed, performed the research and drafted the manuscript; Maeshiro T, Hoshino K, Maruwaka S, Higashiarakawa M, Parrott G, Hirata T and Kinjo K provided technical support; Hokama A and Parrott G reviewed the manuscript; Hokama A and Fujita J supervised the project.

Institutional review board statement: The study design was approved by the Ethics Committee of University of the Ryukyus, Okinawa, Japan.

Informed consent statement: All subjects provided written informed consent for the use of their anonymized data for an epidemiological study.

Conflict-of-interest statement: We have no conflict of interest.

Data sharing statement: No additional data available.

Correspondence to: Akira Hokama, MD, PhD, Clinical Professor, Department of Endoscopy, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-0215, Japan. hokama-a@med.u-ryukyu.ac.jp
Telephone: +81-98-8951144
Fax: +81-98-8951414

Received: January 18, 2016
Peer-review started: January 19, 2016
First decision: February 22, 2016
Revised: February 26, 2016
Accepted: March 14, 2016
Article in press: March 16, 2016
Published online: May 6, 2016

Abstract

AIM: To investigate the clinical and biochemical factors associated with visceral fat accumulation in the general population.

METHODS: We enrolled 1004 subjects who underwent a medical health checkup between April 2008 and March 2009. The medical health checkup included the following tests: Height, body weight, waist circumference (WC), systolic blood pressure, diastolic blood pressure, urinalysis, blood-cell counts, blood chemistry, electrocardiography, chest radiography, and abdominal computed tomography (CT) for visceral fat accumulation. The patients' medical history and lifestyle factors were collected privately by nurses using a self-administered questionnaire, and they included questions regarding physical activity, sleep duration, dietary habits, smoking, and alcohol consumption. Visceral fat area (VFA) was defined as the sum of the intraperitoneal fat area at the level of the umbilicus with CT density in the range of -150 to -50 Hounsfield units.

RESULTS: The mean age and body mass index (BMI) of the study subjects were 57.0 years and 24.4 kg/m^2. In both male and females, VFA was significantly and

positively correlated with WC (r = 0.532, P < 0.01; r = 0.612, P < 0.01). Subjects with high levels of VFA were primarily male with significantly higher age, height, body weight, BMI, systolic blood pressure (BP), diastolic BP, and hemoglobin in all subjects (P < 0.05). A multivariate logistic regression analysis revealed that VFA had a positive relationship with age ⩾ 56, BMI ⩾ 25 kg/m^2, and triglyceride level ⩾ 149 in males (P < 0.05), whereas it had a positive relationship with age ⩾ 58, BMI ⩾ 24.4 kg/m^2, high-density lipoprotein cholesterol level < 40 mg/dL, and current drinking in females (P < 0.05).

CONCLUSION: These results suggest that gender differences exist in the clinical and biochemical parameters associated with visceral fat accumulation.

Key words: Visceral fat accumulation; Computed tomography; Metabolic syndrome; Alcohol consumption; Waist circumference

Core tip: Although close association between visceral fat accumulation and metabolic syndrome has been established, little is known about what clinical and biochemical parameters affect visceral fat accumulation. We analyzed the clinical and biochemical parameters of the health checkup subjects and assessed the visceral fat area (VFA). A multivariate logistic regression analysis revealed that VFA had a positive relationship with age, body mass index (BMI), and triglyceride level in males, whereas it had a positive relationship with age, BMI, and current drinking in females. These results suggest that gender differences exist in the clinical and biochemical parameters associated with visceral fat accumulation.

Arakaki S, Maeshiro T, Hokama A, Hoshino K, Maruwaka S, Higashiarakawa M, Parrott G, Hirata T, Kinjo K, Fujita J. Factors associated with visceral fat accumulation in the general population in Okinawa, Japan. *World J Gastrointest Pharmacol Ther* 2016; 7(2): 261-267 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i2/261.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i2.261

INTRODUCTION

Visceral fat accumulation is closely related to atherogenic disorders and metabolic syndrome (MS), including diabetes mellitus, hypertension, and dyslipidemia[1]. It also leads to obesity-related complications. MS further increases the risks for cardiovascular diseases and thus is an important therapeutic target. Although computed tomography (CT) has been applied widely as the gold standard method to evaluate visceral fat accumulation[2], little is known about what clinical and biochemical parameters affect visceral fat accumulation. The aim of this study is to investigate the clinical and biochemical parameters potentially associated with visceral fat accumulation.

MATERIALS AND METHODS

Study population

We searched the database to find 1151 subjects who underwent abdominal fat CT scans for visceral fat area (VFA), and blood tests for a routine health checkup between 1 April 2008 and 31 March 2009 at Okinawa Health Promotion Foundation, Okinawa, Japan. The health checkups were provided as part of a medical health initiative to promote public health through the early detection of chronic diseases and the evaluation of associated underlying risk factors. Subjects were included if they fulfilled the following criteria: (1) absence of markers for hepatitis B virus infection [hepatitis B surface antigen (HBsAg)] and hepatitis C virus (HCV) infection (anti-HCV antibodies); and (2) absence of excess drinking of alcohol defined as consumption of > 280 g/wk. Among the 1151 subjects, 147 subjects were positive for either HBsAg or anti-HCV, or were defined as excess drinkers. The data for the remaining 1004 people were included in the analysis. All subjects provided written informed consent for the use of their anonymized data for an epidemiological study. The study design was approved by the Ethics Committee of University of the Ryukyus. The study was conducted in accordance with the Declaration of Helsinki.

Measurements of clinical and laboratory parameters

The medical health checkup included the following tests: Height, body weight, waist circumference (WC), systolic blood pressure (SBP), diastolic BP (DBP), urinalysis, blood-cell counts, blood chemistry, electrocardiography, chest radiography, and abdominal CT for VFA. The patients' medical history and lifestyle factors were collected privately by nurses using a self-administered questionnaire, and they included questions regarding physical activity, sleep duration, dietary habits, smoking, and alcohol consumption. For our study purposes, individuals who consumed at least one alcoholic beverage per week were defined as a "current drinker". Patients who reported alcohol consumption of > 280 g/wk were identified as "excess drinkers"[3]. Alcohol consumption was evaluated by asking the participants about the amount and type of alcoholic beverages they consumed per week, an estimated total alcohol intake was calculated in grams. Blood samples were taken after > 10 h of overnight fasting. Laboratory tests were performed with standard laboratory methods and included measurements of hemoglobin (HGB), platelet count (PLT), aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transferase, alkaline phosphatase, cholinesterase, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), triglycerides (TG), fasting plasma glucose, glycosylated hemoglobin

Table 1 Baseline characteristics of the 1004 subjects in this study

Gender (M/F)	540/464
Age (yr)	55.6 ± 11.6
Height (cm)	159.7 ± 9.1
Body weight (kg)	63.8 ± 11.7
BMI (kg/m^2)	24.9 ± 3.4
WC (cm)	88.0 ± 8.4
SBP (mmHg)	123.1 ± 14.5
DBP (mmHg)	76.9 ± 9.6
VFA (cm^2)	99.3 ± 50.5
HGB (g/dL)	14.3 ± 1.4
PLT (× 10^4/μL)	22.2 ± 4.9
AST (IU/L)	23.1 ± 8.3
ALT (IU/L)	25.4 ± 16.0
GGT (IU/L)	34.6 ± 30.7
ALP (IU/L)	226.1 ± 64.2
ChE (IU/L)	355.1 ± 65.9
TC (mg/dL)	206.5 ± 31.7
LDL-C (mg/dL)	126.1 ± 29.0
HDL-C (mg/dL)	56.0 ± 13.2
TG (mg/dL)	121.0 ± 74.3
FPG (g/dL)	99.7 ± 16.1
HbA1c (%)	5.3 ± 0.5
UA (mg/dL)	5.73 ±1.4
Alcohol consumption (non/current drinkers)	561/443

Data are expressed as means ± standard deviation or numbers where appropriate. BMI: Body mass index; WC: Waist circumference; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; VFA: Visceral fat area; HGB: Hemoglobin; PLT: Platelet; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: γ-glutamyl transferase; ALP: Alkaline phosphatase; ChE: Cholinesterase; TC: Total cholesterol; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol; TG: Triglycerides; FPG: Fasting blood glucose; HbA1c: Hemoglobin A1c; UA: Uric acid.

A1c, serum uric acid, HBsAg, and anti-HCV.

Measurement of visceral fat areas

Abdominal visceral fat distribution was assessed with a single-slice CT image taken at the level of the umbilicus by a helical CT scanner (Siemens, Germany). The area of visceral fat was defined as the sum of the intraperitoneal fat area with CT density in the range of -150 to -50 Hounsfield units.

Statistical analysis

Descriptive statistics (means and standard deviations) were calculated for all continuous variables. Differences between the two groups were compared using χ^2 test. The comparisons of continuous variables between the 2 groups were performed using the Student *t* test. The linear association of WC and VFA was evaluated by the Spearman's rank correlation. The statistical analyses were performed using SPSS 19.0 (SPSS Inc, Chicago, IL, United States). Statistical significance was achieved at $P < 0.05$.

RESULTS

Clinical and biochemical characteristics

The clinical and biochemical characteristics of 1004 subjects are summarized in Table 1. The subjects were predominantly middle-aged (55.6 ± 11.6 years; range 25-88 years), and 53.8% were male. The mean BMI of all subjects was 24.9 ± 3.4 kg/m^2, and 44.8% of the subjects met the criteria for obesity (BMI ⩾ 25 kg/m^2).

Table 2 Clinical parameters associated with visceral fat accumulation of all subjects by univariate analysis

	VFA ⩾ 100 (n = 459)	VFA < 100 (n = 545)	P value
Gender (M/F)	384/111	192/353	< 0.001
Age (yr)	56.9 ± 11.5	54.5 ± 11.5	0.319
Height (cm)	162.61 ± 8.6	157.3 ± 8.8	< 0.001
Body weight (kg)	70.1 ± 11.1	58.5 ± 9.4	< 0.001
BMI (kg/m^2)	26.5 ± 3.4	23.6 ± 2.6	< 0.001
WC (cm)	91.9 ± 7.9	84.8 ± 7.3	< 0.001
SBP(mmHg)	126.5 ± 13.6	120.3 ± 14.6	< 0.001
DBP(mmHg)	79.1 ± 9.2	75.0 ± 9.5	< 0.001
HGB (g/dL)	14.8 ± 1.2	13.9 ± 1.3	< 0.001
PLT (× 10^4/μL)	22.0 ± 4.9	23.3 ± 8.5	0.24
AST (IU/L)	22.8 ± 7.9	25.4 ± 10.2	0.32
ALT (IU/L)	24.6 ± 15.2	25.9 ± 16.7	0.19
GGT (IU/L)	32.5 ± 27.3	36.3 ± 33.3	0.048
ALP (IU/L)	226.4 ± 62.6	225.9 ± 65.7	0.88
ChE (IU/L)	357.8 ± 67.2	352.8 ± 64.7	0.23
TC (mg/dL)	208.5 ± 31.7	204.9 ± 31.7	0.07
LDL-C (mg/dL)	126.8 ± 29.6	125.4 ± 28.6	0.45
HDL-C (mg/dL)	56.5 ± 13.9	55.5 ± 12.6	0.23
TG (mg/dL)	119.5 ± 58.2	122.3 ± 79.1	0.56
FPG (g/dL)	98.7 ± 14.5	100.5 ± 17.3	0.07
HbA1c (%)	5.2 ± 0.47	5.3 ± 0.59	0.11
UA (mg/dL)	5.7 ± 1.4	5.8 ± 1.3	0.09
Alcohol consumption (non/current drinkers)	254/205	307/238	0.401

BMI: Body mass index; WC: Waist circumference; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; VFA: Visceral fat area; HGB: Hemoglobin; PLT: Platelet; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: γ-glutamyl transferase; ALP: Alkaline phosphatase; ChE: Cholinesterase; TC: Total cholesterol; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol; TG: Triglycerides; FPG: Fasting blood glucose; HbA1c: Hemoglobin A1c; UA: Uric acid.

Waist circumference and visceral fat accumulation

Figure 1 shows the relationship between WC and VFA of all subjects, indicating a significant positive correlation (Figure 1; $r = 0.536$, $P < 0.01$). In both males and females, VFA was significantly positively correlated with WC ($r = 0.532$, $P < 0.01$; $r = 0.612$, $P < 0.01$), respectively. VFA in females was more strongly correlated with WC than in males.

Comparison between high and low levels of VFA

We divided the sample using a cut-off point of VFA (100 cm^2)[4]. Subjects with high levels of VFA had primarily male with a significantly higher height, body weight, BMI, SBP, DBP, and HGB in all subjects ($P < 0.05$, Table 2). In male subjects, TC were also significantly higher in high VFA group ($P < 0.05$, Table 3), whereas PLT were significantly higher in females with high VFA group ($P < 0.05$, Table 4). Of note, the rate of current drinking was significant lower in females with

Table 3 Clinical parameters associated with visceral fat accumulation of male subjects by univariate analysis

	VFA ⩾ 100 (*n* = 348)	VFA < 100 (*n* = 192)	*P* value
Age (yr)	55.3 ± 11.7	50.6 ± 12.9	< 0.001
Height (cm)	166.1 ± 6.0	166.2 ± 6.4	0.93
Body weight (kg)	72.1 ± 9.9	66.7 ± 8.2	< 0.001
BMI (kg/m^2)	26.1 ± 3.0	24.1 ± 2.4	0.001
WC (cm)	90.7 ± 7.0	84.9 ± 6.7	< 0.001
SBP (mmHg)	126.6 ± 14.1	121.8 ± 14.2	< 0.001
DBP (mmHg)	79.8 ± 9.5	77.0 ± 8.8	0.001
HGB (g/dL)	15.2 ± 1.0	15.1 ± 1.1	0.29
PLT (× 10^4/μL)	21.4 ± 4.4	21.5 ± 4.2	0.76
AST (IU/L)	22.9 ± 8.0	23.4 ± 8.7	0.51
ALT (IU/L)	24.4 ± 15.9	25.8 ± 16.6	0.34
GGT (IU/L)	32.3 ± 29.3	25.8 ± 16.6	0.29
ALP (IU/L)	227.0 ± 64.1	228.2 ± 72.2	0.84
ChE (IU/L)	359.0 ± 68.5	353.9 ± 68.2	0.41
TC (mg/dL)	209.6 ± 32.8	203.4 ± 32.7	0.03
LDL-C (mg/dL)	127.1 ± 31.0	124.5 ± 30.1	0.35
HDL-C (mg/dL)	57.4 ± 13.7	55.3 ± 13.4	0.09
TG (mg/dL)	118.4 ± 68.3	118.8 ± 79.1	0.95
FPG (g/dL)	98.6 ± 15.3	101.4 ± 19.6	0.07
HbA1c (%)	5.2 ± 0.5	5.3 ± 0.7	0.06
UA (mg/dL)	5.6 ± 1.4	5.7 ± 1.2	0.28
Alcohol consumption (non/current drinkers)	209/139	119/73	0.37

BMI: Body mass index; WC: Waist circumference; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; VFA: Visceral fat area; HGB: Hemoglobin; PLT: Platelet; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: γ-glutamyl transferase; ALP: Alkaline phosphatase; ChE: Cholinesterase; TC: Total cholesterol; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol; TG: Triglycerides; FPG: Fasting blood glucose; HbA1c: Hemoglobin A1c; UA: Uric acid.

Table 4 Clinical parameters associated with visceral fat accumulation of female subjects by univariate analysis

	VFA ⩾ 100 (*n* = 111)	VFA < 100 (*n* = 353)	*P* value
Age (yr)	62.1 ± 9.4	56.7 ± 10.0	< 0.001
Height (cm)	151.4 ± 5.6	152.5 ± 5.5	0.07
Body weight (kg)	63.9 ± 12.2	54.1 ± 6.7	< 0.001
BMI (kg/m^2)	27.8 ± 4.4	23.3 ± 2.7	< 0.001
WC (cm)	95.6 ± 9.5	84.7 ± 7.6	< 0.001
SBP (mmHg)	126.0 ± 11.9	119.4 ± 14.8	< 0.001
DBP (mmHg)	76.9 ± 8.0	74.0 ± 9.7	0.004
HGB (g/dL)	13.6 ± 1.1	13.3 ± 0.9	0.02
PLT (× 10^4/μL)	24.1 ± 5.9	22.9 ± 5.1	0.03
AST (IU/L)	22.5 ± 7.6	23.3 ± 8.4	0.38
ALT (IU/L)	25.3 ± 12.8	26.0 ± 16.8	0.67
GGT (IU/L)	33.0 ± 20.2	36.9 ± 30.3	0.21
ALP (IU/L)	224.8 ± 57.8	224.6 ± 61.9	0.97
ChE (IU/L)	354.0 ± 62.8	352.2 ± 62.8	0.79
TC (mg/dL)	204.8 ± 27.9	205.7 ± 31.1	0.77
LDL-C (mg/dL)	125.8 ± 24.7	125.9 ± 27.7	0.98
HDL-C (mg/dL)	53.8 ± 14.2	55.6 ± 12.2	0.2
TG (mg/dL)	123.1 ± 68.0	124.1 ± 79.1	0.9
FPG (g/dL)	98.9 ± 11.7	100.1 ± 15.9	0.5
HbA1c (%)	5.2 ± 0.4	5.3 ± 0.5	0.11
UA (mg/dL)	5.7 ± 1.4	5.8 ± 1.3	0.66
Alcohol consumption (non/current drinkers)	45/66	188/165	0.01

BMI: Body mass index; WC: Waist circumference; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; VFA: Visceral fat area; HGB: Hemoglobin; PLT: Platelet; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: γ-glutamyl transferase; ALP: Alkaline phosphatase; ChE: Cholinesterase; TC: Total cholesterol; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol; TG: Triglycerides; FPG: Fasting blood glucose; HbA1c: Hemoglobin A1c; UA: Uric acid.

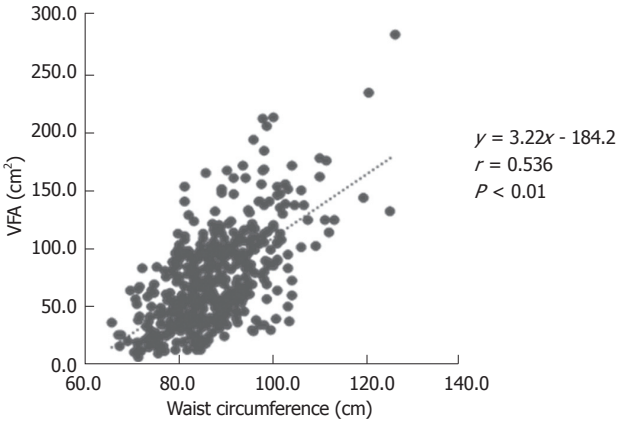

Figure 1 Relationship between waist circumference and visceral fat area of all subjects, showing a significant positive correlation of both parameters (*r* = 0.536, *P* < 0.01). VFA: Visceral fat area.

high VFA group.

Factors affecting visceral fat accumulation

We further evaluated the risk factors for VFA using the multivariate logistic regression analysis. VFA showed positive relationships with age ⩾ 56, male gender, BMI ⩾ 24.4 kg/m^2, BP ⩾ 149 and/or 90 mmHg, TG ⩾ 149 mg/dL, and current drinking in all subjects (*P* < 0.05, Table 5). In male subjects, VFA was positively associated with age ⩾ 56, BMI ⩾ 25.0 kg/m^2, and TG ⩾ 149 mg/dL (*P* < 0.05, Table 6), whereas in female subjects positive associations were observed with age ⩾ 58, BMI ⩾ 24.4 kg/m^2, HDL-C < 40 mg/dL, and current drinking (*P* < 0.05, Table 7).

DISCUSSION

This study was conducted in Okinawa is a subtropical island with 1.4 million population located in the southwest of Japan. BMI of 24.9 ± 3.4 kg/m^2 in this study was higher than that (23.0 ± 3.3 kg/m^2) of a multicenter large health checkup study conducted in the main land Japan[5]. United States ruled Okinawa for 27 years after the World War II, thus a westernized food and life styles have been popular in Okinawa[6]. This westernization may attribute to the obesity (higher BMI) in Okinawa.

The Japanese Visceral Fat Syndrome Study Committee of the Ministry of Health and Welfare of Japan was organized to establish the diagnostic criteria of obesity disease and the importance of visceral fat accumulation among the multiple obesity-related cardiovascular risk factors[4]. They also demonstrated that among the various anthropometric parameters measured in

Table 5 Clinical parameters associated with visceral fat accumulation of all subjects by multivariate logistic regression analysis

Variable	β	SE	Wald	*P* value	OR	95%CI
Age (≥ 56 yr)	0.692	0.165	17.714	< 0.001	1.999	1.448-2.759
Male gender	1.746	0.202	74.406	< 0.001	5.73	3.854-8.52
BMI (≥ 24.4 kg/m^2)	1.621	0.158	105.265	< 0.001	5.06	3.712-6.897
BP (≥ 149/90 mmHg)	0.415	0.196	4.503	0.034	1.515	1.032-2.223
HGB (≥ 14.3 g/dL)	0.251	0.193	1.696	0.193	1.286	0.881-1.876
PLT (≥ 22.2 × 10^4/μL)	-0.014	0.187	0.006	0.938	0.986	0.683-1.422
TC (≥ 219 mg/dL)	-0.206	0.234	0.775	0.379	0.814	0.514-1.288
TG (≥ 149 mg/dL)	0.473	0.203	5.416	0.02	1.604	1.077-2.389
HDL-C (≥ 40 mg/dL)	-0.197	0.279	0.499	0.48	0.821	0.475-1.42
LDL-C (≥ 139 mg/dL)	0.184	0.242	0.574	0.449	1.201	0.747-1.932
AST (≥ 30 IU/L)	-0.191	0.276	0.477	0.49	0.826	0.481-1.42
ALT (≥ 30 IU/L)	0.075	0.238	0.099	0.753	1.078	0.676-1.72
GGT (≥ 51 IU/L)	-0.324	0.233	1.927	0.165	0.724	0.458-1.143
ALP (≥ 325 IU/L)	0.156	0.305	0.263	0.608	1.169	0.643-2.126
ChE (≥ 350 IU/L)	-0.065	0.165	0.155	0.694	0.937	0.678-1.294
UA (≥ 5.8 mg/dL)	-0.117	0.177	0.439	0.508	0.89	0.629-1.257
FPG (≥ 110 g/dL)	-0.473	0.257	3.369	0.066	0.623	0.376-1.033
HbA1c (≥ 6.2%)	-0.205	0.45	0.208	0.649	0.814	0.337-1.969
Current drinking	0.371	0.171	4.713	0.03	1.449	1.037-2.026

BMI: Body mass index; WC: Waist circumference; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; VFA: Visceral fat area; HGB: Hemoglobin; PLT: Platelet; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: γ-glutamyl transferase; ALP: Alkaline phosphatase; ChE: Cholinesterase; TC: Total cholesterol; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol; TG: Triglycerides; FPG: Fasting blood glucose; HbA1c: Hemoglobin A1c; UA: Uric acid; SE: Standard error.

Table 6 Clinical parameters associated with visceral fat accumulation of male subjects by multivariate logistic regression analysis

Variable	β	SE	Wald	*P* value	OR	95%CI
Age (≥ 56 yr)	0.688	0.21	10.734	0.001	1.99	1.319-3.004
BMI (≥ 25 kg/m^2)	1.459	0.204	51.055	< 0.001	4.301	2.883-6.417
BP (≥ 149/90 mmHg	0.418	0.247	2.859	0.091	1.518	0.936-2.464
Hb (≥ 15.1 g/dL)	0.293	0.203	2.098	0.148	1.341	0.902-1.995
Plt (≥ 20.9 × 10^4/μL)	-0.324	0.236	1.874	0.171	0.724	0.455-1.15
TC (≥ 219 mg/dL)	-0.104	0.316	0.108	0.743	0.901	0.485-1.674
TG (≥ 149 mg/dL)	0.602	0.273	4.862	0.027	1.826	1.069-3.117
HDL-C (≥ 40 mg/dL)	0.218	0.341	0.408	0.523	1.243	0.637-2.427
LDL-C (≥ 139 mg/dL)	0.037	0.322	0.013	0.908	1.038	0.552-1.952
AST (≥ 30 IU/L)	-0.161	0.343	0.22	0.639	0.851	0.434-1.669
ALT (≥ 30 IU/L)	0.022	0.302	0.005	0.942	1.022	0.566-1.847
GGT (≥ 51 IU/L)	-0.003	0.309	0	0.992	0.997	0.544-1.828
ALP (≥ 325 IU/L)	0.124	0.37	0.113	0.737	1.132	0.548-2.34
ChE (≥ 350 IU/L)	-0.049	0.217	0.052	0.819	0.952	0.622-1.455
UA (≥ 5.8 mg/dL)	-0.085	0.232	0.133	0.716	0.919	0.583-1.448
FPG (≥ 110 g/dL)	-0.485	0.327	2.201	0.138	0.615	0.324-1.169
HbA1c (≥ 6.2%)	-0.162	0.532	0.093	0.761	0.85	0.3-2.414
Current drinking	0.345	0.222	2.421	0.12	1.412	0.914-2.18

BMI: Body mass index; WC: Waist circumference; BP: Blood pressure; VFA: Visceral fat area; HGB: Hemoglobin; PLT: Platelet; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: γ-glutamyl transferase; ALP: Alkaline phosphatase; ChE: Cholinesterase; TC: Total cholesterol; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol; TG: Triglycerides; FPG: Fasting blood glucose; HbA1c: Hemoglobin A1c; UA: Uric acid; SE: Standard error.

their study, WC showed the closest relationship with VFA in both men and women. WC is used as an index of visceral fat accumulation in the diagnosis of MS because of its ease of measurement. According to the modified National Cholesterol Education Program's Adult Treatment Panel Ⅲ (NCEP-ATP) guidelines[7], the prevalence of MS in Japan and China was 20.6% and 27.6%, respectively[8,9]. In this study, the CT-evaluated visceral fat accumulation also correlated with WC; our findings confirm that WC is a reliable surrogate measurement for visceral fat accumulation, and the findings are in concordance with results from previous studies conducted within the Japanese population[10-12].

We focused on gender differences and the relationship between visceral fat accumulation and other clinical parameters. It is well known that visceral fat accumulation exhibits age, sex, and race differences in both prevalence and severity[13]. Age and gender differences are further affected by country-specific differences in the prevalence of obesity and lifestyle-

Table 7 Clinical parameters associated with visceral fat accumulation of female subjects by multivariate logistic regression analysis

Variable	β	SE	Wald	*P* value	OR	95%CI
Age (≥ 58 yr)	0.793	0.259	9.349	0.002	2.211	1.33-3.676
BMI (≥ 24.4 kg/m^2)	2.074	0.298	48.386	< 0.001	7.957	4.436-14.275
BP (≥ 149/90 mmHg)	0.576	0.346	2.768	0.096	1.78	0.902-3.509
Hb (≥ 14.3 g/dL)	0.39	0.26	2.25	0.134	1.477	0.887-2.457
Plt (≥ 22.2 × 10^4/μL)	0.186	0.295	0.399	0.528	1.205	0.676-2.146
TC (≥ 219 mg/dL)	-0.297	0.388	0.586	0.444	0.743	0.347-1.59
TG (≥ 149 mg/dL)	0.148	0.334	0.196	0.658	1.16	0.602-2.233
HDL-C (≥ 40 mg/dL)	-0.83	0.465	4.463	0.035	0.374	0.15-0.931
LDL-C (≥ 139 mg/dL)	0.355	0.4	0.791	0.374	1.427	0.652-3.122
AST (≥ 30 IU/L)	-0.137	0.489	0.078	0.78	0.872	0.334-2.275
ALT (≥ 30 IU/L)	0.247	0.394	0.392	0.531	1.28	0.591-2.773
GGT (≥ 51 IU/L)	-0.928	0.393	5.567	0.018	0.395	0.183-0.855
ALP (≥ 325 IU/L)	-0.093	0.571	0.027	0.87	0.911	0.298-2.787
ChE (≥ 350 IU/L)	-0.106	0.267	0.158	0.691	0.899	0.533-1.517
UA (≥ 5.8 mg/d)	-0.011	0.285	0.001	0.97	0.989	0.566-1.729
FPG (≥ 110 g/dL)	-0.567	0.412	1.895	0.169	0.567	0.253-1.272
HbA1c (≥ 6.2%)	0.225	0.824	0.075	0.785	1.252	0.249-6.299
Current drinking	0.574	0.285	4.062	0.044	1.776	1.016-3.104

BMI: Body mass index; WC: Waist circumference; BP: Blood pressure; VFA: Visceral fat area; HGB: Hemoglobin; PLT: Platelet; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: γ-glutamyl transferase; ALP: Alkaline phosphatase; ChE: Cholinesterase; TC: Total cholesterol; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol; TG: Triglycerides; FPG: Fasting blood glucose; HbA1c: Hemoglobin A1c; UA: Uric acid; SE: Standard error.

related diseases[14]. In the present multivariate logistic regression analysis, current drinking was significantly associated with VFA in females but not in male subjects. These differences may be a result of alcoholic drink choice, such as beer *vs* liquor. The effect of alcohol on fat metabolism remains controversial. In a cross-sectional study of healthy South Korean men, Kim *et al*[15] reported that alcohol consumption showed a significant association with increased VFA, which was independent of other factors. On the other hand, Fan *et al*[16] have reported that current alcohol consumption was associated with a lower prevalence of MS, irrespective of alcohol intake, and alcohol consumption had a favorable influence on HDL-C and WC in a Shanghai study. Excessive alcohol consumption is known to cause alcoholic liver diseases; however, Moriya *et al*[3] from Japan reported that light to moderate alcohol consumption by men was likely to protect individuals against fatty liver over time. Most recently, Takahashi *et al*[17] from Japan showed clearly in a cohort study that alcohol had a biphasic effect on fatty liver. Although the available evidence is conflicting, moderation of alcohol consumption is still a consistent recommendation for a healthy lifestyle[18].

The strengths of our study are the large sample size and the direct assessment of VFA using a CT scan which allowed for the precise determination of the WC component[12]. In addition, the study subjects were representative of the general population undergoing a health checkup. There are some limitations of this study. First, because of the cross-sectional design of this study, we could not identify the causal relationship between VFA and the various parameters in depth. Second, the self-administered questionnaire on alcohol consumption may have resulted in under-reported alcohol intake for some subjects. Third, this study lacks data regarding patient medication use, nutritional intake, and physical fitness, all variables that can influence visceral fat accumulation. There was a possibility of selection bias because subjects were volunteers who opted to complete a health checkup. Thus, it is possible the study subjects also had an increased awareness of healthy behaviors.

In conclusion, despite these limitations, the present study showed gender differences in the clinical and biochemical parameters associated with visceral fat accumulation in the general population in Okinawa, Japan. Visceral obesity is probably the most important target for future interventions in MS. Future studies are needed to clarify preventive methods among different gender and age groups.

ACKNOWLEDGMENTS

We are grateful to staff members in OHPF for their great contribution.

COMMENTS

Background

Visceral fat accumulation is closely related to atherogenic disorders and metabolic syndrome (MS), including diabetes mellitus, hypertension, and dyslipidemia. It also leads to obesity-related complications. MS further increases the risks for cardiovascular diseases and thus is an important therapeutic target.

Research frontiers

Although computed tomography has been applied widely as the gold standard method to evaluate visceral fat accumulation, little is known about what clinical and biochemical parameters affect visceral fat accumulation. The aim of this study is to investigate the clinical and biochemical parameters potentially associated with visceral fat accumulation.

Innovations and breakthroughs

The present study showed gender differences in the clinical and biochemical

parameters which associated with visceral fat accumulation in the general population in Okinawa, Japan.

Applications

Visceral obesity is probably the most important target for future interventions in MS.

Terminology

Visceral fat accumulation is defined as the sum of the intraperitoneal fat area with computed tomography (CT) density in the range of -150 to -50 Hounsfield units.

Peer-review

The authors retrospectively analyzed data of 1004 check up patients. They found that visceral fat area measured by CT is correlated with waist circumference and metabolic parameters in both sex.

REFERENCES

1 **Matsuzawa Y**, Funahashi T, Nakamura T. The concept of metabolic syndrome: contribution of visceral fat accumulation and its molecular mechanism. *J Atheroscler Thromb* 2011; **18**: 629-639 [PMID: 21737960 DOI: 10.5551/jat.7922]

2 **Ryo M**, Kishida K, Nakamura T, Yoshizumi T, Funahashi T, Shimomura I. Clinical significance of visceral adiposity assessed by computed tomography: A Japanese perspective. *World J Radiol* 2014; **6**: 409-416 [PMID: 25071881 DOI: 10.4329/wjr.v6.i7.409]

3 **Moriya A**, Iwasaki Y, Ohguchi S, Kayashima E, Mitsumune T, Taniguchi H, Ando M, Yamamoto K. Roles of alcohol consumption in fatty liver: a longitudinal study. *J Hepatol* 2015; **62**: 921-927 [PMID: 25433160 DOI: 10.1016/j.jhep.2014.11.025]

4 **Examination Committee of Criteria for 'Obesity Disease' in Japan**; Japan Society for the Study of Obesity. New criteria for 'obesity disease' in Japan. *Circ J* 2002; **66**: 987-992 [PMID: 12419927 DOI: 10.1253/circj.66.987]

5 **Eguchi Y**, Hyogo H, Ono M, Mizuta T, Ono N, Fujimoto K, Chayama K, Saibara T. Prevalence and associated metabolic factors of nonalcoholic fatty liver disease in the general population from 2009 to 2010 in Japan: a multicenter large retrospective study. *J Gastroenterol* 2012; **47**: 586-595 [PMID: 22328022 DOI: 10.1007/s00535-012-0533-z]

6 **Sugama C**, Isa K, Okumura K, Iseki K, Kinjo K, Ohya Y. Trends in the incidence of stroke and cardiovascular risk factors on the isolated island of Okinawa: the Miyakojima study. *J Stroke Cerebrovasc Dis* 2013; **22**: e118-e123 [PMID: 23122721 DOI: 10.1016/j.jstrokecerebrovasdis.2012.08.016]

7 **Grundy SM**, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC, Spertus JA, Costa F. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005; **112**: 2735-2752 [PMID: 16157765 DOI: 10.1161/CIRCULATIONAHA.105.169404]

8 **Takami H**, Nakamoto M, Uemura H, Katsuura S, Yamaguchi M, Hiyoshi M, Sawachika F, Juta T, Arisawa K. Inverse correlation between coffee consumption and prevalence of metabolic syndrome: baseline survey of the Japan Multi-Institutional Collaborative Cohort (J-MICC) Study in Tokushima, Japan. *J Epidemiol* 2013; **23**: 12-20 [PMID: 23047663 DOI: 10.2188/jea.JE20120053]

9 **Xi B**, He D, Hu Y, Zhou D. Prevalence of metabolic syndrome and its influencing factors among the Chinese adults: the China Health and Nutrition Survey in 2009. *Prev Med* 2013; **57**: 867-871 [PMID: 24103567 DOI: 10.1016/j.ypmed.2013.09.023]

10 **Ishibashi E**, Eguchi Y, Eguchi T, Matsunobu A, Oza N, Nakashita S, Kitajima Y, Kuroki S, Ozaki I, Kawaguchi Y, Ide Y, Yasutake T, Iwakiri R, Mizuta T, Ono N, Fujimoto K. Waist circumference correlates with hepatic fat accumulation in male Japanese patients with non-alcoholic fatty liver disease, but not in females. *J Gastroenterol Hepatol* 2008; **23**: 908-913 [PMID: 18373563 DOI: 10.1111/j.1440-1746.2008.05366.x]

11 **Oka R**, Kobayashi J, Yagi K, Tanii H, Miyamoto S, Asano A, Hagishita T, Mori M, Moriuchi T, Kobayashi M, Katsuda S, Kawashiri MA, Nohara A, Takeda Y, Mabuchi H, Yamagishi M. Reassessment of the cutoff values of waist circumference and visceral fat area for identifying Japanese subjects at risk for the metabolic syndrome. *Diabetes Res Clin Pract* 2008; **79**: 474-481 [PMID: 18031862 DOI: 10.1016/j.diabres.2007.10.016]

12 **Matsushita Y**, Nakagawa T, Shinohara M, Yamamoto S, Takahashi Y, Mizoue T, Yokoyama T, Noda M. How can waist circumference predict the body composition? *Diabetol Metab Syndr* 2014; **6**: 11 [PMID: 24472677 DOI: 10.1186/1758-5996-6-11]

13 **Camhi SM**, Bray GA, Bouchard C, Greenway FL, Johnson WD, Newton RL, Ravussin E, Ryan DH, Smith SR, Katzmarzyk PT. The relationship of waist circumference and BMI to visceral, subcutaneous, and total body fat: sex and race differences. *Obesity* (Silver Spring) 2011; **19**: 402-408 [PMID: 20948514 DOI: 10.1038/oby.2010.248]

14 **Yatsuji S**, Hashimoto E, Tobari M, Tokushige K, Shiratori K. Influence of age and gender in Japanese patients with non-alcoholic steatohepatitis. *Hepatol Res* 2007; **37**: 1034-1043 [PMID: 17610504 DOI: 10.1111/j.1872-034X.2007.00156.x]

15 **Kim KH**, Oh SW, Kwon H, Park JH, Choi H, Cho B. Alcohol consumption and its relation to visceral and subcutaneous adipose tissues in healthy male Koreans. *Ann Nutr Metab* 2012; **60**: 52-61 [PMID: 22327000 DOI: 10.1159/000334710]

16 **Fan JG**, Farrell GC. VAT fat is bad for the liver, SAT fat is not! *J Gastroenterol Hepatol* 2008; **23**: 829-832 [PMID: 18565017 DOI: 10.1111/j.1440-1746.2008.05474.x]

17 **Takahashi H**, Ono M, Hyogo H, Tsuji C, Kitajima Y, Ono N, Eguchi T, Fujimoto K, Chayama K, Saibara T, Anzai K, Eguchi Y. Biphasic effect of alcohol intake on the development of fatty liver disease. *J Gastroenterol* 2015; **50**: 1114-1123 [PMID: 25733100 DOI: 10.1007/s00535-015-1058-z]

18 **Traversy G**, Chaput JP. Alcohol Consumption and Obesity: An Update. *Curr Obes Rep* 2015; **4**: 122-130 [PMID: 25741455 DOI: 10.1007/s13679-014-0129-4]

P- Reviewer: Balaban YH, Hallgren T **S- Editor**: Gong ZM
L- Editor: A **E- Editor**: Wu HL

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i2.268

World J Gastrointest Pharmacol Ther 2016 May 6; 7(2): 268-273
ISSN 2150-5349 (online)

ORIGINAL ARTICLE

Observational Study

Digital chromoendoscopy utilization in clinical practice: A survey of gastroenterologists in Connecticut

Karl M Langberg, Neil D Parikh, Yanhong Deng, Maria Ciarlegio, Loren Laine, Harry R Aslanian

Karl M Langberg, Neil D Parikh, Loren Laine, Harry R Aslanian, Section of Digestive Diseases, Yale University School of Medicine, New Haven, CT 06510, United States

Yanhong Deng, Maria Ciarleglio, Department of Biostatistics, Yale School of Public Health, New Haven, CT 06510, United States

Loren Laine, VA Connecticut Healthcare System, West Haven, CT 06516, United States

Author contributions: Parikh ND and Aslanian HR contributed to editing, reviewing, study conception and design; Langberg KM and Parikh ND contributed to data acquisition, background research and writing of article; Deng Y and Ciarleglio M contributed data analysis; Laine L contributed with editing and reviewing the article.

Institutional review board statement: Waiver for IRB review was given by the Human Investigation Committee at Yale University.

Informed consent statement: All study participants provided informed consent prior to study enrollment.

Conflict-of-interest statement: None of the study authors have any conflicts of interest to disclose.

Data sharing statement: No additional data are available.

Correspondence to: Harry R Aslanian, MD, Associate Professor of Medicine, Section of Digestive Diseases, Yale University School of Medicine, PO Box 208019, New Haven, CT 06510, United States. harry.aslanian@yale.edu
Telephone: +1-203-2005083
Fax: +1-203-2002235

Received: February 3, 2016
Peer-review started: February 14, 2016
First decision: March 1, 2016
Revised: March 13, 2016
Accepted: March 24, 2016
Article in press: March 25, 2016
Published online: May 6, 2016

Abstract

AIM: To use a survey to characterize and identify potential barriers to the use of digital chromoendoscopy (DC) by practicing gastroenterologists.

METHODS: An anonymous, internet-based survey was sent to gastroenterologists in Connecticut who were members of one of three national gastrointestinal organizations. The survey collected demographic information, frequency of DC use, types of procedures that the respondent performs, setting of practice (academic *vs* community), years out of training, amount of training in DC, desire to have DC training and perceived barriers to DC use. Responses were collected anonymously. The primary endpoint was the proportion of endoscopists utilizing DC. Associations between the various data collected were analyzed using χ^2 test.

RESULTS: One hundred and twenty-four gastroenterologists (48%) of 261 who received the online survey responded. Seventy-eight percent of surveyed gastroenterologists have used DC during the performance of upper endoscopy and 81% with lower endoscopy. DC was used in more than half of procedures by only 14% of gastroenterologists during upper endoscopy and 12% during lower endoscopy. Twenty-three percent (upper) and 21% (lower) used DC more than one quarter of the time. DC was used for 10% or less of endoscopies by 60% (upper) and

53% (lower) of respondents. Endoscopists reported lack of training as the leading deterrent to DC use with 36% reporting it as their primary deterrent. Eighty-nine percent of endoscopists never received formal training in DC. Lack of time (30% of respondents), lack of evidence (24%) and lack of reimbursement (10%) were additional deterrents. There were no differences in DC use relative to academic *vs* community practice setting or years out of training.

CONCLUSION: DC is used infrequently by most endoscopists, primarily due to a lack of training. Training opportunities should be expanded to meet the interest expressed by the majority of endoscopists.

Key words: Endoscopy; Surveys and questionnaires; Gastrointestinal diseases; Clinical practice patterns; Esophageal neoplasms; Colonic neoplasms; Narrow band imaging; Flexible spectral imaging color enhancement; I-scan

Core tip: Digital chromoendoscopy (DC) is a technology present on most modern endoscopes that provides electronic contrast enhancement of the gastrointestinal mucosa. This survey study assessed the frequency of digital chromoendocopy use and perceived barriers to its use among practicing gastroenterologists in Connecticut. DC was used in ten percent or less of endoscopies by the majority of respondents. Lack of training was the most commonly cited barrier to DC use and most desired formal training. Enhancing training opportunities for DC could increase its use.

Langberg KM, Parikh ND, Deng Y, Ciarlegio M, Laine L, Aslanian HR. Digital chromoendoscopy utilization in clinical practice: A survey of gastroenterologists in Connecticut. *World J Gastrointest Pharmacol Ther* 2016; 7(2): 268-273 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i2/268.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i2.268

INTRODUCTION

Image-enhanced endoscopy allows for *in vivo* characterization of colon polyps and detection of neoplasia in Barrett's esophagus[1,2]. Image-enhanced endoscopy modalities include digital chromoendoscopy (DC), confocal laser endomicroscopy, autofluorescence and optical coherence tomography. DC was incorporated into new endoscope models as a standard feature approximately 10 years ago. DC modalities include narrow band imaging (NBI; Olympus, Tokyo, Japan), i-scan (Pentax, Tokyo, Japan), and flexible spectral imaging color enhancement (Fujinon, Saitama, Japan). These technologies are designed to provide contrast enhancement of the gastrointestinal mucosa as an alternative to dye-based chromoendoscopy[3]. NBI uses blue and green light, in addition to computerized image processing to visualize capillaries[4]. Flexible spectral imaging color enhancement and i-scan use proprietary computerized post-processing software to enhance mucosal surface detail[3,5,6].

DC has recently appeared in documents from the American Society of Gastrointestinal Endoscopy and American Gastroenterological Association for the endoscopic surveillance and management of colorectal cancers. The use of DC is supported in resect-and-discard or inspect-and-do-not-resect strategies for diminutive colorectal polyps as long as endoscopists meet pre-specified performance thresholds[1,7]. Furthermore, decreases in the number of polypectomy specimens sent for histological assessment also may result in important cost savings[8]. European Society for Gastrointestinal Endoscopy guidelines state that DC should be used in patients with Lynch syndrome and serrated polyposis syndrome, in addition to the resect-and-discard strategy[9]. DC has also shown diagnostic utility in Barrett's esophagus[2].

Despite DC's ease of use, lack of additional cost, widespread availability for the past decade, and potential diagnostic benefit, it is unclear how prevalent its use is in clinical practice. The aim of this study was to survey gastroenterologists to assess their use of DC and identify potential barriers.

MATERIALS AND METHODS

Identifying gastroenterologists

We searched the online membership databases for the American Society of Gastrointestinal Endoscopy, American Gastroenterology Association, and the American College of Gastroenterology to identify gastroenterologists in the state of Connecticut. Only members with an active email address were enrolled in the survey study.

Survey

An anonymous, internet based survey (SurveyMonkey, Palo Alto, California) was sent *via* e-mail. Non-responders were contacted up to three times *via* e-mail.

The survey comprised of 17 questions. The first part consisted of demographic information, including gender, practice setting, years as a practicing gastroenterologist, and number of endoscopic procedures performed per month. The second portion of the survey focused on specific types of endoscopic procedures performed and when DC was used in upper and lower endoscopies. The final part of the survey evaluated the physician's interest in learning DC, effort to self-train, and potential deterrents to routine DC use.

Endpoints

The primary endpoint was the proportion of endoscopists utilizing DC. Analyses were performed

Table 1 Demographic characteristics of endoscopists

Gender (n^1 = 124)	
Male	98 (79%)
Female	26 (21%)
Practice setting (n^1 = 123)	
Academic	40 (33%)
Community	83 (67%)
Average years in practice	16.5
Number of endoscopic procedures/month (n^1 = 123)	
Less than 25	17 (14%)
25 to 50	19 (15%)
51 to 75	24 (20%)
Greater than 75	63 (51%)
Number who perform specialized procedures (n = 67)	
Ablation of Barrett's esophagus	31 (46%)
Endoscopic mucosal resection for Barrett's	36 (54%)
Endoscopic mucosal resection for colon polyps	44 (66%)
Dye-based chromoendoscopy	24 (36%)

[1]Numbers vary because number of respondents answering each question varied.

to compare the variation in DC use with different demographic categories: Practice setting, years in practice, and performance of interventional endoscopy procedures. Secondary outcomes included deterrents to DC use, percentage of physicians with formal training in DC, and percentage of physicians interested in additional DC training.

Statistical analysis

Summary statistics including means and standard deviations were calculated for quantitative variables; frequencies and percentages were calculated for categorical variables. Mean values were compared using two-sample *t* tests and associations between categorical variables were explored using χ^2 tests or Fisher's exact test, as appropriate. Statistical analyses were conducted by Deng Y and Ciarleglio M from Yale University School of Public Health using SAS 9.3 (Cary, NC).

RESULTS

Two hundred and sixty-one gastroenterologists with valid e-mail addresses received the online survey request. Of these, 124 (48%) responded. Table 1 summarizes the demographic characteristics of the responding physicians. All had access to DC enabled scopes in their practice.

Seventy-eight percent of surveyed gastroenterologists have used DC in upper endoscopy and 81% have used DC in lower endoscopy while 22% and 20% have never used the technology for upper and lower endoscopy respectively (Figure 1). DC was used some but less than 10% of the time by 38% (upper) and 33% (lower) of respondents. DC was used for 10% or less (combination of the < 10% and never groups) of endoscopies by 60% (upper) and 53% (lower) of respondents. Only 14% for upper endoscopy and 12% for lower endoscopy used DC in more than half of procedures and only 23% (upper) and 21% (lower) use DC more than one quarter of the time (Figure 1). DC usage was similar by academic (82%) and community gastroenterologists (85%). The average number of years of practice was comparable for those who used DC (16.3 years) and those who did not (18.6 years). Gastroenterologists that performed interventional endoscopic procedures were more likely than general gastroenterologists to utilize DC in the upper GI tract (95% *vs* 71%, $P = 0.0034$) and the lower GI tract (91% *vs* 74%, $P = 0.0517$) (Figure 2).

Lack of training was the most commonly identified deterrent to DC use (36%), followed by lack of time (30%), lack of evidence (24%), and lack of reimbursement (10%) (Figure 3). Lack of training and lack of time were the most commonly stated reasons for not using DC irrespective of an academic or community setting (30% *vs* 38% for training, $P = 0.46$; 30% *vs* 30% for time, $P = 0.95$). Gastroenterologists who performed DC in < 10% of all procedures ranked lack of training as their primary deterrent (47% *vs* 19% among those who utilize DC in ⩾ 10% cases, $P < 0.05$), while lack of time was the most common deterrent in those who used DC in ⩾ 10% of procedures (42% *vs* 22% among those who use DC in < 10% of cases, $P < 0.05$). Eighty-nine percent (n = 109) of responding gastroenterologists had received no formal training in DC and 76% (n = 93) were interested in DC training. 82% of respondents reported some degree of self-training in DC, with learning performed *via* conferences (23%), publications (16%) and comparisons with histology (13%).

DISCUSSION

Our survey study demonstrated that DC is infrequently used in clinical practice by gastroenterologists. The majority of respondents (56%) use DC in less than one tenth of cases and one fifth never use DC. Physicians reported lack of training as the leading deterrent to DC use with approximately 90% of them having never received formal training.

DC is a standard option included on all modern endoscopes and was available to all survey respondents. While DC may not be required in endoscopic practice, it does improve diagnostic yield in Barrett's esophagus surveillance and it provides accurate characterization of diminutive colorectal polyps. In addition, training in DC may improve an endoscopist's ability to perform standard white-light endoscopy[8].

In patients referred for Barrett's screening or surveillance, NBI with targeted biopsy of visible lesions identified similar proportions of patients with intestinal metaplasia and more dysplastic areas with fewer biopsies required than standard white light endoscopy with 4-quadrant biopsies plus targeted biopsies[2]. Furthermore, flexible spectral imaging color enhancement was equally effective and less time consuming compared to dye-based chromoendoscopy for detection of high-grade dysplasia in Barrett's[10].

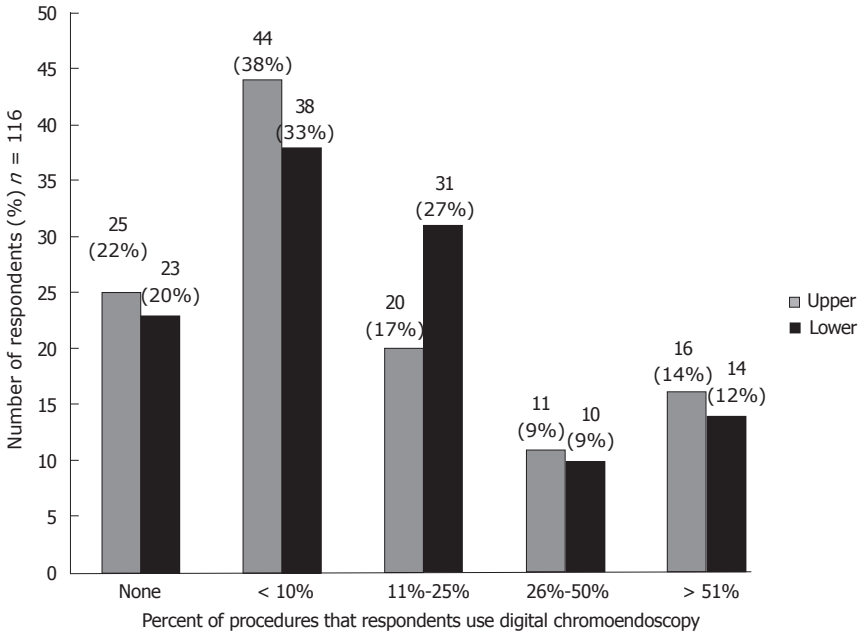

Figure 1 Percentage of digital chromoendoscopy use.

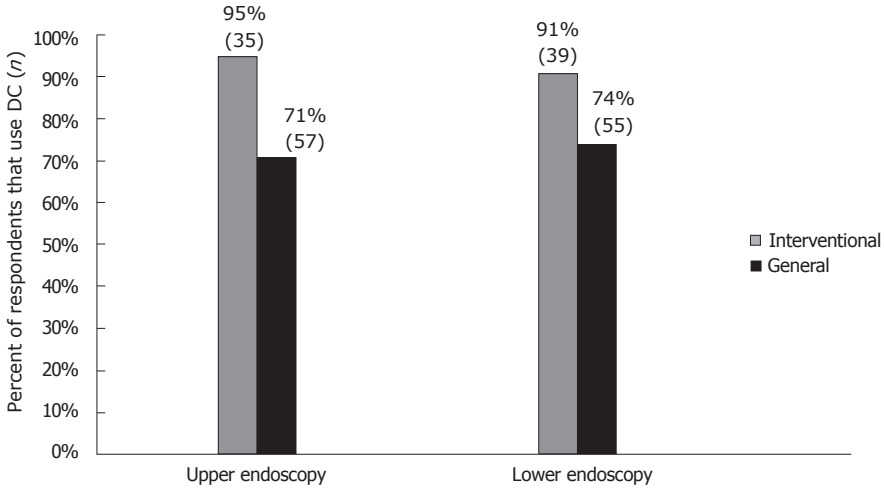

Figure 2 Digital chromoendoscopy and Interventional endoscopy.

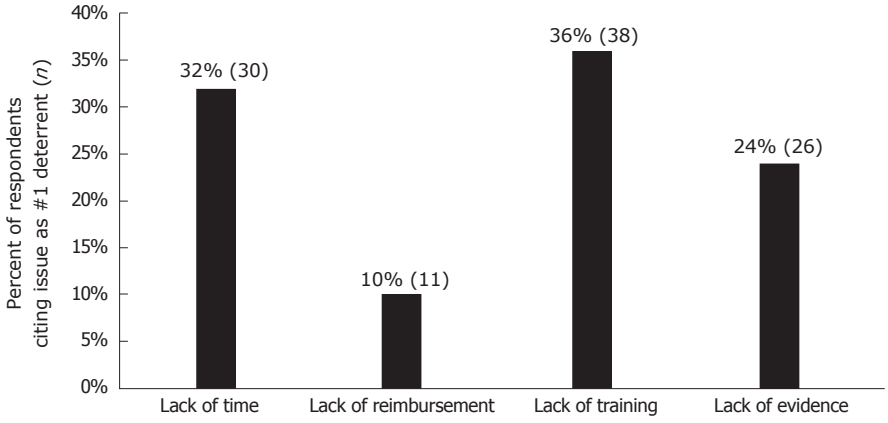

Figure 3 Deterrents to digital chromoendoscopy use.

In the lower GI tract, the majority of DC studies have focused on colorectal neoplasia and both NBI and i-scan have been shown to be useful in the *in vivo* characterization of diminutive (≤ 5 mm) colon

polyps as hyperplastic *vs* adenomatous[11]. Per current recommendations[1,7], diminutive rectosigmoid polyps diagnosed as hyperplastic using NBI[12], i-scan[6,13] or flexible spectral imaging color enhancement[11] may be left in place if endoscopists achieve the threshold of a negative predictive value ⩾ 90% for adenoma or can be resected and discarded if histological assessment based on DC leads to a ⩾ 90% agreement in appropriate post-polypectomy surveillance interval[1]. Estimated savings to the health care system with such a "resect and discard" strategy are up to $33 million annually[14]. DC has not been clearly shown to improve detection of adenomas as compared to standard white-light endoscopy, and this may be a reason that some endoscopists do not employ DC during colonoscopy. Almost a quarter (24%) of endoscopists in our survey reported lack of evidence as a factor in not utilizing DC.

It is not surprising that gastroenterologists who performed interventional procedures such as endoscopic mucosal resection in Barrett's esophagus or colorectal lesions were more likely to use DC, as DC has benefit in Barrett's and may assist in identifying the borders of challenging polyps and residual neoplasia[15]. Interestingly, we found no difference in DC utilization between academic and community physicians. The number of years in clinical practice also did not influence DC usage.

The lack of formal training appears to be one of the primary reasons behind the limited utilization of DC. In our survey, this factor was the most commonly listed deterrent and those that used DC in < 10% of procedures ranked this as the primary deterrent significantly more than those who used DC more frequently. Almost 90% of the surveyed gastroenterologists had never received formal training and three-fourths of them were interested in DC training. Despite its widespread availability, DC is not commonly a part of formal GI fellowship curriculum. Most gastroenterologists tend to self-learn DC through conferences, publications and comparisons with histology. Studies have shown that standardized training modules (computer-based) can significantly improve the endoscopist's diagnostic accuracy in DC[16]. A rapid learning curve for DC has been reported. A 20-min didactic session was shown to significantly increase NBI accuracy for colorectal neoplasia from 48% to 91%[17]. Our findings indicate that additional training opportunities would be of interest to gastroenterologists and could influence their endoscopic practice.

Lack of time (30%) was the second most common deterrent to use of DC and when comparing those gastroenterologists who used DC in ⩾ 10% of procedures *vs* the infrequent users (< 10%), lack of time was the primary deterrent. At present, there is no reimbursement for use of DC for assessment of colon polyp histology or for the potential cost savings associated with resecting and discarding without pathologic evaluation or not resecting diminutive polyps. If DC was billable more gastroenterologists might be willing to spend the additional time needed for examination with DC.

This survey study was not without limitations. While there was a sufficient response rate of 48%, the overall sample size is limited. This study was restricted to the state of Connecticut, potentially limiting generalizability nationally. In addition, like most surveys, results may be biased by self-reporting. Further investigation could include a wider survey area, objective reviews of case logs to determine actual DC use, and evaluation of the influence of training programs on the frequency of DC use and other clinical outcomes.

In conclusion, our study found that DC is used infrequently by most endoscopists despite being widely available. The most common reason for infrequent use was lack of training. The vast majority of those who responded had received no formal training and most stated that they would be interested in such training. Given the potential for DC to enhance diagnostic yield and reduce healthcare costs, the development of additional training opportunities should be considered.

COMMENTS

Background

Digital chromoendoscopy (DC) is a mode available on most modern endoscopes that enhances visual contrast between normal and abnormal gastrointestinal (GI) mucosa using digital image processing. It can be useful in both upper and lower endoscopy. Despite potential advantages the frequency of DC use is unknown.

Research frontiers

Establishing the role of DC, at present, in particular relation to a resect and discard strategy for diminutive colorectal polyps and the targeting of esophageal biopsies for Barrett's esophagus.

Innovations and breakthroughs

The study is the first to assess the frequency of and perceived barriers to DC use.

Applications

The authors' finding that a lack of training is a major barrier to DC use suggests that increased DC training as part of continuing medical education and gastroenterology fellowship may be beneficial.

Terminology

Digital chromoendoscopy: A group of technologies that enhances visual contrast between normal and abnormal GI mucosa using digital image post-processing.

Peer-review

The authors present a scientific paper very interesting, related to a topic of current debate.

REFERENCES

1 **Rex DK**, Kahi C, O'Brien M, Levin TR, Pohl H, Rastogi A, Burgart L, Imperiale T, Ladabaum U, Cohen J, Lieberman DA. The American Society for Gastrointestinal Endoscopy PIVI (Preservation and Incorporation of Valuable Endoscopic Innovations) on real-time endoscopic assessment of the histology of diminutive colorectal polyps. *Gastrointest Endosc* 2011; **73**: 419-422 [PMID: 21353837 DOI: 10.1016/j.gie.2011.01.023]

2 **Sharma P**, Hawes RH, Bansal A, Gupta N, Curvers W, Rastogi

A, Singh M, Hall M, Mathur SC, Wani SB, Hoffman B, Gaddam S, Fockens P, Bergman JJ. Standard endoscopy with random biopsies versus narrow band imaging targeted biopsies in Barrett's oesophagus: a prospective, international, randomised controlled trial. *Gut* 2013; **62**: 15-21 [PMID: 22315471 DOI: 10.1136/gutjnl-2011-300962]

3 **Manfredi MA**, Abu Dayyeh BK, Bhat YM, Chauhan SS, Gottlieb KT, Hwang JH, Komanduri S, Konda V, Lo SK, Maple JT, Murad FM, Siddiqui UD, Wallace MB, Banerjee S. Electronic chromoendoscopy. *Gastrointest Endosc* 2015; **81**: 249-261 [PMID: 25484330 DOI: 10.1016/j.gie.2014.06.020]

4 **Gono K**, Obi T, Yamaguchi M, Ohyama N, Machida H, Sano Y, Yoshida S, Hamamoto Y, Endo T. Appearance of enhanced tissue features in narrow-band endoscopic imaging. *J Biomed Opt* 2004; **9**: 568-577 [PMID: 15189095 DOI: 10.1117/1.1695563]

5 **Pohl J**, May A, Rabenstein T, Pech O, Ell C. Computed virtual chromoendoscopy: a new tool for enhancing tissue surface structures. *Endoscopy* 2007; **39**: 80-83 [PMID: 17252465 DOI: 10.1055/s-2006-945045]

6 **Hoffman A**, Kagel C, Goetz M, Tresch A, Mudter J, Biesterfeld S, Galle PR, Neurath MF, Kiesslich R. Recognition and characterization of small colonic neoplasia with high-definition colonoscopy using i-Scan is as precise as chromoendoscopy. *Dig Liver Dis* 2010; **42**: 45-50 [PMID: 19473893 DOI: 10.1016/j.dld.2009.04.005]

7 **Lieberman D**, Brill J, Canto M, DeMarco D, Fennerty B, Gupta N, Laine L, Lightdale C, Montgomery E, Odze R, Rex D, Sharma P, Kochman M, Tokar J. Management of Diminutive Colon Polyps Based on Endoluminal Imaging. *Clin Gastroenterol Hepatol* 2015; **13**: 1860-1866; quiz 1868-1869 [PMID: 26192139 DOI: 10.1016/j.cgh.2015.07.011]

8 **Kim JS**, Lee BI, Choi H, Kang BK, Kim JI, Lee HM, Im EJ, Kim BW, Kim SW, Choi MG, Choi KY. Brief education on microvasculature and pit pattern for trainees significantly improves estimation of the invasion depth of colorectal tumors. *Gastroenterol Res Pract* 2014; **2014**: 245396 [PMID: 24971089 DOI: 10.1155/2014/245396]

9 **Kamiński MF**, Hassan C, Bisschops R, Pohl J, Pellisé M, Dekker E, Ignjatovic-Wilson A, Hoffman A, Longcroft-Wheaton G, Heresbach D, Dumonceau JM, East JE. Advanced imaging for detection and differentiation of colorectal neoplasia: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. *Endoscopy* 2014; **46**: 435-449 [PMID: 24639382 DOI: 10.1055/s-0034-1365348]

10 **Pohl J**, May A, Rabenstein T, Pech O, Nguyen-Tat M, Fissler-Eckhoff A, Ell C. Comparison of computed virtual chromoendoscopy and conventional chromoendoscopy with acetic acid for detection of neoplasia in Barrett's esophagus. *Endoscopy* 2007; **39**: 594-598 [PMID: 17611913 DOI: 10.1055/s-2007-966649]

11 **Wanders LK**, East JE, Uitentuis SE, Leeflang MM, Dekker E. Diagnostic performance of narrowed spectrum endoscopy, autofluorescence imaging, and confocal laser endomicroscopy for optical diagnosis of colonic polyps: a meta-analysis. *Lancet Oncol* 2013; **14**: 1337-1347 [PMID: 24239209 DOI: 10.1016/S1470-2045(13)70509-6]

12 **Ignjatovic A**, East JE, Suzuki N, Vance M, Guenther T, Saunders BP. Optical diagnosis of small colorectal polyps at routine colonoscopy (Detect InSpect ChAracterise Resect and Discard; DISCARD trial): a prospective cohort study. *Lancet Oncol* 2009; **10**: 1171-1178 [PMID: 19910250 DOI: 10.1016/S1470-2045(09)70329-8]

13 **Basford PJ**, Longcroft-Wheaton G, Higgins B, Bhandari P. High-definition endoscopy with i-Scan for evaluation of small colon polyps: the HiSCOPE study. *Gastrointest Endosc* 2014; **79**: 111-118 [PMID: 23871094 DOI: 10.1016/j.gie.2013.06.013]

14 **Hassan C**, Pickhardt PJ, Rex DK. A resect and discard strategy would improve cost-effectiveness of colorectal cancer screening. *Clin Gastroenterol Hepatol* 2010; **8**: 865-869, 869.e1-e3 [PMID: 20621680 DOI: 10.1016/j.cgh.2010.05.018]

15 **Rogart JN**, Aslanian HR, Siddiqui UD. Narrow band imaging to detect residual or recurrent neoplastic tissue during surveillance endoscopy. *Dig Dis Sci* 2011; **56**: 472-478 [PMID: 20532981 DOI: 10.1007/s10620-010-1289-z]

16 **McGill SK**, Soetikno R, Rastogi A, Rouse RV, Sato T, Bansal A, McQuaid K, Kaltenbach T. Endoscopists can sustain high performance for the optical diagnosis of colorectal polyps following standardized and continued training. *Endoscopy* 2015; **47**: 200-206 [PMID: 25264764 DOI: 10.1055/s-0034-1378096]

17 **Raghavendra M**, Hewett DG, Rex DK. Differentiating adenomas from hyperplastic colorectal polyps: narrow-band imaging can be learned in 20 minutes. *Gastrointest Endosc* 2010; **72**: 572-576 [PMID: 20561618]

P- Reviewer: Braden B, Soria F, Terruzzi V **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Wu HL

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i2.274

World J Gastrointest Pharmacol Ther 2016 May 6; 7(2): 274-282
ISSN 2150-5349 (online)

ORIGINAL ARTICLE

Randomized Clinical Trial

Polyethylene glycol 3350 in occasional constipation: A one-week, randomized, placebo-controlled, double-blind trial

Thomas McGraw

Thomas McGraw, Merck & Co., Inc., Kenilworth, NJ 07033, United States

Author contributions: McGraw T contributed to the conception, design and planning of the study, data acquisition and analysis, interpretation of results, drafting the manuscript, and critically reviewing and revising the manuscript in terms of intellectual content.

Institutional review board statement: The study was reviewed and supervised by Allendale IRB for all sites.

Clinical trial registration: This study is registered at https://clinicaltrials.gov/ct2/show/NCT00770432. The registration identification number is NCT00770432.

Informed consent statement: Written informed consent was obtained from all subjects prior to any study-related procedures.

Conflict-of-interest statement: McGraw T was an employee of Merck & Co., Inc., Kenilworth, NJ, United States when the study was conducted. Merck & Co. was involved in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Data sharing statement: No additional data are available.

Correspondence to: Thomas McGraw, PhD, Merck & Co., Inc., Kenilworth, 2000 Galloping Hill Road Kenilworth, NJ 07033,
United States. thomas_p_mcgraw@yahoo.com
Telephone: +1-908-6044202

Received: October 29, 2015
Peer-review started: October 29, 2015
First decision: December 11, 2015
Revised: December 23, 2015
Accepted: January 9, 2016
Article in press: January 13, 2016
Published online: May 6, 2016

Abstract

AIM: To evaluate the efficacy and safety of polyethylene glycol (PEG) 3350 in subjects with self-reported occasional constipation.

METHODS: Eligible subjects ⩾ 17 years of age were randomized to receive either placebo or PEG 3350 17 g once daily in this multicenter, double-blind trial. Evaluations were conducted before (baseline) and after a 7-d treatment period. The primary efficacy variable was the proportion of subjects reporting complete resolution of straining and hard or lumpy stools. Secondary efficacy variables assessed the severity of the subjects' daily bowel movement (BM) symptoms, and preference of laxatives based on diary entries, visual analog scale scores, and questionnaires.

RESULTS: Of the 203 subjects enrolled in the study, 11 had major protocol violations. Complete resolution was noted by 36/98 (36.7%) subjects in the PEG 3350 group and 23/94 (24.5%) in the placebo group (P = 0.0595). The number of complete BMs without straining or lumpy stools was similar between both groups. Subjects receiving PEG 3350 experienced significant relief in straining and reduction in hardness of stools over a 7-d period (P < 0.0001). Subjects reported that PEG 3350 had a better effect on their daily lives, provided better control over a BM, better relief from constipation,

cramping, and bloating, and was their preferred laxative. Adverse events (AEs) were balanced between the PEG 3350 and the placebo groups. No deaths, serious AEs, or discontinuations due to AEs were reported. This trial is registered at clinicaltrials.gov as NCT00770432.

CONCLUSION: Oral administration of 17 g PEG 3350 once daily for a week is effective, safe, and well tolerated in subjects with occasional constipation.

Key words: Polyethylene glycol 3350; Laxative; Straining; Bowel movements; Occasional constipation

Core tip: Unlike chronic constipation, which typically needs to be diagnosed by a healthcare professional, occasional constipation is a self-diagnosed condition. polyethylene glycol (PEG) 3350 (MiraLAX®) is a Food and Drug Administration-approved, once-daily oral over-the-counter laxative indicated for short-term (1 wk) use to relieve occasional constipation. However, very few data are available on the effectiveness of PEG 3350 for the treatment of occasional constipation. This is the first placebo-controlled study to evaluate the effectiveness and safety of PEG 3350 in subjects with occasional constipation after a week's treatment.

McGraw T. Polyethylene glycol 3350 in occasional constipation: A one-week, randomized, placebo-controlled, double-blind trial. *World J Gastrointest Pharmacol Ther* 2016; 7(2): 274-282 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i2/274.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i2.274

INTRODUCTION

Constipation is a common disorder that affects people of all ages and both sexes, with a predilection for the elderly and female populations[1-3]. Constipation negatively impacts quality of life (QoL), with a magnitude that is comparable to that in patients with chronic allergies, dermatitis, diabetes, and stable ulcerative colitis[4,5].

There is a high level of discrepancy in the diagnosis of constipation between patients and healthcare practitioners. Consequently, the prevalence of constipation differs based on the definition of constipation used. In adults, constipation as defined strictly on the basis of frequency of bowel movements (BMs), such as having a BM fewer than three times a week[6], accounts for a prevalence rate ranging from < 1% to 5.4%[7]. Higher prevalence rates are observed when constipation is self-reported or includes symptoms of constipation such as straining, hard and lumpy stools, bloating, and infrequent defecation[8-10]. The rome III criteria were devised to facilitate a diagnosis of functional constipation, accommodating both frequency of BMs and symptoms of constipation[11]. Results from surveys suggest that the prevalence of constipation perceived by patients is generally higher than that based on Rome criteria-diagnoses[12]. Only 50% of self-reported constipation fulfills the Rome criteria[13]. Constipation represents varying complaints among individuals that often cannot be restricted to frequency and/or two or more symptoms. Thus, a comprehensive definition of constipation needs to account for the patient's perception.

Constipation may persist for 3 mo or more, as in chronic constipation, or may be intermittent, lasting for shorter periods of time before resolving spontaneously as seen in occasional constipation. Unlike chronic constipation, which typically needs to be diagnosed by a healthcare professional, occasional constipation is a self-diagnosed condition that is often relieved by lifestyle changes or over-the-counter (OTC) laxatives[14]. Although there is no validated, agreed-upon definition of occasional constipation, this study evaluated occasional constipation sufferers as being those with constipation (straining with lumpy or hard stools, or the inability to produce a BM in the last 48 h) that does not resolve on its own with time, as opposed to chronic sufferers who need prescription medication and/or medical intervention to resolve their problem. Laxatives with an osmotic effect, such as polyethylene glycol (PEG) and lactulose, are common OTC treatments for constipation[15-17]. Osmotic laxatives aid defecation by increasing the osmotic pressure in the lumen of the gastrointestinal tract. Osmotic laxatives are known to improve frequency, straining, and the form of stools[17-19]. PEG has been shown to be more effective than lactulose in the management of chronic constipation and exhibited lesser side effects than lactulose[15,16]. PEG is a non-absorbable and non-metabolizable polymer that is not fermented by the gut flora and hence does not contribute to gas accumulation[20].

MiraLAX® is PEG 3350 powder, without any excipients, that retains water in the intestinal lumen by forming hydrogen bonds with water[21], resulting in softening of the stools. PEG 3350 is effective in the treatment of constipation in adults, having been shown to significantly increase the frequency of BMs, improve the symptoms of constipation[22-26], and reduce cramping and gas[19]. The Food and Drug Administration (FDA) approved PEG 3350 (MiraLAX®) on October 6, 2006 (NDA 22-015) for use as a nonprescription laxative for relief from occasional constipation (irregularity). However, efficacy and safety trials of PEG 3350 for the treatment of constipation were conducted in subjects with chronic constipation that generally fulfilled the Rome II criteria for constipation, which requires subjects to present symptoms of constipation for at least 12 wk, which need not be consecutive, in the preceding 12 mo.

This study investigated the efficacy, safety, and

preference for PEG 3350 based on the current marketed dose in subjects who occasionally used OTC laxatives.

MATERIALS AND METHODS

Study design

This study was a multicenter, randomized, placebo-controlled, double-blind study; Protocol # CL2007-12; Clinicaltrials.gov registration: NCT00770432. The study complied with US Code of Federal Regulations title 21, parts 50 and 56 for informed subject consent and Institutional Review Board (IRB) approval. The study was reviewed and supervised by Allendale IRB for all sites.

The study comprised two visits: One prior to, and one following, a 7-d at-home double-blind treatment period. Subjects were provided the investigational medications, instructed on study procedures, and required to complete questionnaires on symptoms, QoL, global evaluation, and preference during the visits. Each subject's participation lasted up to 13 d.

Subjects

Key inclusion criteria for the study required subjects to be $\geqslant$ 17 years of age with a current diagnosis of untreated constipation for $\leqslant$ 7 d based on signs/symptoms of straining and hard or lumpy stools or inability to have a BM within 48 h prior to randomization of the trial. Subjects were required to demonstrate their willingness to participate by signing a written informed consent. Subjects had to be users of OTC laxatives for the treatment of occasional constipation (defined as using a nonprescription laxative to treat < 3 episodes of constipation within the last 12 mo prior to randomization). Subjects were required not to use any medication to either treat the constipation or known to cause constipation during the course of the study. Subjects were in otherwise good health as determined by physical examination and medical history. Subjects were excluded from the study if they had a history of chronic constipation or were in the midst of having a constipation episode lasting for more than 1 wk prior to randomization or were under doctor's care for constipation at the time of study. Subjects who previously used PEG as a laxative; subjects with severe abdominal pain as their predominant complaint; subjects who participated in an investigational clinical surgical, drug, or device study within 30 d prior to randomization; subjects who were allergic to PEG or maltodextrin; and subjects with a history of alcohol or drug abuse were also excluded from the study.

Randomization, blinding, and treatments

Subjects who fulfilled the enrollment criteria were randomized in a 1:1 ratio to receive PEG 3350 or placebo according to a computer-generated randomization code. Subjects were randomly assigned the investigational drug or placebo at each site by selection of the next box of medication in the numerical sequence from the shipment provided. Maltrin® M500 (maltodextrin 500) that was identical in appearance and taste to PEG 3350 was used as the placebo and was dispensed in identical bottles as PEG 3350.

PEG 3350 17 g or a similarly sized dose of placebo (maltodextrin 500) was mixed in 4-8 ounces of any hot, cold, or room temperature beverage and was administered orally for a 7-d period. Per protocol, dosage was once (prior to noon) at approximately the same time each day. Subjects were instructed to return all test articles for inventory and accountability.

Compliance

Treatment compliance was based on completion of at least 5 of 7 diary days with answers to questions regarding study drug dosing and primary endpoints. Each subject indicated in the daily diary whether or not a complete or incomplete BM was accompanied by straining and whether the stool was hard or lumpy.

Endpoints

The primary efficacy variable assessed the proportion of subjects who self-reported complete resolution of straining and hard/lumpy stools for the intent-to-treat (ITT) and the per-protocol (PP) populations. Complete resolution was defined by daily diary reports of a complete BM with no straining or hard/lumpy stools for at least 48 h without a recurrence of $\geqslant$ 2 consecutive BM episodes with straining or hard/lumpy stools. Secondary endpoints included diary ratings of visual analog scale (VAS) format (BM control, gas bloating, abdominal discomfort/cramping, and well-being) and binary outcomes (BM satisfaction and BM sense of completion). Response to treatment based on laxative preference (Likert scale and VAS) was assessed.

The safety endpoint was based on tabulation and analysis of adverse events (AEs) and measurement of vital signs at the first visit.

Study populations

The ITT population received at least one dose of the assigned drug and underwent a baseline and post-baseline evaluation performed at the visits. The PP population included all ITT subjects who additionally had no major protocol violations or other events biasing their study outcome (*e.g.*, use of prohibited medications or excessive missing data).

The safety population included all subjects that took one or more dose of study medication, and was equivalent in numbers to the ITT populations.

Sample size

Detection of a 25% difference in a binomial endpoint (P = 0.05), which may be considered clinically significant, required a sample size of 85 per group for 90% power. Allowing for a 15% rate of early discontinuation, a

Table 1 Patient demographics and baseline characteristics *n* (%)

Summary	Treatment group		All subjects	*P* value[1]
	PEG 3350	Placebo		
ITT subjects	102	101	203	
Gender				0.2165
Male	25 (24.5)	33 (32.7)	58 (28.6)	
Female	77 (75.5)	68 (67.3)	145 (71.4)	
Age (yr)				0.7361
n	102	101	203	
Mean ± SD	45.8 ± 12.52	45 ± 14.15	45.4 ± 13.33	
Race				0.3340
American Indian	0	2 (2.0)	2 (1.0)	
Alaskan native	0	0	0	
Asian	0	0	0	
Black/African American	7 (6.9)	9 (8.9)	16 (7.9)	
Native Hawaiian	0	0	0	
White	81 (79.4)	71 (70.3)	152 (74.9)	
Hispanic or Latino	14 (13.7)	19 (18.8)	33 (16.3)	
Weight (kg)				0.5257
n	102	101	203	
Mean ± SD	80.2 ± 19.11	79.7 ± 21.19	79.9 ± 20.12	
On average, how many successful bowel movements does the subject have per week?				0.8232
0-2	36 (35.3)	35 (34.7)	71 (35.0)	
3-5	44 (43.1)	40 (39.6)	84 (41.4)	
6-8	22 (21.6)	25 (24.8)	47 (23.2)	
> 9	0	1 (1.0)	1 (0.5)	
What type of laxative is this?				0.6775
Stimulant	57 (55.9)	63 (62.4)	120 (59.1)	
Bulk forming fiber	12 (11.8)	15 (14.9)	27 (13.3)	
Lubricant	3 (2.9)	2 (2.0)	5 (2.5)	
Osmotic	8 (7.8)	4 (4.0)	12 (5.9)	
Carbon dioxide releasing	0	0	0	
Stool softener	18 (17.6)	15 (14.9)	33 (16.3)	
Combination	2 (2.0)	0	2 (1.0)	
Other	2 (2.0)	2 (2.0)	4 (2.0)	

[1]Wilcoxon Rank-Sum test was used to assess homogeneity among treatment groups of baseline continuous variables, and Fisher's exact test was used to evaluate categorical variables. ITT: Intent to treat; PEG: Polyethylene glycol.

sample of 196 subjects was estimated to complete with 170 subjects.

Statistical analysis

Statistical analysis was performed for the primary efficacy variable on the ITT and PP populations between the two treatment groups using Cochran-Mantel-Haenszel (CMH) analysis adjusting for investigational site. VAS and Likert ratings were analyzed between the treatment groups using analysis of variance (ANOVA) with factors for treatment, site, and treatment-site interaction. The VAS rating scales were assessed as percent change from baseline over time. Preference questionnaires were presented as binary outcomes and analyzed using CMH and ANOVA.

RESULTS

Demographics and baseline characteristics

A total of 203 subjects - 102 in the PEG 3350 group and 101 in the placebo group - from 7 investigational sites were enrolled in the study from November 28, 2007, with the last subject's visit on January 18, 2008. The study was completed by 98 subjects (96.1%) in the PEG 3350 group and 94 subjects (93.1%) in the placebo group (Figure 1). A total of 11 subjects (7 in the placebo group and 4 in the PEG 3350 group) had major protocol violations prior to breaking of the randomization code, and were excluded from the PP group analysis. All randomized subjects were enrolled in the ITT population (*i.e.*, subjects who received at least one dose of the assigned drug and had a baseline and post-baseline evaluation performed at the visits). Subjects' ages ranged between 17 and 79 years. Approximately three quarters of subjects were White, and 71% were female (29% male) (Table 1). Subjects in both treatment groups were well matched with regards to medical history, baseline physical examinations, vital signs, and other baseline characteristics (Table 1). Similar percentages of treatment compliance were observed for both the treatment groups; 99% for the PEG 3350 group and 98% for the placebo group. Because only 11 (5.4%) subjects of the ITT population were not part of the PP population, the results are presented for the PP population to assess the robustness of the study.

Endpoints

Analysis of the primary efficacy variable showed that in the PP population, 36 subjects (36.7%) on PEG 3350 reported complete resolution compared with 23 (24.5%) receiving placebo ($P = 0.0595$) (Figure 2).

Secondary efficacy analysis showed statistically significant superiority of PEG 3350 over placebo. Subjects using PEG 3350 experienced significant relief in straining and reduction in the hardness of stools over the 7-d treatment period ($P < 0.0001$). Figure 3 shows that subjects using PEG 3350 recorded a VAS score of 29.0 *vs* 45.3 with placebo, for symptoms of hardness, and 21.1 *vs* 37.8, respectively, for straining. The percentage of all BMs that was complete without straining or lumpy stools was also significantly higher for the PEG 3350 group; 34% for PEG 3350 compared to 18.4% for the placebo group ($P < 0.0002$) (Table 2).

No statistical significance was observed for the number of complete BMs without straining or lumpy stools (Table 2). There was no significant decrease in the symptoms of constipation (bloating, gas, control, and cramping) with PEG 3350 over placebo, indicating that PEG 3350 did not improve or alleviate these symptoms (Figure 3). Global assessment of the effect of constipation on their daily lives showed that subjects randomized to PEG 3350 perceived a significantly better impact on their daily lives than did those randomized to placebo in all categories tested,

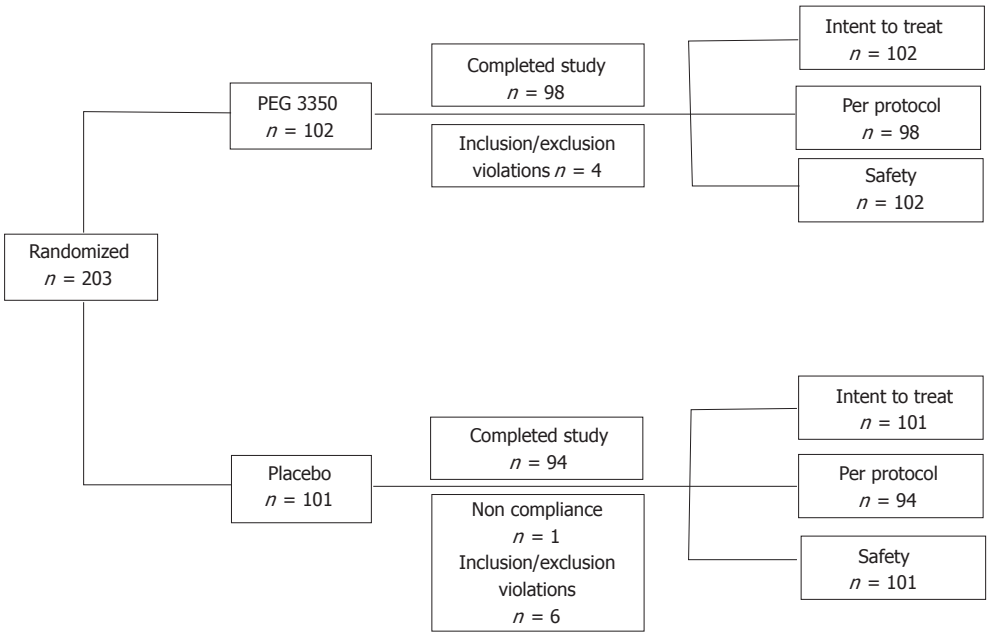

Figure 1 Disposition of subjects. The ITT population included all subjects randomized to a study treatment and receiving at least one dose of the assigned drug. The per-protocol population included all ITT subjects who additionally exhibited no major protocol violations or other events considered biasing the study outcome. The safety population included all subjects who received one or more dose of the study medication. ITT: Intent-to-treat; PEG: Polyethylene glycol.

Table 2 Summary of average daily diary bowel movement assessments of the subjects *n* (%)

Mean (SD)	Placebo *n* = 94	PEG 3350 *n* = 98	*P* value[1]
Average BMs per day	1.0 (0.63)	1.1 (0.84)	0.46
Average BMs per day that were complete without straining or lumpy stool	0.5 (0.46)	0.6 (0.46)	0.57
Percent of all BMs that were complete without straining or lumpy stool	18.4 (25.00)	34 (35.84)	0.0002
Percent of all BMs that were failures	10.2 (17.64)	10.1 (19.31)	0.94
Percent of all BMs that were incomplete	34.6 (27.75)	25.7 (28.49)	0.16

[1]ANOVA controlling for site and site by treatment interaction was used to test homogeneity of response across treatment arms. BM: Bowel movement; ANOVA: Analysis of variance; PFG: Polyethylene glycol.

and over 40% (ratio, 2:1) of subjects considered PEG 3350 to be the best laxative they had tried (Table 3). PEG 3350 was preferred over their usual laxative based on VAS rating, and when subjects were queried on the following: Recommendation (P = 0.0069), better relief from constipation (P = 0.0206), better relief from cramping (P = 0.0112), better relief from bloating (P = 0.0002), and better control over a BM (P = 0.0349) using the Likert scale (Figure 4).

The most frequently observed AEs included headache and back and neck pain and were similar for both treatment groups, indicating that this was not a drug-related effect (Table 4). No deaths or serious AEs were recorded from the therapy, and there were no discontinuations due to a drug-related AE.

DISCUSSION

PEG 3350 (MiraLAX®) is an FDA-approved, once-daily, oral OTC laxative indicated for short-term (1 wk) use to relieve occasional constipation/irregularity. Subjects and healthcare practitioners have a wide range of treatment options for relieving symptoms of constipation. Large-scale randomized trials support lactulose[27,28], tegaserod[29], prucalopride, lubiprostone, and linaclotide[30], as well as PEG[19,22-25] for the treatment of chronic constipation. However, there are no reports on the effectiveness of PEG 3350 for the treatment of occasional constipation. This is the first placebo-controlled study to evaluate the effectiveness and safety of PEG 3350 in subjects with occasional constipation after a week's treatment. The current study demonstrated that in subjects with self-reported occasional constipation, there is no significant difference between PEG 3350 and placebo in achieving relief from straining and reduction in hardness of stools experienced during BMs. PEG 3350 was generally well tolerated; AEs were similar in incidence and severity (mostly mild) between treatment groups, in line with previous reports[19,22-26].

The present study used the subject's own estimation of treatment effect to evaluate whether treatment with PEG 3350 led to resolution of straining and lumpy/hard stools compared with placebo. Although subjects on PEG 3350 had more successful BMs than did those on placebo during the treatment period, a statistically significant difference between PEG 3350 and placebo was achieved only on day 3 of treatment. In subjects receiving placebo, constipation resolved by day 5 as expected in occasional constipation, which may have

Table 3 Summary of global assessments *n* (%)

Summary	Treatment group		*P* value[1]
	PEG 3350	Placebo	
Per-protocol subjects, *n*	98	94	
It works better than other laxatives I have tried			
Strongly agree	16 (16.3)	8 (8.5)	0.0037
Agree	34 (34.7)	27 (28.7)	
Neither agree nor disagree	24 (24.5)	15 (16.0)	
Disagree	19 (19.4)	35 (37.2)	
Strongly disagree	5 (5.1)	9 (9.6)	
Not applicable/missing	0	0	
It is the best laxative I have tried			
Strongly agree	18 (18.4)	7 (7.4)	0.0005
Agree	22 (22.4)	13 (13.8)	
Neither agree nor disagree	26 (26.5)	22 (23.4)	
Disagree	23 (23.5)	36 (38.3)	
Strongly disagree	9 (9.2)	16 (17.0)	
Not applicable/missing	0	0	
It helps me feel less irritable			
Strongly agree	6 (6.1)	7 (7.4)	0.0414
Agree	32 (32.7)	26 (27.7)	
Neither agree nor disagree	38 (38.8)	25 (26.6)	
Disagree	18 (18.4)	21 (22.3)	
Strongly disagree	4 (4.1)	14 (14.9)	
Not applicable/missing	0	1 (1.1)	
It helps me feel more confident			
Strongly agree	7 (7.1)	7 (7.4)	0.0262
Agree	25 (25.5)	15 (16.0)	
Neither agree nor disagree	38 (38.8)	28 (29.8)	
Disagree	19 (19.4)	28 (29.8)	
Strongly disagree	8 (8.2)	14 (14.9)	
Not applicable/missing	1 (1.0)	2 (2.1)	
It feels better for my body than other laxatives I have tried			
Strongly agree	13 (13.3)	8 (8.5)	0.0215
Agree	34 (34.7)	27 (28.7)	
Neither agree nor disagree	24 (24.5)	15 (16.0)	
Disagree	18 (18.4)	30 (31.9)	
Strongly disagree	9 (9.2)	14 (14.9)	
Not applicable/ missing	0	0	

[1]The *P* value obtained from Cochran-Mantel-Haenszel row mean scores difference χ^2 test adjusted for study site was used to compare homogeneity of results in treatment arms.

resulted in a loss of significance between treatment arms. In the study of PEG 3350[25] for the treatment of chronic constipation, PEG 3350 was successful in treating constipation, where success was defined as a subject experiencing ⩾ 3 satisfactory BMs per week and having no more than one of the remaining three Rome III symptoms. The same study also reported that PEG-treated subjects had higher incidences of complete spontaneous BMs over placebo[25]. In the present study, PEG 3350 was better, but not significantly different (*P* = 0.595) than placebo in producing a complete BM devoid of straining and hard/lumpy stools. However, significant results were obtained in favor of PEG 3350 with secondary measures that assessed subjective symptoms such as straining and hardness of stools. Because most subjects describe constipation as hard and lumpy stools and/or straining, the results of the study are clinically meaningful, despite the lack of statistical significance for the primary endpoint.

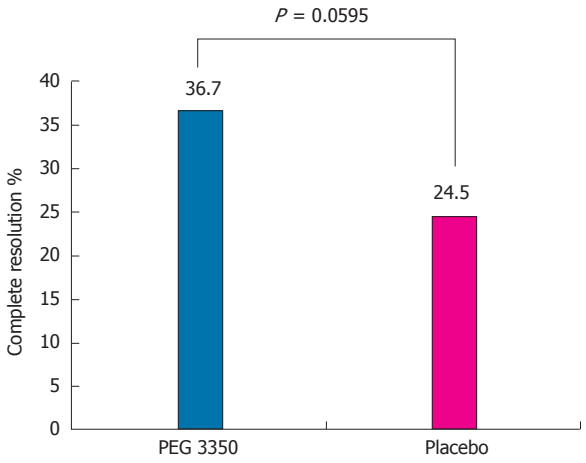

Figure 2 Assessment of primary outcome between polyethylene glycol 3350 and placebo (per protocol population). Resolution was recorded if the subject reported no occurrence of two or more consecutive unsuccessful bowel movements (BMs) for the rest of the study following the first successful BM. PEG: Polyethylene glycol.

Most subjects suffering from constipation are likely, at least initially, to use OTC laxatives for relieving their symptoms, and are generally not satisfied with the results[3,7,31]. PEG 3350 has been reported to be effective in relieving cramping and gas symptoms in subjects with chronic constipation[19,26], yet other secondary measures evaluating the effect of PEG 3350 on cramping and gas showed no significant difference between PEG 3350 and placebo. This may be due to the fact that the beneficial effect of PEG relative to placebo is confined to improved stool consistency, which may be due to increased osmotic action of intraluminal content, and hence does not affect cramping and gas; it may also be due to the relatively milder symptoms of subjects with occasional constipation and the limited duration of treatment. The latter explanation is supported by the DiPalma study[19], which evaluated PEG 3350 in subjects with chronic constipation, and reported significantly less gas and cramping with PEG 3350. However, it should also be noted that the current study evaluated subjects with occasional constipation, a self-limiting condition, unlike the DiPalma study which evaluated patients with chronic constipation and who were enrolled strictly on the basis of stool frequency, *i.e.*, ⩽ 2 stools during the week and were treated with PEG for 2 wk. While it is plausible that significant differences in cramping and gas may be detected in the second week of treatment with PEG 3350, other long-term studies[22,23] have indicated no significant effect of PEG 3350 on flatulence, consistent with results of the present study.

Lifestyle modifications, diet, and defecation practices were not evaluated in the study. Lifestyle modifications and dietary fiber may help resolve occasional constipation symptoms, and bulk/roughage in the diet could have been measured to determine if these factors had an effect on the outcome. Also, bad defecation practices such as deferring the urge to defecate could

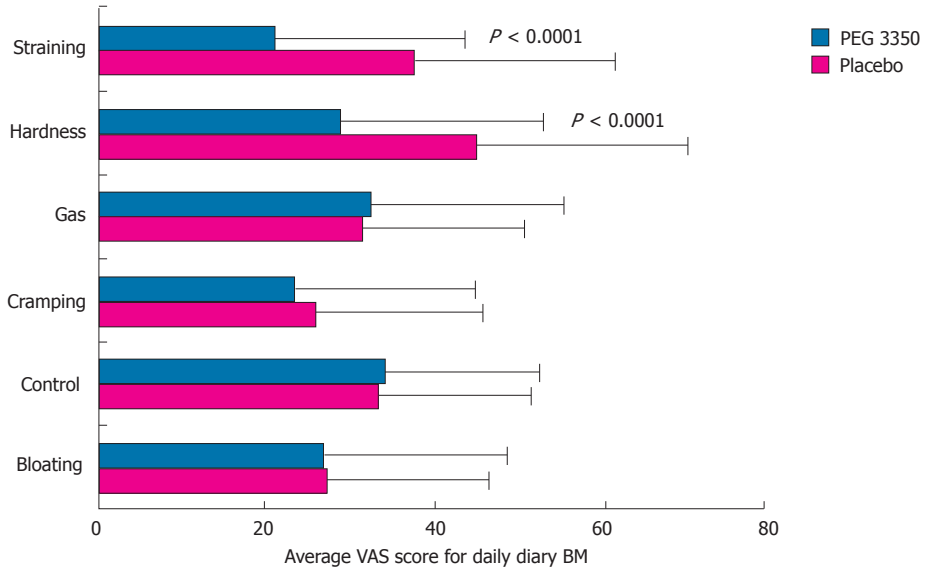

Figure 3 Average visual analog scale scores for daily diary entries of bowel movements of subjects receiving polyethylene glycol 3350 or placebo (PP population). Error bars represent standard deviations of mean. BM: Bowel movement; PEG: Polyethylene glycol; VAS: Visual analog scale.

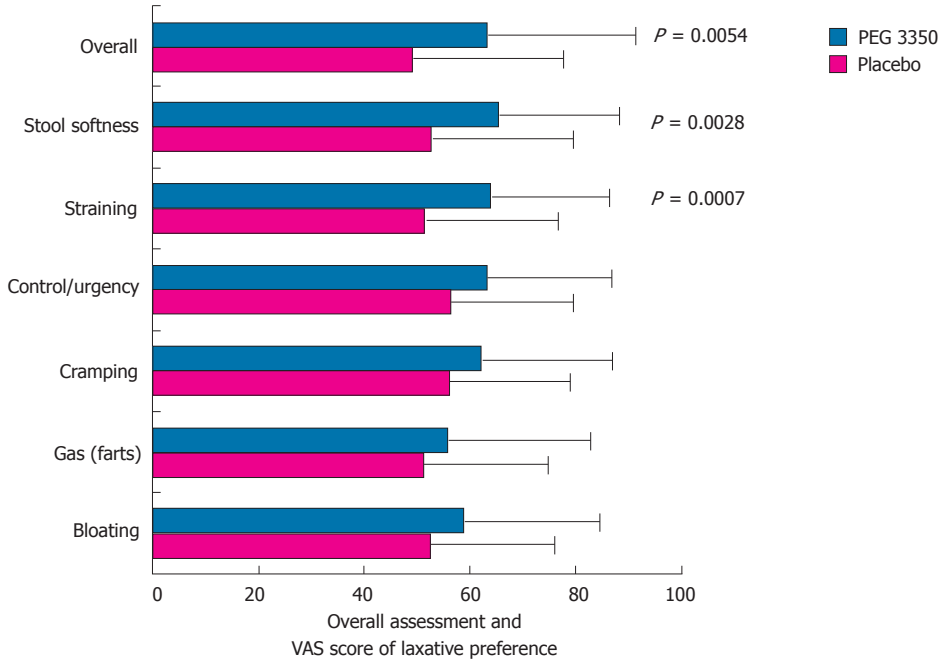

Figure 4 Visual analog scale scores for laxative preference of subjects receiving polyethylene glycol 3350 or placebo (per protocol population). Error bars represent standard deviations of mean. PEG: Polyethylene glycol; VAS: Visual analog scale.

have been monitored to determine if they affected or led to constipation. However, it is unlikely that these factors could have influenced the results of the study as the effects would be expected to be evenly distributed between the groups.

This study has important limitations. The sample size was estimated based on studies[22] conducted in subjects with chronic constipation, which showed an 11% success for subjects in the placebo group. The current study design used a primary endpoint in the first week of treatment that had not been previously reported, and hence there were very little clinical data available upon which to base sample size calculations. In the present study, the success rate for the placebo group with occasional constipation was 24.5%. Based on the differences observed between the two study groups, an additional 40 subjects per group would have been required to observe a statistically significant difference over placebo for the primary endpoint at $P < 0.05$. This underestimation of the sample size may have led to the introduction of a type Ⅱ (false negative) error, which in turn might have led to the non-significance between the groups for the primary endpoint.

The self-limiting nature of occasional constipation

Table 4 Summary of treatment-emergent adverse events, safety population *n* (%)

Summary	Treatment group	
	PEG 3350	Placebo
Total subjects	102	101
Total subjects with an adverse event	14 (13.7)	17 (16.8)
Nervous system disorders	11 (10.8)	11 (10.9)
Headache	11 (10.8)	11 (10.9)
Migraine	1 (< 1.0)	0
Musculoskeletal and connective tissue disorders	3 (2.9)	2 (2.0)
Back pain	0	2 (2.0)
Neck pain	2 (2.0)	0
Arthralgia	1 (< 1.0)	0
Gastrointestinal disorders	0	4 (4.0)
Nausea	0	2 (2.0)
Abdominal distension	0	1 (< 1.0)
Dyspepsia	0	1 (< 1.0)
Infections and infestations	1 (< 1.0)	1 (< 1.0)
Nasopharyngitis	1 (< 1.0)	0
Sinusitis	0	1 (< 1.0)
Psychiatric disorders	1 (< 1.0)	1 (< 1.0)
Insomnia	1 (< 1.0)	1 (< 1.0)
Respiratory, thoracic, and mediastinal disorders	2 (2.0)	0
Nasal congestion	1 (< 1.0)	0
Pharyngolaryngeal pain	1 (< 1.0)	0

PEG: Polyethylene glycol.

may sometimes result in spontaneous improvement, which could be perceived by the subjects as a drug-related effect, especially over the short one-week treatment duration. This could also have had an effect on the global assessment of the impact of constipation on the subjects' daily lives and their laxative preferences, which were based on the subjects' perceptions of symptom alleviation.

Subjects receiving placebo had to take 17 g maltodextrin in 4-8 ounces of water. This could have led to a lack of standardization of the liquid medium as the volume for placebo was not constant, which in turn could have affected the final osmolality. Also, the patients could have mixed the placebo in hyperosmolar liquids such as fruit juices, which could have led to further variance in the molality of the liquid medium.

The above-mentioned limitations of the current study could be taken into consideration when designing future trials evaluating the effectiveness of PEG 3350 in subjects with occasional constipation.

PEG 3350 at a dose of 17 g, administered once daily for a week, is safe, effective, well tolerated, and may be preferred by subjects over other laxatives in the treatment of occasional constipation.

ACKNOWLEDGMENTS

The author wishes to express his appreciation to all the trial investigators (Table 5). Medical writing and/or editorial assistance was provided by Elphine Telles, PhD, of Cactus Communications. This assistance was funded by Merck & Co., Inc., Kenilworth, NJ, United States.

Table 5 List of study sites[1] and primary investigators

Site	Primary investigator
Product Investigations, Inc., Conshohocken, PA	Morris Shelanski, MD
Site 2, Turnpike Levittown, NY	Maurice Gunsberger, MD
Site 3, South Windsor, CT	Raymond Kurker, MD
International Research Services, Inc., Port Chester, NY	Roger A. Villi, MD
Hartford Research Center	Anthony Roselli, MD
Product Investigations, Inc., Modesto, CA	Clinton E. Prescott, MD
International Research Services, Inc, Rockland, ME	Robert Jorden, MD
Avon Family Medical Group, Avon, CT	Anthony Roselli, MD

[1]Of the 8 participating sites, only 7 sites enrolled ⩾ 1 subject.

COMMENTS

Background

Polyethylene glycol (PEG) 3350 has been shown to be effective in the treatment of chronic constipation in large-scale randomized trials. PEG 3350 (MiraLAX®), an oral over-the-counter (OTC) laxative, administered once-daily over a week, has been approved by the Food and Drug Administration to relieve occasional constipation.

Research frontiers

Very few data are available regarding the effectiveness of PEG 3350 for the treatment of occasional constipation.

Innovations and breakthroughs

This is the first placebo-controlled study to evaluate the effectiveness and safety of PEG 3350 in subjects with occasional constipation after a week's treatment. PEG 3350, at a dose of 17 g, administered once daily for a week, was safe, effective, and well tolerated, and may be preferred by patients over other laxatives.

Applications

The findings and limitations of the current study could be taken into consideration while designing future trials evaluating the effectiveness of PEG 3350 in subjects with occasional constipation.

Terminology

Occasional constipation is a self-diagnosed condition that is often relieved by lifestyle changes or OTC laxatives. Occasional constipation was defined here as constipation (straining with lumpy or hard stools, or the inability to produce a bowel movement in the last 48 h) that does not resolve on its own with time.

Peer-review

The present study addresses a scarcely investigated topic, occasional constipation. In addition, it reflects a common use of PEG 3350 in clinical practice and therefore it is of considerable interest to determine whether this common off-label use of PEG 3350 is justified.

REFERENCES

1 **Higgins PD**, Johanson JF. Epidemiology of constipation in North America: a systematic review. *Am J Gastroenterol* 2004; **99**: 750-759 [PMID: 15089911 DOI: 10.1111/j.1572-0241.2004.04114.x]

2 **Johanson JF**, Sonnenberg A, Koch TR. Clinical epidemiology of chronic constipation. *J Clin Gastroenterol* 1989; **11**: 525-536 [PMID: 2551954 DOI: 10.1097/00004836-198910000-00008]

3 **Pare P**, Ferrazzi S, Thompson WG, Irvine EJ, Rance L. An epidemiological survey of constipation in canada: definitions, rates, demographics, and predictors of health care seeking. *Am J Gastroenterol* 2001; **96**: 3130-3137 [PMID: 11721760 DOI: 10.1111/j.1572-0241.2001.05259.x]

4 **Belsey J**, Greenfield S, Candy D, Geraint M. Systematic review: impact of constipation on quality of life in adults and children. *Aliment Pharmacol Ther* 2010; **31**: 938-949 [PMID: 20180788 DOI: 10.1111/j.1365-2036.2010.04273.x]

5 **Wald A**, Scarpignato C, Kamm MA, Mueller-Lissner S, Helfrich I, Schuijt C, Bubeck J, Limoni C, Petrini O. The burden of constipation on quality of life: results of a multinational survey. *Aliment Pharmacol Ther* 2007; **26**: 227-236 [PMID: 17593068 DOI: 10.1111/j.1365-2036.2007.03376.x]

6 **Connell AM**, Hilton C, Irvine G, Lennard-Jones JE, Misiewicz JJ. Variation of bowel habit in two population samples. *Br Med J* 1965; **2**: 1095-1099 [PMID: 5838411 DOI: 10.1136/bmj.2.5470.1095]

7 **Wald A**, Scarpignato C, Mueller-Lissner S, Kamm MA, Hinkel U, Helfrich I, Schuijt C, Mandel KG. A multinational survey of prevalence and patterns of laxative use among adults with self-defined constipation. *Aliment Pharmacol Ther* 2008; **28**: 917-930 [PMID: 18644012 DOI: 10.1111/j.1365-2036.2008.03806.x]

8 **Sandler RS**, Drossman DA. Bowel habits in young adults not seeking health care. *Dig Dis Sci* 1987; **32**: 841-845 [PMID: 3608732 DOI: 10.1007/BF01296706]

9 **Stewart WF**, Liberman JN, Sandler RS, Woods MS, Stemhagen A, Chee E, Lipton RB, Farup CE. Epidemiology of constipation (EPOC) study in the United States: relation of clinical subtypes to sociodemographic features. *Am J Gastroenterol* 1999; **94**: 3530-3540 [PMID: 10606315 DOI: 10.1111/j.1572-0241.1999.01642.x]

10 **Walter S**, Hallböök O, Gotthard R, Bergmark M, Sjödahl R. A population-based study on bowel habits in a Swedish community: prevalence of faecal incontinence and constipation. *Scand J Gastroenterol* 2002; **37**: 911-916 [PMID: 12229965 DOI: 10.1080/003655202760230865]

11 **Longstreth GF**, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC. Functional bowel disorders. *Gastroenterology* 2006; **130**: 1480-1491 [PMID: 16678561 DOI: 10.1053/j.gastro.2005.11.061]

12 **Cottone C**, Tosetti C, Disclafani G, Ubaldi E, Cogliandro R, Stanghellini V. Clinical features of constipation in general practice in Italy. *United European Gastroenterol J* 2014; **2**: 232-238 [PMID: 25360307 DOI: 10.1177/2050640614527283]

13 **Digesu GA**, Panayi D, Kundi N, Tekkis P, Fernando R, Khullar V. Validity of the Rome III Criteria in assessing constipation in women. *Int Urogynecol J* 2010; **21**: 1185-1193 [PMID: 20495784 DOI: 10.1007/s00192-010-1179-0]

14 Website AGA. Accessed December 15, 2014. Available from: URL: http://www.gastro.org/patient-center/digestive-conditions/constipation

15 **Dupont C**, Leluyer B, Maamri N, Morali A, Joye JP, Fiorini JM, Abdelatif A, Baranes C, Benoît S, Benssoussan A, Boussioux JL, Boyer P, Brunet E, Delorme J, François-Cecchin S, Gottrand F, Grassart M, Hadji S, Kalidjian A, Languepin J, Leissler C, Lejay D, Livon D, Lopez JP, Mougenot JF, Risse JC, Rizk C, Roumaneix D, Schirrer J, Thoron B, Kalach N. Double-blind randomized evaluation of clinical and biological tolerance of polyethylene glycol 4000 versus lactulose in constipated children. *J Pediatr Gastroenterol Nutr* 2005; **41**: 625-633 [PMID: 16254521 DOI: 10.1097/01.mpg.0000181188.01887.78]

16 **Petticrew M**, Rodgers M, Booth A. Effectiveness of laxatives in adults. *Qual Health Care* 2001; **10**: 268-273 [PMID: 11743157 DOI: 10.1136/qhc.0100268]

17 **Petticrew M**, Watt I, Sheldon T. Systematic review of the effectiveness of laxatives in the elderly. *Health Technol Assess* 1997; **1**: i-iv, 1-52 [PMID: 9483163]

18 **Corazziari E**, Badiali D, Bazzocchi G, Bassotti G, Roselli P, Mastropaolo G, Lucà MG, Galeazzi R, Peruzzi E. Long term efficacy, safety, and tolerabilitity of low daily doses of isosmotic polyethylene glycol electrolyte balanced solution (PMF-100) in the treatment of functional chronic constipation. *Gut* 2000; **46**: 522-526 [PMID: 10716682 DOI: 10.1136/gut.46.4.522]

19 **DiPalma JA**, DeRidder PH, Orlando RC, Kolts BE, Cleveland MB. A randomized, placebo-controlled, multicenter study of the safety and efficacy of a new polyethylene glycol laxative. *Am J Gastroenterol* 2000; **95**: 446-450 [PMID: 10685748 DOI: 10.1111/j.1572-0241.2000.01765.x]

20 **Bouhnik Y**, Neut C, Raskine L, Michel C, Riottot M, Andrieux C, Guillemot F, Dyard F, Flourié B. Prospective, randomized, parallel-group trial to evaluate the effects of lactulose and polyethylene glycol-4000 on colonic flora in chronic idiopathic constipation. *Aliment Pharmacol Ther* 2004; **19**: 889-899 [PMID: 15080850 DOI: 10.1111/j.1365-2036.2004.01918.x]

21 **Schiller LR**, Emmett M, Santa Ana CA, Fordtran JS. Osmotic effects of polyethylene glycol. *Gastroenterology* 1988; **94**: 933-941 [PMID: 3345895]

22 **Di Palma JA**, Cleveland MV, McGowan J, Herrera JL. A randomized, multicenter comparison of polyethylene glycol laxative and tegaserod in treatment of patients with chronic constipation. *Am J Gastroenterol* 2007; **102**: 1964-1971 [PMID: 17573794 DOI: 10.1111/j.1572-0241.2007.01365.x]

23 **Di Palma JA**, Cleveland MV, McGowan J, Herrera JL. An open-label study of chronic polyethylene glycol laxative use in chronic constipation. *Aliment Pharmacol Ther* 2007; **25**: 703-708 [PMID: 17311603 DOI: 10.1111/j.1365-2036.2006.03228.x]

24 **DiPalma JA**, Cleveland MB, McGowan J, Herrera JL. A comparison of polyethylene glycol laxative and placebo for relief of constipation from constipating medications. *South Med J* 2007; **100**: 1085-1090 [PMID: 17984738 DOI: 10.1097/SMJ.0b013e318157ec8f]

25 **Dipalma JA**, Cleveland MV, McGowan J, Herrera JL. A randomized, multicenter, placebo-controlled trial of polyethylene glycol laxative for chronic treatment of chronic constipation. *Am J Gastroenterol* 2007; **102**: 1436-1441 [PMID: 17403074 DOI: 10.1111/j.1572-0241.2007.01199.x]

26 **Cleveland MV**, Flavin DP, Ruben RA, Epstein RM, Clark GE. New polyethylene glycol laxative for treatment of constipation in adults: a randomized, double-blind, placebo-controlled study. *South Med J* 2001; **94**: 478-481 [PMID: 11372795 DOI: 10.1097/00007611-200105000-00006]

27 **Sanders JF**. Lactulose syrup assessed in a double-blind study of elderly constipated patients. *J Am Geriatr Soc* 1978; **26**: 236-239 [PMID: 346631 DOI: 10.1111/j.1532-5415.1978.tb01967.x]

28 **Wesselius-De Casparis A**, Braadbaart S, Bergh-Bohlken GE, Mimica M. Treatment of chronic constipation with lactulose syrup: results of a double-blind study. *Gut* 1968; **9**: 84-86 [PMID: 4867936 DOI: 10.1136/gut.9.1.84]

29 **Quigley EM**, Wald A, Fidelholtz J, Boivin M, Pecher E, Earnest D. Safety and tolerability of tegaserod in patients with chronic constipation: pooled data from two phase III studies. *Clin Gastroenterol Hepatol* 2006; **4**: 605-613 [PMID: 16678076 DOI: 10.1016/j.cgh.2006.02.017]

30 **Ford AC**, Suares NC. Effect of laxatives and pharmacological therapies in chronic idiopathic constipation: systematic review and meta-analysis. *Gut* 2011; **60**: 209-218 [PMID: 21205879 DOI: 10.1136/gut.2010.227132]

31 **Johanson JF**, Kralstein J. Chronic constipation: a survey of the patient perspective. *Aliment Pharmacol Ther* 2007; **25**: 599-608 [PMID: 17305761 DOI: 10.1111/j.1365-2036.2006.03238.x]

P- Reviewer: Bassotti G, Soares RLS, Tandon RK **S- Editor**: Ma YJ
L- Editor: A **E- Editor**: Wu HL

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i2.283

World J Gastrointest Pharmacol Ther 2016 May 6; 7(2): 283-293
ISSN 2150-5349 (online)

SYSTEMATIC REVIEWS

Disaccharidase activity in children undergoing esophagogastroduodenoscopy: A systematic review

Taylor Daileda, Peter Baek, Morgan E Sutter, Kalpesh Thakkar

Taylor Daileda, Peter Baek, Morgan E Sutter, Kalpesh Thakkar, the SONIC Gut Research Group, Houston, TX 77479, United States

Author contributions: Daileda T, Sutter ME and Thakkar K contributed to the collection of data, study design, analysis of data, drafting the manuscript, and editing the manuscript; Baek P and contributed to the collection of data, study design, and editing the manuscript; Sutter ME contributed to the study design and editing the manuscript; all authors approved the final version of the manuscript.

Conflict-of-interest statement: No funding or conflicts of interest to report.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at Dryad repository, will provide a permanent, citable and open-access home for the dataset. All data for this systematic review was gathered from included studies.

Correspondence to: Kalpesh Thakkar, MD, the SONIC Gut Research Group, 1803 Cambria Lane, Sugar Land, Houston, TX 77479, United States. thakkarsmail@gmail.com
Telephone: +1-281-7255970
Fax: +1-281-7255971

Received: July 24, 2015
Peer-review started: July 27, 2015
First decision: August 25, 2015
Revised: January 15, 2016
Accepted: January 27, 2016
Article in press: January 29, 2016
Published online: May 6, 2016

Abstract

AIM: To investigate the utility of intestinal disaccharide analysis during esophagogastroduodenoscopy (EGD) in children, we performed a systematic review of studies examining disaccharide activity.

METHODS: All full-length articles published in English during 1966-2014 were included if: (1) participants had small intestinal biopsy evaluation of disaccharide activity; (2) levels of lactase, sucrase, maltase or palatinase were reported; and (3) age of participants was under 18 years.

RESULTS: Thirty articles examining 34753 disaccharide assays fulfilled the specific search, inclusion, and exclusion criteria. All of the studies were observational in design and 57% (17) were prospective. Sixteen studies were conducted in the United States and 9 European studies were identified. The biggest study enrolled about 30, 314 procedures and 13 studies investigated fewer than 50 procedures. Eleven studies examined Caucasian subjects, 3 studies examined Asian subjects, and 6 examined African subjects. Only one Hispanic subject was included. In studies reporting disaccharide deficiency, the overall proportion of lactase deficiency was 39.2%, sucrase deficiency was 9.0%, maltase deficiency was 12.6% and palatinase deficiency was 9.1%. The prevalence of duodenal inflammatory changes ranged from 6% to 24% for non-specific histological lesions (*e.g.*, duodenitis). Sixteen studies examined the association of histologic findings with disaccharide activities, and 12 studies reported an inverse association between degree of histologic inflammation and disaccharide levels.

CONCLUSION: We reviewed 30 studies including 34753 biopsy specimens with disaccharide analysis from children undergoing EGD. Our findings advocate a large study is to further illuminate the importance of EGD

with disaccharide analysis in children.

Key words: Disaccharidase; Endoscopy; Children

Core tip: Intestinal disaccharide analysis of duodenal biopsy specimens are often obtained during esophagogastroduodenoscopy (EGD) in children. In our review examining 34753 disaccharide assays the overall proportion of lactase deficiency was 39.2%, sucrase deficiency was 9.0%, maltase deficiency was 12.6% and palatinase deficiency was 9.1% in children. The impact of EGD with disaccharide analysis on treatment plans, quality of life, improvement of gastrointestinal symptoms, and cost-effectiveness has not been well studied. There is also little published data on Hispanic children undergoing EGD with disaccharide analysis.

Daileda T, Baek P, Sutter ME, Thakkar K. Disaccharidase activity in children undergoing esophagogastroduodenoscopy: A systematic review. *World J Gastrointest Pharmacol Ther* 2016; 7(2): 283-293 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i2/283.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i2.283

INTRODUCTION

Intestinal disaccharide analysis of duodenal biopsy specimens are often obtained during esophagogastroduodenoscopy (EGD) in children. Options for disaccharide evaluation include stool analysis, hydrogen breath tests, and sugar tolerance testing. However, the "gold-standard" to accomplish a diagnosis of disaccharide deficiency is a small intestinal biopsy and enzyme assay[1,2]. The four enzyme complexes commonly assessed for disaccharide hydrolysis (disaccharidases) are lactase, sucrase, maltase and palatinase. In pediatrics, disaccharide deficiency has a wide clinical presentation with symptoms possibly including diarrhea, bloating, flatulence, abdominal pain, borborygmi, and failure to thrive. Therefore, it can be challenging to select patients undergoing EGD to complete the additional disaccharide evaluation which generally requires at least two additional duodenal biopsy specimens.

Many clinical investigators have attempted to characterize the prevalence of disaccharide deficiency and explore disaccharide activity in select pediatric populations. However, for most children with nonspecific symptoms, clinical guidelines do not clearly express indications for disaccharide measurement during diagnostic EGD. Clear indications for disaccharide analysis might include chronic diarrhea and failure to thrive of unclear etiology. However, in patients with other clinical features such as abdominal pain, bloating, or gastroesophageal reflux it is not clear when disaccharide analysis should be pursued. For example, the diagnosis of functional gastrointestinal disease usually is made without evaluations of disaccharide activity, although symptoms from carbohydrate intolerance can overlap.

We completed a systematic review of the medical literature to appraise the evidence regarding intestinal disaccharide activity reported in duodenal biopsy specimens from children undergoing EGD. We sought to review the effect of ethnicity, underlying conditions, presenting symptoms, histological findings, and region of origin on disaccharide activities in children. Finally, we searched for studies examining clinical outcomes (treatment changes, quality of life, impact on gastrointestinal symptoms, cost-effectiveness) following EGD with disaccharide analysis.

MATERIALS AND METHODS

Literature search

A query of the medical literature was performed for clinical studies examining subjects undergoing EGD with small intestinal biopsy evaluation of disaccharide activity using MEDLINE (1966-March 2014), EMBASE (1995-March 2014), and the Cochrane Database (March 2014). Manuscripts were identified with the Medical Subject Heading (MeSH) and free text terms Disaccharidases/analysis, Duodenum/enzymology, Disaccharidases/deficiency, Disaccharidases/metabolism, Intestinal Mucosa/enzymology (all MeSH heading and free text terms). PubMed was utilized to query MEDLINE and the limits were applied to restrict the search to manuscripts written in the English language and including subjects under 18 years. Bibliographies of manuscripts that met inclusion criteria were reviewed for pertinent articles (Figure 1).

Selection criteria and analysis

Manuscripts were chosen if they examined disaccharide levels after EGD in pediatric patients. The specific inclusion criteria for the studies were: (1) participants had small intestinal biopsy evaluation of disaccharide activity; (2) levels of lactase, sucrase, maltase or palatinase were reported; and (3) age of participants was under 18 years. The exclusion criteria were: (1) subjects over 18 years of age; and (2) omission of specific results (activity levels or deficiency) of disaccharide analysis of intestinal biopsy.

The specific data exported from each study included: The year and country of enrollment, sample size, ethnicity, underlying conditions, study design, presenting symptoms, disaccharide activity level, and histology reports. Included manuscripts were reviewed for examination of the following elements: (1) relationship of clinical symptoms with diagnostic yield; (2) relationship of the particulars regarding abdominal pain (severity, site) with specific pathology; (3) patient outcomes (quality of life, improvement of symptoms);

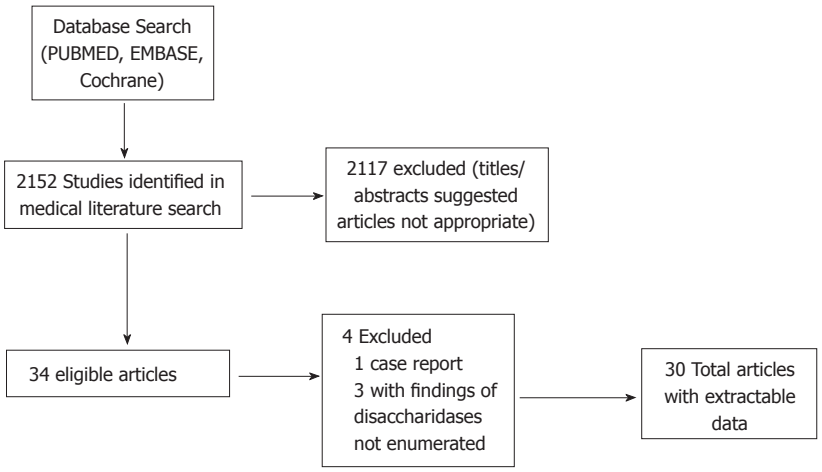

Figure 1 Literature search strategy.

or cost-effectiveness; and (4) patient-centered clinical outcomes (quality of life, symptom abatement), or cost-effectiveness. Several specific quality measures were searched for in each study: Whether (1) the participants were enrolled consecutively; (2) the clinical outcomes was measured precisely; and (3) confounding factors were recognized and adjusted for.

We computed prevalence estimates by combining data from studies that achieved the inclusion criteria and calculating sample-size - weighted mean values. We calculated pooled means for disaccharide levels using the weighted means equation. Our findings are included in tabular format.

RESULTS

Overall retrieval was 2152 manuscripts based on the search criteria described in the Methods (Figure 1). We reviewed all the titles and abstracts from the overall retrieval and 30 studies met both the inclusion and the exclusion criteria (Tables 1-3). The included articles were all observational or cohort studies and the majority were prospective (17). The studies were completed between 1966 and 2012. 77% of the studies (n = 23) occurred in European or American subjects. 11 studies examined Caucasian subjects, 3 studies examined Asian subjects, 6 examined African subjects, and one study examined Native Americans. Hispanic subjects were only included in one study which had only one Hispanic subject[3]. Only one study did not report histologic features of participating subjects[4]. The studies did not examined resource utilization, cost-effectiveness, or quality of life related to disaccharide analysis after EGD. The biggest study included about 30000 endoscopies and 10 studies included fewer than 100 endoscopies.

The largest study examined 30314 biopsy specimens. A sum of 4439 subjects were participants in the remaining 29 studies. In studies reporting specific disaccharide deficiency, the overall proportion of lactase deficiency was 39.2%, sucrase deficiency was 9.0%, maltase deficiency was 12.6% and palatinase deficiency was 9.1%.

Twenty-nine studies included histological analysis. Among histologic findings, eight studies reported no histopathologic abnormalities and 21 studies reported abnormal histopathology. Subjects classified as "abnormal" usually had varying amounts of villous atrophy or histological mucosal inflammation. The occurrence of duodenal inflammatory changes ranged findings ranged from 6% to 24% for non-specific histological gastrointestinal inflammatory lesions such as duodenitis. The prevalence of villous atrophy ranged from 8.7% to 100%. Sixteen studies examined the association of histologic findings with disaccharide activities. Four studies reported no clear association between histopathology findings and disaccharide activity[5-8]. However, 12 studies reported some degree of inverse association between degree of histologic inflammation and disaccharide levels. Among these 12 studies, 3 studies reported an association between all disaccharide levels and histologic findings[9-11]. Five studies reported correlation between lactase, sucrose, and malatase and histoligc inflammation[12-16]. O'Grady *et al*[17] study reported association between lactase and sucrose with histology inflammation. Heitlinger *et al*[18] reported an inverse correlation with lactase and maltase with inflammatory changes. Finally, two studies reported a correlation with lactase only[19,20].

Seven studies specifically examined patients with celiac disease and enumerated the results accordingly[6,14,16,17,21-23]. These studies examined a total of 269 EGDs in 224 patients were performed with celiac disease. Among these 269 procedures, 181 had significant microscopic inflammation/villious atrophy and 88 patients had no significant inflammation. Six studies reported mean disaccharide levels and found that mean lactase levels were 6.9 μmol/min per gram protein in patients with significant inflammation and 20.69 6.9 μmol/min per gram protein in patients without significant histologic changes. Mean sucrase levels were 18.3 6.9 μmol/min per gram protein in patients with significant inflammation and 45.14 6.9 μmol/min per gram protein in patients without significant histologic changes. Mean maltase levels were reported in 5 studies, 6.9 μmol/min per gram protein in patients with significant inflammation and 102.4 6.9 μmol/min

Table 1 Description of 13 studies examining intestinal disaccharides in children undergoing esophagogastroduodenoscopy with sample size less than 50

Ref.	Histology association between histology and disaccharidase activity	Other underlying condition	Sample size, % male	Age range, mean	Ethnicity	Lactase (μmol/min per gram)	Sucrase (μmol/min per gram) mean	Maltase(μmol/min per gram) mean	Palatinase (μmol/min per gram) mean
Disaccharidase values in iron-deficient infants (1981) Lanzkowsky *et al*[35] United States	No abnormalities	Severe nutritional iron deficiency anemia	10 (5 biopsied), sex not reported	8-30 mo, 14.9 mo	Not reported	Initial: 10 Post-iron treatment: 40	Initial: 140 Post-iron treatment: 230	Initial: 190 Post-iron treatment: 400	Not reported
Diamine oxidase and disaccharidase activities in small intestinal biopsies of children (1984) Forget *et al*[24] Belgium	No abnormalities	Bronchitis, vomiting, chronic diarrhea, failure to thrive	18, 61%	0.2-6 yr, 2.32 yr	Not reported	1.78 (measured in U/g wet weight)	4.34 (measured in U/g wet weight)	15.48 (measured in U/g wet weight)	Not reported
Dissacharidase deficiency in children with immunologic deficits (1970) Dubois *et al*[30] United States	Villous atrophy in two patients with idiopathic acquired hypogammaglobulinemia. No abnormalities in all other patients; Association not reported	Congenital hypogammaglobulinemia; Idiopathic acquired hypogammaglobulinemia; Isolated IgA deficiency; Thymic Dysplasia, Thymic alymphoplasia	18, 77.80%	3 mo-16 yr, 8.72 yr	Caucasian	14.2 (21 biopsies)	40.1 (20 biopsies)	150 (20 biopsies)	Not reported
Histologic findings are not correlated with disaccharidase activities in infants with protracted diarrhea (1991) Shulman *et al*[5] United States	Mucosal inflammation; No association	Diarrhea of approximately 2 wk, mild to severe malnutrition	21, sex not reported	1.0-6.0 mo, 2.5 ± 1.5 mo	Not reported	17.1	71.1	224.3	Not reported
Intestinal disaccharidase deficiency in children with coeliac disease (1966) Arthur *et al*[22] United Kingdom	Mucosal inflammation and increased cells in lamina propria; Association not reported	Coeliac disease, post-gastroenteritis, monosaccharide intolerance, and pancreatic hypoplasia	22, 50% Normal: 6 Celiac disease: 12 Miscellaneous conditions: 4	7 mo-10.58 yr, 2.84 yr	Not reported	3.17 (measured in U/g wet weight)	3.42 (measured in U/g wet weight)	9.98 (measured in U/g wet weight)	1.34 (measured in U/g wet weight)
Lactose absorption and mucosal disaccharidases in covalescent pellagra and kwashiorkor children (1971) Prinsloo *et al*[26] South Africa	No abnormalities	Kwashiorkor, classical pellagra without oedema	22, 68.18%	15 mo-12 yr, 8.758 yr	Bantu	5.28	50.4	173.5	Not reported
Intestinal disaccharidase and alkaline phosphatase activity in giadiasis (1984) Welsh *et al*[7] United States	Villous atrophy in two patients; No association	Giardiasis, diarrhea of 2 wk to 12 mo duration	23, 43%	11 mo-14 yr, 3.39 yr	Caucasian	46	82	269	Not reported
Moderate and severe protein energy malnutrition in childhood: Effects on jejunal mucosal morphology and disaccharidase activites (1983) Römer *et al*[25] Venezuela	Villous atrophy; Association not reported	Degrees of Malnutrition: I Degree (10%-24% deficit), II Degree (25%-39% deficit), III Degree (over 40% deficit), marasmic kwashiorkor	33, 60.60%	0.7-5.6 yr Mean not reported	Not reported	11.4	59	191.5	Not reported

Disaccharidase activities in jejunal fluid (1983) Aramayo *et al*[12] United Kingdom	Villous atrophy and parasitic infection; Association present for lactase, sucrase, and maltase	STVA, unspecified gastrointestinal symptoms	29, sex not reported	10 mo-14 yr, 5.9 yr	Not reported	3.67 (measured in U/g wet weight) (approximated from figure)	6.39 (measured in U/g wet weight) (approximated from figure)	23 (measured in U/g wet weight) (approximated from figure)	Not reported
Reinvestigation of lactose intolerant children: lack of correlation between continuing lactose intolerance and small intestinal morphology, disaccharidase activity, and lactose tolerance tests (1977) Harrison *et al*[8] United Kingdom	Mucosal inflammation and villous atrophy; No association	Secondary lactose intolerance	30, 69%	2-38 mo, 10.4 mo	Not reported	2.3 (data from 4 patients) (measured in U/g wet weight)	4.4 (data from 4 patients) (measured in U/g wet weight)	16.4 (data from 4 patients) (measured in U/g wet weight)	Not reported
Disaccharidases in coeliac disease (1983) Horvath *et al*[6] Hungary	Villous atrophy; No association	Confirmed and suspected coeliac disease	30, sex not reported	8 mo-10 yr, 2.4 yr	Not reported	2.1 (measured in U/g wet weight)	Not reported	16 (measured in U/g wet weight)	Not reported
Quantitative assay of disaccharidase activities of small intestinal mucosal biopsy specimens in infancy and childhood (1965) Townley *et al*[23] United States	Villous atrophy; Association not reported	Urinary tract infection, cystic fibrosis, diabetes mellitus, hepatosplenomegaly, hypoproteinemia, protein-losing enteropathy, anemia, celiac disease	36, 58.30%	1/12-16 11/12 yr, 3.62 yr	Not reported	1.91 (measured in U/g wet weight)	3.6 (measured in U/g wet weight)	13.2 (measured in U/g wet weight)	4.03 (measured in U/g wet weight)
Disaccharidase activities in dyspeptic children: Biochemical and molecular investigations of maltase-glucoamylase activity (2002) Karnsakul *et al*[3] United States	No abnormalities	Dyspepsia, abdominal pain, reflux, vomiting	44, 66.70%	0.5-18 yr, 9.5 yr	37 Caucasian (84%) 1 Hispanic (2%) 2 African (5%)	7.01 (data from 12 patients)	19.2 (data from 11 patients)	92.66 (data from 8 patients)	Not reported

per gram protein in patients without significant histologic changes. Mean palatinase levels were reported in 2 studies 3.44 6.9 μmol/min per gram protein in patients with significant inflammation and 8.27 6.9 μmol/min per gram protein in patients without significant histologic changes. Among 4 studies reporting the proportion of subjects with lactase deficiency, lactase deficiency was found in 54/61 (88.5%) patients with untreated celiac disease as compared to 8/51 (15.7%) patients with treated celiac disease. Among 3 studies reporting the proportion of subjects with maltase deficiency, maltase deficiency was found in 54/61 (88.5%) patients with untreated celiac disease as compared to 2/6 (33.3%) patients with treated celiac disease. Among 3 studies reporting the proportion of subjects with sucrase deficiency, sucrase deficiency was found in 27/76 (35.5%) patients with untreated celiac disease as compared to patients with 2/6 (33.3%) treated celiac disease. Among 2 studies reporting the proportion of subjects with palatinase deficiency, palatinase deficiency was found in 27/31 (87.1%) patients with untreated celiac disease as compared to 2/6 (33.3%) patients with treated celiac disease.

Five studies specifically examined patients with chronic diarrhea and enumerated the results accordingly[5,9,14,23,24]. These studies examined a total of 214 patients were performed with chronic diarrhea undergoing EGD. All studies reported mean disch levels and found that mean lactase levels were 27.5 μmol/min per gram protein in patients with normal histology, 15.3 μmol/min per gram protein in patients with mild inflammatory changes, 8.7 μmol/min per gram protein in patients with moderate/severe inflammation. Mean sucrase levels were 59.7 μmol/min per gram protein in patients with normal histology, 44.2 μmol/min per gram protein in patients with mild inflammatory changes, 27.3 μmol/min per gram protein in patients with moderate/severe inflammation. Mean maltase levels were 201.5 μmol/min per gram protein in patients with normal histology, 177.2 μmol/min per gram protein in patients with mild inflammatory changes, 110.3 μmol/min per gram protein in patients with moderate/

Table 2 Description of 8 studies examining intestinal disaccharides in children undergoing esophagogastroduodenoscopy with sample size between 50 and 200

Ref.	Histology; association between histology and disaccharidase activity	Other underlying condition	Sample size, % male	Age range, mean	Ethnicity	Lactase (μmol/min per gram) mean	Sucrase (μmol/min per gram) mean	Maltase (μmol/min per gram) mean	Palatinase (μmol/min per gram) mean
Disaccharidase deficiency in pediatric patients with celiac disease and intact villi (2011) Mones *et al*[21] United States	Increased lymphocytes and crypt hypertrophy; Association not reported	Celiac disease	51 CD: 25, 54% Control: 26, 50%	Ranges not reported, CD: 11.3 yr Control: 12.3 yr	Not reported	11.6	34.1	104.7	7.25
Disaccharidase activities, jeujunal morphology, and carbohydrate tolerance in children with chronic diarrhea (1985) Calvin *et al*[14] United States	Villous atrophy; Association present for lactase, sucrase, and maltase	Chronic Diarrhea	88, sex not reported	1-16 yr, 25 mo	Not reported	23.7	56.3	214.6	Not reported
Brush border enzyme activities in relation to histological lesion in pediatric celiac disease (2008) Prasad *et al*[16] India	Villous atrophy; Association present for lactase, sucrase, and maltase	GERD, celiac disease	GERD: 29, 62.09% CD: 71, 60.56%	CD: 15 mo-14 yr, 6.0 yr Control: 18 mo-14 yr, 6.2 yr	North Indian	15.7	30.7	62.3	Not reported
Activity of duodenal disaccharidases in relation to normal and abnormal mucosal morphology (1990) Langman *et al*[15] Australia	Mucosal inflammation and villous atrophy; Association present for lactase, sucrase, and maltase	Suspected celiac disease, giardiasis, diarrhea, weight loss, abdominal pain, low folate concentrations	100, 41%	7-76 yr, Four patients under 15 yr, data is from all pts including adults 39 yr	98 Caucasian (98%) 1 African (1%) 1 Indian (1%)	5.7	16	28.5	Not reported
Glucoamylase and dissacharidase activities in normal subjectsand in patients with mucosal injury of the small intestine (1980) Lebenthal *et al*[29] United States	Villous atrophy; Association present for lactase, sucrase, maltase, and palatinase	Chronic diarrhea, failure to thrive	124, sex not reported	1 mo-18 yr, mean not reported	Not reported	19.7	42.4	141	10.8
Disaccharidase activity in infants and comparison based on symptoms and histological changes (2007) Tori *et al*[11] United States	Villous atrophy; Association present for lactase, sucrase, maltase, and palatinase	Diarrhea, failure to thrive	131, 57%	20 d-364 d, 180 d	111 Caucasian (85%) 14 African (11%)	29.2	42.1	138.2	10
Correlation of Lactase activity, lactose tolerance and milk consumption in different age groups (1975) Lebenthal *et al*[10] United Kingdom	No abnormalities	Failure to thrive with no organic cause, irritable colon syndrome	160 sex not reported	6 wk-50 yr, mean not reported	6 Other (4%) Caucasian	29.8	56.2	189.6	15.5

Intestinal Disaccharidase activity in patients with autism (2011) Kushak *et al*[20] United States	Mucosal inflammation (6%); Association present for lactase.	Autism (abdominal pain, flatulence, constipation, vomiting, weight loss, food allergy, suspected GERD)	199, 82.40%	22 mo-28 yr, 5.75 yr	195 Caucasian (98%) 2 Asian (1%) 2 Indian (1%)	14.7	45.6	209.2	Not reported

GERD: Gastroesophageal reflux disease.

Table 3 Description of 9 studies examining intestinal disaccharides in children undergoing esophagogastroduodenoscopy with sample size greater than 200

Ref.	Histology; association between histology and disaccharidase activity	Underlying condition/symptoms	Sample size, % male	Age range, mean	Ethnicity	Lactase (μmol/min per gram) mean	Sucrase (μmol/min per gram) mean	Maltase (μmol/min per gram) mean	Palatinase (μmol/min per gram) mean
Ethnic differences in intestinal disaccharidase values in children in Finland (2000) Kolho *et al*[28] Finland	No abnormalities	Abdominal pain, vomiting, suspected celiac disease, suspected inflammatory bowel disease, asthma, constipation, diarrhea, feeding problems, anemia, other	223, 55.20%	Finnish: 0.2-18 yr, median: 8.0 yr African: 1-13 yr, median: 5.0 yr Other: 4.5-15 yr, median: 12 yr	188 Finnish (84%) 27 African (12%) 8 other (4%)	23.4	48.1	186.3	Not reported
Intestinal lactase, sucrase, and alkaline phosphatase in 373 patients with coeliac disease (1984) O'Grady *et al*[17] Ireland	Mucosal inflammation; Association present for lactase and sucrase	Celiac disease	230 sex not reported	1-18 yr, Mean not reported	Not reported	14.05 (data from 45 patients)	33.9 (data from 45 patients)	Not reported	Not reported
Disaccharidase activities in children: Normal values and comparison based on symptoms and histological changes (1999) Gupta *et al*[9] United States	Villous atrophy; Association present for lactase, sucrase, maltase, and palatinase	Group 1: Recurrent abdominal pain, vomiting, gastroesophageal reflux, hematemesis, failure to thrive Group 2: All had diarrhea (included patients with celiac disease, inflammatory bowel disease, parasitic infestation, and congenital sucrase-isomaltase deficiency)	232, 47.80%	0.08-17 yr 5.9 yr	Not reported	18.3	37.9	169.1	12.7
Disaccharidase activities in small intestinal mucosa in patients with cystic fibrosis (1978) Antonowicz *et al*[19] United States	Villous atrophy; Association present for lactase	Cystic fibrosis Chronic diarrhea, failure to thrive, abdominal pain, vomiting, crying baby, other	240 sex not reported	Range and mean not reported	Caucasian	31.1	64	241	19.8
Disaccharidase activites in Belgian children: Reference intervals and comparison with non-Belgian Caucasian children (2003) Blomme *et al*[13] Belgium	Mucosal inflammation and villous atrophy; Association present for lactase, sucrase, and maltase		185 60.50%	Belgian: 0.1-12 yr, Median: 1.3 yr Caucasian: 0.2-8 yr, Median: 1.3 yr	151 Belgian (82%) 34 non-Belgian Caucasian (18%)	(median values) Group A: 40 (6-122) Group B: 28 Group C: 7 Non-Belgian: 33 (5-70)	(median values) Group A: 69 (18-184) Group B: 54 Group C: 25 Non-Belgian: 63 (10-125)	(median values) Group A: 208 Group B: 181 Group C: 96 Non-Belgian: 186	Not reported

Intestinal Disaccharidase Activites in Relation to Age, Race, and Mucosal Damage (1978) Welsh *et al*[7] United States	No abnormalities	Mucosal damage	399 sex not reported	1 mo-93 yr, mean not reported	339 Caucasian (85%) 53 African (13%)	35.9	76.5	262.2	26.6
"Normal" disaccharidase levels in children (1988) Barnes *et al*[34] Australia	No abnormalities	Failure to thrive, chronic diarrhea, short stature, family history of celiac disease, iron deficiency anemia, food hypersensitivity	580 sex not reported	0-12 yr, mean not reported	7 Native American (2%) Not reported	3.9 (measured in U/g wet weight)	6.4 (measured in U/g wet weight)	19.1 (measured in U/g wet weight)	Not reported
Human intestinal disaccharidase activities: Correlations with age, biopsy technique, and degree of villus atrophy (1991) Heitlinger *et al*[18] United States	Mucosal inflammation and villous atrophy; Association present for lactase and maltase	Not reported	798 sex not reported	0-18 yr, Mean not reported	Not reported	23.3	52.5	145.8	11.6
Frequency of sucrase deficiency in mucosal biopsies (2012) Nichols *et al*[4] United States	Not reported	Not reported	27875 % male not reported	0-93.5 yr, 11 yr	Not reported	21.8	56.5	167.6	11.3

severe inflammation. Two study reported palatinase levels and found mean palatinase levels were 12.1 μmol/min per gram protein in patients with normal histology, 5.8 μmol/min per gram protein in patients with mild inflammatory changes, 3.0 μmol/min per gram protein in patients with moderate/severe inflammation[9,23]. Among two studies reporting the proportion of subjects with lactase deficiency in subjects with chronic diarrhea, lactase deficiency was found in 73/190 (38.4%)[9,14]. One study reported sucrose deficiency in subjects with chronic diarrhea and found 10/88 (11.4%)[14].

Two studies examining 7 patients reported disaccharide activities in subjects with failure to thrive[23,24]. All studies reported mean disaccharide levels and found that mean lactase levels were 1.97 μmol/min per gram protein, mean sucrase levels were 4.6 μmol/min per gram protein, mean maltase levels were 15.5 μmol/min per gram protein. All 7 patients had pan disaccharide deficiency.

Two studies examining 14 patients reported disaccharide activities in subjects with kwashiorkor[25,26]. All studies reported mean disaccharide levels and found that mean lactase levels were 6.4 μmol/min per gram protein, mean sucrase levels were 52.9 μmol/min per gram protein, mean maltase levels were 190.9 μmol/min per gram protein. One article reported the specific proportion of disaccharide deficient patients and found 8/10 (80%) were lactase deficient, 2/10 were sucrose deficient, and 3/10 were maltase deficient[26].

Three studies compared disaccharide activity across ethnic populations[13,27,28]. One study performed in the United States found that lactase deficiency is rare in Caucasian children as compared to native American and African populations as all 117 Caucasian children under age 5 had normal lactase levels[27]. Another study from Finland found that the mean activities of lactase, sucrose, and maltase were significantly higher in Finnish children as compared to African children[28]. The study further found that 31% (59/188) of Finnish children had low lactase as compared to 67% (18/27) of African children. The final study was performed in Belgium and compared Belgian children to non-Belgian Caucasian children and found that median values for lactase levels were lower in non-Belgian children (33 μmol/L per gram) as compared to Belgian children (40 μmol/L per gram) ($P = 0.02$).

Eleven studies examined Caucasian subjects, but only 4 focused on Caucasian subjects and enumerated results accordingly. Among these 4 studies, a total of 441 patients were included with variable underlying conditions including failure to thrive, irritable bowel syndrome, cystic fibrosis, immunologic deficits and giardiasis[7,19,29,30]. All 4 studies reported the proportion of lactase deficiency in subgroup populations and the overall prevalence was 43/162 (26.5%). Two studies report the proportion of sucrose and maltase deficiency and sucrose deficiency was reported in 12/39 (30.8%) and maltase deficiency was reported in 16/39 (41.0%). Combined mean disaccharide levels from studies examining Caucasian populations were: 30.6 μmol/L per gram for lactase, 61.0 μmol/L per gram for sucrose, 204.0 μmol/L per gram for maltase and 16.7 μmol/L per gram for palatinase. When stratified by histologic inflammation, Caucasian patients with normal histology had mean levels of 31.8 μmol/L per

gram for lactase, 61.4 sucrase, 204.8 for maltase, and 16.8 for palatinase. Those with mild inflammation had levels of 17.9 lactase, 62.0 sucrase, 219.5 maltase, 17.3 for palatinase. Moderate severe inflammation was associated with levels of 7.3 lactase, 39.1 sucrase, 125.5 maltase, 11.3 palatinase.

One study focused on 100 Indian subjects with celiac or GERD and did not report the proportion of disaccharide deficiency in the cohort[16]. Overall levels were reported at 15.7 for lactase, 30.7 sucrase, 62.2 for maltase. When stratified by histologic inflammation, Caucasian patients with normal histology had mean levels of 23.3 μmol/L per gram for lactase, 39.9 sucrase, 72.8 for maltase. Those with mild inflammation had levels of 18.4 lactase, 28.7 sucrase, 64.3 maltase. Moderate severe inflammation was associated with levels of 11.0 lactase, 25.3 sucrase, 55.7 maltase.

Prinsloo *et al*[26] reported disaccharide levels in an exclusively African cohort of 22 subjects with kwashiorkor or pellagra. The proportion of patients who had disaccharide deficiency was only reported for lactase with 7/10 subjects with kwashiorkor and 10/10 for pellagra for an overall prevalence of 17/20 (85%). For kwashiorkor, the levels were 8.4 for lactase, 50.1 for sucrose, 185.7 for maltase, 67.9 for palatinase. For pellagra, the levels were 2.73 lactase, 50.7 sucrase, 163.4 maltase and 70.0 for palatinase. Combined levels were 5.3 lactase, 50.4 sucrase, 173.5 maltase, 69.1 for palatinase.

Three studies examined Asian subjects, 6 examined African subjects, and one study examined Native Americans. Studies focused on specific ethnic populations.

The largest study reported findings in 30314 samples received over a 5 year period in a reference laboratory[4]. This study found that the most common deficiency was lactase occurring in 8963 (32%), followed by pandisaccharide deficiency in 2347 (8%). Congential sucrose-isomaltase deficiency was extremely rare, occurring in just 0.1% of the samples.

Only one study examined management changes as a result of intestinal disaccharide analysis[9]. Gupta *et al*[9] conducted a questionnaire to evaluate the usefulness of diet changes in patients with lactase deficiency and found that 81.5% (22/27) of patients responded to dietary modification.

DISCUSSION

Our systematic review of 30 studies of intestinal disaccharide analysis, including over 30000 samples, found that lactase deficiency was most common (39.2%), followed by maltase deficiency (12.6%), palatinase deficiency (9.1%), and sucrase deficiency (9.0%). Histopathology was reported in most studies and the primary findings included duodenal inflammation (6% to 24%) or villious atropy (9% to 100%). A large multi-center study including 30314 disaccharide analysis was performed in 2012, however, this study did not include information on the underlying conditions, histology, or ethnicity of its subjects.

In the articles reviewed, many did not specifically enumerate the indication or underlying condition for the EGD with disaccharide analysis. The most common conditions examined included celiac disease, chronic diarrhea, and malnutrition. In clinical practice, chronic diarrhea and malnutrition are common indications for disaccharide analysis. However, generally enzyme levels are not routinely measured in patients with celiac disease, as intestinal function usually normalizes with a gluten free diet. The most common indications for EGD in children include abdominal pain, vomiting, and reflux symptoms[31,32]. However, subjects with abdominal pain, reflux symptoms or vomiting were specifically examined in just 2 studies. Karnsakul *et al*[3] examined 33 children with abdominal pain, 11 with vomiting, and with reflux. Overall, half of all enrolled children had low activity of one or more disaccharidases[3]. The study also found that vomiting was related to low lactase, but no other associations between symptoms and disaccharide levels were found. Prasad *et al*[16] enrolled 29 children with GERD symptoms and found normal disaccharide levels in this small cohort. Four remaining studies include patients with abdominal pain, vomiting, and reflux but did not specifically analyze the relationship between disaccharide activity and these indications for EGD.

The majority of studies (29/30) included analysis of histopathology. Histology is critical to examine because it can be a factor leading to differentiation of primary from secondary disaccharidase deficiencies. The majority of studies (12/16) examining the association between enzyme levels and histopathology found that inflammatory changes were associated with enzyme deficiencies. It has been argued that specimens should be considered unsatisfactory when all the enzymes assayed are low and the histology appears normal. However, four studies included in this review reported no clear association between histopathology findings and disaccharide activity. Additionally, data in adult patients suggests that the disaccharidase deficiency is not confined to patients with abnormal histology[33]. Therefore, we conclude that although enzymes levels are lower in the majority of patients with duodenal inflammation, enzyme levels may be affected even with normal histopathology.

While ethnicity was reported in many studies, only three studies compared disaccharide activity between ethnic cohorts. The primary finding was lower levels of disacharidase activity in children of African descent[27,28]. Although current studies includes over 30000 biopsy specimens, only 1 Hispanic subject was included.

Change in clinical management after EGD with disaccharide analysis was reported only in relation to dietary treatment low lactase levels. No studies explored management changes after the discovery of low sucrase, maltase or palatinase. Studies also did not report on the use of enzyme or dietary supplements.

Our review is limited somewhat by the heterogeneity

and variability of the included studies. Our review of the current evidence also did not contain any clinical trials, and included primarily observational studies.

In summary, the present medical literature examining the utility of disaccharide analysis during EGD for children is limited primarily by inadequate investigation of clinical presentation to disaccharide levels. The prevalence and outcomes of disaccharide deficiency in pediatric patients with abdominal pain, reflux, vomiting has not been well-studied. Also, the majority of clinical outcomes after EGD with disaccharide analysis (*e.g.*, patient management impact, quality of life improvement, symptom abatement, cost) are not well described in the current literature. Prior studies do not sufficiently include Hispanic subjects. However, our findings may have applicability to routine clinical practice. We recommend large studies examining the association between clinical factors and disaccharide levels with detailed elucidation of histopathology reports to illuminate the utility of disaccharide analysis in children undergoing EGD. Further investigation should include a large sample size and explore the value of specific management options after low disaccharide levels are discovered by small intestinal biopsies.

COMMENTS

Background

For children with non-specific symptoms, clinical guidelines and current data do not clearly express indications for disaccharide measurement during diagnostic esophagogastroduodenoscopy (EGD).

Research frontiers

The impact of disaccharide measurement on clinical management and the cost-effectiveness of disaccharide assays are important areas of future research.

Innovations and breakthroughs

This study is the first to compile the medical literature examining the utility of disaccharide analysis during EGD for children and reveal significant gaps in the current clinical data on this subject.

Applications

This review directs future investigations to include a large sample size and explore the value of specific management options after low disaccharide levels are discovered by small intestinal biopsies.

Terminology

Disaccharide: Class of sugars, such as maltose, lactose, and sucrose, having two linked monosaccharide units per molecule; EGD: Test to examine the lining of the esophagus, stomach, and first part of the small intestine.

Peer-review

The report describes the usefulness of disaccharide analysis from children undergoing EGD. The quality of manuscript is very good.

REFERENCES

1 **Robayo-Torres CC**, Quezada-Calvillo R, Nichols BL. Disaccharide digestion: clinical and molecular aspects. *Clin Gastroenterol Hepatol* 2006; **4**: 276-287 [PMID: 16527688 DOI: 10.1016/j.cgh.2005.12.023]

2 **Lee SH**, Park YK, Cho SM, Kang JK, Lee DJ. Technical skills and training of upper gastrointestinal endoscopy for new beginners. *World J Gastroenterol* 2015; **21**: 759-785 [PMID: 25624710 DOI: 10.3748/wjg.v21.i3.759]

3 **Karnsakul W**, Luginbuehl U, Hahn D, Sterchi E, Avery S, Sen P, Swallow D, Nichols B. Disaccharidase activities in dyspeptic children: biochemical and molecular investigations of maltase-glucoamylase activity. *J Pediatr Gastroenterol Nutr* 2002; **35**: 551-556 [PMID: 12394383 DOI: 10.1097/00005176-200210000-00017]

4 **Nichols BL**, Adams B, Roach CM, Ma CX, Baker SS. Frequency of sucrase deficiency in mucosal biopsies. *J Pediatr Gastroenterol Nutr* 2012; **55** Suppl 2: S28-S30 [PMID: 23103647 DOI: 10.1097/01.mpg.0000421405.42386.64]

5 **Shulman RJ**, Langston C, Lifschitz CH. Histologic findings are not correlated with disaccharidase activities in infants with protracted diarrhea. *J Pediatr Gastroenterol Nutr* 1991; **12**: 70-75 [PMID: 1905750 DOI: 10.1097/00005176-199101000-00015]

6 **Horváth K**, Horn G, Bodánszky H, Tóth K, Váradi S. Disaccharidases in coeliac disease. *Acta Paediatr Hung* 1983; **24**: 131-136 [PMID: 6416272]

7 **Welsh JD**, Poley JR, Hensley J, Bhatia M. Intestinal disaccharidase and alkaline phosphatase activity in giardiasis. *J Pediatr Gastroenterol Nutr* 1984; **3**: 37-40 [PMID: 6420534 DOI: 10.1097/00005176-198401000-00010]

8 **Harrison M**, Walker-Smith JA. Reinvestigation of lactose intolerant children: lack of correlation between continuing lactose intolerance and small intestinal morphology, disaccharidase activity, and lactose tolerance tests. *Gut* 1977; **18**: 48-52 [PMID: 838402 DOI: 10.1136/gut.18.1.48]

9 **Gupta SK**, Chong SK, Fitzgerald JF. Disaccharidase activities in children: normal values and comparison based on symptoms and histologic changes. *J Pediatr Gastroenterol Nutr* 1999; **28**: 246-251 [PMID: 10067723 DOI: 10.1097/00005176-199903000-00007]

10 **Lebenthal E**, Lee PC. Glucoamylase and disaccharidase activities in normal subjects and in patients with mucosal injury of the small intestine. *J Pediatr* 1980; **97**: 389-393 [PMID: 6774072 DOI: 10.1016/S0022-3476(80)80187-9]

11 **Tori AJ**, Carroll AE, Gupta SK. Disaccharidase activity in infants and comparison based on symptoms and histological changes. *J Pediatr Gastroenterol Nutr* 2007; **45**: 194-198 [PMID: 17667715 DOI: 10.1097/MPG.0b013e31804c98cc]

12 **Aramayo LA**, De Silva DG, Hughes CA, Brown GA, McNeish AS. Disaccharidase activities in jejunal fluid. *Arch Dis Child* 1983; **58**: 686-691 [PMID: 6414385 DOI: 10.1136/adc.58.9.686]

13 **Blomme B**, Gerlo E, Hauser B, Vandenplas Y. Disaccharidase activities in Belgian children: reference intervals and comparison with non-Belgian Caucasian children. *Acta Paediatr* 2003; **92**: 806-810 [PMID: 12892159 DOI: 10.1111/j.1651-2227.2003.tb02537.x]

14 **Calvin RT**, Klish WJ, Nichols BL. Disaccharidase activities, jejunal morphology, and carbohydrate tolerance in children with chronic diarrhea. *J Pediatr Gastroenterol Nutr* 1985; **4**: 949-953 [PMID: 4067784 DOI: 10.1097/00005176-198512000-00016]

15 **Langman JM**, Rowland R. Activity of duodenal disaccharidases in relation to normal and abnormal mucosal morphology. *J Clin Pathol* 1990; **43**: 537-540 [PMID: 2116456 DOI: 10.1136/jcp.43.7.537]

16 **Prasad KK**, Thapa BR, Nain CK, Sharma AK, Singh K. Brush border enzyme activities in relation to histological lesion in pediatric celiac disease. *J Gastroenterol Hepatol* 2008; **23**: e348-e352 [PMID: 18070009 DOI: 10.1111/j.1440-1746.2007.05248.x]

17 **O'Grady JG**, Stevens FM, Keane R, Cryan EM, Egan-Mitchell B, McNicholl B, McCarthy CF, Fottrell PF. Intestinal lactase, sucrase, and alkaline phosphatase in 373 patients with coeliac disease. *J Clin Pathol* 1984; **37**: 298-301 [PMID: 6421895 DOI: 10.1136/jcp.37.3.298]

18 **Heitlinger LA**, Rossi TM, Lee PC, Lebenthal E. Human intestinal disaccharidase activities: correlations with age, biopsy technique, and degree of villus atrophy. *J Pediatr Gastroenterol Nutr* 1991; **12**: 204-208 [PMID: 1904933 DOI: 10.1097/00005176-199102000-00011]

19 **Antonowicz I**, Lebenthal E, Shwachman H. Disaccharidase activities in small intestinal mucosa in patients with cystic fibrosis.

J Pediatr 1978; **92**: 214-219 [PMID: 413896 DOI: 10.1016/S0022-3476(78)80007-9]
20 **Kushak RI**, Lauwers GY, Winter HS, Buie TM. Intestinal disaccharidase activity in patients with autism: effect of age, gender, and intestinal inflammation. *Autism* 2011; **15**: 285-294 [PMID: 21415091 DOI: 10.1177/1362361310369142]
21 **Mones RL**, Yankah A, Duelfer D, Bustami R, Mercer G. Disaccharidase deficiency in pediatric patients with celiac disease and intact villi. *Scand J Gastroenterol* 2011; **46**: 1429-1434 [PMID: 21936724 DOI: 10.3109/00365521.2011.619276]
22 **Arthur AB**. Intestinal disaccharidase deficiency in children with coeliac disease. *Arch Dis Child* 1966; **41**: 519-524 [PMID: 5957725 DOI: 10.1136/adc.41.219.519]
23 **Townley RR**, Khaw KT, Shwachman H. Quantitative assay of disaccharidase activities of small intestinal mucosal biopsy specimens in infancy and childhood. *Pediatrics* 1965; **36**: 911-921 [PMID: 5846834]
24 **Forget P**, Grandfils C, van Cutsem JL, Dandrifosse G. Diamine oxidase and disaccharidase activities in small intestinal biopsies of children. *Pediatr Res* 1984; **18**: 647-649 [PMID: 6433312 DOI: 10.1203/00006450-198407000-00016]
25 **Römer H**, Urbach R, Gomez MA, Lopez A, Perozo-Ruggeri G, Vegas ME. Moderate and severe protein energy malnutrition in childhood: effects on jejunal mucosal morphology and disaccharidase activities. *J Pediatr Gastroenterol Nutr* 1983; **2**: 459-464 [PMID: 6413668 DOI: 10.1097/00005176-198302030-00011]
26 **Prinsloo JG**, Wittmann W, Kruger H, Freier E. Lactose absorption and mucosal disaccharidases in convalescent pellagra and kwashiorkor children. *Arch Dis Child* 1971; **46**: 474-478 [PMID: 5109409 DOI: 10.1136/adc.46.248.474]
27 **Welsh JD**, Poley JR, Bhatia M, Stevenson DE. Intestinal disaccharidase activities in relation to age, race, and mucosal damage. *Gastroenterology* 1978; **75**: 847-855 [PMID: 100368]
28 **Kolho KL**, Savilahti E. Ethnic differences in intestinal disaccharidase values in children in Finland. *J Pediatr Gastroenterol Nutr* 2000; **30**: 283-287 [PMID: 10749412 DOI: 10.1097/00005176-200003000-00013]
29 **Lebenthal E**, Antonowicz I, Shwachman H. Correlation of lactase activity, lactose tolerance and milk consumption in different age groups. *Am J Clin Nutr* 1975; **28**: 595-600 [PMID: 1173320]
30 **Dubois RS**, Roy CC, Fulginiti VA, Merrill DA, Murray RL. Disaccharidase deficiency in children with immunologic deficits. *J Pediatr* 1970; **76**: 377-385 [PMID: 4189883 DOI: 10.1016/S0022-3476(70)80476-0]
31 **Thakkar K**, El-Serag HB, Mattek N, Gilger MA. Complications of pediatric EGD: a 4-year experience in PEDS-CORI. *Gastrointest Endosc* 2007; **65**: 213-221 [PMID: 17258979 DOI: 10.1016/j.gie.2006.03.015]
32 **Gilger MA**, Gold BD. Pediatric endoscopy: new information from the PEDS-CORI project. *Curr Gastroenterol Rep* 2005; **7**: 234-239 [PMID: 15913484 DOI: 10.1007/s11894-005-0040-y]
33 **Wilson IR**, Oxner RB, Frampton CM, Tisch G, Chapman BA, Cook HB. Comparison of endoscopic forceps biopsies and capsule biopsies in determining disaccharidase activity in the duodenum. *Gastrointest Endosc* 1991; **37**: 527-530 [PMID: 1936829 DOI: 10.1016/S0016-5107(91)70821-3]
34 **Barnes GL**, Ford RP, Dawson S, Lawrance S. 'Normal' disaccharidase levels in children. *Aust Paediatr J* 1988; **24**: 31-33 [PMID: 3128260]
35 **Lanzkowsky P**, Karayalcin G, Miller F, Lane BP. Disaccharidase values in iron-deficient infants. *J Pediatr* 1981; **99**: 605-608 [PMID: 7277105]

P- Reviewer: Amornyotin S, Lee SH **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Wu HL

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i2.294

World J Gastrointest Pharmacol Ther 2016 May 6; 7(2): 294-305
ISSN 2150-5349 (online)

SYSTEMATIC REVIEWS

Adverse events of sacral neuromodulation for fecal incontinence reported to the federal drug administration

Klaus Bielefeldt

Klaus Bielefeldt, Division of Gastroenterology, University of Pittsburgh Medical Center, Pittsburgh, PA 15217, United States

Author contributions: Bielefeldt K contributed to the manuscript.

Conflict-of-interest statement: The author has no conflict of interest.

Data sharing statement: Technical appendix and dataset are available from the corresponding author at bielefeldtk@upmc.edu.

Correspondence to: Klaus Bielefeldt, MD, PhD, Division of Gastroenterology, University of Pittsburgh Medical Center, 200 Lothrop St. Pittsburgh, PA 15217,
United States. bielefeldtk@upmc.edu
Telephone: +1-412-8647087
Fax: +1-412-6489378

Received: December 19, 2015
Peer-review started: December 22, 2015
First decision: February 22, 2016
Revised: February 23, 2016
Accepted: March 14, 2016
Article in press: March 16, 2016
Published online: May 6, 2016

Abstract

AIM: To investigate the nature and severity of AE related to sacral neurostimulation (SNS).

METHODS: Based on Pubmed and Embase searches, we identified published trials and case series of SNS for fecal incontinence (FI) and extracted data on adverse events, requiring an active intervention. Those problems were operationally defined as infection, device removal explant or need for lead and/or generator replacement. In addition, we analyzed the Manufacturer and User Device Experience registry of the Federal Drug Administration for the months of August - October of 2015. Events were included if the report specifically mentioned gastrointestinal (GI), bowel and FI as indication and if the narrative did not focus on bladder symptoms. The classification, reporter, the date of the recorded complaint, time between initial implant and report, the type of AE, steps taken and outcome were extracted from the report. In cases of device removal or replacement, we looked for confirmatory comments by healthcare providers or the manufacturer.

RESULTS: Published studies reported adverse events and reoperation rates for 1954 patients, followed for 27 (1-117) mo. Reoperation rates were 18.6% (14.2-23.9) with device explants accounting for 10.0% (7.8-12.7) of secondary surgeries; rates of device replacement or explant or pocket site and electrode revisions increased with longer follow up. During the period examined, the FDA received 1684 reports of AE related to SNS with FI or GI listed as indication. A total of 652 reports met the inclusion criteria, with 52.7% specifically listing FI. Lack or loss of benefit (48.9%), pain or dysesthesia (27.8%) and complication at the generator implantation site (8.7%) were most commonly listed. Complaints led to secondary surgeries in 29.7% of the AE. Reoperations were performed to explant (38.2%) or replace (46.5%) the device or a lead, or revise the generator pocket (14.6%). Conservative management changes mostly involved changes in stimulation parameters (44.5%), which successfully addressed concerns in 35.2% of cases that included information about treatment results.

CONCLUSION: With reoperation rates around 20%, physicians need to fully disclose the high likelihood of

complications and secondary interventions and exhaust non-invasive treatments, including transcutaneous stimulation paradigms.

Key words: Sacral nerve stimulation; Implanted medical devices; Treatment complications; Defecation disorders; Device registry

Core tip: Sacral neuromodulation can improve fecal incontinence refractory to other treatments. However, adverse events are very common and often require additional operations. Many of the reported patient concerns surface early after stimulator implantation, respond to changes in stimulation parameters and may thus be considered a part of the routine maintenance of this treatment modality. Nonetheless, rates of surgical re-interventions are high and increase over time. Physicians counseling patients about this treatment for fecal incontinence should emphasize the likely need for such secondary surgeries and consider emerging non-invasive treatment options. In addition, prospective studies should compare less invasive paradigms, such as transcutaneous stimulation, with permanently implanted devices to more clearly define their differential impact.

Bielefeldt K. Adverse events of sacral neuromodulation for fecal incontinence reported to the federal drug administration. *World J Gastrointest Pharmacol Ther* 2016; 7(2): 294-305 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i2/294.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i2.294

INTRODUCTION

Fecal incontinence affects millions of Americans with an estimated prevalence between 6%-10% in adults[1]. While dietary changes, physical therapy with or without biofeedback, bulking agents or medications alleviate or control symptoms[2-5], a significant number of patients will not respond to these interventions. In March of 2011, the Federal Drug Administration (FDA) approved sacral neurostimulation (SNS) for patients with otherwise refractory fecal incontinence. Short-term studies consistently show high response rates[6,7]. However, the lasting benefit of SNS is difficult to define with an apparent increase in device explants over time[8]. Consistent with this impression, an early analysis of published data suggested efficacy in select groups, but emphasized the high likelihood of secondary surgeries[9]. A recent systematic review of data from published studies concluded that differences in endpoints and reporting complicated the overall assessment; despite remaining conceptual concerns about the validity of their estimates, the authors pooled trial data for 518 patients with a rate of complete continence of 36.5%[10].

Research frontier

With lasting improvement rates around 50% or even less, a better understanding of adverse effects is essential to appropriately weigh risks and benefits.

Innovation and breakthrough

The aim of this study was to systematically analyze published data on side effects based on a systematic literature search and define the type, relative frequency and resulting interventions of adverse events. We also performed an assessment of adverse events described in the Manufacturer and User Device Experience (MAUDE) databank of the FDA, which offers detailed descriptions of device-related problems and concerns, but has been underutilized in outcomes research related to gastrointestinal disorders.

MATERIALS AND METHODS

Literature search

We queried the PubMed and Embase databanks using sacral neurostimulation or sacral nerve stimulation and fecal incontinence as search terms. We retrieved full length articles if the title and abstract described SNS as treatment modality and if cohorts included at least 5 patients with fecal incontinence. We abstracted sample size, definition of endpoints, results defined as responders or changes in symptom scores, duration of follow up and adverse events, separately examining pocket site infections, pain or displacement at the generator pocket, pain in other areas, device erosion, device explant, lead migration or break, battery depletion and lack of effectiveness. Whenever possible, we assessed the total number of reoperation. Only data focusing on the use definite rather than temporary stimulation were included.

MAUDE databank

We used the electronic search option of the MAUDE database (http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfmaude/TextResults.cfm) for the years 2005 to 2010 as a reference time period prior to FDA approval of SNS for fecal incontinence, and extended our queries to October 2015, the most recent month with completed reporting at the time of the study. We recorded the number of reports and reviewed the detailed descriptions of adverse events for the period between August 1 and October 31, 2015, using the key word *Interstim* as trade name of the device. The MAUDE databank collects voluntarily reported adverse events related to the use of medical devices. Unlike users or healthcare providers, manufacturers are required to forward all information about side effects to the FDA. Prior work demonstrated that information obtained from this site enables the identification of rare, but potentially important adverse events, and provides insight into the type and relative frequency of complications related to medical devices marketed in the United States[11-13]. Reports include information provided by manufacturers,

distributors, healthcare providers, and patients, list device type, date of event report and a narrative of the event as well as information about follow up interactions and potential responses of the manufacturer. Patient identifiers have been removed, and dates are limited to the reporting day and year of initial implant or other intervention, thus protecting privacy and not allowing linkage between the publically available records and other data banks. As SNS received FDA approval for fecal incontinence in 2011, we only included reports that provided information about the year of implantation and that fell between 2011 and 2015. We searched the narrative for indications and key symptoms and extracted data only if the terms fecal incontinence, bowel or gastrointestinal dysfunction were listed and if the narrative did not focus on urinary symptoms. We abstracted the date of the report, year of permanent stimulator implantation, the reporter (healthcare provider, manufacturer representative or patient/patient's relative or partner), and the classification of the complaint as entered into the database, the nature of the problem, steps taken and outcome. Reported outcomes were summarized as resolved, persisting or unknown. We separately coded operative interventions, assessed whether they were confirmed by the company, dividing them into groups based on the nature of the steps as explant, replacement, lead or pocket site revision.

Statistical analysis

We used Comprehensive Meta-Analysis version 2.0 (Biostat, Englewood, NJ) to calculate incidence of adverse events based on the more conservative random effect method. Unless mentioned otherwise, data are given as mean with 95%CI. Categorical variables were compared with the χ^2 test.

RESULTS

Published data

Using the predefined terms and filtering information based on English language of human studies and publication as full length article, the search yielded a total of 45 articles describing distinct patient cohorts from different institutions or collaborative groups, which provided information about adverse events and included more than 5 patients treated fecal incontinence a permanently implanted system (Table 1). The total number of patients was 1953 followed for a median time period of 27 mo (range: 1-117 mo). Definition and description of variables differed significantly between studies as did follow up times. The most detailed information was found in 2 studies that prospectively monitored a total of 201 patients and recorded 828 incident adverse events over a period 5 years, most of which were related to loss of benefit[8,14]. While most of these side effects responded to conservative therapy, one fifth of the cohorts had their devices explanted at the time of the last follow up with additional surgeries in a significant number of patients. Only five additional publications discussed adverse events with some detail, but reported a significantly lower incidence[15-19]. We therefore focused on serious adverse events that typically prompted secondary interventions and were more consistently described. We found information about infectious complications after implantation of a permanent device in 44 studies with 1953 patients. The pooled rate of infection was 5.1% (4.1-6.4) (Figure 1) without significant heterogeneity between trials (I^2 = 0%). Device explants were largely due to infection, but were also caused by generator erosion through the skin or other local complications at the pocket site and lack of benefit, thus leading to a higher rate of reoperation. A total of 39 studies covering 1810 patients provided information about explant rates at the end of their follow

Table 1 Studies included

Sample	Follow-up[1]	Response	Adverse Events			Ref.
			Infection	Explant	Reoperation	
61	13		0	1	2	[60]
16			0	0	3	[61]
85	24		0	3	13	[45]
39	12		2	2	2	[62]
60	47	17	2	9	15	[18]
101	60	26	2	20	20	[14]
50	55.5		2	6	11	[63]
42			0	2	5	[64]
23	114		2	3	5	[65]
101	62		1	10	39	[66]
37	17	27	0	0	3	[50]
41	51		0	0	6	[67]
34	24	83	1	2	4	[19]
37	6		3	5	5	[68]
58	74	37	0	6	15	[49]
12	117		0	3	14	[69]
29	34		0	0	0	[70]
7	32		1	1	2	[71]
53	12		0	0	0	[72]
10			0	0	1	[73]
29	35		2	2	8	[74]
37	13		2	4	10	[75]
14	6		0	0	0	[76]
87	48.5		4	12	36	[77]
40			3	3	7	[78]
15	6	10	0	0	0	[47]
85	12	31	0	1	1	[54]
145	12	137	5	6	15	[6]
29			0	1	1	[79]
27	10.7		3	1	4	[80]
9	12		0	0	1	[81]
120	60	90	12	30	72	[8]
57	63		2	6	12	[82]
8			0	0	1	[83]
18	17		2	3	4	[84]
50	74		4	11	24	[85]
11	14	6	0	1	1	[51]
55	37		1	1	11	[16]
23	38		0	0	4	[15]
32	33		3	7	10	[17]
53	12		0	0	0	[7]
50	12		2	4	10	[86]
10	29.5	10	2	8	8	[53]
33	27	0		5	11	[87]
16	15.5		0	0	0	[88]

[1]Follow up in months.

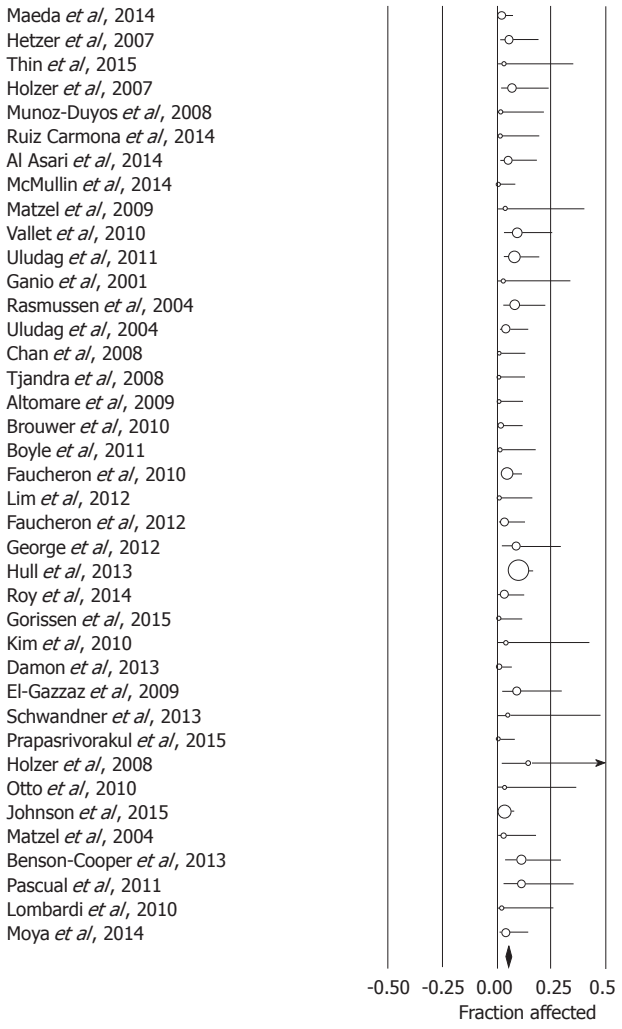

Figure 1 Forrest plot showing infection rates as fraction of the sample size in cohorts treated with sacral neurostimulation for fecal incontinence.

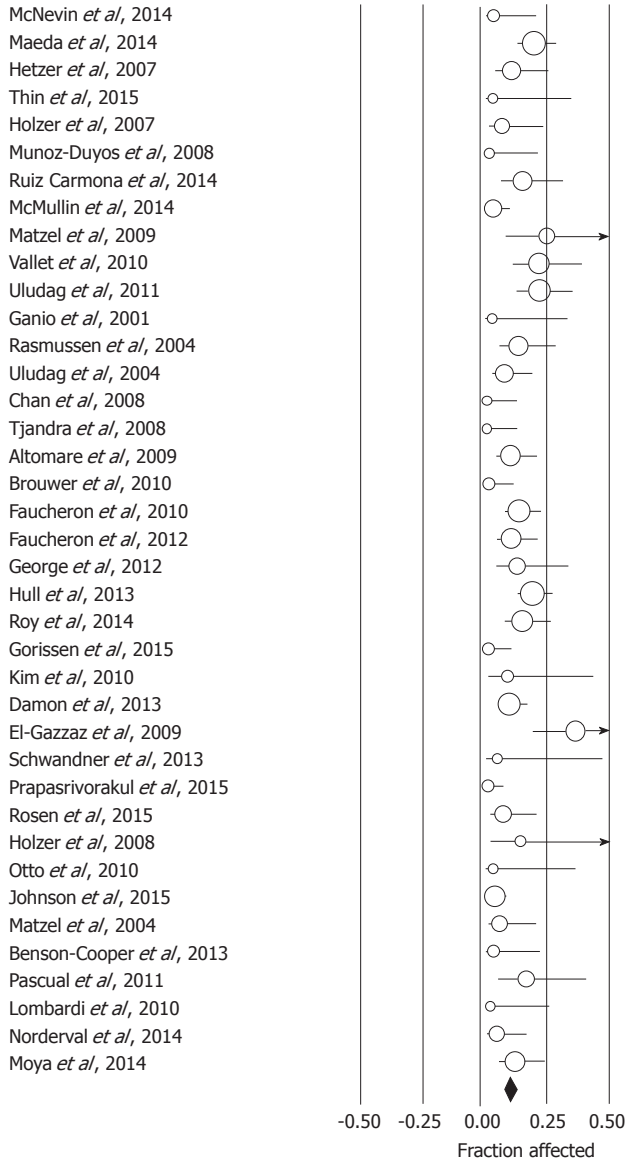

Figure 2 Forrest plot showing explant rates as fraction of the sample size in cohorts treated with sacral neurostimulation for fecal incontinence.

up period, with of an average of 10.0% (7.8-12.7) (I^2 = 54.0%; Figure 2) and a significant increase with the duration of follow up (Figure 3A). Lead complications, battery depletion or pain all contribute to additional intervention, with an overall re-operation rate of 18.6% (14.2-23.9) (I^2 = 80.5%) based on cohorts with a total of 1784 patients (Figure 4). Reoperation rates rose with longer follow-up times (Figure 3B).

MAUDE databank

Monthly reporting of adverse events related to the Interstim device ranged around 10 incidents per month in 2005, rose about tenfold within the next 3 years, and then remained stable until in the year prior to FDA approval of SNS for fecal incontinence [114.5 (65.2-163.8)]. As shown in Figure 5A, reported concerns more than tripled since. Focusing on the time from August to October 2015, the FDA received a total of 1684 reports of problems related to SNS, with 652 reports meeting the inclusion criteria as they mentioned GI as an indication for SNS therapy and did not focus on urinary symptoms. The narrative specifically referred to bowel dysfunction or fecal incontinence in 278 (42.6%) case forms. Within the same time period of 2014, only 4% of 1250 complaints listed GI or fecal incontinence as indication for stimulator implantation. In contrast to narratives reviewed for 2015, most reports did not include any information about treatment indications or targets, suggesting a change in recording of data as a potential confounder. The majority of adverse events were reported within the first two years after stimulator implantation without differences between the entire cohort and subgroup undergoing SNS for fecal incontinence (Figure 5B). The majority of reports came from patients, their partners or family members (71.6%). Company representatives (15.7%) or healthcare providers (12.7%) accounted for the remaining reports. Reports were classified as injury (65.0%) or malfunction (35.0%) with a significant overlap in adverse events described (Table 2).

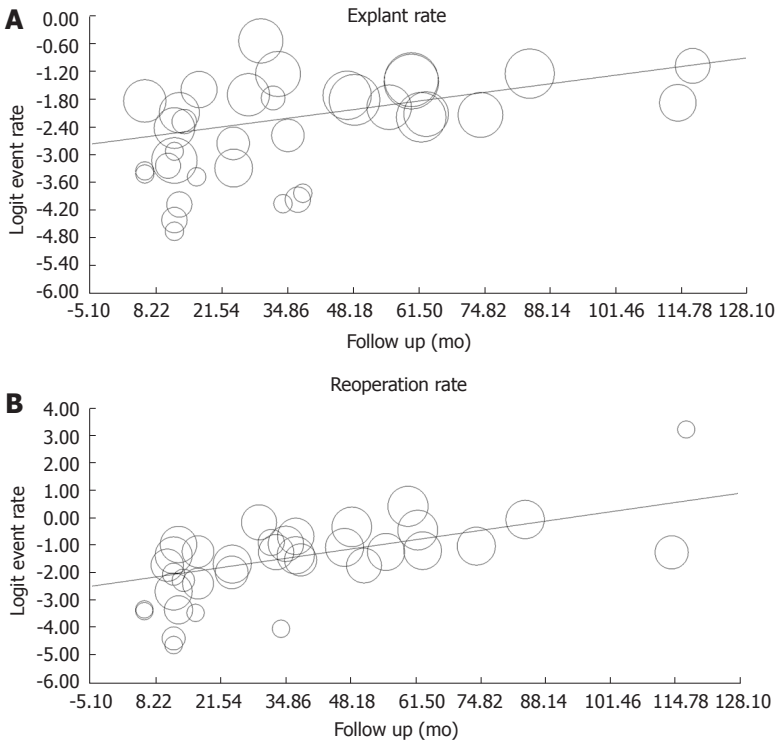

Figure 3 Meta-regression showing the time dependent increase in explant. A: $Q = 9.35$; $P = 0.002$ and reoperation panel; B: $Q = 20.3$; $P < 0.001$ rates after sacral neurostimulation initiation.

Table 2 Reported problems based on classification of adverse effects

Reported problem	Injury (n = 228)	Malfunction (n = 424)	P value
Battery depletion	9	6	0.057
Generator displacement	11	5	0.007
Pocket erosion	9	0	< 0.001
Infection	21	0	< 0.001
Pain	41	102	0.091
Lack of benefit	70	243	< 0.001
Lead problems	40	30	< 0.001
Other	27	9	

The identification of appropriate candidates for SNS proceeds in a two-step process with the initial phase utilizing insertion of temporary electrodes and an external device, followed by the implantation of a permanent stimulator only if patients experience at least 50% improvement during the 2 wk trial. Despite these stringent criteria, nearly half of the reported problems focused on lack or loss of benefit (49.7%). Results were similar in the cohort with fecal incontinence listed as the primary indication (51.8%). As was true for the overall pattern, 51.1% of the reports were filed within the first year after implantation, 24% in year 2, 8.6% in year 3, 10.8% in year 4 and 5.5% in year 5. Figure 6A shows the primary causes of concern. Pain or paresthesias accounted for 14.9% of the complaints (year 1: 78.4%; year 2: 3.1%; year 3: 13.4%; year 4: 5.1%), with 35.1% of these reports specifically referring to the generator site as affected area. Lead-related problems accounted for 10.7% of the reports and similarly surfaced primarily in the first year (62.9%), with a subsequent decrease over time (15.7% in year 2, 17.1% in year 3, 4.3% in year 4). Thirty reports (4.6%) described programming problems (year 1: 46.7%; year 2: 23.3%; year 3: 13.3%; year 4: 13.3%; year 5: 3.3%), which were related to the patient programmer in 43.3% of the cases. When reports listed a second concern (130; 19.9%), pain was most commonly brought up accounting for 40.8% of the problems, followed by limited benefit (16.1%) and concerns about possible electromagnetic interference (13.1%). Table 3 depicts secondary adverse events for the most common primary concerns.

The narrative did not include any information about corrective actions in 270 cases. As depicted in Figure 6B, most reported problems prompted adjustment of stimulation parameter (35.0%). Exactly one quarter of the concerns led to secondary operative interventions (n = 163; year 1: 37.4%; year 2: 24.5%; year 3: 17.8%; year 4: 11.7%; year 5: 8.6%). Re-interventions were nearly equally split between explants (39.3%) and replacements (41.7%). Isolated lead replacement accounted for only 4.3% of the reports. Pocket revisions were responsible for 14.1%, and minor operative revisions in 0.6%. Table 4 lists steps taken in relation to the reported primary concerns. While the majority of reports fell within the first two years after the original implant, the relative likelihood of undergoing secondary operative interventions increased significantly over time ($\chi^2 = 18.5$, $P < 0.001$; Figure 6C).

The narratives included some information about the outcome in 249 cases (38.2%). Dichotomizing assess-

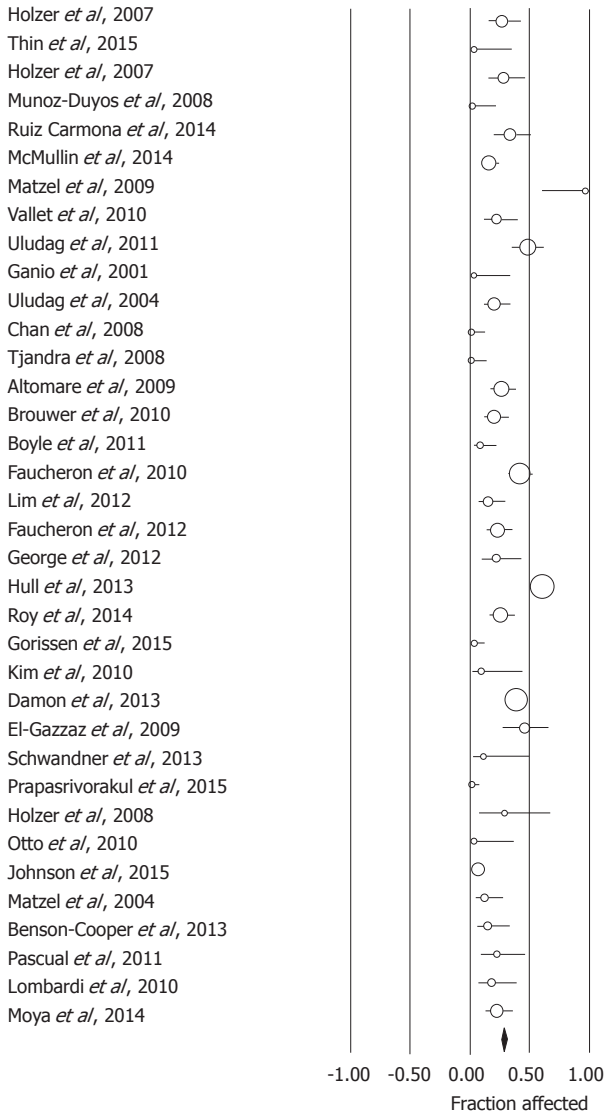

Figure 4 Forrest plot showing reoperation rates as fraction of the sample size in cohorts treated with sacral neurostimulation for fecal incontinence.

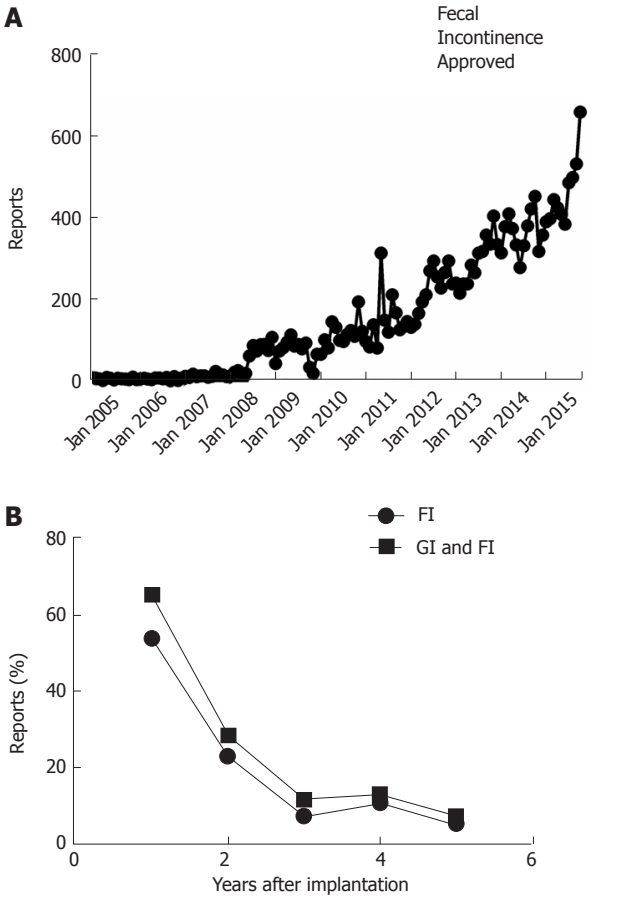

Figure 5 Manufacturer and user device experience databank. A: Reports about adverse events related to therapy with Interstim are plotted as a function of month of posting by the Federal Drug Administration; B: The lower panel depicts the percentage of reports mentioning gastrointestinal problems (squares) or specifically fecal incontinence (circles) as indication for sacral neurostimulation.

ments as persisting or solved, all 27 intraoperative problems had been successfully addressed. Reports described adjustments of stimulation parameters in 176 cases, with sufficient benefit in 35.2%. Secondary surgeries solved problems in 17 (68%) of 25 patients based on follow up information. In the remaining cases, medications (n = 7 of 9), exchange of the personal programmer (n = 2 of 2) or no specific intervention (n = 3 of 9) led to resolution of reported problems.

DISCUSSION

Fecal incontinence carries a significant burden with impaired quality of life due to stigmatization and subjective fears about embarrassing situation, leading to withdrawal and isolation[20]. Dietary, medical and surgical intervention often leave patients with significant residual problems[21-24], thus prompting the need to look for novel and more effective treatments. Sacral nerve stimulation for urinary incontinence had been introduced more than 20 years ago[25] and led to systematic studies in fecal incontinence soon afterwards[26]. More than 10 years after FDA approval, Laudano *et al*[27] observed a significant increase in implantations for urological indications in Medicare recipients. During this time period, more providers adopted this technique, suggesting more widespread acceptance of SNS as an appropriate treatment modality[28]. This apparent rise correlated with an increase in the number of adverse events listed in MAUDE. A similar pattern may have contributed to the tripling in concerns received by the FDA since approval of SNS for fecal incontinence as shown in this study. However, narratives only recently include information about indications for SNS therapy, thus not allowing us to address to what extend fecal incontinence therapy contributed to these changes.

Even though response to temporary stimulation is a prerequisite for permanent stimulator implantation, most of the concerns focused on lack or loss of benefit, which accounted for half of the primary problems described in the narrative. Conceptually, one may question whether lack or loss of benefit is truly an adverse event. Adjustment of stimulation parameters effectively resolved many of the reported problems, which

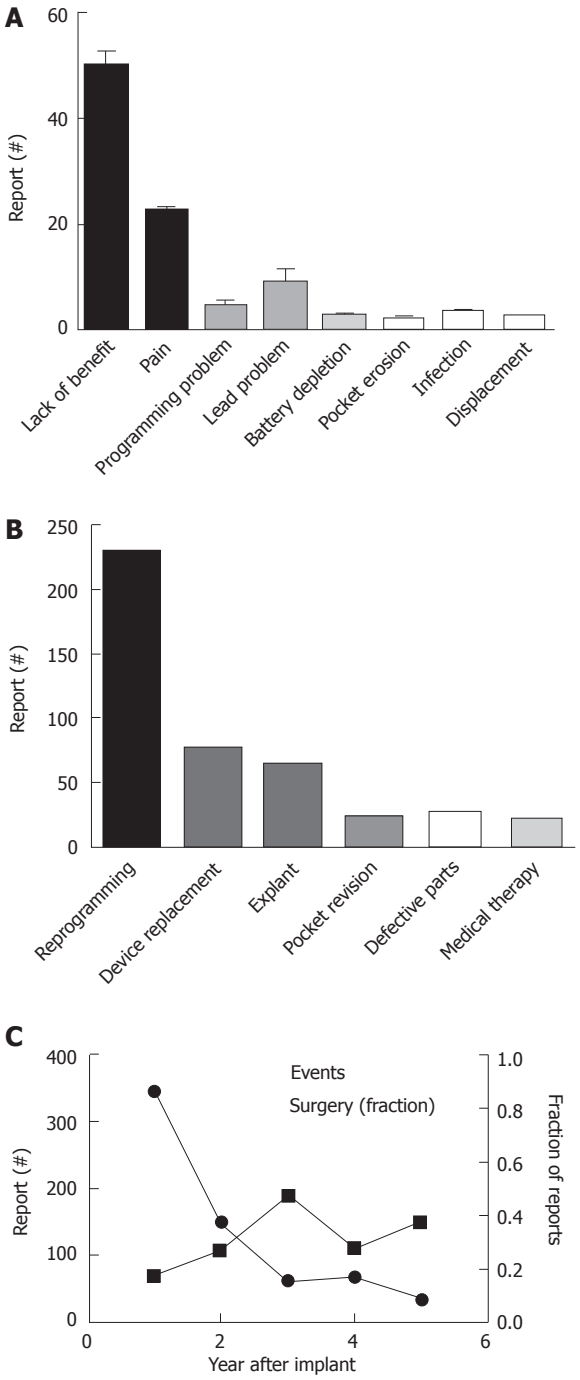

Figure 6 Concerns and interventions related to Interstim treatment and described in manufacturer and user device experience. A: Depicts the number of reported events sorted based on the primary concerns; B: Shows the main corrective steps taken ("Defective Parts" refers to intra-operative problems with device components requiring exchange); C: The proportions of operatively treated interventions is plotted in relation to the overall number of reported adverse events.

Table 3 Relationship between common primary and secondary concerns

Primary concern	No. of reports	Secondary concern
Lack of benefit	74	Battery depletion: $n = 2$ Generator displacement: $n = 3$ Electromagnetic interference: $n = 7$ Lead problem: $n = 3$ Pain: $n = 43$ Programming problems: $n = 14$ Other: $n = 2$
Pain or discomfort	32	Generator displacement: $n = 1$ Electromagnetic interference: $n = 8$ Lack of benefit: $n = 11$ Lead problem: $n = 1$ Pain (second type): $n = 4$ Programming problems: $n = 5$ Other: $n = 2$
Pocket site complications	8	Lack of benefit: $n = 1$ Lead problem: $n = 2$ Pain: $n = 5$
Lead problem	5	Generator displacement: $n = 1$ Lack of benefit: $n = 3$ Pain: $n = 1$
Programming problem	4	Electromagnetic interference: $n = 2$ Lack of benefit: $n = 1$ Pain: $n = 1$

Table 4 Reported interventions based on patient problems

Primary concern	Sample	Conservative therapy	Operative therapy
Lack of benefit	325	Stimulation adjusted: 160 Medication: 3 System check: 7	Explant: 22 Replacement: 17 Pocket revision: 3
Pain or discomfort	97	Stimulation adjusted: 34 Medication: 4 System check: 2	Explant: 8 Replacement: 1 Pocket revision: 7
Lead problem	70	Stimulation adjusted: 3 System check: 2	Explant: 1 Replacement: 36
Programming problems	30	Stimulation adjusted: 11	Replacement: 1

The most commonly described corrective actions are listed for key concerns addressed in the narratives. Limited or lacking information accounts for the differences between total sample size of the subgroups defined by their primary concerns.

could thus be seen as analogous to dosing changes in pharmacotherapy. Similarly, any treatment based on electrical stimulation will require energy and will, therefore, deplete the battery over time. Unless the need for battery replacement surfaces very early after stimulator implantation, it may also be considered routine maintenance of electrotherapy. The inclusion of such anticipated and often correctable problems as adverse events obviously inflates the number of side effects and may paint a more negative picture of SNS. As already indicated, the clinical relevance of some of the episodes registered was limited, whether it was transient loss of benefit due to programming issues or an expired lead that had been discovered and replaced during surgery. Despite this theoretical shortcoming, we chose an approach that still accepts these occurrences as adverse events, as they were specifically reported to the FDA as an unanticipated problem that was sufficiently relevant to the affected individual to contact the agency. Especially in treatments that target quality of life, such as management of fecal incontinence, the perceived distress of an undesired outcome should count. Such a strategy also falls in line with operational definitions

of side effects used in prospective studies. One large cohort study that followed 101 patients over 5 years reported more than 500 adverse events, most of which revolved around loss efficacy and prompted changes in stimulation parameters in about 80%[14]. Interestingly, only about one third of the changes in stimulation parameters led to a resolution of concerns, which correlates well with our results. Similarly high rates of adverse events were seen in a large consortium study of SNS for fecal incontinence[8] and in a cohort treated with mixed indications[29]. During 5 years of follow up, 46 of 120 patients underwent 72 additional operative interventions[8]. These numbers are significantly higher than data posted by Medtronics based on a prospective registry following 490 patients treated for urinary incontinence with a total number of 134 adverse events[30]. This apparent discrepancy may in part be due to the relatively short period of observation in the device maker's registry. Consistent with this interpretation, preliminary data on lead survival in this cohort suggest rising rates of problems, which exceed 20% after 33 mo. Interestingly, physicians assessed details of the adverse events in this registry and judged less than 30% as device-related. This classification was based on the analysis of device components rather than the nature of the adverse event and, for example, considered infections or other localized complications at the implant site as unrelated, if the explanted device was functional.

Data captured in MAUDE do not enable us to estimate incidence rates, which requires sufficient information about the total number of devices implanted for the indication of interest. In addition, data are de-identified and do not allow to differentiate repeat concerns by a single individual from similar concerns coming from multiple persons. Despite this caveat, our findings with descriptions of reoperation accounting for 25% of the reports highlight the potentially significant burden of SNS. This number falls into the range seen in larger cohort studies of spinal cord stimulators or gastric electrical stimulators with sufficient follow up[31-33] and corresponds with the calculated annual rate of repeat surgeries after cardiac pacemaker implantation of about 5%[34]. Large cohort studies of SNS for urinary problems showed even higher rates of about 40%[35,36]. A more detailed assessment of indications for re-interventions demonstrated comparable infection rates that require removal in 1%-4% of patients treated with spinal stimulators, intrathecal drug delivery systems, cardiac devices or gastric electrical stimulators[33,37-40], lead fractures or related problems in 2%-4% of cardiac devices[41] or a need for replacement due to device malfunction with annual replacement rates around 2% for implantable defibrillators[42]. Battery depletion and replacement played a relatively minor role in our analysis, largely due to the relatively recent approval of SNS for fecal incontinence and the expected battery lifetime of more than 4 years[43,44].

Operative re-intervention are obviously costly. However, even if patient concerns do not necessitate additional surgical steps, the required evaluations and treatment changes come with a significant financial burden[45]. This burden needs to be seen in the context of the perceived benefit. Consistent with a recently published meta-analysis of SNS for urinary and gastrointestinal problems, the lack of control groups, poorly defined or differing endpoints and limited information about the variance of reported results do not allow a sufficiently detailed meta-analysis[10]. Results typically used a more than 50% improvement of incontinence rating scales as cutoff for treatment success, which also functions as response definitions during temporary stimulation and was supported by a recently published analysis of patient data[46]. Accepting a 50% improvement or less stringent definitions for symptom improvement as response definition[6,8,18,19,47-54], we found improvement rates for fecal incontinence of about 60% (data not shown). Despite the general acceptance of this threshold definition, a comparison of overall treatment satisfaction with changes in symptom severity scales revealed only minor differences between 25%-50% and 50%-75% improvements, but a significant increase in global satisfaction above this range in a large cohort of SNS treated patients[55]. Especially assessments relying primarily on event frequency may underestimate disgust and emotional factors which contribute significantly to the subjective disease burden[56]. Finally, a small study demonstrated continued benefit after temporary discontinuation of SNS in about 50%[57], raising questions about non-specific or placebo effects of this treatment. None of these points question the benefit many patients experience. Nonetheless, the potential for improvement has to be weighed against a high likelihood of at least some residual symptoms and a high chance of adverse effects with secondary surgeries. Knowing about the less serious, but common unanticipated or undesired effects may also guide us as we can educate patients about such problems and their often successful solutions. Beyond open discussions with patients, we need controlled trials using less invasive approaches, such as medications, biofeedback or the transcutaneously applied stimulation[58,59].

Our study has several important limitations. Any study that relies on databanks collecting reports of adverse events will by definition paint a negative image of the intervention studied. In addition, relying on the MAUDE data repository cannot truly define the incidence of adverse events. However, we tried to address these important caveats by systematically examining published trials and cohort studies of SNS for fecal incontinence. Findings were strikingly similar, supporting the validity of our conclusions. As described above, the variable and at times incompletely described endpoints did not enable us to systematically assess the benefit in SNS for fecal incontinence. In addition, we need to keep in mind that the reported response rates of about 60% are skewed as they typically exclude patients who failed treatment trials with temporary

stimulation. The retrospective design of many studies leaves questions about the reliable recording of adverse effects. We therefore limited our systematic analysis to more easily defined endpoints that required secondary interventions. Finally, we tried to gain more detailed insight into the time course of adverse event recordings related to SNS for fecal incontinence. However, more than 2 years after FDA approval, only 2%-4% of the narratives provided information about the underlying indication for SNS, thereby limiting our ability to extend our analysis to earlier times.

Taken together, our findings highlight the high rate of adverse events after SNS with common need for reoperations, which should be clearly discussed with patients considering this treatment. This point is even more relevant as criteria defining treatment responses may leave patients with potentially significant residual symptoms. Considering the emergence of less invasive stimulation paradigms, comparative effectiveness trials are essential to define true therapeutic gains of permanently implanted devices. Lastly, the results show the important insight we can gain by analyzing reports about adverse events received by the FDA.

COMMENTS

Background

Fecal incontinence is a stigmatizing and unfortunately at times difficult to treat problem. After the recent approval by regulatory agencies in the United States, electrical stimulation of nerves that control anal closing muscle control has become a treatment option for patients not responding to other therapies. It does involve surgery and requires ongoing use of an implanted device, thus increasing the risk of complications and the potential need for repeat operations.

Research frontiers

The key questions in such more invasive treatments relate to ratios of risk *vs* benefit. In the context of a treatment that targets the symptom of fecal incontinence, how does improvement of perceived benefit compare to the frequency and severity of perceived problems or even harm due to this very treatment?

Innovations and breakthroughs

This study took a different approach than previously published reports, which typically described side effects in patients treated by the various investigators. The data presented here are based on problems reported to the regulatory agencies in the United States. The results show that complaints are common. While many of the perceived problems may be correctable through reprogramming or other steps, they were disturbing enough to the affected persons to convey their concerns to federal regulators. Beyond such temporary problems, repeat surgeries are common and often involve device removal or replacement. Knowing about these adverse effects will help patients and healthcare providers when weighing decisions related to the use of this treatment.

Terminology

Fecal incontinence is defined as involuntary loss of fecal matter. Sacral neuromodulation is a surgical treatment of incontinence. It includes the implantation of electrodes, connected to a stimulator, which then electrically activates nerves that control anal closing muscle function.

Peer-review

The reviewers emphasized the fact that many of the perceived side effects represented expected problems that are part of any treatment. If stimulation intensity is too high or too low, patients may feel shocks or experience no or little benefits. Both scenarios can often be managed by changing stimulation parameter. Similarly, battery replacement should be expected in any therapy that relies on electrical stimulation. While perhaps more complex in electrotherapy, the reviewers emphasized that these steps may well be equivalent to changes in medication dosing or prescription refills.

REFERENCES

1 **Ng KS**, Sivakumaran Y, Nassar N, Gladman MA. Fecal Incontinence: Community Prevalence and Associated Factors--A Systematic Review. *Dis Colon Rectum* 2015; **58**: 1194-1209 [PMID: 26544818 DOI: 10.1097/dcr.0000000000000514]

2 **Norton C**, Chelvanayagam S, Wilson-Barnett J, Redfern S, Kamm MA. Randomized controlled trial of biofeedback for fecal incontinence. *Gastroenterology* 2003; **125**: 1320-1329 [PMID: 14598248 DOI: 10.1016/j.gastro.2003.09.039]

3 **Heymen S**, Scarlett Y, Jones K, Ringel Y, Drossman D, Whitehead WE. Randomized controlled trial shows biofeedback to be superior to pelvic floor exercises for fecal incontinence. *Dis Colon Rectum* 2009; **52**: 1730-1737 [PMID: 19966605 DOI: 10.1007/DCR.0b013e3181b55455]

4 **Fowler CJ**. The perspective of a neurologist on treatment-related research in fecal and urinary incontinence. *Gastroenterology* 2004; **126**: S172-S174 [PMID: 14978657 DOI: 10.1053/j.gastro.2003.10.006]

5 **Rao SS**. Diagnosis and management of fecal incontinence. American College of Gastroenterology Practice Parameters Committee. *Am J Gastroenterol* 2004; **99**: 1585-1604 [PMID: 15307881 DOI: 10.1111/j.1572-0241.2004.40105.x]

6 **Johnson BL**, Abodeely A, Ferguson MA, Davis BR, Rafferty JF, Paquette IM. Is sacral neuromodulation here to stay? Clinical outcomes of a new treatment for fecal incontinence. *J Gastrointest Surg* 2015; **19**: 15-19; discussion 19-20 [PMID: 25115325 DOI: 10.1007/s11605-014-2611-4]

7 **Tjandra JJ**, Chan MK, Yeh CH, Murray-Green C. Sacral nerve stimulation is more effective than optimal medical therapy for severe fecal incontinence: a randomized, controlled study. *Dis Colon Rectum* 2008; **51**: 494-502 [PMID: 18278532 DOI: 10.1007/s10350-007-9103-5]

8 **Hull T**, Giese C, Wexner SD, Mellgren A, Devroede G, Madoff RD, Stromberg K, Coller JA. Long-term durability of sacral nerve stimulation therapy for chronic fecal incontinence. *Dis Colon Rectum* 2013; **56**: 234-245 [PMID: 23303153 DOI: 10.1097/DCR.0b013e318276b24c]

9 **Herbison GP**, Arnold EP. Sacral neuromodulation with implanted devices for urinary storage and voiding dysfunction in adults. *Cochrane Database Syst Rev* 2009; **(2)**: CD004202 [PMID: 19370596 DOI: 10.1002/14651858.CD004202.pub2]

10 **Mirbagheri N**, Sivakumaran Y, Nassar N, Gladman MA. Systematic review of the impact of sacral neuromodulation on clinical symptoms and gastrointestinal physiology. *ANZ J Surg* 2016; **86**: 232-236 [PMID: 26245170 DOI: 10.1111/ans.13257]

11 **Overbey DM**, Townsend NT, Chapman BC, Bennett DT, Foley LS, Rau AS, Yi JA, Jones EL, Stiegmann GV, Robinson TN. Surgical Energy-Based Device Injuries and Fatalities Reported to the Food and Drug Administration. *J Am Coll Surg* 2015; **221**: 197-205.e1 [PMID: 26095572 DOI: 10.1016/j.jamcollsurg.2015.03.031]

12 **Naumann RW**, Brown J. Complications of Electromechanical Morcellation Reported in the Manufacturer and User Facility Device Experience (MAUDE) Database. *J Minim Invasive Gynecol* 1989; **22**: 1018-1021 [PMID: 25987522 DOI: 10.1016/j.jmig.2015.05.008]

13 **Tremaine AM**, Avram MM. FDA MAUDE data on complications with lasers, light sources, and energy-based devices. *Lasers Surg Med* 2015; **47**: 133-140 [PMID: 25655709 DOI: 10.1002/lsm.22328]

14 **Maeda Y**, Lundby L, Buntzen S, Laurberg S. Outcome of sacral nerve stimulation for fecal incontinence at 5 years. *Ann Surg* 2014; **259**: 1126-1131 [PMID: 23817505 DOI: 10.1097/SLA.0b013e31829d3969]

15 **Lombardi G**, Del Popolo G, Cecconi F, Surrenti E, Macchiarella A. Clinical outcome of sacral neuromodulation in incomplete spinal

cord-injured patients suffering from neurogenic bowel dysfunctions. *Spinal Cord* 2010; **48**: 154-159 [PMID: 19668257 DOI: 10.1038/sc.2009.101]

16 **Brouwer R**, Duthie G. Sacral nerve neuromodulation is effective treatment for fecal incontinence in the presence of a sphincter defect, pudendal neuropathy, or previous sphincter repair. *Dis Colon Rectum* 2010; **53**: 273-278 [PMID: 20173472 DOI: 10.1007/DCR.0b013e3181ceeb22]

17 **Vallet C**, Parc Y, Lupinacci R, Shields C, Parc R, Tiret E. Sacral nerve stimulation for faecal incontinence: response rate, satisfaction and the value of preoperative investigation in patient selection. *Colorectal Dis* 2010; **12**: 247-253 [PMID: 19508523 DOI: 10.1111/j.1463-1318.2009.01899.x]

18 **Roy AL**, Gourcerol G, Menard JF, Michot F, Leroi AM, Bridoux V. Predictive factors for successful sacral nerve stimulation in the treatment of fecal incontinence: lessons from a comprehensive treatment assessment. *Dis Colon Rectum* 2014; **57**: 772-780 [PMID: 24807603 DOI: 10.1097/dcr.0000000000000115]

19 **Matzel KE**, Kamm MA, Stösser M, Baeten CG, Christiansen J, Madoff R, Mellgren A, Nicholls RJ, Rius J, Rosen H. Sacral spinal nerve stimulation for faecal incontinence: multicentre study. *Lancet* 2004; **363**: 1270-1276 [PMID: 15094271 DOI: 10.1016/s0140-6736(04)15999-0]

20 **Bharucha AE**, Zinsmeister AR, Locke GR, Seide BM, McKeon K, Schleck CD, Melton LJ. Prevalence and burden of fecal incontinence: a population-based study in women. *Gastroenterology* 2005; **129**: 42-49 [PMID: 16012933 DOI: 10.1053/j.gastro.2005.04.006]

21 **Madoff RD**. Surgical treatment options for fecal incontinence. *Gastroenterology* 2004; **126** (Suppl 1): S48-S54 [DOI: 10.1053/j.gastro.2003.10.015]

22 **Enck P**, Van der Voort IR, Klosterhalfen S. Biofeedback therapy in fecal incontinence and constipation. *Neurogastroenterol Motil* 2009; **21**: 1133-1141 [PMID: 19566591 DOI: 10.1111/j.1365-2982.2009.01345.x]

23 **Markland AD**, Burgio KL, Whitehead WE, Richter HE, Wilcox CM, Redden DT, Beasley TM, Goode PS. Loperamide Versus Psyllium Fiber for Treatment of Fecal Incontinence: The Fecal Incontinence Prescription (Rx) Management (FIRM) Randomized Clinical Trial. *Dis Colon Rectum* 2015; **58**: 983-993 [PMID: 26347971 DOI: 10.1097/dcr.0000000000000442]

24 **Norton C**, Cody JD, Hosker G. Biofeedback and/or sphincter exercises for the treatment of faecal incontinence in adults. *Cochr Data Syst Rev* 2006; **(3)**: CD002111 [DOI: 10.1002/14651858.cd002111.pub2]

25 **Dijkema HE**, Weil EH, Mijs PT, Janknegt RA. Neuromodulation of sacral nerves for incontinence and voiding dysfunctions. Clinical results and complications. *Eur Urol* 1993; **24**: 72-76 [PMID: 8396034]

26 **Vaizey CJ**, Kamm MA, Turner IC, Nicholls RJ, Woloszko J. Effects of short term sacral nerve stimulation on anal and rectal function in patients with anal incontinence. *Gut* 1999; **44**: 407-412 [PMID: 10026329 DOI: 10.1136/gut.44.3.407]

27 **Laudano MA**, Seklehner S, Sandhu J, Reynolds WS, Garrett KA, Milsom JW, Te AE, Kaplan SA, Chughtai B, Lee RK. Disparities in the Use of Sacral Neuromodulation among Medicare Beneficiaries. *J Urol* 2015; **194**: 449-453 [PMID: 25846418 DOI: 10.1016/j.juro.2015.03.111]

28 **Suskind AM**, Clemens JQ, Zhang Y, Hollenbeck BK. Physician Use of Sacral Neuromodulation Among Medicare Beneficiaries With Overactive Bladder and Urinary Retention. *Urology* 2015; **86**: 30-34 [PMID: 26026856 DOI: 10.1016/j.urology.2015.04.003]

29 **Leroi AM**, Lenne X, Dervaux B, Chartier-Kastler E, Mauroy B, Normand LL, Grise P, Faucheron JL, Parc Y, Lehur PA, Mion F, Damon H, Barth X, Leriche A, Saussine C, Guy L, Haab F, Bresler L, Sarramon JP, Bensadoun H, Rullier E, Slim K, Sielezneff I, Mourey E, Ballanger P, Michot F. Outcome and cost analysis of sacral nerve modulation for treating urinary and/or fecal incontinence. *Ann Surg* 2011; **253**: 720-732 [PMID: 21475012 DOI: 10.1097/SLA.0b013e318210f1f4]

30 **Medtronic**. Medtronic Product Performance Report 2014. Available from: URL: http://professional.medtronic.com/wcm/groups/mdtcom_sg/@mdt/@neuro/documents/documents/mdt_product_performance_2014.pdf

31 **Hayek SM**, Veizi E, Hanes M. Treatment-Limiting Complications of Percutaneous Spinal Cord Stimulator Implants: A Review of Eight Years of Experience From an Academic Center Database. *Neuromodulation* 2015; **18**: 603-608; discussion 608-609 [PMID: 26053499 DOI: 10.1111/ner.12312]

32 **Abell T**, McCallum R, Hocking M, Koch K, Abrahamsson H, Leblanc I, Lindberg G, Konturek J, Nowak T, Quigley EM, Tougas G, Starkebaum W. Gastric electrical stimulation for medically refractory gastroparesis. *Gastroenterology* 2003; **125**: 421-428 [PMID: 12891544]

33 **Zehetner J**, Ravari F, Ayazi S, Skibba A, Darehzereshki A, Pelipad D, Mason RJ, Katkhouda N, Lipham JC. Minimally invasive surgical approach for the treatment of gastroparesis. *Surg Endosc* 2013; **27**: 61-66 [PMID: 22752276 DOI: 10.1007/s00464-012-2407-0]

34 **Duray GZ**, Schmitt J, Cicek-Hartvig S, Hohnloser SH, Israel CW. Complications leading to surgical revision in implantable cardioverter defibrillator patients: comparison of patients with single-chamber, dual-chamber, and biventricular devices. *Europace* 2009; **11**: 297-302 [PMID: 19054788 DOI: 10.1093/europace/eun322]

35 **Peeters K**, Sahai A, De Ridder D, Van Der Aa F. Long-term follow-up of sacral neuromodulation for lower urinary tract dysfunction. *BJU Int* 2014; **113**: 789-794 [PMID: 24238278 DOI: 10.1111/bju.12571]

36 **Al-zahrani AA**, Elzayat EA, Gajewski JB. Long-term outcome and surgical interventions after sacral neuromodulation implant for lower urinary tract symptoms: 14-year experience at 1 center. *J Urol* 2011; **185**: 981-986 [PMID: 21247597 DOI: 10.1016/j.juro.2010.10.054]

37 **Engle MP**, Vinh BP, Harun N, Koyyalagunta D. Infectious complications related to intrathecal drug delivery system and spinal cord stimulator system implantations at a comprehensive cancer pain center. *Pain Physician* 2013; **16**: 251-257 [PMID: 23703411]

38 **Kahlow H**, Olivecrona M. Complications of vagal nerve stimulation for drug-resistant epilepsy: a single center longitudinal study of 143 patients. *Seizure* 2013; **22**: 827-833 [PMID: 23867218 DOI: 10.1016/j.seizure.2013.06.011]

39 **Palmisano P**, Accogli M, Zaccaria M, Luzzi G, Nacci F, Anaclerio M, Favale S. Rate, causes, and impact on patient outcome of implantable device complications requiring surgical revision: large population survey from two centres in Italy. *Europace* 2013; **15**: 531-540 [PMID: 23407627 DOI: 10.1093/europace/eus337]

40 **Krahn AD**, Lee DS, Birnie D, Healey JS, Crystal E, Dorian P, Simpson CS, Khaykin Y, Cameron D, Janmohamed A, Yee R, Austin PC, Chen Z, Hardy J, Tu JV. Predictors of short-term complications after implantable cardioverter-defibrillator replacement: results from the Ontario ICD Database. *Circ Arrhythm Electrophysiol* 2011; **4**: 136-142 [PMID: 21325209 DOI: 10.1161/circep.110.959791]

41 **Armaganijan LV**, Toff WD, Nielsen JC, Andersen HR, Connolly SJ, Ellenbogen KA, Healey JS. Are elderly patients at increased risk of complications following pacemaker implantation? A meta-analysis of randomized trials. *Pacing Clin Electrophysiol* 2012; **35**: 131-134 [PMID: 22040168 DOI: 10.1111/j.1540-8159.2011.03240.x]

42 **Maisel WH**, Moynahan M, Zuckerman BD, Gross TP, Tovar OH, Tillman DB, Schultz DB. Pacemaker and ICD generator malfunctions: analysis of Food and Drug Administration annual reports. *JAMA* 2006; **295**: 1901-1906 [PMID: 16639048 DOI: 10.1001/jama.295.16.1901]

43 **Blandon RE**, Gebhart JB, Lightner DJ, Klingele CJ. Re-operation rates after permanent sacral nerve stimulation for refractory voiding dysfunction in women. *BJU Int* 2008; **101**: 1119-1123 [PMID: 18190624 DOI: 10.1111/j.1464-410X.2007.07426.x]

44 **North RB**, Brigham DD, Khalessi A, Calkins SK, Piantadosi S, Campbell DS, Daly MJ, Dey PB, Barolat G, Taylor R. Spinal cord stimulator adjustment to maximize implanted battery longevity: a randomized, controlled trial using a computerized, patient-interactive programmer. *Neuromodulation* 2004; **7**: 13-25 [PMID: 22151122 DOI: 10.1111/j.1525-1403.2004.04002.x]

45 **McMullin CM**, Jadav AM, Hanwell C, Brown SR. Resource implications of running a sacral neuromodulation service: a 10-year

experience. *Colorectal Dis* 2014; **16**: 719-722 [PMID: 24930568 DOI: 10.1111/codi.12686]

46 **Jelovsek JE**, Chen Z, Markland AD, Brubaker L, Dyer KY, Meikle S, Rahn DD, Siddiqui NY, Tuteja A, Barber MD. Minimum important differences for scales assessing symptom severity and quality of life in patients with fecal incontinence. *Female Pelvic Med Reconstr Surg* 2014; **20**: 342-348 [PMID: 25185630 DOI: 10.1097/spv.0000000000000078]

47 **Thin NN**, Taylor SJ, Bremner SA, Emmanuel AV, Hounsome N, Williams NS, Knowles CH. Randomized clinical trial of sacral versus percutaneous tibial nerve stimulation in patients with faecal incontinence. *Br J Surg* 2015; **102**: 349-358 [PMID: 25644291 DOI: 10.1002/bjs.9695]

48 **Mishra A**, Prapasrivorakul S, Gosselink MP, Gorissen KJ, Hompes R, Jones O, Cunningham C, Matzel KE, Lindsey I. Sacral neuromodulation for persistent faecal incontinence after laparoscopic ventral rectopexy for high-grade internal rectal prolapse. *Colorectal Dis* 2016; **18**: 273-278 [PMID: 26391837 DOI: 10.1111/codi.13125]

49 **Altomare DF**, Ratto C, Ganio E, Lolli P, Masin A, Villani RD. Long-term outcome of sacral nerve stimulation for fecal incontinence. *Dis Colon Rectum* 2009; **52**: 11-17 [PMID: 19273950 DOI: 10.1007/DCR.0b013e3181974444]

50 **Boyle DJ**, Murphy J, Gooneratne ML, Grimmer K, Allison ME, Chan CL, Williams NS. Efficacy of sacral nerve stimulation for the treatment of fecal incontinence. *Dis Colon Rectum* 2011; **54**: 1271-1278 [PMID: 21904142 DOI: 10.1097/DCR.0b013e3182270af1]

51 **Kim DH**, Faruqui N, Ghoniem GM. Sacral neuromodulation outcomes in patients with urge urinary incontinence and concomitant urge fecal incontinence. *Female Pelvic Med Reconstr Surg* 2010; **16**: 171-178 [PMID: 22453282 DOI: 10.1097/SPV.0b013e3181d67c24]

52 **Duelund-Jakobsen J**, van Wunnik B, Buntzen S, Lundby L, Laurberg S, Baeten C. Baseline factors predictive of patient satisfaction with sacral neuromodulation for idiopathic fecal incontinence. *Int J Colorectal Dis* 2014; **29**: 793-798 [PMID: 24743849 DOI: 10.1007/s00384-014-1870-6]

53 **El-Gazzaz G**, Zutshi M, Salcedo L, Hammel J, Rackley R, Hull T. Sacral neuromodulation for the treatment of fecal incontinence and urinary incontinence in female patients: long-term follow-up. *Int J Colorectal Dis* 2009; **24**: 1377-1381 [PMID: 19488765 DOI: 10.1007/s00384-009-0745-8]

54 **Prapasrivorakul S**, Gosselink MP, Gorissen KJ, Fourie S, Hompes R, Jones OM, Cunningham C, Lindsey I. Sacral neuromodulation for faecal incontinence: is the outcome compromised in patients with high-grade internal rectal prolapse? *Int J Colorectal Dis* 2015; **30**: 229-234 [PMID: 25433818 DOI: 10.1007/s00384-014-2078-5]

55 **Duelund-Jakobsen J**, van Wunnik B, Buntzen S, Lundby L, Baeten C, Laurberg S. Functional results and patient satisfaction with sacral nerve stimulation for idiopathic faecal incontinence. *Colorectal Dis* 2012; **14**: 753-759 [PMID: 21883814 DOI: 10.1111/j.1463-1318.2011.02800.x]

56 **Reynolds LM**, Bissett IP, Consedine NS. Predicting the patients who will struggle with anal incontinence: sensitivity to disgust matters. *Colorectal Dis* 2015; **17**: 73-80 [PMID: 25234697 DOI: 10.1111/codi.12781]

57 **Altomare DF**, Giannini I, Giuratrabocchetta S, Digennaro R. The effects of sacral nerve stimulation on continence are temporarily maintained after turning the stimulator off. *Colorectal Dis* 2013; **15**: e741-e748 [PMID: 24102954 DOI: 10.1111/codi.12418]

58 **Rimmer CJ**, Knowles CH, Lamparelli M, Durdey P, Lindsey I, Hunt L, Nugent K, Gill KA. Short-term Outcomes of a Randomized Pilot Trial of 2 Treatment Regimens of Transcutaneous Tibial Nerve Stimulation for Fecal Incontinence. *Dis Colon Rectum* 2015; **58**: 974-982 [PMID: 26347970 DOI: 10.1097/dcr.0000000000000444]

59 **Thomas GP**, Norton C, Nicholls RJ, Vaizey CJ. A pilot study of transcutaneous sacral nerve stimulation for faecal incontinence. *Colorectal Dis* 2013; **15**: 1406-1409 [PMID: 23910042 DOI: 10.1111/codi.12371]

60 **Gorissen KJ**, Bloemendaal AL, Prapasrivorakul S, Gosselink MP, Jones OM, Cunningham C, Lindsey I, Hompes R. Dynamic Article: Permanent Sacral Nerve Stimulation Under Local Anesthesia: Feasibility, Best Practice, and Patient Satisfaction. *Dis Colon Rectum* 2015; **58**: 1182-1185 [PMID: 26544816 DOI: 10.1097/dcr.0000000000000489]

61 **Kahlke V**, Topic H, Peleikis HG, Jongen J. Sacral nerve modulation for fecal incontinence: results of a prospective single-center randomized crossover study. *Dis Colon Rectum* 2015; **58**: 235-240 [PMID: 25585083 DOI: 10.1097/dcr.0000000000000295]

62 **Al Asari S**, Meurette G, Mantoo S, Kubis C, Wyart V, Lehur PA. Percutaneous tibial nerve stimulation vs sacral nerve stimulation for faecal incontinence: a comparative case-matched study. *Colorectal Dis* 2014; **16**: O393-O399 [PMID: 24911219 DOI: 10.1111/codi.12680]

63 **Moya P**, Arroyo A, Lacueva J, Candela F, Soriano-Irigaray L, López A, Gómez MA, Galindo I, Calpena R. Sacral nerve stimulation in the treatment of severe faecal incontinence: long-term clinical, manometric and quality of life results. *Tech Coloproctol* 2014; **18**: 179-185 [PMID: 23624794 DOI: 10.1007/s10151-013-1022-y]

64 **Norderval S**, Behrenbruch C, Brouwer R, Keck JO. Efficacy of cyclic sacral nerve stimulation for faecal incontinence. *Tech Coloproctol* 2013; **17**: 511-516 [PMID: 23525966 DOI: 10.1007/s10151-013-0999-6]

65 **George AT**, Kalmar K, Panarese A, Dudding TC, Nicholls RJ, Vaizey CJ. Long-term outcomes of sacral nerve stimulation for fecal incontinence. *Dis Colon Rectum* 2012; **55**: 302-306 [PMID: 22469797 DOI: 10.1097/DCR.0b013e3182401ecd]

66 **Damon H**, Barth X, Roman S, Mion F. Sacral nerve stimulation for fecal incontinence improves symptoms, quality of life and patients' satisfaction: results of a monocentric series of 119 patients. *Int J Colorectal Dis* 2013; **28**: 227-233 [PMID: 22885883 DOI: 10.1007/s00384-012-1558-8]

67 **Lim JT**, Hastie IA, Hiscock RJ, Shedda SM. Sacral nerve stimulation for fecal incontinence: long-term outcomes. *Dis Colon Rectum* 2011; **54**: 969-974 [PMID: 21730785 DOI: 10.1097/DCR.0b013e31821e57c2]

68 **Rasmussen OO**, Buntzen S, Sørensen M, Laurberg S, Christiansen J. Sacral nerve stimulation in fecal incontinence. *Dis Colon Rectum* 2004; **47**: 1158-1162; discussion 1162-1163 [PMID: 15216409 DOI: 10.1007/s10350-004-0553-8]

69 **Matzel KE**, Lux P, Heuer S, Besendörfer M, Zhang W. Sacral nerve stimulation for faecal incontinence: long-term outcome. *Colorectal Dis* 2009; **11**: 636-641 [PMID: 18727721 DOI: 10.1111/j.1463-1318.2008.01673.x]

70 **Muñoz-Duyos A**, Navarro-Luna A, Brosa M, Pando JA, Sitges-Serra A, Marco-Molina C. Clinical and cost effectiveness of sacral nerve stimulation for faecal incontinence. *Br J Surg* 2008; **95**: 1037-1043 [PMID: 18574847 DOI: 10.1002/bjs.6140]

71 **Holzer B**, Rosen HR, Zaglmaier W, Klug R, Beer B, Novi G, Schiessel R. Sacral nerve stimulation in patients after rectal resection--preliminary report. *J Gastrointest Surg* 2008; **12**: 921-925 [PMID: 18278538 DOI: 10.1007/s11605-008-0485-z]

72 **Chan MK**, Tjandra JJ. Sacral nerve stimulation for fecal incontinence: external anal sphincter defect vs. intact anal sphincter. *Dis Colon Rectum* 2008; **51**: 1015-1024; discussion 1024-1025 [PMID: 18484136 DOI: 10.1007/s10350-008-9326-0]

73 **O'Riordan JM**, Healy CF, McLoughlin D, Cassidy M, Brannigan AE, O'Connell PR. Sacral nerve stimulation for faecal incontinence. *Ir J Med Sci* 2008; **177**: 117-119 [PMID: 18392782 DOI: 10.1007/s11845-008-0145-z]

74 **Mattioli S**, Lugaresi ML, Costantini M, Del Genio A, Di Martino N, Fei L, Fumagalli U, Maffettone V, Monaco L, Morino M, Rebecchi F, Rosati R, Rossi M, Santi S, Trapani V, Zaninotto G. The short esophagus: intraoperative assessment of esophageal length. *J Thorac Cardiovasc Surg* 2008; **136**: 834-841 [PMID: 18954619]

75 **Hetzer FH**, Hahnloser D, Clavien PA, Demartines N. Quality of life and morbidity after permanent sacral nerve stimulation for fecal incontinence. *Arch Surg* 2007; **142**: 8-13 [PMID: 17224495 DOI: 10.1001/archsurg.142.1.8]

76 **Otto SD**, Burmeister S, Buhr HJ, Kroesen A. Sacral nerve stimulation induces changes in the pelvic floor and rectum that improve continence and quality of life. *J Gastrointest Surg* 2010; **14**: 636-644 [PMID: 20058096 DOI: 10.1007/s11605-009-1122-1]

77 **Faucheron JL**, Voirin D, Badic B. Sacral nerve stimulation for fecal incontinence: causes of surgical revision from a series of 87 consecutive patients operated on in a single institution. *Dis Colon Rectum* 2010; **53**: 1501-1507 [PMID: 20940598 DOI: 10.1007/DCR.0b013e3181f1cf14]
78 **Rosen A**, Taragano L, Condrea A, Sidi A, Ron Y, Ginath S. Effects of Sacral Neuromodulation on Urinary and Fecal Incontinence. *Isr Med Assoc J* 2015; **17**: 351-355 [PMID: 26233993]
79 **McNevin MS**, Moore M, Bax T. Outcomes associated with Interstim therapy for medically refractory fecal incontinence. *Am J Surg* 2014; **207**: 735-737; discussion 737-738 [PMID: 24791636 DOI: 10.1016/j.amjsurg.2014.01.003]
80 **Benson-Cooper S**, Davenport E, Bissett IP. Introduction of sacral neuromodulation for the treatment of faecal incontinence. *N Z Med J* 2013; **126**: 47-53 [PMID: 24217590]
81 **Schwandner O**. Sacral neuromodulation for fecal incontinence and "low anterior resection syndrome" following neoadjuvant therapy for rectal cancer. *Int J Colorectal Dis* 2013; **28**: 665-669 [PMID: 23559414 DOI: 10.1007/s00384-013-1687-8]
82 **Faucheron JL**, Chodez M, Boillot B. Neuromodulation for fecal and urinary incontinence: functional results in 57 consecutive patients from a single institution. *Dis Colon Rectum* 2012; **55**: 1278-1283 [PMID: 23135587 DOI: 10.1097/DCR.0b013e31826c7789]
83 **Simpson JA**, Peacock J, Maxwell-Armstrong C. Use of a gentamicin-impregnated collagen sheet (Collatamp(®)) following implantation of a sacral nerve stimulator for faecal incontinence. *Colorectal Dis* 2012; **14**: e200-e202 [PMID: 22093106 DOI: 10.1111/j.1463-1318.2011.02886.x]
84 **Pascual I**, Gómez Cde C, Ortega R, Toscano MJ, Marijuán JL, Espadas ML, Cebrián JM, Olmo DG, Montero JA. Sacral nerve stimulation for fecal incontinence. *Rev Esp Enferm Dig* 2011; **103**: 355-359 [PMID: 21770681]
85 **Uludağ O**, Melenhorst J, Koch SM, van Gemert WG, Dejong CH, Baeten CG. Sacral neuromodulation: long-term outcome and quality of life in patients with faecal incontinence. *Colorectal Dis* 2011; **13**: 1162-1166 [PMID: 20955512 DOI: 10.1111/j.1463-1318.2010.02447.x]
86 **Uludağ O**, Koch SM, van Gemert WG, Dejong CH, Baeten CG. Sacral neuromodulation in patients with fecal incontinence: a single-center study. *Dis Colon Rectum* 2004; **47**: 1350-1357 [PMID: 15484349]
87 **Ruiz Carmona MD**, Martín Arévalo J, Moro Valdezate D, Plá Martí V, Checa Ayet F. Sacral nerve stimulation for the treatment of severe faecal incontinence: results after 10 years experience. *Cir Esp* 2014; **92**: 329-335 [PMID: 24594318 DOI: 10.1016/j.ciresp.2013.04.014]
88 **Ganio E**, Ratto C, Masin A, Luc AR, Doglietto GB, Dodi G, Ripetti V, Arullani A, Frascio M, BertiRiboli E, Landolfi V, DelGenio A, Altomare DF, Memeo V, Bertapelle P, Carone R, Spinelli M, Zanollo A, Spreafico L, Giardiello G, de Seta F. Neuromodulation for fecal incontinence: outcome in 16 patients with definitive implant. The initial Italian Sacral Neurostimulation Group (GINS) experience. *Dis Colon Rectum* 2001; **44**: 965-970 [PMID: 11496076]

P- Reviewer: Carter D, Falletto E, Santoro GA **S- Editor**: Qi Y
L- Editor: A **E- Editor**: Wu HL

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i2.306

World J Gastrointest Pharmacol Ther 2016 May 6; 7(2): 306-319
ISSN 2150-5349 (online)

s

SYSTEMATIC REVIEWS

Nutritional and health benefits of semi-elemental diets: A comprehensive summary of the literature

Dominik D Alexander, Lauren C Bylsma, Laura Elkayam, Douglas L Nguyen

Dominik D Alexander, Lauren C Bylsma, EpidStat Institute, Ann Arbor, MI 48105, United States

Laura Elkayam, Exponent Inc., Health Sciences, Chicago, IL 60661, United States

Douglas L Nguyen, Department of Medicine, Irvine School of Medicine, University of California, Orange, CA 92868, United States

Author contributions: Alexander DD and Nguyen DL contributed to conception, design, analysis and interpretation of the data; Alexander DD, Bylsma LC, Elkayam L and Nguyen DL contributed to drafting of the article; Alexander DD, Bylsma LC and Nguyen DL contributed to critical revision of the article for important intellectual conten; all authors approved the final version of the article.

Conflict-of-interest statement: Dominik D Alexander, Lauren C Bylsma, Laura Elkayam, and Douglas L Nguyen have served as consultants to Nestle Health Science.

Data sharing statement: The data set and search algorithms are available from the corresponding author at douglaln@.uci.edu.

Correspondence to: Douglas L Nguyen, MD, Assistant Clinical Professor of Medicine, Department of Medicine, Irvine School of Medicine, University of California, 333 City Blvd. West, Suite 400, Orange, CA 92868, United States. douglaln@uci.edu
Telephone: +1-714-4566745
Fax: +1-714-4567753

Received: August 10, 2015
Peer-review started: August 10, 2015
First decision: September 11, 2015
Revised: December 19, 2015
Accepted: January 27, 2016
Article in press: January 29, 2016
Published online: May 6, 2016

Abstract

AIM: To critically review and summarize the literature on nutritional and health outcomes of semi-elemental formulations on various nutritionally vulnerable patient populations who are unable to achieve adequate nutrition from standard oral diets.

METHODS: We conducted a comprehensive literature search of Pubmed and Embase databases. We manually screened articles that examined nutritional and health outcomes (*e.g.*, growth, disease activity, gastrointestinal impairment, mortality, and economic impact) among various patient groups receiving semi-elemental diets. This review focused on full-text articles of randomized controlled clinical trials and other intervention studies, but pertinent abstracts and case studies were also included. Results pertaining primarily to tolerance, digestion, and absorption were summarized for each patient population in this systematic review.

RESULTS: Results pertaining primarily to tolerance, digestion, and absorption were summarized for each patient population. The efficacy of semi-elemental whey hydrolyzed protein (WHP) diet have been reported in various nutritionally high risk patient populations including - Crohn's disease, short bowel syndrome, acute and chronic pancreatitis, cerebral palsy, cystic fibrosis, cerebrovascular accidents, human immunodeficiency virus, critically ill, and geriatrics. Collectively, the evidence from the medical literature indicates that feeding with a semi-elemental diet performs as well or better than parenteral or amino acid based diets in terms of toler-

ance, digestion, and nutrient assimilation measures across various disease conditions.

CONCLUSION: Based on this comprehensive review of the literature, patient populations who have difficulty digesting or absorbing standard diets may be able to achieve improved health and nutritional outcomes through the use of semi-elemental WHP diets.

Key words: Semi-elemental diet; Malnutrition; 100% hydrolyzed whey protein; Nutrition; Malabsorption

Core tip: Patients with major chronic illnesses may not be able to achieve adequate macronutrient or micronutrient requirements through standard oral diet because of difficulties tolerating, digesting, or absorbing whole foods. In our systematic review, we summarized the literature on the numerous nutritional and health benefits of semi-elemental formulations across various nutritionally vulnerable patient populations. Overall, the literature demonstrates that semi-elemental diet performs consistently as well or better than parenteral or amino acid based diets in terms of tolerance, digestion, and nutrient assimilation measures across various disease conditions.

Alexander DD, Bylsma LC, Elkayam L, Nguyen DL. Nutritional and health benefits of semi-elemental diets: A comprehensive summary of the literature. *World J Gastrointest Pharmacol Ther* 2016; 7(2): 306-319 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i2/306.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i2.306

INTRODUCTION

Nutrition plays a significant role in achieving optimal health, but in certain high risk populations with significant systemic illnesses, achieving adequate nutrition with a traditional oral diet maybe difficult secondary to inability to tolerate, digest, and absorb whole foods. In these nutritionally-vulnerable populations, additional nutritional support *via* parenteral nutrition (PN) or enteral nutrition (EN) is necessary. When feasible, EN is clearly favored over PN because of fewer infectious complications, reduced healthcare costs, improved return of gut function, and reduced length of hospital stay[1].

Elemental diet formulas are used to provide liquid nutrients in a form that is easily and readily assimilated. Such diets provide protein in the form of individual amino acids and may provide a portion of the fat calories as medium chain triglycerides (MCT). These diets are typically reserved for individuals transitioning off of PN or with severe gastrointestinal pathology that prevents normal digestion, absorption or motility. Semi-elemental formulas, however, contain peptides of varying chain length, and fat primarily as MCT[2,3]. While semi-elemental diets are slightly more expensive then polymeric diets (formulas containing intact protein, complex carbohydrates, and long chain triglycerides), they are widely used because it is suggested that they are better absorbed and tolerated in patients with malabsorptive conditions and are more palatable than conventional elemental formulations[2].

A large volume of clinical studies have demonstrated significant health benefits with semi-elemental diets in all phases of the dietary process[4-6]. Indeed, such diet formulas have been shown to reduce the degree of regurgitation, gastric emptying times, and gagging while improving tolerance[7,8]. As a result, studies have suggested improved growth and development patterns, fewer gastrointestinal complications, improved visceral protein levels, and decreased rates of mortality. Studies of patients with Crohn's disease, pancreatitis, and human immnodeficiency virus (HIV) among other conditions have shown improved nutrition status and clinical outcomes from supplemental semi-elemental formulas[9-12]. The purpose of this review is to comprehensively summarize the scientific and clinical evidence of 100% whey-hydrolyzed protein (WHP) semi-elemental diets and nutritional and health outcomes across various nutritionally-vulnerable populations.

MATERIALS AND METHODS

We conducted a comprehensive literature search using the MEDLINE biomedical literature database, accessed from PubMed and the Embase database. The literature search and study identification process utilized in this review was different and more complex than typical literature reviews. This is a broad and dynamic topic area that covers many formula comparisons (*e.g.*, semi-elemental WHP diets *vs* amino acid based diets), nutritional and health outcomes (*e.g.*, growth, disease activity, gastrointestinal impairment, mortality, economic impact), and patient populations (*e.g.*, Crohn's disease, pancreatitis, stroke, critically ill patients). Given this diversity, we incorporated an all-inclusive approach to study designs such that we included all lines of human health evidence. Specifically, we focused on results from randomized controlled clinical trials and prospective intervention studies. In addition, results from relevant observational studies, case reports and series, and abstracts were included.

Relevant studies were identified through a comprehensive series of individual literature searches using a wide variety of keywords and search terms, such as - but not limited to - "semi-elemental diet", "semi-elemental formula", "peptide based diet", and "enteral nutrition". The literature search was limited to English-language publications with no prior date truncations. We supplemented our literature search by manually reviewing the reference lists of relevant articles to

identify any additional studies. Studies that combined treatment of semi-elemental WHP diets with other treatments, such as corticosteroids, or included patient populations less than one year of age were excluded from this review. Given the between study variation across the literature, we did not attempt to combine data quantitatively in a meta-analysis format.

RESULTS

Crohn's disease

Studies of 100% WHP and Crohn's disease are characterized in Table 1[5,10,11,13-25]. In a one year prospective study, six Crohn's disease patients (median age, 13.6 years) were treated with an isotonic, 100% WHP semi-elemental diet to evaluate growth parameters and disease activity measures[11]. Significant increases in height and weight velocity as well as significant improvement in clinical disease activity as measured by the Crohn's disease activity index, albumin, somatomedin C, and improvement in growth failure were observed in all patients. Similarly, in an open-label pilot study at two pediatric centers, Hussey *et al*[13] observed excellent tolerance and efficacy of a six-week tube feeding regimen of a 100% WHP semi-elemental diet among active Crohn's disease patients (mean age 11.4 ± 2.3 years). Throughout the study, the formula was well-tolerated, and subjects demonstrated significant gains in weight, height and achieved improved nutritional status. In addition, inflammation and disease activity was decreased with a resulting improved quality of life as measured by pediatric inflammatory bowel disease questionnaire[13].

Royall *et al*[14] conducted a randomized controlled trial among patients with active Crohn's disease to evaluate clinical and nutritional outcomes comparing a peptide-based 100% WHP diet with an amino acid-based elemental diet. After three weeks, clinical remission rates were similar in the amino acid group compared with the peptide group. The authors concluded that peptide-based diets are equally efficacious as amino acid-based diets in terms of high rates of clinical remission and is better tolerated orally[14]. In another randomized controlled study, Mansfield *et al*[15] compared the efficacy of a 100% WHP diet with an amino acid-based diet to achieve remission in 44 patients with active Crohn's disease. After four weeks of treatment, exactly similar clinical remission rates of 36% were achieved in both the 100% WHP diet group and the elemental diet group, but the 100% WHP diet was much better tolerated orally[15].

In another trial, 22 patients suffering from moderately active Crohn's disease were randomized to receive treatment with a 100% WHP semi-elemental diet as monotherapy (n = 10) or corticosteroids (n = 10)[17]. After two weeks of treatment, there were significant improvements in the Crohn's disease activity index, body mass index, and prealbumin level among patients treated with the 100% WHP diet and the results were statistically similar to corticosteroids across all measured parameters. However, the 100% WHP diet was well-tolerated with less side effects. Collectively, these studies demonstrate that in patients with moderate to severely active Crohn's disease, semi-elemental formula may be a viable alternative to corticosteroids at inducing clinical remission, improving lean body mass, reducing risk for growth failure, and enhancing the probability of maintaining clinical remission.

Short bowel syndrome and intestinal failure

Several studies have evaluated the role of semi-elemental feedings as primary nutritional therapy among patients who have undergone extensive gastrointestinal resection. In an initial case report, Rodriguez *et al*[26] reported a 62-year-old male who underwent extensive bowel resection with resulting short bowel syndrome. He was treated with a 100% WHP semi-elemental tube feeding regimen for 112 d and demonstrated an improved nutritional state with improvement in visceral protein levels without need for parenteral nutrition. In a retrospective study of 85 pediatrics patients with short bowel syndrome who underwent an intestinal transplantation (median age at transplant, 2.7 years), patients on semi-elemental product reached full feeds faster than patients who were started on an amino acid formula (3 mo *vs* 5 mo) because of better oral tolerance. In a crossover study among six children with short bowel syndrome, patients were treated with a semi-elemental diet followed by a free amino acid (FAA) diet[24]. The results showed that while fat excretion was identical in both formulas and stool electrolyte excretion was not significantly different, trace element analysis demonstrated that copper (P = 0.0002) and sulfur (P = 0.02) excretion was much greater for the FAA diet, suggesting a benefit from semi-elemental formulation with regards to micronutrient absorption. The authors concluded that treatment with peptide-based enteral formula after an intestinal transplant may provide more nutritional benefits among pediatric patients compared to patients who receive an amino-based formula, likely through more efficient micronutrient and nitrogen absorption.

Pancreatitis

Studies of 100% WHP and pancreatitis are characterized in Table 2[6,9,27-32]. In a prospective pilot study conducted by Tiengou *et al*[27], patients with severe acute pancreatitis who required nasojejunal nutrition were randomized to receive a 100% WHP semi-elemental diet (n = 15) or a standard polymeric formula (n = 15) for seven days. Both formulas were well tolerated in patients with acute pancreatitis, though the group on semi-elemental 100% WHP formula provided a more favorable clinical course because it was associated with less weight loss (P = 0.001), a significantly shorter hospital duration (P = 0.006), and a trend towards

Table 1 Selected studies of semi-elemental whey hydrolyzed protein diets and Crohn's disease and other gastrointestinal complications

Ref.	Study population	Design	Feeding mode (comparison)	No. patients (comparison)	Feeding duration	Relevant results[1]
Polk *et al*[11]	Children, tanner stage I-II, mean age 13.6	Prospective cross-over	Isotonic hydrolyzed whey formula administered *via* nocturnal nasogastric infusion (patients served as their controls based on observations at least a year before the study)	6 (6, served as own controls)	Intermittent diet program for 1 yr	Height increased 2.6 ± 0.8 to 9.3 ± 0.9 cm/yr ($P < 0.0001$) Weight increased 3.0 ± 1.2 to 6.63 ± 1.2 kg/yr ($P < 0.02$) Somatomedin C increase 0.7 ± 0.1 to 1.8 ± 0.3 UL ($P < 0.0001$) Albumin increase 3.4 ± 0.2 to 4.0 ± 0.1 g/dL ($P < 0.0003$) CDAI increase 64 ± 3.4 to 80.1 ± 2.2 ($P < 0.01$) (disease activity inversely correlates with numerical score)
Hussey *et al*[13]	Children with active CD, mean age 11.4	Prospective, NR, open-label pilot	Peptamen with Prebio *via* nasogastric tubes	10 - single group	6 wk	Height increased 143.8 ± 13 to 144.5 ± 13.1 cm ($P < 0.01$) Weight increases 31.9 ± 7.2 to 36.5 ± 8.1 kg ($P < 0.0001$) PCDAI decrease 40 ± 13 to 5 ± 6 ($P < 0.0001$) (lower score corresponds to lower disease activity) Albumin increase 3.1 ± 0.4 to 3.8 ± 0.4 g/dL ($P < 0.01$) PEDIBDQ increase 198 ± 31 to 243 ± 34 ($P < 0.01$) (higher score indicating better quality of life)
Royall *et al*[14]	Adults with moderate to severely active CD	RCT	Peptamen administered *via* a nasoduodenal feeding tube (Vivonex-TEN, amino acid based formula)	21 (19)	3 wk	Remission rates after 3 wk: 75% in the peptide group, 84% in the amino acid group Remission rates after 1 yr: 40% in the peptide group, 31% in the amino acid group Weight increased 2.0 ± 0.5 kg in the peptide group and 1.7 ± 0.3 kg in the amino acid group ($P < 0.0005$ within group differences after 3 wk) Total phospholipids (mg/mL) concentration increase in the peptide group (1.37 ± 0.1 to 1.71 ± 0.15) ($P < 0.025$) (no difference in amino acid group)
Mansfield *et al*[15]	Adults with active CD	RCT	Pepti-2000 LF Liquid received through nasogastric tube (Elemental 028)	22 (22)	4 wk	Remission rates after 4 wk: 36% in the Pepti-2000 group and 36% in the E028 group Mean percent ideal body weight: Pepti-2000 group increased from 92 ± 4 to 95 ± 4 and E028 group remained the same at 83 ± 5
Middleton *et al*[16]	Adults with active CD	RCT	Pepdite 2+ given orally or through nasogastric tube if necessary (Elemental 028/Elemental 028 + LCT/ Elemental 028 + MCT)	18 (17/22/19)	3 wk	Remission rates after 3 wk: 87% in Pepdite 2+ group, 92% in the E028 group, 55% in the E028 LCT group, and 92% in the E028 MCT group Mean CRP: Decreased significantly in E028 group and E028 MCT group, but non-significantly decreased in Pepdite 2+ group and E028 LCT group (values not provided)
Zoli *et al*[17]	Adults with moderately active CD	RCT	Peptamen received orally (0.5 mg/kg per day prednisolone)	10 (10)	2 wk	Peptide group: CD activity score (CDAS): 5.6 ± 0.8 to 2 ± 1.4 ($P < 0.01$) ESR: 21.4 ± 6 to 16.7 ± 6.7 ($P < 0.05$) Permeability index: 4.9 ± 5.3 to 2.1 ± 2 ($P < 0.01$) BMI: 18.5 ± 3 to 19.2 ± 3.1 ($P < 0.02$) Prealbumin: 22.2 ± 8 to 23.5 ± 7.8 ($P < 0.01$) Retinol binding protein: 3.7 ± 0.7 to 4 ± 0.8 ($P < 0.02$) *In vivo* cell-mediated immunity (Multitest IMC): 4.2 ± 2.1 to 5.9 ± 2.3 ($P < 0.01$) (in the corticosteroid group, there were significant findings for improvement of simple CD activity index and fat free mass)

Pereira *et al*[18]	Adults with mildly active CD and healthy laboratory staff	Follow-up study (secondary study)	Peptamen received orally (0.5 mg/kg per day prednisolone)	13 CD patients (17 healthy controls)	2 wk	No significant differences between groups in clinical response to treatment, markers of disease activity, or plasma phospholipid classes (data not reported)
Malchow *et al*[19]	Adults with active CD	RCT	Survimed given orally (12-48 mg/d 6-methyl prednisolone and 3 g/d sulfasalazine)	51 (44)	6 wk	Percent underweight after 3 wk: 15.1% in Survimed group and 13.4% in steroid group Crohn's disease activity index after 3 wk: 87.2 in Survimed group and 88.8 in steroid group Number of soft stools per week after 3 wk: 43.2 in Survimed group and 60.0 in steroid group
Lochs *et al*[20]	Adults with acute active CD	RCT	Peptisorb received through nasogastric tube (12-48 mg/d 6-methyl prednisolone and 3 g/d sulfasalazine)	55 (52)	6 wk	Remission rates after 6 wk: 52.7% in the Peptisorb group and 78.8% in the steroid group ($P < 0.01$) Body weight: increased in Peptisorb group from 55.6 ± 1.8 kg to 58.9 ± 1.6 kg, and increased in steroid group from 53.5 ± 1.3 kg to 56.8 ± 1.2 kg after treatment Number of soft stools per week: Decreased in Peptisorb group from 31.9 ± 4.3 to 9.7 ± 1.8 and decreased in steroid group from 37.1 ± 2.9 to 9.4 ± 1.5 after treatment
Lindor *et al*[21]	Adults with active CD	RCT	Vital HN received orally or through nasogastric tube if necessary (0.75 mg/kg per day prednisone)	9 (10)	1 mo	Decrease in Crohn's disease activity index of 50 points or more after 1 mo: 33% in vital HN group and 70% in steroid group
Sakurai *et al*[22]	Adults with active CD	RCT	Twinline received through nasogastric tube (Elental)	18 (18)	6 wk	Remission rates after 6 wk: 72% (47%-90%) in Twinline group and 67% (41%-87%) in the Elental group Crohn's disease activity index after 6 wk: 82 in Twinline group and 102 in Elental group
Khoshoo *et al*[5]	Children with gastrointestinal dysmotility ($n = 9$), CD ($n = 3$), mild short bowel syndrome ($n = 2$)	Randomized, double-blind, cross-over clinical study	Peptamen Junior and Peptamen Junior with fiber and prebiotics	14	1 formula for 2 wk, 5 d washout, 2nd formula for 2 wk	Flatulence/gas among 9 children with a neurological disorder: Significantly less for the fiber formula ($P < 0.05$) Frequency of bowel movements: No difference between groups ($P > 0.05$) Stool frequency in the CD group: Higher with the fiber formula but no change in consistency (data not reported)
Parekh *et al*[23]	Patients undergoing intestinal rehab with varying diseases [radiation enteritis ($n = 5$), ulcerative colitis ($n = 1$), bowel volvulus ($n = 1$), mesenteric ischemia ($n = 1$)]	Cross-over study	Semi-elemental/polymeric diet with a switch to an isocaloric, isotonic, semi-elemental formula with prebiotics	2 (6)	4.9 mo after an initiation of 60 d post-abdominal resection; second diet for a mean of 2.9 mo	Weight change: Mean loss of 5.1% in the semi-elemental/polymeric group, mean gain of 5.7% in the isocaloric, isotonic, semi-elemental formula with prebiotics group
Hamaoui *et al*[10]	Patients undergoing major abdominal surgery	Randomized prospective study	Reabilan HN, small peptide based formula *via* jejunostomy (equicaloric isonitrogenous total PN)	11 (8)	Primary analyses within 1 wk of enrollment	Mean daily stool output: 93.1 ± 68.5 g/d in the Reabilan group, 22.2 ± 35.3 g/d in the PN group ($P < 0.05$) No significant differences between groups for serum albumin, prealbumin, or plasma transferrin Average daily cost of supplies: $44.36 ± 8.50 for the Reabilan group, $102.10 ± 11.77 for the PN group ($P < 0.001$); non-nutrient supplies accounted for 13% of the cost in the Reabilan group *vs* 43% in the PN group
Kowalski *et al*[25]	Patients who received a post ITx	Retrospective case review	Peptide product (amino acid product)	34 (15)	Primary analyses within 6 mo	Time to full feedings post ITx from baseline to 1 to 2 yr: Peptide group z-scores: -2.71, -2.36, -2.32 (monotonic trend) Amino acid group z-scores: -2.46, -2.29, -2.35 Time to reaching full feeds (among those receiving rATG therapy): 3 mo in the peptide group, 5 mo in the amino acid group ($P > 0.05$)

Murray *et al*[24]	Children with short bowel syndrome	Randomized cross-over study	Peptamen (Vivonex TEN, high carbohydrate)	6	Two, 7 d periods	Mean ostomy output: 39 cc/kg per day in the Peptamen group, 49 cc/kg per day in the Vivonex TEN group Fat excretion: Identical in both groups (P = 0.9) Trace element analysis: Greater excretion of copper (P = 0.0002) and sulfur (P = 0.02) in the Vivonex TEN group

[1]Numerous results are reported within individual studies, please refer to the studies for a full summary of results. RCT: Randomized controlled clinical trial; NR: Non-randomized; CD: Crohn's disease; CDAI: CD activity index; PCDAI: Pediatric CD activity index; PEDIBDQ: Pediatric inflammatory bowel disease questionnaire; LCT: Long-chain triglycerides; MCT: Medium-chain triglycerides; ESR: Erythrocyte sedimentation rate; BMI: Body mass index; PN: Parenteral nutrition; ITx: Intestinal transplant.

Table 2 Selected studies of semi-elemental whey hydrolyzed protein diets and pancreatitis

Ref.	Study population	Design	Feeding mode (comparison)	No. patients (comparison)	Feeding duration	Relevant results[1]
Tiengou *et al*[27]	Consecutive patients with acute pancreatitis admitted to a gastroenterology and nutrition department	Randomized prospective pilot study	Peptamen (polymeric diet group, sondalis-Iso)	15 (15)	1 wk	Weight (kg): -1.3 ± 1.1 in the peptide group, -2.4 ± 0 in the polymeric group (P < 0.01) Total hospital stay (d): 23 ± 2 in the peptide group, 27 ± 1 in the polymeric group (P = 0.006) Infection: 1/15 in the peptide group, 3/15 in the polymeric group (NS)
Louie *et al*[9]	Consecutive patients with acute pancreatitis in an academic, multi-institutional, tertiary care system	RCT	Peptamen administered *via* nasojejunal feeding tubes (parenteral nutrition, intralipid administered *via* long-term vascular catheters)	10 (18)	Primary analyses within 1 wk of enrollment	C-reactive protein: Reduced 50% at a median of 5 d faster for the peptide group (6 d) *vs* the PN group (11 d) (P = 0.09) Serum cholecystokinin: 56 pmol/L to 55 pmol/L (P = 0.2) in the peptide group, 42 pmol/L to 32 pmol/L in the PN group (P = 0.5) Mortality: 0 deaths in the peptide group, 3 deaths in the PN group (attributable to complications of pancreatitis) Economic cost: Peptide group = $1375, PN group = $2608 ($P$ = 0.08); when 1 NJ tube used: Peptide group = $1086, PN group = $2608 ($P$ = 0.03)
McClave *et al*[28]	Patients with acute pancreatitis or an acute flare of chronic pancreatitis	RCT	Peptamen infused through a nasojejunal tube (total parenteral nutrition infused through a central or peripheral line)	16 (16) (30 patients over 32 admissions)	Primary analyses within 1 wk of enrollment	Length of ICU stay (d): 1.3 ± 0.9 in the peptide group, 2.8 ± 1.3 in the PN group (NS) Length of hospital stay (d): 9.7 ± 1.3 in the peptide group, 11.9 ± 2.6 in the PN group (NS) Economic cost: $761 ± 50.3 in the peptide group, $3294 ± 551.9 in the PN group (P < 0.005)
Kalfarentzos *et al*[32]	Patients with acute pancreatitis admitted to surgery unit	Randomized prospective trial	Reabilan HN administered *via* nasoenteric feeding tube (parenteral nutrition as all-in-one continuous subclavian polyurethane catheter infusion)	18 (20)	Mean: 34.8 d (mean: 32.8 d)	Septic complications: 27.8% in peptide group, 50% in PN group (P < 0.01) Any complications: 44.4% in peptide group, 75% in PN group (P < 0.05) Mean stay in ICU: 11 d in peptide group, 12 d in PN group (significance not provided)
Oláh *et al*[29]	Patients admitted to surgical ward with a diagnosis of acute pancreatitis	Two-phase controlled prospective trial	Survimed administered *via* NJ tube (parenteral nutrition as an all-in-one venous admixture)	41 (48)	5-9 d (5-16 d)	Necrosis: 29% in peptide group, 33% in PN group (NS) Septic complications: 12% in peptide group, 27% in PN group (P = 0.08) Surgery: 12% in peptide group, 23% in PN group (NS) Severe pancreatitis: 17% in peptide group, 21% in PN group (NS) Death: 4.9% in peptide group, 8.3% in PN group (NS)

Petrov *et al*[30]	Patients with severe acute pancreatitis	RCT	Peptamen administered through NJ tube (parenteral nutrition administered through central venous catheter)	35 (34)	Assessment on day of feed commencement, fourth and seventh days	Pancreatic infection: 20% in peptide group, 47% in PN group (P = 0.022) Noninfectious complications: 42.9% in peptide group, 17.6% in PN group (P = 0.036) Serum CRP concentration: 195 (164-216) mg/L on admission to 94 (56-117) mg/L on day 7 in peptide group, 210 (177-246) mg/L on admission to 93 (60-134) on day 7 in PN group (NS) Mortality: 6% in peptide group, 35% in PN group (P = 0.003)
Kumar *et al*[4]	Consecutive patients with severe acute pancreatitis	Randomized pilot study	Peptamen administered through enteral tubes in both groups; patients were randomly allocated to NG or NJ feeding	15 NG, 16 NJ	1 wk	Hospital stay (d): 29.93 ± 25.54 in NJ group, 24.06 ± 14.35 in NG group (P = 0.437) Mortality: 4/14 in NJ group, 5/16 in NG group Recurrence of pain: 1/14 in NJ group, 1/16 in NG group Serum albumin: No significant differences Anthropometric measurements: No significant differences in BMI, mid upper arm circumference, and triceps skin fold thickness)
Shea *et al*[6]	Patients with chronic pancreatitis; healthy control subjects	Follow-up study	Consumption of 3 cans of Peptamen (the same patients completed a daily pain assessment form for 2 wk prior to initiation of enteral formulation)	8, EN evaluated within this group; 6 healthy control subjects receiving EN also evaluated	2 wk baseline period, 10 wk formula period; healthy controls evaluated on a daily basis	Healthy controls: Postprandial plasma CCK: Mean basal CCK levels = 0.46 ± 0.29 pmol/L High fat solid meal = 10.75 ± 0.45 pmol/L Liquid meal full-length triglycerides and intact proteins = 7.9 ± 1.25 pmol/L Liquid meal Peptamen = 1.43 ± 0.72 pmol/L (P < 0.05 compared with other meals)
Freedman[31]	Healthy volunteers	Prospective cross-over	Peptamen, one can over 2 min following an overnight fast (1/4lb hamburger; one can of Ensure)	6 (6, served as own controls)	Assessment immediately after consumption	Chronic pancreatitis patients: Median improvement in pain scores from baseline = 68.5% (P = 0.011) 5 of 8 patients had statistically significant decreases in pain scores Mean basal CCK levels = 0.46 ± 0.29 pmol/L Hamburger = 10.75 ± 0.45 pmol/L Ensure = 7.9 ± 1.25 pmol/L Peptamen = 1.43 ± 0.72 pmol/L (P < 0.0001 compared with other meals)

[1]Numerous results are reported within individual studies, please refer to the studies for a full summary of results. RCT: Randomized controlled clinical trial; NS: Not significant; PN: Parenteral nutrition; EN: Enteral nutrition; NJ: Nasojejunal; NG: Nasogastric; CCK: Cholecystokinin.

reduced risk of infection[27].

In a randomized controlled trial, adult patients (age > 18 years) with acute pancreatitis were randomized to receive an EN regimen of a 100% WHP semi-elemental diet (n = 18) through a nasojejunal feeding tube *vs* parenteral nutrition (n = 10)[9,33]. Overall, the authors indicated that both treatment regimens provided adequate nutritional value and did not trigger significant changes in cholecystokinin (CCK) levels, but the authors noted that patients treated with EN semi-elemental regimen demonstrated a 50% reduction in C-reactive protein, fewer septic complications, a reduction in mortality, and a marked decline in total healthcare costs when compared to patients treated with the parenteral nutrition. These studies suggest that a semi-elemental formula confers more anti-inflammatory effects and promotes a more rapid resolution of the stress response associated with acute pancreatitis.

McClave *et al*[28] randomized patients with acute pancreatitis to receive an EN regimen of a 100% WHP semi-elemental diet through a nasojejunal tube (n = 16) *vs* a parenteral nutrition (n = 16) diet designed to provide a similar carbohydrate-to-fat ratio. While the EN regimen was shown to be as safe and effective as PN, EN was less expensive ($761 *vs* $3294). Based on these findings, it can be concluded that early EN with semi-elemental formulas may be used preferentially over parenteral nutrition among patients with acute pancreatitis due to reduced healthcare cost and improved clinical outcomes.

The effects of semi-elemental formulas appear to extend beyond patients with acute pancreatitis. Freedman[31], in a three day crossover study among six healthy volunteers treated with a 100% WHP semi-elemental diet compared to a standard polymeric formula, reported minimal stimulation of the exocrine pancreas in the semi-elemental group as assessed by CCK levels. This clinically important observation supports the role of a semi-elemental 100% WHP formula in patients with chronic pancreatitis. Shea *et al*[6], in a study among chronic pancreatitis patients, reported that treatment with a 100% WHP semi-elemental diet, compared with a high fat meal (hamburger) or a polymeric supplemental formula containing long-chain triglycerides and intact proteins, minimally increased plasma CCK levels, and decreased postprandial pain associated with chronic pancreatitis. Furthermore, the authors suggested that the reduction in CCK may minimize activation of pancreatic enzyme secretion during digestion, thereby minimizing stress on the pancreas during meals[6]. A case report was published of a 62-year-old male suffering from chronic pancreatitis treated with a 100% WHP semi-elemental diet. After 50 wk of follow-up, normalization of liver function tests, energy level, significant weight gain, as well as significant cost savings was observed[34].

Cerebral palsy (with gastrointestinal dysfunction)

Studies in children with cerebral palsy and gastrointestinal dysfunction have illustrated a benefit with the use of semi-elemental 100% WHP formula on gastric emptying rates. A randomized, double-blind crossover trial was conducted to evaluate the influence of protein composition on the rate of gastric emptying in 15 children (ages 4-15 years) with cerebral palsy using gastrostomy as their main route of nutrition[35]. Each child randomly received one of four isocaloric liquid test meals that contained a standard carbohydrate and fat base plus one of four protein modules: 100% casein, hydrolyzed whey, amino acids, or 40% casein/60% whey. Based on the ^{13}C octanoic acid breath test to assess gastric emptying, the fastest emptying meal was 40% casein/60% whey (median half-emptying time = 63.3 min), followed by amino acids (74.4 min), hydrolyzed whey (82.0 min), and 100% casein (153.9 min). Faster gastric emptying, in turn, was associated with a higher prevalence of adverse postprandial symptoms such as nausea, diarrhea, sweating, and retching. Based on these results, the authors concluded that in children with cerebral palsy the protein composition of a liquid meal influences the rate of gastric emptying, which might affect postprandial symptoms. Thus, the choice of an appropriate meal formula should achieve a balance between promoting slightly delayed gastric emptying times to reduce postprandial symptoms.

In another randomized, double-blind crossover trial that enrolled 13 enterally-fed children with severe cerebral palsy, subjects received a casein-based enteral formula for one week and either a 50% whey/50% casein whole-protein formula or a 100% WHP formula for another week[36]. The three formulas were similar with respect to calories, protein, carbohydrates, fat concentration, and osmolality. No significant differences in total gastroesophageal reflux episodes, reflux pH index, or daily stool frequency were observed between the casein and whey formulas or between the two whey formulas. As found in the study by Brun *et al*[37], median gastric half-emptying time as measured by the ^{13}C octanoic acid breath test was faster with a whey formula (33.9 min for both whey formulas combined) than the casein formula (56.6 min). In contrast to the Brun *et al*[37]'s findings, individual and combined symptoms of gagging, regurgitation, irritability, pain, and constipation did not differ significantly between the casein and whey formulas, nor was a significant correlation observed between gastric emptying time and gastrointestinal symptoms. Overall, their study showed that children who have severe cerebral palsy had significantly faster gastric emptying with WHP compared with casein based formulas. Five of 13 children with delayed gastric emptying in the casein formula group normalized with one of the whey-based formulas leading the authors to conclude that slight acceleration in gastric emptying with whey-based enteral formula relatively to the casein formula might be beneficial in some children with severe cerebral palsy and significantly delayed gastric emptying[36].

In a study evaluating nine consecutive outpatients with spastic quadriplegia, subjects (age range, 3-18 years) were fed a formula that contained casein (80%) and soy (20%) through a gastronomy tube. After gastric emptying was confirmed, each patient participated in a one month, double-blind randomized controlled trial that compared the effects of the casein-predominant formula with three different whey-based formulas: (1) whey dominant; (2) whey hydrolysate; and (3) whey hydrolysate with 70% of the fat as medium-chain triglycerides. The mean percentage of gastric radioactivity at 120 min was significantly lower in the whey-predominant formulas compared with the casein-predominant formula (whey predominant = 48% ± 19%, whey hydrolysate = 56% ± 23%, whey hydrolysate with medium-chain triglycerides = 59% ± 19%; casein-predominant formula = 69% ± 14%; $P < 0.001$), confirming that whey-predominant formulas provided faster gastric emptying times than casein-predominant formulas. Furthermore, there was also a reduction in vomiting episodes when the whey-based formula feedings were compared to the casein-based feedings[8].

Cystic fibrosis

Poor growth and limited weight gain is a significant concern among persons with cystic fibrosis. Erskine *et al*[38] conducted a study among 16 pediatric patients (age range: 4 to 20 years) with cystic fibrosis who

were pancreatic insufficient and treated with either a semi-elemental 100% WHP nutritional formula without enzyme replacement *vs* a polymeric formula with enzyme replacement for six days. An improvement in fat absorption was observed for both groups, and no appreciable differences between groups were reported in terms of fat percentage increase (polymeric formula = 82.3% ± 3.1% *vs* semi-elemental formula = 80.2% ± 2.9%). However, the patient burden could be potentially decreased with the semi-elemental formula due to the elimination of large enzyme pills that are often uncomfortable to swallow[39]. In another study among 10 cystic fibrosis patients who were undernourished, Shepherd *et al*[39] reported that a one year course of nutrient supplementation with a 100% WHP formula resulted in long-term improvement in energy and protein intake and maintenance of net anabolism. Based on these limited studies with short-term follow-up, it still remains unclear if semi-elemental formulas provide additional benefit long-term compared to conventional polymeric formulas in this population.

Stroke

Studies of semi-elemental formula and stroke are summarized in Table 3[40,41]. In a double-blind randomized trial of early enteral nutrition among 31 elderly patients (age ⩾ 65 years) who were admitted within 48 h after acute ischemic stroke, 16 patients were randomized to receive five days of nasogastric feeding with a hydrolyzed casein formula and 15 were randomized to receive a 100% WHP semi-elemental diet[40]. After five days of treatment, there was no difference in mortality, lactic acid, serum albumin, and C-reactive protein between the two groups, though the study may not have been adequately powered to detect these differences. However, serum levels of interleukin-6 (a cytokine that modulates inflammation) were significantly lower and levels of glutathione peroxidase (an enzyme that scavenges free radicals) were significantly higher in the semi-elemental group[41]. Similarly, in a retrospective study of 72 severe acute stroke patients admitted to a single hospital in Japan, 37 patients began enteral nutrition with a 100% WHP semi-elemental diet within 3 d of admission, while the other 35 patients received a standard polymeric formula[41]. Baseline patient and clinical characteristics were similar between the groups. However, the in-hospital mortality rate was significantly lower among patients who received 100% WHP diet (2.7%) than those who received the standard formula (22.9%)[41]. Collectively, these results implies that enteral formula containing 100% WHP may have beneficial short-term anti-inflammatory effects than polymeric formulas in hospitalized patients with acute ischemic stroke.

HIV

Maintaining adequate nutrition and bolstering nutritional parameters is of particular importance in persons living with HIV. Extreme weight loss, infections, diarrhea, and fat distribution changes may occur as a result of the disease itself or because of the potential myriad of medications used to treat complications associated with HIV. In a study conducted among 23 HIV patients with chronic diarrhea, a 100% WHP semi-elemental diet was well tolerated and demonstrated a significant decrease in the number of stools compared to consumption of a regular oral diet (3.6 stools/d *vs* 1.1 stools/d, respectively, $P < 0.01$), and a 53% reduction in fecal fat concentration (0.021 ± 0.025 g of stool, $P < 0.019$)[42,43]. Similarly, in 35 HIV patients suffering from malabsorption syndrome, treatment with a 100% WHP semi-elemental diet for eight weeks was effective in promoting weight gain and managing diarrhea among HIV patients[12]. In a randomized trial comparing total PN ($n = 12$) and an oral semi-elemental diet ($n = 13$) among HIV patients suffering from severe malabsorption, the PN group consumed more calories ($P < 0.05$) and gained more weight ($P = 0.057$) than patients treated with a semi-elemental diet; however, the semi-elemental group scored significantly better than the PN group on a physical functioning subscale of quality of life ($P < 0.01$)[44]. Collectively, these studies supports the concept that optimal use of enteral nutrition using 100% WHP formulas may improve functional status, reduce diarrhea, and reduce HIV-related cachexia.

Critically ill and intensive care unit

In a prospective trial conducted by Borlase *et al*[45], hospitalized critically-ill geriatric patients (Mean age, 66 years) with compromised gastrointestinal function were given tube feeding formula for either a primary or secondary gastrointestinal disorders. Patients were randomized to receive either a 100% WHP semi-elemental diet ($n = 8$) or FAA formula ($n = 8$), and tolerance was evaluated in enteral tube feeding. No significant differences between the groups were observed in terms of compliance with prescribed tube feeding, caloric goals, diarrhea, or abdominal discomfort, though a higher number of stools was reported in the FAA group. Additionally, Heimburger *et al*[46] conducted a trial among intensive care unit patients who were randomized to receive treatment with a 100% WHP diet ($n = 26$) or a standard polymeric diet ($n = 24$) for ten days. The authors reported increases in serum prealbumin and fibronectin in both groups but levels reached statistical significance in the 100% WHP diet group only, indicating improved nutrient assimilation in the semi-elemental group.

In a double-blind randomized trial pilot study, intensive care unit (ICU) patients randomized to enteral treatment with a 100% WHP semi-elemental diet ($n = 5$) demonstrated less gastrointestinal bleeding than those receiving a standard polymeric diet ($n = 5$), suggesting that a semi-elemental diet may be sufficient to reduce ICU-stressed related peptic ulcer disease without need for acid-blocking agents[47].

Table 3 Selected studies of semi-elemental whey hydrolyzed protein diets and stroke

Ref.	Study population	Design	Feeding mode (comparison)	No. patients (comparison)	Feeding duration	Relevant results[1]
de Aguilar-Nascimento *et al*[40]	Elderly patients with acute ischemic stroke	RCT	NG feeding with Peptamen (Hiper-diet Energy Plus, standard formula containing hydrolyzed casein)	10 (15)	5 d	Mortality: 3/10 in Peptamen group, 4/15 in the casein group (P = 1.0) ICU length of stay: 16 ± 8 d in the Peptamen group, 16 ± 5 d in the casein group (P = 0.97) IL-6 (pg/dL): Peptamen group: 62.7 ± 56.2 to 20.6 ± 10.3 Casein group: 64.3 ± 40.3 to 42.0 ± 26.7 (P = 0.03 between group difference) Glutathione (U/G Hb): Peptamen group: 32.2 ± 2.1 to 39.9 ± 4.8 Casein group: 30.0 ± 5.0 to 26.2 ± 6.7 (P = 0.03 between group difference) Glucose (mg/dL) Peptamen group: 132 ± 19 to 139 ± 18 Casein group: 148 ± 20 to 214 ± 43 (P = 0.17 between group difference)
Miyazaki *et al*[41]	Severe acute stroke patients requiring tube feeding	Retrospective follow-up study	Peptamen through an enteral feeding tube (mein, normal protein enteral formula)	37 (35)	1 wk	In hospital mortality: 2.7% in the Peptamen group, 22.9% in the Mein group (P < 0.05) Blood urea nitrogen (BUN, median): 35 mg/dL in the Peptamen group, 23 mg/dL in the Mein group (P < 0.05)

[1]Numerous results are reported within individual studies, please refer to the studies for a full summary of results. RCT: Randomized controlled clinical trial; NG: Nasogastric; ICU: Intensive care unit.

Geriatric patients

Protein-calorie malnutrition is a common problem among nursing home residents and the aging population. Thus, Feller *et al*[48] investigated the nutritional efficacy and tolerance of two different formulas (a 100% WHP semi-elemental diet and an FAA formula) among chronically tube-fed elderly patients. Patients were started on either formula for four weeks and then crossed over to the other study formula. Overall, the 100% WHP diet was superior to the elemental diet in terms of maintaining total protein and albumin levels of the tube-feeding dependent geriatric patients. However, studies are lacking on evaluating the role of 100% WHP in the general geriatrics population with regards to maintenance of muscle mass, improvement of nutritional and functional status.

DISCUSSION

Patients with a heterogeneous array of acute, chronic, and genetic conditions may suffer from feeding complications and as a result, may not be able to achieve or maintain adequate or appropriate energy, macronutrient, and micronutrient requirements with a standard oral diet because of difficulties tolerating, digesting, or absorbing whole foods. Fortunately, accumulating clinical evidence indicates that patients with feeding difficulties may be able to achieve improved health and nutritional outcomes through the use of 100% WHP semi-elemental diets. These types of diets, which are composed of peptides, essential fatty acids, medium chain triglycerides, vitamins, and minerals, are designed to be easily assimilated and well-tolerated. Thus, our objective was to summarize the studies that evaluated semi-elemental WHP diets and nutritional and health outcomes among all patient populations in the scientific literature.

Overall, and as summarized above, the totality of available scientific and clinical evidence indicates that semi-elemental WHP diets are well-tolerated, digested, and absorbed among various patient groups, including those with Crohn's disease, acute and chronic pancreatitis, stroke, HIV, and critically ill. Specifically, the results across the studies show that semi-elemental WHP diets perform as well or better than comparison diets (*e.g.*, amino acid based formulas, parenteral nutrition, regular oral diets) in terms of weight gain and growth, reduction of the systemic inflammatory response, efficiency of nutrient assimilation, lower mortality rates, and lower healthcare costs. Importantly, advantages of a semi-elemental WHP diet are observed across a multitude of patient populations with various health conditions and across all age ranges. The robustness of findings across all patient groups illustrates the efficacy and effectiveness of such dietary regimens.

There are several lines of mechanistic evidence supporting a beneficial role of peptide-based hydrolyzed whey proteins for feeding and nutritional support. In a review of peptide-based diets compared with intact protein or free amino acid formulations among patients with impaired digestion or absorption, DeLegge[35] cited several potential advantages, including improved nitrogen absorption and utilization, maintenance of gut integrity, reduction of bacterial translocation, improved visceral protein synthesis, and enhanced immune support. Peptide-based formulas may facilitate an

Table 4 Suggestions for future semi-elemental whey hydrolyzed protein diet studies

Level of study process	Suggestions for future semi-elemental WHP diet studies
Study development and initiation	Clearly defined study population with reported response rates and loss-to-follow-up data
Study development and initiation	Identification and inclusion of a study population with sufficient statistical power to determine a difference between the formulas under study. The estimated number of subjects based on power calculations should be included in the methods section
Study development and initiation	Stated goals and objectives of the analytical research. Given the multitude of possible outcomes, researchers should strive to clearly state the objective endpoints of the analysis
Analytical comparisons	Clearly define the dietary formulas and product names under study to facilitate a more complete and accurate summary of the findings across studies
Results	Present results with levels of variance such that future systematic reviews and quantitative assessments can combine data across studies
	Present results by intake level and duration of follow-up such that future assessments can evaluate quantitatively these important factors when weighing the evidence
Discussion	Identify important study design, analytical, or other research limitations and challenges so subsequent researchers can endeavor to address these challenges

WHP: Whey hydrolyzed protein.

optimum digestive process ultimately leading to an absorptive advantage compared with free amino acid and intact protein based formulas. Indeed, several studies have suggested that the majority of nitrogen from protein is absorbed as peptides and that amino acids may be absorbed more efficiently in the form of peptides than free amino acids[49-51]. Amino acids infused into the intestine in peptide form are more readily absorbed than free amino acids, secondary to the PepT1 transporter system. The PepT1 transporter is located in the microvillus membrane and has a well-established role as a transporter for di- and tri-peptides. Dietary intake and amino acid composition of the dietary protein increases the expression of PepT1[52]. Primarily, expression of PepT1 is prevalent in the small intestine, but limited in the colon. In patients with short bowel syndrome, Crohn's disease and ulcerative colitis, colonic PepT1 is increased, thereby increasing protein absorption[53]. Combined characteristics of efficient uptake of di- and tri-peptides and low osmolality may be advantageous for enteral nutrition solutions and have a significant role in nutritional management of various disease states. Furthermore, in terms of tolerance, it has been suggested that peptide-based protein may have improved nitrogen retention compared to free amino acids or intact protein, possibly resulting from the peptide's ability to enhance intestinal microcirculation, thereby improving absorption[35,54].

In addition, several health benefits pertaining to the functional and therapeutic aspects of whey protein have been cited extensively in the literature. A growing body of studies recognizes that whey protein has a broad range of possible beneficial impacts on bone health, muscle growth, immune support, infection, wound healing, and aging[55-58]. For example, in a recently published meta-analysis of randomized clinical trials of whey protein and body composition, body weight (-4.20 kg, 95%CI: -7.67, -0.73) and body fat (-3.74 kg, 95%CI: -5.98, -1.50) were significantly decreased from baseline when whey protein was used as a meal replacement[58]. In addition, a statistically significant increase in lean body mass (2.24 kg, 95%CI: 0.66, 3.81) was observed among studies that included a resistance exercise component along with whey protein.

Results from several experimental animal studies of semi-elemental WHP diets have augmented the evidence base of human studies. Tappenden *et al*[59] evaluated the effects of a semi-elemental diet among piglets that underwent gastrostomy placement and banding of the superior mesenteric artery to restrict blood flow to baseline fasting levels, and found that a whey peptide-based diet stimulated the structure and function of the piglets' compromised intestines, and reduced gastrointestinal inflammation. In a study conducted by Zonta *et al*[60], female piglets that underwent bowel transplantation were divided into four study groups: Standard swine chow ad libitum in the postoperative period (group 1, $n = 5$); polymeric enteral solution (group 2, $n = 5$); and a 100% WHP semi-elemental formula (group 3, $n = 5$). None of the transplanted pigs in the semi-elemental formula died before the end of the study and it was suggested that this nutritional regimen may provide faster recovery for the mucosal barrier as well as limit the hypercatabolic state. In a comprehensive animal study evaluating sepsis and septic shock pertaining to critically ill states, rats were allocated to: (1) a soy-based diet high in cysteine and crude fiber (CHOW) and devoid of EPA-DHA; (2) a whey-peptide based liquid diet high in cysteine, EPA-DHA, and FOS (CYSPUFA); or (3) a casein-based liquid isonitrogenous diet low in cysteine and devoid of EPA-DHA-FOS (CASN)[61]. Rats were fed these diets for six days following injection with lipopolysaccharide to mimic sepsis and septic shock. The CYSPUFA group lost significantly less weight (*vs* CASN or CHOW, $P < 0.05$) and had improved levels of liver enzyme concentration, suggesting that a diet rich in CYSPUFA protects against induced systemic inflammatory responses. Previously, the authors reported that intrahepatic levels of cysteine, adenosine and

GSSG were significantly improved in rats on CYSPUFA compared with CASN ($P < 0.05$)[62]. Moinard *et al*[63] evaluated the role of a 100% WHP semi-elemental formula compared with the standard polymeric formula among rats with traumatic brain injury. The authors reported an improvement in glutamine concentration among rats receiving a 100% WHP formula, suggesting that the use of a semi-elemental formula may limit response to injury after suffering a traumatic brain injury[63].

Research gaps and advancing the science

Despite the abundance of evidence illustrating the many benefits of a semi-elemental WHP diet, several factors should be considered when interpreting the totality of evidence. This review is a comprehensive summary of findings across various patient populations, and serves as a foundation for the evidence across the total body of scientific and clinical literature. However, each patient population discussed in this summary may warrant its own specific review manuscript. To that end, future papers, whether based on data analysis or reviews of the literature, should strive to harmonize the methodological approach to critically examine the evidence.

The overall quality of evidence is strong as most studies were randomized controlled clinical trials or intervention studies conducted among various study populations. However, interpretation of findings is somewhat limited by small sample sizes and the inability for some studies to achieve statistically significant differences (if apparent) because of low statistical power. In fact, many studies evaluated analytical study populations of fewer than 10 patients. Thus, the generalizability of study findings should be made in light of possible issues with selection bias. To be sure, it is difficult to identify viable study populations given the nature of the conditions under study, such as acute illness and/or complex genetic or chronic diseases.

Future studies should attempt to standardize the study design, methodological, and analytical procedures when conducting evaluations of semi-elemental WHP diets (Table 4). Currently, a considerable amount of evidence comes from published abstracts, conference proceedings, and case studies. More analytical, peer-reviewed research studies are needed to foster more comprehensive quantitative analyses, such as meta- or pooled analyses. Thus, the consistency in the design and methodological approach with uniform results reporting is of fundamental importance to systematically summarize and interpret the evidence.

To our knowledge, this is the largest and most comprehensive summary of semi-elemental WHP diet studies conducted. Our goal was to summarize the evidence for all types of comparisons among all types of study populations. Based on this review and in terms of digestion, absorption, and tolerance outcomes, the totality of evidence from the scientific and medical literature indicates that feeding with a semi-elemental WHP diet performs as well as or better than parenteral or amino acid based diets. In addition, other beneficial outcomes, such as improved mortality and economic advantages, have been reported. In conclusion, patient populations that have difficulty digesting or absorbing standard diets, or those who are unable to attain adequate nutrition, may be able to achieve improved health outcomes and nutritional goals through the use of semi-elemental WHP diets.

COMMENTS

Background

Nutrition plays a significant role in achieving optimal health, but in certain high risk populations with significant systemic illnesses, achieving adequate nutrition with a traditional oral diet maybe difficult secondary to inability to tolerate, digest, and absorb whole foods. Numerous clinical studies have demonstrated significant health benefits with semi-elemental diets in all phases of the dietary process. These studies have suggested improved growth and development patterns, fewer gastrointestinal complications, improved visceral protein levels, and decreased rates of mortality across multiple disease states.

Research frontiers

Semi-elemental formulas have been shown to reduce the degree of regurgitation, gastric emptying times, and gagging while improving tolerance. As a result, studies have suggested improved growth and development patterns, fewer gastrointestinal complications, improved visceral protein levels, and decreased rates of mortality.

Innovations and breakthroughs

This paper is one of the first in the literature to comprehensively summarize the role of semi-elemental formulas across various patient populations. This paper serves as a foundation for the evidence across the total body of scientific and clinical literature for the routine use of semi-elemental formulas in various nutritionally vulnerable populations.

Applications

This paper serves as a foundation for the evidence across the total body of scientific and clinical literature for the routine use of semi-elemental formulas in various nutritionally vulnerable patient populations such as Crohn's disease, chronic pancreatitis, human immunodeficiency virus, cerebral palsy, and acute cerebral vascular accidents.

Terminology

Semi-elemental formulas, however, contain peptides of varying chain length and primarily medium chain fatty acids. While semi-elemental diets are slightly more expensive then polymeric diets (formulas containing intact protein, complex carbohydrates, and long chain triglycerides), they are widely used because it is suggested that they are better absorbed and tolerated in patients with malabsorptive conditions and are more palatable than conventional elemental formulations.

Peer-review

This is an excellent article. The information is extremely helpful for the practice of medicine.

REFERENCES

1 **Seres DS**, Valcarcel M, Guillaume A. Advantages of enteral nutrition over parenteral nutrition. *Therap Adv Gastroenterol* 2013; **6**: 157-167 [PMID: 23503324 DOI: 10.1177/1756283X12467564]

2 **Makola D**. Elemental and Semi-Elemental Formulas: Are They Superior to Polymeric Formulas? *Practical Gastroenterology* 2005;

34: 59-72 [DOI 10.1002/14651858]
3 **Chen QP**. Enteral nutrition and acute pancreatitis. *World J Gastroenterol* 2001; **7**: 185-192 [PMID: 11819758 DOI: 10.3748/wjg.v7.i2.185]
4 **Kumar A**, Singh N, Prakash S, Saraya A, Joshi YK. Early enteral nutrition in severe acute pancreatitis: a prospective randomized controlled trial comparing nasojejunal and nasogastric routes. *J Clin Gastroenterol* 2006; **40**: 431-434 [PMID: 16721226 DOI: 10.1097/00004836-200605000-00013]
5 **Khoshoo V**, Sun SS, Storm H. Tolerance of an enteral formula with insoluble and prebiotic fiber in children with compromised gastrointestinal function. *J Am Diet Assoc* 2010; **110**: 1728-1733 [PMID: 21034888 DOI: 10.1016/j.jada.2010.08.011]
6 **Shea JC**, Bishop MD, Parker EM, Gelrud A, Freedman SD. An enteral therapy containing medium-chain triglycerides and hydrolyzed peptides reduces postprandial pain associated with chronic pancreatitis. *Pancreatology* 2003; **3**: 36-40 [PMID: 12649562 DOI: 10.1159/000069144]
7 **Billeaud C**, Guillet J, Sandler B. Gastric emptying in infants with or without gastro-oesophageal reflux according to the type of milk. *Eur J Clin Nutr* 1990; **44**: 577-583 [PMID: 2209513]
8 **Fried MD**, Khoshoo V, Secker DJ, Gilday DL, Ash JM, Pencharz PB. Decrease in gastric emptying time and episodes of regurgitation in children with spastic quadriplegia fed a whey-based formula. *J Pediatr* 1992; **120**: 569-572 [PMID: 1552396 DOI: 10.1016/S0022-3476(10)80003-4]
9 **Louie BE**, Noseworthy T, Hailey D, Gramlich LM, Jacobs P, Warnock GL. 2004 MacLean-Mueller prize enteral or parenteral nutrition for severe pancreatitis: a randomized controlled trial and health technology assessment. *Can J Surg* 2005; **48**: 298-306 [PMID: 16149365]
10 **Hamaoui E**, Lefkowitz R, Olender L, Krasnopolsky-Levine E, Favale M, Webb H, Hoover EL. Enteral nutrition in the early postoperative period: a new semi-elemental formula versus total parenteral nutrition. *JPEN J Parenter Enteral Nutr* 1990; **14**: 501-507 [PMID: 2122024 DOI: 10.1177/014860719001400550]
11 **Polk DB**, Hattner JA, Kerner JA. Improved growth and disease activity after intermittent administration of a defined formula diet in children with Crohn's disease. *JPEN J Parenter Enteral Nutr* 1992; **16**: 499-504 [PMID: 1494204 DOI: 10.1177/014860719201600649]
12 **Voss T**, Rowe B, Graf L, Keyes C, Beal J. Management of HIV-related weight loss and diarrhea with an enteral formula containing whey peptides and medium-chain triglycerides. In: Abstract book of the VII International Conference on AIDS. Italy: Florence, 1991
13 **Hussey TA**, Issenman RM, Persad R, Otley AR, Christensen BA. Nutrition therapy in pediatric Crohn's disease patients improves nutritional status and decreases inflammation. *J Pediatr Gastr Nutr* 2003; **37**: 341-342
14 **Royall D**, Jeejeebhoy KN, Baker JP, Allard JP, Habal FM, Cunnane SC, Greenberg GR. Comparison of amino acid v peptide based enteral diets in active Crohn's disease: clinical and nutritional outcome. *Gut* 1994; **35**: 783-787 [PMID: 8020806 DOI: 10.1136/gut.35.6.783]
15 **Mansfield JC**, Giaffer MH, Holdsworth CD. Controlled trial of oligopeptide versus amino acid diet in treatment of active Crohn's disease. *Gut* 1995; **36**: 60-66 [PMID: 7890238 DOI: 10.1136/gut.36.1.60]
16 **Middleton SJ**, Rucker JT, Kirby GA, Riordan AM, Hunter JO. Long-chain triglycerides reduce the efficacy of enteral feeds in patients with active Crohn's disease. *Clin Nutr* 1995; **14**: 229-236 [PMID: 16843936 DOI: 10.1016/S0261-5614(95)80004-2]
17 **Zoli G**, Carè M, Parazza M, Spanò C, Biagi PL, Bernardi M, Gasbarrini G. A randomized controlled study comparing elemental diet and steroid treatment in Crohn's disease. *Aliment Pharmacol Ther* 1997; **11**: 735-740 [PMID: 9305483 DOI: 10.1046/j.1365-2036.1997.t01-1-00192.x]
18 **Pereira SP**, Cassell TB, Engelman JL, Sladen GE, Murphy GM, Dowling RH. Plasma arachidonic acid-rich phospholipids in Crohn's disease: response to treatment. *Clin Sci* (Lond) 1996; **91**: 509-512 [PMID: 8983878 DOI: 10.1042/cs0910509]
19 **Malchow H**, Steinhardt HJ, Lorenz-Meyer H, Strohm WD, Rasmussen S, Sommer H, Jarnum S, Brandes JW, Leonhardt H, Ewe K. Feasibility and effectiveness of a defined-formula diet regimen in treating active Crohn's disease. European Cooperative Crohn's Disease Study III. *Scand J Gastroenterol* 1990; **25**: 235-244 [PMID: 1969678]
20 **Lochs H**, Steinhardt HJ, Klaus-Wentz B, Zeitz M, Vogelsang H, Sommer H, Fleig WE, Bauer P, Schirrmeister J, Malchow H. Comparison of enteral nutrition and drug treatment in active Crohn's disease. Results of the European Cooperative Crohn's Disease Study. IV. *Gastroenterology* 1991; **101**: 881-888 [PMID: 1679736]
21 **Lindor KD**, Fleming CR, Burnes JU, Nelson JK, Ilstrup DM. A randomized prospective trial comparing a defined formula diet, corticosteroids, and a defined formula diet plus corticosteroids in active Crohn's disease. *Mayo Clin Proc* 1992; **67**: 328-333 [PMID: 1548947 DOI: 10.1016/S0025-6196(12)61547-X]
22 **Sakurai T**, Matsui T, Yao T, Takagi Y, Hirai F, Aoyagi K, Okada M. Short-term efficacy of enteral nutrition in the treatment of active Crohn's disease: a randomized, controlled trial comparing nutrient formulas. *JPEN J Parenter Enteral Nutr* 2002; **26**: 98-103 [PMID: 11871742 DOI: 10.1177/014860710202600298]
23 **Parekh NR**, Su LC, Steiger E, Seidner DL. A semi-elemental enteral formula with prebiotics is associated with weight gain in intestinal failure patients undergoing intestinal rehabilitation. *AM J Gastroenterol* 2006; **101** (Supp 2): S313-S314
24 **Murray ND**, Vanderhoof JA. Evaluation of fat containing elemental diet for treatment of shortbowel syndrome in children. *J Parenter Enteral Nutr* 1988; **12** (Supp 3): 21S
25 **Kowalski LA**, Nucci A, Seward L. Nutritional autonomy after pediatric intestinal transplantation. NP01: Abstract 242. Clinical Nutrition Week; 2006 Feb 12-15; Dallas, Texas
26 **Rodriguez DJ**, Clevenger FW. Successful enteral refeeding after massive small bowel resection. *West J Med* 1993; **159**: 192-194 [PMID: 8212691]
27 **Tiengou LE**, Gloro R, Pouzoulet J, Bouhier K, Read MH, Arnaud-Battandier F, Plaze JM, Blaizot X, Dao T, Piquet MA. Semi-elemental formula or polymeric formula: is there a better choice for enteral nutrition in acute pancreatitis? Randomized comparative study. *JPEN J Parenter Enteral Nutr* 2006; **30**: 1-5 [PMID: 16387891 DOI: 10.1177/014860710603000101]
28 **McClave SA**, Greene LM, Snider HL, Makk LJ, Cheadle WG, Owens NA, Dukes LG, Goldsmith LJ. Comparison of the safety of early enteral vs parenteral nutrition in mild acute pancreatitis. *JPEN J Parenter Enteral Nutr* 1997; **21**: 14-20 [PMID: 9002079 DOI: 10.1177/014860719702100114]
29 **Oláh A**, Pardavi G, Belágyi T, Nagy A, Issekutz A, Mohamed GE. Early nasojejunal feeding in acute pancreatitis is associated with a lower complication rate. *Nutrition* 2002; **18**: 259-262 [PMID: 11882400 DOI: 10.1016/S0899-9007(01)00755-9]
30 **Petrov MS**, Kukosh MV, Emelyanov NV. A randomized controlled trial of enteral versus parenteral feeding in patients with predicted severe acute pancreatitis shows a significant reduction in mortality and in infected pancreatic complications with total enteral nutrition. *Dig Surg* 2006; **23**: 336-344; discussion 344-345 [PMID: 17164546 DOI: 10.1159/000097949]
31 **Freedman SD**. Peptamen: A novel therapy in patients with chronic pancreatitis. *Gastroenterology* 1997; **112**: A267
32 **Kalfarentzos F**, Kehagias J, Mead N, Kokkinis K, Gogos CA. Enteral nutrition is superior to parenteral nutrition in severe acute pancreatitis: results of a randomized prospective trial. *Br J Surg* 1997; **84**: 1665-1669 [PMID: 9448611 DOI: 10.1002/bjs.1800841207]
33 **Louie B**, Noseworthy T, Hailey D, Gramlich L, Jacobs P, Warnock G. A randomized controlled trial and cost effectiveness analysis of enteral vs parenteral nutrition in severe pancreatitis. *Gastroenterology* 2002; **122** (Supp 4): A-369
34 **Meyer J**. Home enteral nutrition in chronic pancreatitis: A case report. *Support Line* 2002; **22**: 7-10
35 **DeLegge MH**. Enteral feeding. *Curr Opin Gastroenterol* 2008; **24**: 184-189 [PMID: 18301269 DOI: 10.1097/MOG.0b013e3282f4dbab]
36 **Savage K**, Kritas S, Schwarzer A, Davidson G, Omari T. Whey-

vs casein-based enteral formula and gastrointestinal function in children with cerebral palsy. *JPEN J Parenter Enteral Nutr* 2012; **36**: 118S-123S [PMID: 22237871 DOI: 10.1177/0148607111428139]
37 **Brun AC**, Størdal K, Johannesdottir GB, Bentsen BS, Medhus AW. The effect of protein composition in liquid meals on gastric emptying rate in children with cerebral palsy. *Clin Nutr* 2012; **31**: 108-112 [PMID: 21835514 DOI: 10.101`6/j.clnu.2011.07.009]
38 **Erskine JM**, Lingard CD, Sontag MK, Accurso FJ. Enteral nutrition for patients with cystic fibrosis: comparison of a semi-elemental and nonelemental formula. *J Pediatr* 1998; **132**: 265-269 [PMID: 9506639 DOI: 10.1016/S0022-3476(98)70443-3]
39 **Shepherd RW**, Holt TL, Thomas BJ, Kay L, Isles A, Francis PJ, Ward LC. Nutritional rehabilitation in cystic fibrosis: controlled studies of effects on nutritional growth retardation, body protein turnover, and course of pulmonary disease. *J Pediatr* 1986; **109**: 788-794 [PMID: 3095520 DOI: 10.1016/S0022-3476(86)80695-3]
40 **de Aguilar-Nascimento JE**, Prado Silveira BR, Dock-Nascimento DB. Early enteral nutrition with whey protein or casein in elderly patients with acute ischemic stroke: a double-blind randomized trial. *Nutrition* 2011; **27**: 440-444 [PMID: 21167685 DOI: 10./1016/j.nut.2010.02.013]
41 **Miyazaki Y**, Mori T, Mizokami K, Iwata T, Nakazaki M. Comparison of Peptamen AF® (High-Protein Enteral Formula) With Mein® (Normal-Protein Enteral Formula) in sever acute stroke patients requiring tube feeding. *Clin Nutr Supp* 2012; **7**: 211
42 **Salomon SB**, Jung J, Voss T, Suguitan A, Rowe WB, Madsen DC. An elemental diet containing medium-chain triglycerides and enzymatically hydrolyzed protein can improve gastrointestinal tolerance in people infected with HIV. *J Am Diet Assoc* 1998; **98**: 460-462 [PMID: 9550172 DOI: 10.1016/S0002-8223(98)00104-7]
43 **Glauser M**, Jung J, Voss T, Rowe B. Fat Tolerance in HIV-infected subjects ingesting a semi-elemental diet containing medium-chain triglycerides (MCT) and whey peptides. In: Abstract book of VII International Conference on AIDS. Italy, 1991
44 **Kotler DP**, Fogleman L, Tierney AR. Comparison of total parenteral nutrition and an oral, semielemental diet on body composition, physical function, and nutrition-related costs in patients with malabsorption due to acquired immunodeficiency syndrome. *JPEN J Parenter Enteral Nutr* 1998; **22**: 120-126 [PMID: 9586788 DOI: 10.1177/014860719802003120]
45 **Borlase BC**, Bell SJ, Lewis EJ, Swails W, Bistrian BR, Forse RA, Blackburn GL. Tolerance to enteral tube feeding diets in hypoalbuminemic critically ill, geriatric patients. *Surg Gynecol Obstet* 1992; **174**: 181-188 [PMID: 1542832]
46 **Heimburger DC**, Geels VJ, Bilbrey J, Redden DT, Keeney C. Effects of small-peptide and whole-protein enteral feedings on serum proteins and diarrhea in critically ill patients: a randomized trial. *JPEN J Parenter Enteral Nutr* 1997; **21**: 162-167 [PMID: 9168369 DOI: 10.1177/014860719702100316 2]
47 **McClave SA**, Spain DA, Lukan JK, Lowen CC, Dryden GW. Can enteral nutrition provide adequate stress prophylaxis on patients on mechanical ventilation? *Gastroenterology* 2004; **126** (Supp 2): A-647
48 **Feller A**, Rudman D, Caindec N, Story K, Herring N. Clinical trial of peptamen liquid elemental diet in geriatric patients. In: Kinney JM, Borum PR, editors. Perspectives in clinical nutrition. Baltimore: Urban and Schwarzenberg, 1989: 339-350
49 Branched chain amino and keto acids in health and disease. New York: Karger, 1984
50 **Silk DB**, Grimble GK, Rees RG. Protein digestion and amino acid and peptide absorption. *Proc Nutr Soc* 1985; **44**: 63-72 [PMID: 3885229 DOI: 10.1079/PNS19850011]
51 **Webb KE**. Amino acid and peptide absorption from the gastro-intestinal tract. *Fed Proc* 1986; **45**: 2268-2271 [PMID: 3720965]
52 **Freeman HJ**. Clinical relevance of intestinal peptide uptake. *World J Gastrointest Pharmacol Ther* 2015; **6**: 22-27 [PMID: 25949847 DOI: 10.4292/wjgpt.v6.i2.22]
53 **Daniel H**. Molecular and integrative physiology of intestinal peptide transport. *Annu Rev Physiol* 2004; **66**: 361-384 [PMID: 14977407 DOI: 10.1146/annurev.physiol.66.032102.144149]
54 **Brinson RR**. Fiber-containing enteral formulas: clinical importance. *Nutr Clin Pract* 1990; **5**: 97 [PMID: 2164133 DOI: 10.1177/011542659000500397]
55 **Krissansen GW**. Emerging health properties of whey proteins and their clinical implications. *J Am Coll Nutr* 2007; **26**: 713S-723S [PMID: 18187438 DOI: 10.1080/07315724.2007.10719652]
56 **Ha E**, Zemel MB. Functional properties of whey, whey components, and essential amino acids: mechanisms underlying health benefits for active people (review). *J Nutr Biochem* 2003; **14**: 251-258 [PMID: 12832028 DOI: 10.1016/S0955-2863(03)00030-5]
57 **Marshall K**. Therapeutic applications of whey protein. *Altern Med Rev* 2004; **9**: 136-156 [PMID: 15253675]
58 **Miller PE**, Alexander DD, Perez V. Effects of whey protein and resistance exercise on body composition: a meta-analysis of randomized controlled trials. *J Am Coll Nutr* 2014; **33**: 163-175 [PMID: 24724774]
59 **Tappenden KA**, Maples BA, Chung BM. Whey-based peptide diet alters gastrointestinal immunity and inflammation in piglets with a compromised gastrointestinal tract. *J Pediatr Gastroenterol Nutr* 2005; **41**: 509-510 [DOI: 10.1097/01.mpg.0000181910.08779.37]
60 **Zonta S**, Doni M, Alessiani M, Lovisetto F, Vigano J, Mazzilli M, Dominioni T, Podetta M, De Martino M, Scaglione M, Vicini E, Bottazzi A, Villa C, Morbini P, Dionigi P. Elemental enteral nutrition preserves the mucosal barrier and improves the trophism of the villi after small bowel transplantation in piglets. *Transplant Proc* 1991; **39**: 2024-2027 [PMID: 17692682 DOI: 10.1016/j.transproceed.2007.05.048]
61 **Oz HS**, Chen TS, Neuman M. Nutrition intervention: a strategy against systemic inflammatory syndrome. *JPEN J Parenter Enteral Nutr* 2009; **33**: 380-389 [PMID: 19380752]
62 **Oz HS**, Chen TS, de Villiers WJS. Nutrition intervention against septic shock syndrome: A whey peptide-based diet rich in EPA and DHA. *J Parenter Enteral Nutr* 2007; **31** (Supp 1): S23
63 **Moinard C**, Delpierre E, Loï C, Neveux N, Butel MJ, Cynober L, Charrueau C. An oligomeric diet limits the response to injury in traumatic brain-injured rats. *J Neurotrauma* 2013; **30**: 975-980 [PMID: 23186230 DOI: 10.1089/neu.2012.2707]

P- Reviewer: Abdel-Salam OME, Actis GC, Lee SH, Parker W
S- Editor: Gong XM **L- Editor**: A **E- Editor**: Wu HL

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i2.320

World J Gastrointest Pharmacol Ther 2016 May 6; 7(2): 320-333
ISSN 2150-5349 (online)

SYSTEMATIC REVIEWS

Neuroimaging the brain-gut axis in patients with irritable bowel syndrome

Kristen R Weaver, LeeAnne B Sherwin, Brian Walitt, Gail D'Eramo Melkus, Wendy A Henderson

Kristen R Weaver, LeeAnne B Sherwin, Wendy A Henderson, Digestive Disorders Unit, Biobehavioral Branch, Division of Intramural Research, National Institute of Nursing Research, National Institutes of Health, Department of Health and Human Services, Bethesda, MD 20892, United States

Kristen R Weaver, Gail D'Eramo Melkus, College of Nursing, New York University, New York, NY 10010, United States

Brian Walitt, Division of Intramural Research, National Institute of Nursing Research, National Institutes of Health, Department of Health and Human Services, Bethesda, MD 20892, United States

Author contributions: Weaver KR and Henderson WA participated in the conception and design; all authors contributed to data interpretation in addition to the drafts and revisions of the manuscript; all authors have approved the final version of the manuscript to be published.

Supported by Division of Intramural Research, National Institute of Nursing Research to W.A.H., No. 1ZIANR000018-01-05.

Conflict-of-interest statement: All of the authors have no potential conflicts of interest to disclose.

Data sharing statement: No additional data are available.

Correspondence to: Dr. Wendy A Henderson, Digestive Disorders Unit, Biobehavioral Branch, Division of Intramural Research, National Institute of Nursing Research, National Institutes of Health, Department of Health and Human Services, 10 Center Drive, 2-1341, Bethesda, MD 20892, United States. hendersw@mail.nih.gov
Telephone: +1-301-4519534
Fax: +1-301-4801413

Received: May 9, 2015
Peer-review started: May 11, 2015
First decision: September 8, 2015
Revised: January 8, 2016
Accepted: February 23, 2016
Article in press: February 24, 2016
Published online: May 6, 2016

Abstract

AIM: To summarize and synthesize current literature on neuroimaging the brain-gut axis in patients with irritable bowel syndrome (IBS).

METHODS: A database search for relevant literature was conducted using PubMed, Scopus and Embase in February 2015. Date filters were applied from the year 2009 and onward, and studies were limited to those written in the English language and those performed upon human subjects. The initial search yielded 797 articles, out of which 38 were pulled for full text review and 27 were included for study analysis. Investigations were reviewed to determine study design, methodology and results, and data points were placed in tabular format to facilitate analysis of study findings across disparate investigations.

RESULTS: Analysis of study data resulted in the abstraction of four key themes: Neurohormonal differences, anatomic measurements of brain structure and connectivity, differences in functional responsiveness of the brain during rectal distention, and confounding/correlating patient factors. Studies in this review noted alterations of glutamate in the left hippocampus (HIPP), commonalities across IBS subjects in terms of brain oscillation patterns, cortical thickness/gray matter volume differences, and neuroanatomical regions with

increased activation in patients with IBS: Anterior cingulate cortex, mid cingulate cortex, amygdala, anterior insula, posterior insula and prefrontal cortex. A striking finding among interventions was the substantial influence that patient variables (*e.g.*, sex, psychological and disease related factors) had upon the identification of neuroanatomical differences in structure and connectivity.

CONCLUSION: The field of neuroimaging can provide insight into underlying physiological differences that distinguish patients with IBS from a healthy population.

Key words: Irritable bowel syndrome; Neuroimaging; Brain-gut axis; Functional magnetic resonance imaging

Core tip: The present study reports replicable evidence that persons with irritable bowel syndrome have differences in brain structure and function when compared to healthy volunteers. Gender, psychological factors, and gastrointestinal symptom distress substantially influence these findings.

Weaver KR, Sherwin LB, Walitt B, Melkus GD, Henderson WA. Neuroimaging the brain-gut axis in patients with irritable bowel syndrome. *World J Gastrointest Pharmacol Ther* 2016; 7(2): 320-333 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i2/320.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i2.320

INTRODUCTION

Irritable bowel syndrome (IBS) is the most common condition encountered by gastroenterologists, and yet efficacious treatments have not been discovered that provide patients with satisfactory relief[1]. The diagnosis of IBS is based according to Rome Ⅲ criteria, which requires patients to experience recurrent abdominal pain or discomfort for at least three days per month in the last three months, in addition to their pain being associated with two or more of the following: Improvement in pain with defecation, pain onset associated with a change in the form of stool, or pain onset associated with a change in the frequency of stool[2]. Worldwide prevalence rates of IBS are approximately 11%, with the following countries reporting highest prevalence estimates of over 20%: United States, Taiwan, United Kingdom, Greece, Peru, Croatia, Iceland and Nigeria[3]. The prevalence of IBS is slightly higher in women than in men, and patients are typically categorized into a subgroup based upon their predominant bowel habit: Diarrhea-predominant, constipation-predominant, or a mixture of both[4]. Although IBS occurs in all age groups, the prevalence of IBS is lower in those persons over 50 years of age[5].

The disorder of IBS is associated with adverse physical, psychosocial and socioeconomic consequences for patients and society at large. IBS is not associated with increased rates of mortality but is associated with physical distress, often co-occurring with other debilitating conditions such as fibromyalgia, chronic pelvic pain and chronic fatigue syndrome[3]. Patients with IBS often suffer from psychological disorders such as depression and anxiety, with reported comorbidity rates of 40%-60% and above[6]. Numerous investigations have found IBS to exert a negative impact upon patients' quality of life, as well as result in the disproportionate utilization of health care resources[7]. In addition, financial estimates of managing the disorder are upwards of one billion dollars in the United States; a figure compounded by costs of lost productivity and reduced leisure time[8]. Although significant advances in understanding the pathophysiology of this disorder have been gained by research endeavors, exact mechanisms underlying symptom generation in IBS remain incompletely understood[9].

The "brain-gut axis" (BGA), is a collective term describing pathways between physiological systems noted to be altered in patients with IBS, and is composed of the enteric nervous system, autonomic nervous system and/or the central nervous system[10]. The BGA is a comprehensive framework within which symptom etiology can be evaluated in patients with IBS, as it accounts for the crosstalk or bidirectional communication that occurs between systems[11]. Alterations in the BGA of IBS patients have been shown to include peripheral factors, central and autonomic neural functions, hormones, amines and peptides[12]. Research efforts have also incorporated neuroimaging techniques to evaluate central mechanisms within the BGA, investigating neuroanatomical differences that may shed light on IBS symptomatology.

A Rome Working Team Report in 2009, focusing upon brain imaging and functional gastrointestinal disorders, summarized neuroimaging studies from 1997 onwards that evaluated IBS patients and healthy controls in response to visceral stimuli[13]. This review reported IBS patients experience greater activation of the insula (INS), anterior cingulate cortex (ACC), thalamus (THAL), and prefrontal cortex (PFC) in response to rectal distension. These findings are similar to the reproducible findings seen in response to pain stimuli across a variety of clinical disorders[14]. However, the majority of studies did not control for relevant factors such as affective comorbidity, patient sex, or anxiety related to symptoms that may have impacted these findings.

In an attempt to create a consensus regarding differential regions of brain activation between IBS patients and healthy controls, Tillisch *et al*[15] performed a meta-analysis of pooled neuroimaging data across eighteen rectal distension studies. These authors

reported that IBS patients and healthy controls activate similar brain regions involved in visceral afferent processing [*e.g.*, INS, anterior midcingulate cortex (aMCC), THAL], but noted IBS patients showed greater activation of the emotional arousal regions [*e.g.*, sgACC, amygdala (AMG)], as well as engagement of the midbrain. Although this investigation provided synthesis of neuroimaging data from disparate studies, the authors did not account for the influence of patient variables (*e.g.*, sex, bowel habit, psychological comorbidities) due to the nature of their analyses. The influence of patient variables, heterogeneity in the IBS patient population, variations in study methodology, and neuroimaging findings which overlap with other pain and psychological disorders, makes the discovery of IBS-specific neuroanatomical findings a complex endeavor.

The field of neuroimaging continues to rapidly advance, however, with new techniques to investigate the BGA in patients with IBS. Tillisch *et al*[16]'s article on advances in neuroimaging, provided an overview of 5 techniques that were pertinent to this review: Functional magnetic resonance imaging (fMRI), connectivity analysis, resting state fMRI (rs-fMRI), structural MRI (sMRI) and diffusion tensor imaging (DTI). First the authors reported that fMRI was able to measure moment-to-moment alterations in the oxygen content of blood (the blood oxygen level dependent or BOLD signal). Increases in BOLD activity correlated with increases in neuronal activity, making it possible to estimate the amount and anatomic location of brain activity that occurred during a particular stimulus, such as sorting cards, facial matching, or pain induction with rectal distention. Second, connectivity analysis utilized the same fMRI technique, however, focused on network patterns or circuitry between different brain regions. Third, rs-fMRI evaluated spontaneous brain activity of intrinsic brain networks that took place during a wakeful state. Fourth, sMRI evaluated cortical thickness as well as white and gray matter (GM) volume or density on a global, regional and local scale. Fifth, the authors reported DTI took advantage of the diffusivity of water that evaluated white matter (WM) anatomy and integrity as a function of fractional anisotropy (FA). Some of the networks investigated in neuroimaging studies included salience/executive control, cognitive control, cerebellar, left frontal parietal, right frontal parietal and default mode networks[17]. Lastly, it is important to mention magnetic resonance (MR) spectroscopy that measured relative concentrations of metabolite and neurotransmitter in areas of the brain[18].

Persistent symptomatology in the IBS patient population and ineffective current therapies, require ongoing efforts to understand the etiology and perpetuation of symptoms in patients with IBS. Neuroimaging is one investigative technique to assess central mechanisms in patients with IBS, and may shed light on BGA functioning and relate its relationship to symptom expression. Significant findings have been reported by prior investigations, but ongoing research efforts and technological advances warrant a reappraisal of progress made in the field. Therefore, a literature review was undertaken with the following goal in mind: To summarize and synthesize the literature on neuroimaging the BGA in patients with IBS.

MATERIALS AND METHODS

The following databases were searched in February of 2015 to explore articles on the topic of interest: PubMed, Scopus and Embase. Publication dates were filtered from the year 2009 and onward, as the Rome Working Team Report was published in 2009, and the field of neuroimaging is rapidly advancing. Studies were limited to those written in the English language and those conducted upon human subjects. The following terms were selected as keywords to conduct this review: "irritable bowel syndrome" or "IBS" AND "brain gut axis" or "BGA", "autonomic nervous system" or "ANS", "hypothalamic pituitary adrenal axis" or "HPA axis", "neuroimaging" or "brain scans" or "MRI". Each of the four pairs of terms (BGA, ANS, HPA and neuroimaging) were run separately with IBS as search terms in order to comprehensively cover the subject area of interest. Searches were then re-run without date constraints or filters, in order to capture articles published online ahead of print that otherwise would not be displayed.

The initial search yielded a total of 797 articles: PubMed (193), Scopus (302) and Embase (302). All titles and relevant abstracts were scanned for consideration of inclusion, with review articles being excluded as the interest was upon primary research investigations conducted in patients with IBS. Thirty-eight articles were pulled for full text review, with articles subsequently excluded if the investigation did not include a healthy control group against which to compare patients with IBS, if an article detailed data from an investigation previously accepted for review, and if the article (n = 1) was previously included in the meta-analysis by Tillisch *et al*[15]. Twenty-seven articles fulfilled the inclusion criteria and were reviewed to determine study design, methodology and results. The following data points were then placed in table format to elicit analysis and synthesis of data across studies: 1st Author, Year, Country, Sample Size, Participant Stratification, Imaging Modality, Study Details, Interventions, Major Findings Across Studies, Individual Findings, Physiological Correlations, Significant Inventory Differences, Confounding and Correlating Factors. Major findings that focused on anatomical areas were then tabulated across studies to determine the most prevalent findings. Assessment of study findings included not only isolated areas of brain activation or deactivation, but also differences in patterns of connectivity across brain regions between patients with

IBS and healthy controls. Lastly, individual study findings that included the identification of confounding or correlating patient factors were examined for commonalities in efforts to integrate findings from disparate investigations.

RESULTS

Analysis of study data resulted in the abstraction of four key themes: Neurohormonal differences, anatomic measurements of brain structure and connectivity, differences in the functional responsiveness of the brain during rectal distention, and confounding/correlating patient factors (Table 1). The first three themes identify differences between patients with IBS and healthy controls, whereas the fourth illuminates factors that influence the identification of such differences or interpretation of findings. Patient inventories that differed significantly between patients with IBS and healthy controls are displayed in tabular format. All 27 studies utilized neuroimaging techniques to assess the BGA in patients with IBS, and are grouped according to imaging modality to facilitate comparisons across disparate investigations.

Magnetic resonance spectroscopy

Niddam *et al*[19] utilized proton magnetic resonance spectroscopy (H-MRS) in an investigation of HPA axis dysregulation in patients with IBS. To gain insight of the stress response system, metabolite concentrations of excitatory Glutamate (Glu) and Glutamine (Gln) were measured to assess the HIPP inhibitory feedback to the HPA axis. Reduced concentration of metabolites Glu-Gln (Glx) were reported in the left HIPP of IBS patients (n = 15) *vs* healthy controls (n = 15), P = 0.04. Further, Glx metabolite concentrations in IBS patients were negatively correlated with state anxiety (r = -0.741) and catastrophizing (r = -0.633).

sMRI

In an investigation that explored the contribution of patient variables to altered brain structure, Blankstein *et al*[20] compared patients with IBS (n = 11) to healthy controls (n = 16) using sMRI with voxel-based morphometry (VBM) and cortical thickness (CT) analysis. IBS patients were found to have increased HYP GM and decreased cortical thickness in the aMCC in comparison to controls. When age was accounted for, a negative correlation was found between the thickness of the dorsolateral prefrontal cortex (dlPFC) and scores on the pain catastrophizing scale (PCS) (r = -0.66), in contrary a positive correlation was found between the thickness of the aINS and the duration of patient pain (r = 0.78).

In a larger sample, Seminowicz *et al*[21] utilized sMRI with VBM and CT analysis to investigate anatomical brain differences in female patients with IBS. Initially these researchers found numerous differences in GM density between IBS patients (n = 56) and healthy controls (n = 49). However, after adjusting for anxiety and depression, GM density decreased in the left posterior parietal cortex, left MFG, and bilateral temporal cortices in IBS patients. These differences in GM density were largely consistent across subgroups that categorized patients based on predominant bowel habit as well as pain *vs* no-pain as most bothersome IBS symptom. They also reported a small negative correlation (r = -0.35) between IBS duration and GMD in the dorsolateral PFC, but only in the non-pain predominant group.

In a study that compared healthy subjects to those with inflammatory *vs* non-inflammatory types of visceral abdominal pain, Hong *et al*[22] utilized sMRI to examine IBS subjects with diarrhea (n = 11), subjects with ulcerative colitis (UC) (n = 16) and healthy controls (n = 41). In comparison to healthy controls, IBS subjects were found to have significantly lower grey matter CT in the right aINS; a difference that remained significant when anxiety and depression were controlled for in statistical analyses. This regional difference was distinct from those reported between subjects with UC and healthy controls, and significant correlations were not found between CT and patient variables in the IBS group.

Jiang *et al*[23] evaluated regional GM changes as a function of sex in patients with IBS using sMRI. Comparing patients with IBS (n = 90) to healthy controls (n = 176), initially no differences in whole brain CT were found. However, when separating groups by sex and disease, female patients with IBS were found to have diminished CT in bilateral sgACC, as well as greater CT in pre and post central gyrus (PoCG) in comparison to female healthy controls. Region of interest (ROI) analysis revealed female IBS patients have significantly smaller CT in bilateral aINS, medial INS, pINS and left sgACC compared to female healthy controls. Differences in CT were found in female IBS patients in the right pINS that were dependent on their bowel subtype (diarrhea *vs* constipation), as well as by sex (males having greater CT in right sgACC). Correlations of patient variables with CT revealed female IBS patients to have negative correlations between length of disease and CT in right mINS (r = -0.293), as well as anxiety symptoms with CT in right aINS (r = -0.273) and right mINS (r = -0.248). Correlations of patient variables with CT in male IBS patients included symptom severity with CT in left mINS (r = -0.673), trait anxiety with CT in sgACC (r = - 0.525) and in right pINS (r = 0.469), as well as early trauma inventory (ETI) with CT in left mINS (r = 0.556).

The relevance of patient variables was also reported by Labus *et al*[24] in an investigation of pooled sMRI studies examining GM volume and regional brain network alterations in female patients with IBS. Initially numerous differences were identified between IBS patients (n = 82) and healthy controls (n = 119) when

Table 1 Neuroimaging the brain-gut axis in patients with irritable bowel syndrome

Ref.	Year	Country	Imaging	NI	F	M	CN	ACC	MCC	AMG	HIP	HYP	aINS	pINS	PFC	PoCG	THAL	Inven Diff	Conf/corr
Niddam *et al*[19]	2011	Taiwan	MRS	15	8	7	15				-							-	A (st), PCS
Blankstein *et al*[20]	2010	Canada	sMRI	11	11	0	16		-			+						-	Dz, PCS
Seminowicz *et al*[21]	2010	United States	sMRI	56	56	0	49											A, D	A, D, Dz
Hong *et al*[22]	2014	United States	sMRI	11	9	2	41						-R					A, C, D, S, V	-
Jiang *et al*[23]	2014	United States	sMRI	90	70	20	176	- B					-B	-B		+L		A, C, D, E, S	A, Dz, ETI, Sb, Sx
Labus *et al*[24]	2014	United States	sMRI	82	82	0	119			-R						+L		A, C, D, E	Dz, ETI
Piché *et al*[25]	2013	Canada	sMRI	14	14	0	14							+R				A, C, D	Dz
Berman *et al*[26]	2012	United States	sMRI/PET	11	5	6	11	+B		+R				+R				N, VSI	ETISF
Chen *et al*[27]	2011	Canada	MRI-DTI	10	10	0	16							+R				-	Dz, N, PCS
Ellingston *et al*[28]	2013	United States	MRI-DTI	33	21	12	93	+B									-B	-	Dz, Sx
Ma *et al*[29]	2014	China	rs-fMRI	21	7	14	21				-R					-B		NA	Dz
Hong *et al*[30]	2013	United States	rs-fMRI	60	31	29	118			+L	+R		+B					A, D, N, V	Dz, Sx
Hong *et al*[31]	2014	United States	rs-fMRI	48	24	24	48						-L					A, D, V	Dz, VSI
Gupta *et al*[17]	2014	United States	rs-fMRI	58	28	30	110	+B	+R				+B[1]	+R			+L	A, D, E, N	ETI, Sx
Aizawa *et al*[41]	2012	Japan	fMRI	30	15	15	30				-R			+L	-R			-	-
Labus *et al*[42]	2013	United States	fMRI	47	27	20	67			+R								A, D	A, D, Sx
Hubbard *et al*[43]	2011	United States	fMRI	14	14	0	17		+L	+L		+L						A (tr)	A (st & tr)
Kilpatrick *et al*[44]	2011	United States	fMRI	26	26	0	29			+L	+L							-	Genotype
Hall *et al*[32]	2009	Canada	fMRI-RD	7	7	0	6	+R					+L[1]		+B			NA	-
Larsson *et al*[33]	2012	Sweden	fMRI-RD	44	44	0	20	+L			+R		+R	+L	+L		+L	A, D	Subgrps of Pain
Elsenbruch *et al*[34]	2010	Germany	fMRI-RD	15	15	0	12		+L				+L[1]		+L			A	A
Icenhour *et al*[35]	2015	Germany	fMRI-RD	17	15	2	21	+R	+L	+L					+		+	A, C, D, N	A
Bouhassira *et al*[36]	2013	France	fMRI-RD	20	20	0	11											HAD -t	HAD, PCS
Lee *et al*[37]	2012	Taiwan	fMRI-RD	17	11	6	17		+B				+L[1]		+B			A, HAD -t	A
Schmid *et al*[38]	2015	Germany	fMRI-RD	17	15	2	17		+B		+L							A, D, N	D
Lowén *et al*[39]	2013	Sweden	fMRI-RD	44	44	0	18	+B	+L		-L		+R	+R	+L			-	-
Labus *et al*[40]	2011	Netherlands	fMRI- RD	14	14	0	12			+					+			A, D	-

[1]Results reported for insula *vs* aINS; +: Activation/increased connectivity; -: Deactivation/decreased connectivity; A: Anxiety; A (st): State anxiety; A (tr): Trait anxiety; ACC: Anterior cingulate cortex; AMG: Amygdala; aINS: Anterior insular cortex; B: Bilateral; C: Catastrophizing; CN: Control group sample size; Conf/Corr: Confounding/correlating variables; D: Depression; Dz: Disease variables; E or ETI: Early trauma inventory; ETISF: Early trauma inventory short form; F: Female; fMRI: Functional magnetic resonance imaging; HAD: Hospital Anxiety and Depression Index; HAD-t: Hospital Anxiety and Depression Index Total Score; HIP: Hippocampus, HYP: Hypothalamus; Inven Diff: Inventory differences; L: Left; M: Male; MCC: Mid cingulate cortex; MRI-DTI: Magnetic resonance imaging - diffusion tensor imaging; MRS: Magnetic resonance spectroscopy; NI: Sample size of IBS patients; N: Neuroticism measured *via* NEO-PI (five factor inventory for personality); NA: Not assessed; PCS: Pain Catastrophizing Scale; PET: Positron emission tomography; PFC: Prefrontal cortex; pINS: Posterior insular cortex; PoCG: Post central gyrus; R: Right; RD: Rectal distension; S: Somatization - PHQ score (Patient Health Questionnaire); rs-fMRI: Resting state functional magnetic resonance imaging; Sb: Bowel subgroup; Sx: Patient sex; sMRI: Structural magnetic resonance imaging; THAL: Thalamus; VSI: Visceral sensitivity index.

aggregating data across seven neuroimaging studies. However, once the authors accounted for early life events *via* the ETI, the majority of group differences between IBS patients and controls were no longer statistically significant. Only the findings of reduced GM in the right AMG and higher GM in the left PoCG remained to differentiate patients with IBS from healthy controls. In addition, small correlations were reported between IBS symptoms and GM volume changes, that included associations between left INS and symptom severity (r = -0.26), as well as right inferior frontal gyri and disease duration (r = -0.26).

Piché *et al*[25] conducted an investigation that explored brain morphology, pain inhibition, and associations with disease in female patients with diarrhea-predominant

IBS. The study compared IBS patients (n = 14) to healthy controls (n = 14), and used sMRI to evaluate the reaction to electrical stimulation and heterotopic noxious counter-stimulation (HNCS). IBS patients did not differ from controls in terms of shock pain ratings nor cold pain stimulus (HNCS), but did differ in terms of decreased pain inhibition (P = 0.02). In addition, IBS patients were found to have a thicker right pINS; a finding that remained significant when patient variables were controlled, and was positively correlated with duration of IBS (r = 0.67), but not the severity of IBS.

Berman *et al*[26] used noradrenergic (NE) receptor antagonists, agonists, and placebo, and assessed differences between IBS patients (n = 11) and healthy controls (n = 11) in central NE signaling during an auditory vigilance task. IBS patients were found to have higher baseline levels of plasma NE, and through positron emission tomography and sMRI, were shown to experience less reduction of activity in a central arousal circuit (bilateral sgACC, left pregenual ACC, right AMG, left dorsal brainstem and right pINS) by Yohimbine (YOH), a NE antagonist, in comparison to healthy controls. Although IBS patients and healthy controls were not found to differ in pre-ingestion levels of anxiety, YOH increased anxiety more in IBS patients (P = 0.011), and the increased anxiety correlated with plasma NE levels in IBS patients (r = 0.61) but not in healthy controls. Clonidine (CLO), a NE agonist, was found to increase left sgACC activity more in patients with IBS than in healthy controls. Lastly, the inhibitory effect of YOH was found to inversely correlate with early life trauma in the left dorsal brain stem and left AMG, although group differences could not be assessed due to missing data.

Across investigations employing sMRI, the two most consistent anatomical findings were reduced CT in the aINS and increased CT/GM volume in the PoCG in female patients with IBS. Anatomic alterations commonly correlated with patient variables such as sex, anxiety, depression, IBS bowel subtype and early life trauma. Negative correlations were found between dlPFC with PCS scores and with IBS symptom duration, and between the INS with anxiety and with IBS symptom severity.

DTI

Chen *et al*[27] used MRI DTI and investigated WM abnormalities in female patients with IBS. FA was used as a measure of microstructural WM integrity. The researchers evaluated pre-selected areas associated with nociception, and reported that patients with IBS (n = 10) had an increased FA in the fornix and in the external capsule bordering the right pINS in comparison to healthy controls (n = 16). Additionally, the relationship between FA and patients' disease and personality characteristics was explored with the following correlations reported: Pain severity and FA in bilateral aINS (r = 0.71) and right ventral posterior lateral nucleus of the THAL (r = 0.576), unpleasantness and FA in left aINS (r = 0.790), duration and FA in left pINS (r = 0.662), neuroticism and FA in left medial dorsal nucleus of the THAL (r = 0.685), as well as PCS scores and FA in right mACC (r = -0.764).

Ellingson *et al*[28] also utilized DTI in an investigation of alterations in brain microstructure in patients with IBS (n = 33) in comparison to healthy control subjects (n = 93). These researchers found IBS patients to have diminished FA (low directional coherence) in bilateral THAL, bilateral globus pallidus, bilateral putamen, bilateral primary and secondary somatosensory and motor regions, as well as in bilateral PCC, and found increased FA (high directional coherence) in bilateral frontal lobe, bilateral ACC and corpus callosum. Similar to study findings previously reviewed, these investigators found differences in surrogate measures of brain microstructure dependent upon IBS and patient sex. Although such differences were not found among healthy controls, female IBS patients were found to have lower FA in the THAL and primary sensory and motor regions in comparison to male patients with IBS. Correlations between symptom severity and DTI were also examined: No significant correlations were found for female patients with respect to FA, although male patients were found to have negative correlations between symptom severity and average FA in the ACC (R^2 = 0.4199), BG (R^2 = 0.4793) and WM regions near the INS (R^2 = 0.4086).

Across the two investigations employing DTI, consistent areas of FA were not reported. In terms of patient variables, sex was noted by the second investigation to influence study findings. Lastly, although correlations were noted for female patients in the first investigation between FA with patient variables, significant correlations for female patients were not reported by the second.

rs-fMRI

rs-fMRI was employed by Ma *et al*[29] in an investigation of brain alterations in patients with IBS. Through investigation of the differences between IBS patients (n = 21) and healthy controls (n = 21) in amplitude of low-frequency fluctuation (ALFF) and ROI based functional connectivity, IBS patients were reported to have diminished amplitude in the left SFG, right HIPP, right MFG, bilateral PoCG and right superior temporal pole, as well as increased ALFF in the left calcarine and left MCG. Correlations were found between disease duration and ALFF values in the left MCG (r = -0.477) as well as in the right MFG (r = 0.517). Lastly, in comparison to healthy controls, IBS patients were found to have increased connectivity between the left MCG and left SFG, revealing increased connectivity between the cingulate and frontal cortex.

Hong *et al*[30] utilized rs-fMRI to investigate the dynamics of resting brain activity in patients with chronic visceral pain. Additionally, the effect of sex on

brain activity in patients with IBS (n = 60) and healthy controls (n = 118) was assessed. Similar to findings of previously reviewed investigations, no differences were initially found when males and females were grouped together; however, significant differences between groups were noted once separated by sex. The study also evaluated differences in resting-state brain function (spontaneous brain frequency oscillations) with fractional amplitude of low-frequency fluctuation (fALFF) at three different frequencies: Low (LF), medium (MF) and high (HF). Female IBS patients were found to have increased frequency power distribution in the left AMG, right HIPP and bilateral aINS, and LF power distribution in the sensorimotor regions when compared with female healthy controls and with male patients with IBS. In addition, a correlation between abdominal discomfort and HF power distribution in the left aINS (r = 0.506) was found for female subjects with IBS.

Building upon these findings, Hong *et al*[31] performed an rs-fMRI investigation of patients with IBS (n = 48) in comparison to healthy controls (n = 48), exploring the effect of sex and disease upon brain connectivity patterns of the dorsal aINS. Connectivity differences were reported between the sexes, as were reports of differences as a function of sex and disease. For instance, in comparison to female healthy controls and male patients with IBS, female IBS patients were found to exhibit a greater negative connectivity between bilateral dorsal aINS to bilateral PFC regions, as well as between the left dorsal aINS to the left precuneus. Correlations were reported for male IBS patients, between connectivity of the bilateral dorsal aINS with the bilateral dorsal medial PFC and the Visceral Sensitivity Index, (left r = 0.442, right r = 0.405), and between the left dorsal aINS and left mPFC (r = 0.41). Correlations were also reported for female IBS patients, between connectivity of the left dorsal aINS to the precuneus with symptom intensity (r = 0.597), and between the bilateral dorsal aINS to the bilateral dorsal mPFC (left r = 0.497, right r = 0.466).

The influence of patient variables was also evaluated by Gupta *et al*[17] in a pooled rs-fMRI investigation of early adverse life events (EAL) and sex in patients with IBS and healthy controls. Comparing 58 IBS patients with 110 healthy controls, increased BOLD oscillation intrinsic connectivity patterns in the left frontal parietal, salience, and default mode networks [regions including, but limited to bilateral INS, bilateral ACC, right MCC, left THAL, right supramarginal gyrus (SMG) and right precuneus] were found. The effect of EAL was examined and the authors reported reduced connectivity in the salience/executive control network in female and male patients with IBS compared to healthy controls: Bilateral INS, bilateral ACC, left supplementary motor area (SMA) and left parietal and frontal regions, as well as increased connectivity in the left putamen. Lastly, male patients with IBS and EALs were found to have alterations of increased connectivity in the cerebellar network: Left THAL, right pINS, bilateral ACC, right cerebellum and left middle temporal gyrus. These patterns of connectivity were greater than in male healthy controls with EALs, and were not found in female participants (IBS or healthy controls).

Across rs-fMRI investigations, congruence was found with increased oscillation patterns in the INS, although conflicting findings were reported for the right HIPP. Patient variables of sex and early life trauma were found to influence study findings. In addition, correlations were reported between patient variables and neuroimaging findings, primarily relating to disease characteristics and psychological factors.

fMRI using rectal distension paradigms

Since 2009, there have been additional fMRI studies that utilized rectal distension protocols to investigate differences between IBS patients and healthy controls, 9 of which met the inclusion criteria for this review. Hall *et al*[32] utilized a single rectal distension protocol and fMRI to evaluate female patients with IBS (n = 7) in comparison to healthy controls (n = 6). IBS patients were found to experience heightened activation during painful rectal distension in the right ACC, left INS and ventral medial prefrontal regions, as well as a failure to down-regulate activation of bilateral vmPFC during the tonic phase (constant state) of the distension protocol.

Larsson *et al*[33] investigated differences between female subjects with IBS (n = 44) and healthy controls (n = 20), using fMRI and a high/low rectal distension paradigm. IBS subjects were found to experience greater activation in the left vlPFC during high distension, greater activation in left mINS during low distension, and greater activation in right aINS, right mINS and right HIPP during expectation of high distension. Further differences were noted within IBS patients when stratifying according to subtype of pain (normosensitive *vs* hypersensitive), although the two groups did not differ in scores of anxiety, depression, disease duration or severity. For example, normosensitive patients did not differ significantly from healthy controls in brain responses during active distension, but did experience greater activation in the right HIPP during expectation of high stimulus distension. Hypersensitive patients, in contrast, were found to differ significantly from healthy controls in brain responses during high rectal distension, showing increased activation in the left pINS, left THAL and left pACC.

Elsenbruch *et al*[34] investigated the role of emotional context on visceral stimuli response in patients with IBS, utilizing fMRI, painful/non-painful rectal distensions and environmental manipulation (progressive muscle relaxation and a public speaking stressor). Analysis of group responses to stress revealed female IBS patients (n = 15) with increased activation in the left aMCC and left vlPFC during nonpainful distension, and with greater activation in the right INS and left vlPFC during painful distensions in comparison to healthy controls (n = 12).

These findings remained significant after including anxiety as a covariate, and anxiety was noted to explain diminished relaxation response.

Alterations in fear learning and extinction in IBS patients was evaluated by Icenhour *et al*[35], using a rectal distension protocol to promote learning, extinction and reinstatement. Study analysis of IBS patients (n = 17) and healthy controls (n = 21) also included fMRI, skin conductance responses as well as salivary analysis for cortisol and alpha-amylase. The authors report group differences to be greatly affected by the inclusion of anxiety as a covariate, and yet significant findings remained. In comparison to healthy controls, IBS patients exhibited increased activation of the right ACC, left MCC and left AMG during fear learning, the THAL during extinction, and the dmPFC, right ACC and left MCC during reinstatement. The influence of sex upon findings was also investigated in an exploratory analysis between females with IBS (n = 15) and healthy controls (n = 10), with the authors confirming differences noted between groups in the full sample.

Bouhassira *et al*[36] investigated pain processes in female patients with IBS, constipation predominant (n = 20), in comparison to healthy controls (n = 11), using slow-ramp rectal distension. These investigators used fMRI, assessed diffuse noxious inhibitory control of pain, and categorized IBS patients into two groups based upon the following: Inhibition (I) or facilitation (F) of RIII nociceptive spinal reflex (measured on the left lower limb). The two IBS groups did not differ in terms of clinical characteristics, although the Group F patients reported significantly higher IBS severity scores. When pooled together as a group a positive correlation was found between rectal sensation and two psychological inventories: HAD (r = 0.61) and PCS (r = 0.42). In addition, when pooled together as a group to analyze fMRI data, patients with IBS did not differ from healthy controls in areas of brain activation during non-painful distension, but did exhibit increased activation of various regions during painful rectal distension including the ACC, MCC, aINS, pINS, and THAL. However, once scores on the HAD or PCS were included as covariates, fMRI differences between groups were no longer significant.

Lee *et al*[37] investigated the effect of psychological factors upon patterns of brain activation during placebo analgesia, in a rectal distension protocol that compared IBS patients (n = 17) with healthy controls (n = 17). fMRI was used to analyze patterns of activity during anticipation and rectal distension while research subjects received inert substances along with suggestions of pain relief. Investigators reported comparable placebo effects among patients with IBS and healthy controls, however areas of brain activation during placebo analgesia differed between groups. For instance, upon rectal distension during placebo analgesia, IBS patients showed greater activation of the left INS, bilateral MCC, bilateral vlPFC and right precuneus in comparison to healthy controls. In addition, IBS patients had greater activation of the left vlPFC during anticipation of placebo. Lastly, in IBS patients, the HAD score for anxiety was negatively correlated with activation of the left vlPFC/INS (z-score = 3.69) during placebo analgesia, and was predictive of a weaker placebo effect.

Schmid *et al*[38] similarly investigated placebo analgesia in the context of visceral pain, and also found that patient variables influenced the placebo response. A rectal distension protocol was used in patients with IBS (n = 17), UC (n = 15) and healthy controls (n = 17), and fMRI assessed areas of brain activation during placebo analgesia. A negative correlation was found between patient variables and placebo analgesia response; however in this study, depression emerged as the relevant variable (r = -0.30). Unlike the prior investigation, however, IBS patients had dysregulated placebo response during rectal pain in comparison to healthy controls. Including depression scores as a covariate, IBS patients were found to have reduced down-regulation (or increased activation) in bilateral MCC, left HIPP, right PCC and right somatosensory cortex during rectal distension while undergoing placebo analgesia. Patients with UC did not experience this same dysregulated down-modulation. This study also assessed salivary cortisol levels as a marker of HPA axis dysfunction, but was unable to standardize collection times and did not find significant differences between groups.

Lowén *et al*[39] evaluated the neuroanatomical mechanisms of gut-directed hypnotherapy, in a rectal distension fMRI investigation of female IBS patients (n = 25) compared to healthy controls (n = 18). Prior to therapy initiation (either hypnotherapy or education intervention), patients with IBS who responded to treatment were found to have increased activation of the following regions in response to rectal distension: Left vlPFC, left aMCC, bilateral pACC and bilateral sgACC, as well as reduced activation of the left HIPP. During expectation of rectal distension, IBS patients, in comparison to healthy control subjects, had greater activation of the right ventral aINS and right dorsal pINS.

Alterations in the serotonin signaling system was investigated by Labus *et al*[40], comparing IBS constipation-predominant female patients (n = 14) to healthy control subjects (n = 12), who were administered an amino acid mixture to induce acute dietary tryptophan depletion (ATD). According to the authors, ATD has been shown as a reliable method to induce lower central and peripheral serotonin [5-hydroxytryptamine (5-HT)] levels. With the use of fMRI during a rectal distension protocol, healthy control subjects who underwent ATD were found to exhibit similar patterns of brain connectivity in an emotional arousal circuit as patients with IBS. Although subjects were studied under different

conditions, both groups during rectal distension were found to experience similarities of increased connectivity from the orbital medial prefrontal cortex (omPFC) to the AMG, and from the AMG to infragenual ACC.

Across fMRI investigations using rectal distension protocols, the following brain regions were consistently activated: ACC, MCC, AMG, aINS, pINS and PFC (primarily vlPFC). Patient variables that influenced study findings include anxiety, catastrophizing, and bowel subtype. In addition, patient psychological variables of depression and anxiety were reported to moderate the placebo response.

fMRI using other testing paradigms

Aizawa *et al*[41] utilized the Wisconsin Card Sorting Test (WCST) and fMRI, to explore associations between cognitive flexibility and neuroanatomical alterations in patients with IBS. No differences were found in WCST overall performance based upon IBS group or patient sex. In terms of fMRI results, patients with IBS were found to have decreased activity of right dlPFC, right HIPP and increased activity of left pINS at error feedback during set-shifting, as well as less connectivity from the dlPFC to the pre SMA in comparison to healthy controls. Duration of IBS symptomatology was not found to significantly correlate with either neuroimaging or WSCT results.

The influence of sex and disease was evaluated by Labus *et al*[42] using fMRI and a negative emotion face viewing paradigm to investigate emotion-related cognitive processes in patients with IBS. Neuroanatomical differences were not found between IBS patients (n = 47) and healthy controls (n = 67) until separating groups by sex. Male subjects with IBS were then found to experience greater activation in the right AMG compared to male healthy controls, whereas differences were not detected between females. IBS patients as a group showed lesser engagement of the HIPP to AMG circuit, although they exhibited stronger positive connectivity from the AMG to HIPP (with female IBS patients demonstrating the greatest connection). When accounting for patient variables of depression and anxiety, differences between male and female IBS patients revealed augmented activity for males in the HYP and aINS.

Hubbard *et al*[43] used a corticotropin-releasing factor antagonist, painful stimulus (transcutaneous abdominal electrical stimulation), and fMRI to look for evidence of HPA axis dysfunction in female patients with IBS. Baseline levels of ACTH were reduced in IBS patients (n = 14) *vs* healthy controls (n = 17), and although peripheral effects of HPA axis activity were not found after drug administration, fMRI data suggested evidence of central effects. In comparison to healthy controls, IBS patients were found to experience increased activation after placebo administration in the left HYP and left LCC during expectation of pain, as well as greater activity reduction in the left HYP after antagonist administration (both at low and high doses of drug). After inclusion of trait anxiety as a covariate into the analyses, differences in the left HYP remained significant only at the low dose of the drug. State anxiety was also found to moderate drug induced BOLD signal changes in the left HYP for average to high levels of anxiety. IBS patients were also found to exhibit a strong positive connectivity in an emotional-arousal circuit (*e.g.*, from aMCC to AMG and from AMG to HIPP). Antagonist administration significantly altered such connectivity, in that some IBS patients' path coefficients (*e.g.*, from HYP to AMG) resembled that of healthy controls' after placebo administration.

Alterations in the 5-HT signaling pathway in patients with IBS was investigated by Kilpatrick *et al*[44], in a study of serotonin gene polymorphism (HTR3A, c.-42C > T) assessed through salivary samples. These investigators used fMRI to evaluate AMG responsiveness during an affective matching paradigm in both female patients with IBS (n = 26) and healthy controls (n = 29). During a neutral visual task, IBS subjects were found to have greater activity in the left AMG and left HIPP in comparison to healthy controls, as well as increased left HIPP activity during the emotional task. In both IBS patients and healthy controls that carried the homogenous genotype, an association was found with increased anxiety and increased AMG responsiveness to neutral and emotional stimuli. Lastly, in patients with IBS who carried the homogenous genotype, an association was found with higher IBS symptom ratings, as well as a subset of patients who experienced low differential AMG activation, or difficulty discriminating between stimuli due to heightened engagement.

These fMRI studies did not reveal consistent neuroimaging differences between IBS patients and healthy controls. Likely this was due to variations in investigative focus and disparate study design. However, the influence of patient variables upon neuroimaging findings was noted across studies, and included patient sex, depression, anxiety as well as genotype.

DISCUSSION

The findings of this review reveal new developments in neuroimaging the BGA in patients with IBS. The field has continued to provide insight into underlying physiological differences that distinguish patients with IBS from a healthy population. These differences are wide-ranging, including differences in neurotransmitter concentrations, in gross and functional anatomic structure, and in functional brain responsiveness to rectal-distention. These cross-sectional observations and their correlation with sex, psychological factors, and gastrointestinal symptoms are an important scientific step towards understanding the pathophysiology of IBS.

As noted in prior reviews, there is a consistency in anatomical brain regions that are structurally and functionally different in patients with IBS than in healthy

volunteers. The greatest amount of consistency was seen with fMRI rectal distension studies, with increased BOLD activation of the ACC, MCC, AMG, aINS, pINS and PFC in IBS patients. This pattern of brain regions appear to represent a rectal-distention pain-related network and are similar to other pain-related networks[45]. These regions have been implicated in autonomic and neuroendocrine functioning, memory, interoception, consciousness, cognition and emotional arousal, among others[46]. The observations of heightened sensitivity of activation of the rectal-distension pain-related network in IBS also parallels the observations seen in other pain disorders[47,48]. The rectal distension paradigm demonstrates that IBS symptoms have a biologic foundation, but not one that is specific only to the IBS patient population.

Studies of anatomic structure are less consistent than seen with the rectal distention paradigm. While the two most consistent anatomical findings were reduced CT in aINS and increased CT/GM volume in PoCG in female patients with IBS, there is variability of structural results between the different studies. To date, only a small number of IBS studies focused on determining differences in the neurochemical constituents and functional connectivity patterns have been performed.

One striking finding was the influence of patient sex, psychological status, and disease related variables upon the identification of neuroanatomical differences between patients with IBS and healthy controls. These factors influenced results across the full spectrum of imaging modalities reviewed. Many of the studies reported results that were either sex-specific or sex-disease subtype specific. Sex hormones are increasingly recognized as influencing the BGA and contributing to the development of IBS symptomatology[49]. Many investigators in this review accounted for hormonal fluctuations in terms of scanner timing. However, the substantial differences in neuroimaging results suggest that IBS pathophysiology may not be uniform across gender.

Psychological factors, in particular anxiety, depression, early life trauma and catastrophizing, had substantial influence on the neuroimaging correlates of IBS. Of the 27 studies included in this review, 25 used validated inventories to assess the psychological characteristics of their study population, with 18 finding group differences in IBS patients. This stratification was of tremendous importance. Psychological variables confound the identification of IBS -specific neuroimaging group differences, as many of the imaging findings either positively or negatively correlated with psychological symptoms and tendencies. The majority of the reviewed studies reported associations between psychological inventories and neuroimaging findings, more so in fact than associations between disease characteristics and neuroimaging findings. The strength of such correlations varied between investigations, and although does not aid in determining causality of patient symptomatology, does reiterate the relevance of psychological factors in patients with IBS.

Many of the investigations also stratified subjects based upon their bowel subtype. The finding of decreased grey matter density of various areas of the brain was associated with IBS subtypes based on predominant bowel habit and predominance of pain symptoms. One innovation in IBS neuroimaging is the inclusion of UC patients as a comparison group for patients with IBS. Including a group that suffers from an organic disorder manifested by similar gastrointestinal symptomatology may help parcel out the various influences that contribute to IBS.

The ability to interpret the neuroimaging correlates of IBS and generalize them to the population is hindered by a number of limitations of the studies themselves. The studies were performed in nine different countries: United States (12), Canada (4), Germany (3), Sweden (2), Taiwan (2), China (1), Japan (1), France (1) and The Netherlands (1). Although this heterogeneity increases the generalizability of the results, and is relevant given the international prevalence of IBS, consideration must be given to the cultural context in which these studies are conducted. Cultural norms, at the very least, influence the acceptability of expressing symptomatology, the willingness to seek assistance for medical or psychological disorders, faith or distrust of the healthcare system, and the likelihood of participation in medical research. This leads to another issue that deserves mention: The self-selection of subjects who willingly agreed to participate in the aforementioned investigations. The study results are therefore reflective of this patient population, who may or may not represent the general IBS population at large. In addition, the sample size of the studies was noted to vary greatly, ranging from 13 to 266 participants. Therefore, the findings of these investigations need to be viewed in terms of their subject numbers and subsequent study power. All of the studies included in this review, except for two[34,35], matched IBS participants with a healthy control population of similar sex and age. The interpretation of the results of these two studies may be impacted by unmeasured age-related brain changes. Further aspects of sampling that need to be addressed include the stratification of participants by sex, IBS subtype, pain-type, and psychological characteristics. Nearly half of the studies in this review recruited female participants exclusively (n = 13). While using a homogeneous gender cohort typically decreases research variability, the gender-specific differences highlighted above suggest that such results cannot be generalized.

A final point for discussion is the inconsistencies that continue to permeate the results of neuroimaging studies, even when the best efforts are made to analyze a homogenous IBS patient population. As previously

mentioned, many neuroanatomical findings consistently replicated in the IBS patient population, are not specific to the IBS patient population. The heterogeneity of the patient population and the overlapping influence of psychological and physical pain disorders have made the identification of clinically relevant neuroanatomical findings a challenge. As neuroimaging results may display central changes between patients with IBS and healthy controls, we have yet to develop a consensus of the downstream effects of this structural snap shot or connectivity pattern as it relates to BGA functioning. The aforementioned studies provide evidence of the utility of neuroimaging in a disorder with innumerable intricacies.

The limitations of this review must be addressed in like fashion as the studies it has reviewed. First, only accepted literature for publication was included in this analysis, therefore results are subject to publication bias. Second, this review aimed to cover investigations that evaluated neuroimaging the BGA in patients with IBS. Additional neuroimaging pain literature may prove pertinent to this topic, but was outside the scope of this review. One final limitation is that this review is subject to the bias of the persons conducting it, from selection of articles, assessment and interpretation of findings as well as presentation of results.

IBS is a multi-factorial disorder, ridden with complexities, which exacts an exorbitant toll upon society' s financial and human resources. The manifestation of IBS symptomatology may occur anywhere along the BGA continuum, and has proven resistant, thus far, to curative medical treatment. Although great gains have been made by research endeavors in BGA dysfunction in patients with IBS, the development of symptomatology has remained elusive to our understanding.

The field of neuroimaging has provided insight into the underlying physiological differences that distinguish patients with IBS from a healthy population. Differences in neurotransmitter concentrations, differences in gross and functional anatomic structure, and the improving description of a rectal-distention pain-related network are important scientific steps to understand the pathophysiology of IBS. Use of relevant comparator groups, such as those with inflammatory bowel disease and psychological disorders, will be essential in expanding this understanding.

Future directions should include efforts to simultaneously assess peripheral and central aspects of the BGA to better understand how these brain findings relate to circumstances in the gut. Coupling brain imaging to measurements of gut motility and permeability, autonomic and endocrine function, individual microbiome, and host transcriptome data would enrich our understanding of the BGA. In addition, joint efforts and collaborations developed to investigate the neurological correlates of IBS, such as the Pain and Interoception Imaging Network[50], and the ENIGMA Consortium[51], will enhance future understanding of the role of the brain-gut axis in patients with IBS.

COMMENTS

Background

Irritable bowel syndrome (IBS) is the most commonly encountered condition by gastroenterologists, yet understanding of disease evolution and treatment interventions remain suboptimal. The "brain-gut axis" (BGA), composed of the enteric nervous system, autonomic nervous system and/or the central nervous system, has been noted as dysregulated in patients with IBS. Neuroimaging is one technique to assess central processing mechanisms in patients with IBS, and may shed light on BGA functioning and its relationship to symptom expression.

Research frontiers

Neuroimaging techniques provide insight as to differences between patients with IBS and healthy volunteers in anatomical measurements of brain structure and connectivity, as well as functional responsiveness of the brain. Neuroimaging modalities in this review include magnetic resonance spectroscopy, diffusion tensor imaging, structural magnetic resonance imaging (MRI), resting state MRI and functional MRI.

Innovations and breakthroughs

The 27 investigations in this review validate prior findings regarding structural and functional differences in brain regions between patients with IBS and healthy volunteers, and reveal new findings related to metabolite concentrations, oscillation patterns, and neurohormones. In addition, an important finding noted across investigations is the confounding influence of patient variables such as sex, psychological factors, and gastrointestinal symptoms, upon the identification of neuroimaging differences between groups.

Applications

Neuroimaging investigations reveal central processing differences between patients with IBS and healthy controls, yet many neuroanatomical findings replicated in the IBS patient population are not specific to the IBS patient population. Further applications should recognize the substantial influence of psychological factors upon the neuroimaging correlates of IBS, use relevant comparator groups, and simultaneously assess peripheral and central aspects of the BGA, in order to optimize understanding as to how brain findings relate to IBS symptomatology.

Terminology

Functional MRI: Measures changes in the oxygen content of blood (through magnetic properties of hemoglobin); increases in oxygenation correlate with increases in neuronal activity; Resting state MRI: Evaluates spontaneous brain activity of intrinsic brain networks, taking place during a wakeful state; Structural MRI: Evaluates cortical thickness as well as white and gray matter volume or density on a global, regional and local scale; Diffusion tensor imaging: Takes advantage of the diffusivity of water to evaluate white matter anatomy and integrity as a function of fractional anisotropy; Magnetic resonance spectroscopy: Measures relative concentrations of metabolites and neurotransmitters in areas of the brain.

Peer-review

In this manuscript, the authors summarize and synthesize current literatures on Neuroimaging the Brain-Gut Axis in patients with IBS.This provided a deep insight into the physiological differences that distinguish patients with IBS from a healthy population.

REFERENCES

1 **Halmos EP**, Power VA, Shepherd SJ, Gibson PR, Muir JG. A diet low in FODMAPs reduces symptoms of irritable bowel syndrome. *Gastroenterology* 2014; **146**: 67-75.e5 [PMID: 24076059 DOI: 10.1053/j.gastro.2013.09.046]

2 **Longstreth GF**, Thompson WG, Chey WD, Houghton LA, Mearin F,

Spiller RC. Functional bowel disorders. *Gastroenterology* 2006; **130**: 1480-1491 [PMID: 16678561 DOI: 10.1053/j.gastro.2005.11.061]
3 **Canavan C**, West J, Card T. The epidemiology of irritable bowel syndrome. *Clin Epidemiol* 2014; **6**: 71-80 [PMID: 24523597 DOI: 10.2147/CLEP.S40245]
4 **Vaiopoulou A**, Karamanolis G, Psaltopoulou T, Karatzias G, Gazouli M. Molecular basis of the irritable bowel syndrome. *World J Gastroenterol* 2014; **20**: 376-383 [PMID: 24574707 DOI: 10.3748/wjg.v20.i2.376]
5 **Lovell RM**, Ford AC. Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. *Clin Gastroenterol Hepatol* 2012; **10**: 712-721.e4 [PMID: 22426087 DOI: 10.1016/j.cgh.2012.02.029]
6 **Dekel R**, Drossman DA, Sperber AD. The use of psychotropic drugs in irritable bowel syndrome. *Expert Opin Investig Drugs* 2013; **22**: 329-339 [PMID: 23316916 DOI: 10.1517/13543784.2013.761205]
7 **Agarwal N**, Spiegel BM. The effect of irritable bowel syndrome on health-related quality of life and health care expenditures. *Gastroenterol Clin North Am* 2011; **40**: 11-19 [PMID: 21333898 DOI: 10.1016/j.gtc.2010.12.013]
8 **Everhart JE**, Ruhl CE. Burden of digestive diseases in the United States Part III: Liver, biliary tract, and pancreas. *Gastroenterology* 2009; **136**: 1134-1144 [PMID: 19245868 DOI: 10.1053/j.gastro.2009.02.038]
9 **Mayer EA**, Tillisch K. The brain-gut axis in abdominal pain syndromes. *Annu Rev Med* 2011; **62**: 381-396 [PMID: 21090962 DOI: 10.1146/annurev-med-012309-103958]
10 **Ohman L**, Simrén M. New insights into the pathogenesis and pathophysiology of irritable bowel syndrome. *Dig Liver Dis* 2007; **39**: 201-215 [PMID: 17267314 DOI: 10.1016/j.dld.2006.10.014]
11 **Kennedy PJ**, Clarke G, Quigley EM, Groeger JA, Dinan TG, Cryan JF. Gut memories: towards a cognitive neurobiology of irritable bowel syndrome. *Neurosci Biobehav Rev* 2012; **36**: 310-340 [PMID: 21777613 DOI: 10.1016/j.neubiorev.2011.07.001]
12 **Camilleri M**. Physiological underpinnings of irritable bowel syndrome: neurohormonal mechanisms. *J Physiol* 2014; **592**: 2967-2980 [PMID: 24665101 DOI: 10.1113/jphysiol.2014.270892]
13 **Mayer EA**, Aziz Q, Coen S, Kern M, Labus JS, Lane R, Kuo B, Naliboff B, Tracey I. Brain imaging approaches to the study of functional GI disorders: a Rome working team report. *Neurogastroenterol Motil* 2009; **21**: 579-596 [PMID: 19646070 DOI: 10.1111/j.1365-2982.2009.01304.x]
14 **Apkarian AV**, Bushnell MC, Treede RD, Zubieta JK. Human brain mechanisms of pain perception and regulation in health and disease. *Eur J Pain* 2005; **9**: 463-484 [PMID: 15979027 DOI: 10.1016/j.ejpain.2004.11.001]
15 **Tillisch K**, Mayer EA, Labus JS. Quantitative meta-analysis identifies brain regions activated during rectal distension in irritable bowel syndrome. *Gastroenterology* 2011; **140**: 91-100 [PMID: 20696168 DOI: 10.1053/j.gastro.2010.07.053]
16 **Tillisch K**, Labus JS. Advances in imaging the brain-gut axis: functional gastrointestinal disorders. *Gastroenterology* 2011; **140**: 407-411.e1 [PMID: 21167161 DOI: 10.1053/j.gastro.2010.12.014]
17 **Gupta A**, Kilpatrick L, Labus J, Tillisch K, Braun A, Hong JY, Ashe-McNalley C, Naliboff B, Mayer EA. Early adverse life events and resting state neural networks in patients with chronic abdominal pain: evidence for sex differences. *Psychosom Med* 2014; **76**: 404-412 [PMID: 25003944 DOI: 10.1097/PSY.0000000000000089]
18 **Saab C**. Visualizing the complex brain dynamics of chronic pain. *J Neuroimmune Pharmacol* 2013; **8**: 510-517 [PMID: 22684310 DOI: 10.1007/s11481-012-9378-8]
19 **Niddam DM**, Tsai SY, Lu CL, Ko CW, Hsieh JC. Reduced hippocampal glutamate-glutamine levels in irritable bowel syndrome: preliminary findings using magnetic resonance spectroscopy. *Am J Gastroenterol* 2011; **106**: 1503-1511 [PMID: 21502999 DOI: 10.1038/ajg.2011.120]
20 **Blankstein U**, Chen J, Diamant NE, Davis KD. Altered brain structure in irritable bowel syndrome: potential contributions of pre-existing and disease-driven factors. *Gastroenterology* 2010; **138**: 1783-1789 [PMID: 20045701 DOI: 10.1053/j.gastro.2009.12.043]
21 **Seminowicz DA**, Labus JS, Bueller JA, Tillisch K, Naliboff BD, Bushnell MC, Mayer EA. Regional gray matter density changes in brains of patients with irritable bowel syndrome. *Gastroenterology* 2010; **139**: 48-57.e2 [PMID: 20347816 DOI: 10.1053/j.gastro.2010.03.049]
22 **Hong JY**, Labus JS, Jiang Z, Ashe-Mcnalley C, Dinov I, Gupta A, Shi Y, Stains J, Heendeniya N, Smith SR, Tillisch K, Mayer EA. Regional neuroplastic brain changes in patients with chronic inflammatory and non-inflammatory visceral pain. *PLoS One* 2014; **9**: e84564 [PMID: 24416245 DOI: 10.1371/journal.pone.0084564]
23 **Jiang Z**, Dinov ID, Labus J, Shi Y, Zamanyan A, Gupta A, Ashe-McNalley C, Hong JY, Tillisch K, Toga AW, Mayer EA. Sex-related differences of cortical thickness in patients with chronic abdominal pain. *PLoS One* 2013; **8**: e73932 [PMID: 24040118 DOI: 10.1371/journal.pone.0073932]
24 **Labus JS**, Dinov ID, Jiang Z, Ashe-McNalley C, Zamanyan A, Shi Y, Hong JY, Gupta A, Tillisch K, Ebrat B, Hobel S, Gutman BA, Joshi S, Thompson PM, Toga AW, Mayer EA. Irritable bowel syndrome in female patients is associated with alterations in structural brain networks. *Pain* 2014; **155**: 137-149 [PMID: 24076048 DOI: 10.1016/j.pain.2013.09.020]
25 **Piché M**, Chen JI, Roy M, Poitras P, Bouin M, Rainville P. Thicker posterior insula is associated with disease duration in women with irritable bowel syndrome (IBS) whereas thicker orbitofrontal cortex predicts reduced pain inhibition in both IBS patients and controls. *J Pain* 2013; **14**: 1217-1226 [PMID: 23871603 DOI: 10.1016/j.jpain.2013.05.009]
26 **Berman S**, Suyenobu B, Naliboff BD, Bueller J, Stains J, Wong H, Mandelkern M, Fitzgerald L, Ohning G, Gupta A, Labus JS, Tillisch K, Mayer EA. Evidence for alterations in central noradrenergic signaling in irritable bowel syndrome. *Neuroimage* 2012; **63**: 1854-1863 [PMID: 22917679 DOI: 10.1016/j.neuroimage.2012.08.028]
27 **Chen JY**, Blankstein U, Diamant NE, Davis KD. White matter abnormalities in irritable bowel syndrome and relation to individual factors. *Brain Res* 2011; **1392**: 121-131 [PMID: 21466788 DOI: 10.1016/j.brainres.2011.03.069]
28 **Ellingson BM**, Mayer E, Harris RJ, Ashe-McNally C, Naliboff BD, Labus JS, Tillisch K. Diffusion tensor imaging detects microstructural reorganization in the brain associated with chronic irritable bowel syndrome. *Pain* 2013; **154**: 1528-1541 [PMID: 23721972 DOI: 10.1016/j.pain.2013.04.010]
29 **Ma X**, Li S, Tian J, Jiang G, Wen H, Wang T, Fang J, Zhan W, Xu Y. Altered brain spontaneous activity and connectivity network in irritable bowel syndrome patients: A resting-state fMRI study. *Clin Neurophysiol* 2015; **126**: 1190-1197 [PMID: 25454279 DOI: 10.1016/j.clinph.2014.10.004]
30 **Hong JY**, Kilpatrick LA, Labus J, Gupta A, Jiang Z, Ashe-McNalley C, Stains J, Heendeniya N, Ebrat B, Smith S, Tillisch K, Naliboff B, Mayer EA. Patients with chronic visceral pain show sex-related alterations in intrinsic oscillations of the resting brain. *J Neurosci* 2013; **33**: 11994-12002 [PMID: 23864686 DOI: 10.1523/JNEUROSCI.5733-12.2013]
31 **Hong JY**, Kilpatrick LA, Labus JS, Gupta A, Katibian D, Ashe-McNalley C, Stains J, Heendeniya N, Smith SR, Tillisch K, Naliboff B, Mayer EA. Sex and disease-related alterations of anterior insula functional connectivity in chronic abdominal pain. *J Neurosci* 2014; **34**: 14252-14259 [PMID: 25339739 DOI: 10.1523/JNEUROSCI.1683-14.2014]
32 **Hall GB**, Kamath MV, Collins S, Ganguli S, Spaziani R, Miranda KL, Bayati A, Bienenstock J. Heightened central affective response to visceral sensations of pain and discomfort in IBS. *Neurogastroenterol Motil* 2010; **22**: 276-e80 [PMID: 20003075 DOI: 10.1111/j.1365-2982.2009.01436.x]
33 **Larsson MB**, Tillisch K, Craig AD, Engström M, Labus J, Naliboff B, Lundberg P, Ström M, Mayer EA, Walter SA. Brain responses to visceral stimuli reflect visceral sensitivity thresholds in patients with

irritable bowel syndrome. *Gastroenterology* 2012; **142**: 463-472.e3 [PMID: 22108191 DOI: 10.1053/j.gastro.2011.11.022]
34 **Elsenbruch S**, Rosenberger C, Enck P, Forsting M, Schedlowski M, Gizewski ER. Affective disturbances modulate the neural processing of visceral pain stimuli in irritable bowel syndrome: an fMRI study. *Gut* 2010; **59**: 489-495 [PMID: 19651629 DOI: 10.1136/gut.2008.175000]
35 **Icenhour A**, Langhorst J, Benson S, Schlamann M, Hampel S, Engler H, Forsting M, Elsenbruch S. Neural circuitry of abdominal pain-related fear learning and reinstatement in irritable bowel syndrome. *Neurogastroenterol Motil* 2015; **27**: 114-127 [PMID: 25557224 DOI: 10.1111/nmo.12489]
36 **Bouhassira D**, Moisset X, Jouet P, Duboc H, Coffin B, Sabate JM. Changes in the modulation of spinal pain processing are related to severity in irritable bowel syndrome. *Neurogastroenterol Motil* 2013; **25**: 623-e468 [PMID: 23551988 DOI: 10.1111/nmo.12123]
37 **Lee HF**, Hsieh JC, Lu CL, Yeh TC, Tu CH, Cheng CM, Niddam DM, Lin HC, Lee FY, Chang FY. Enhanced affect/cognition-related brain responses during visceral placebo analgesia in irritable bowel syndrome patients. *Pain* 2012; **153**: 1301-1310 [PMID: 22541443 DOI: 10.1016/j.pain.2012.03.018]
38 **Schmid J**, Langhorst J, Gaß F, Theysohn N, Benson S, Engler H, Gizewski ER, Forsting M, Elsenbruch S. Placebo analgesia in patients with functional and organic abdominal pain: a fMRI study in IBS, UC and healthy volunteers. *Gut* 2015; **64**: 418-427 [PMID: 24833636 DOI: 10.1136/gutjnl-2013-306648]
39 **Lowén MB**, Mayer EA, Sjöberg M, Tillisch K, Naliboff B, Labus J, Lundberg P, Ström M, Engström M, Walter SA. Effect of hypnotherapy and educational intervention on brain response to visceral stimulus in the irritable bowel syndrome. *Aliment Pharmacol Ther* 2013; **37**: 1184-1197 [PMID: 23617618 DOI: 10.1111/apt.12319]
40 **Labus JS**, Mayer EA, Jarcho J, Kilpatrick LA, Kilkens TO, Evers EA, Backes WH, Brummer RJ, van Nieuwenhoven MA. Acute tryptophan depletion alters the effective connectivity of emotional arousal circuitry during visceral stimuli in healthy women. *Gut* 2011; **60**: 1196-1203 [PMID: 21402618 DOI: 10.1136/gut.2010.213447]
41 **Aizawa E**, Sato Y, Kochiyama T, Saito N, Izumiyama M, Morishita J, Kanazawa M, Shima K, Mushiake H, Hongo M, Fukudo S. Altered cognitive function of prefrontal cortex during error feedback in patients with irritable bowel syndrome, based on FMRI and dynamic causal modeling. *Gastroenterology* 2012; **143**: 1188-1198 [PMID: 22841782 DOI: 10.1053/j.gastro.2012.07.104]
42 **Labus JS**, Gupta A, Coveleskie K, Tillisch K, Kilpatrick L, Jarcho J, Feier N, Bueller J, Stains J, Smith S, Suyenobu B, Naliboff B, Mayer EA. Sex differences in emotion-related cognitive processes in irritable bowel syndrome and healthy control subjects. *Pain* 2013; **154**: 2088-2099 [PMID: 23791896 DOI: 10.1016/j.pain.2013.06.024]
43 **Hubbard CS**, Labus JS, Bueller J, Stains J, Suyenobu B, Dukes GE, Kelleher DL, Tillisch K, Naliboff BD, Mayer EA. Corticotropin-releasing factor receptor 1 antagonist alters regional activation and effective connectivity in an emotional-arousal circuit during expectation of abdominal pain. *J Neurosci* 2011; **31**: 12491-12500 [PMID: 21880911 DOI: 10.1523/JNEUROSCI.1860-11.2011]
44 **Kilpatrick LA**, Labus JS, Coveleskie K, Hammer C, Rappold G, Tillisch K, Bueller JA, Suyenobu B, Jarcho JM, McRoberts JA, Niesler B, Mayer EA. The HTR3A polymorphism c. -42C& gt; T is associated with amygdala responsiveness in patients with irritable bowel syndrome. *Gastroenterology* 2011; **140**: 1943-1951 [PMID: 21420406 DOI: 10.1053/j.gastro.2011.03.011]
45 **Talbot JD**, Marrett S, Evans AC, Meyer E, Bushnell MC, Duncan GH. Multiple representations of pain in human cerebral cortex. *Science* 1991; **251**: 1355-1358 [PMID: 2003220]
46 **Crossman AR**, Neary D. Neuroanatomy: an illustrated colour text. 2nd ed. London: Harcourt, 2000: 133-149, 167-175
47 **Giesecke T**, Gracely RH, Grant MA, Nachemson A, Petzke F, Williams DA, Clauw DJ. Evidence of augmented central pain processing in idiopathic chronic low back pain. *Arthritis Rheum* 2004; **50**: 613-623 [PMID: 14872506 DOI: 10.1002/art.20063]
48 **Gracely RH**, Petzke F, Wolf JM, Clauw DJ. Functional magnetic resonance imaging evidence of augmented pain processing in fibromyalgia. *Arthritis Rheum* 2002; **46**: 1333-1343 [PMID: 12115241 DOI: 10.1002/art.10225]
49 **Mulak A**, Taché Y, Larauche M. Sex hormones in the modulation of irritable bowel syndrome. *World J Gastroenterol* 2014; **20**: 2433-2448 [PMID: 24627581 DOI: 10.3748/wjg.v20.i10.2433]
50 **Labus JS**, Naliboff B, Kilpatrick L, Liu C, Ashe-McNalley C, dos Santos IR, Alaverdyan M, Woodworth D, Gupta A, Ellingson BM, Tillisch K, Mayer EA. Pain and Interoception Imaging Network (PAIN): A multimodal, multisite, brain-imaging repository for chronic somatic and visceral pain disorders. *Neuroimage* 2016; **124**: 1232-1237 [PMID: 25902408 DOI: 10.1016/j.neuroimage.2015.04.018]
51 **Thompson PM**, Stein JL, Medland SE, Hibar DP, Vasquez AA, Renteria ME, Toro R, Jahanshad N, Schumann G, Franke B, Wright MJ, Martin NG, Agartz I, Alda M, Alhusaini S, Almasy L, Almeida J, Alpert K, Andreasen NC, Andreassen OA, Apostolova LG, Appel K, Armstrong NJ, Aribisala B, Bastin ME, Bauer M, Bearden CE, Bergmann O, Binder EB, Blangero J, Bockholt HJ, Bøen E, Bois C, Boomsma DI, Booth T, Bowman IJ, Bralten J, Brouwer RM, Brunner HG, Brohawn DG, Buckner RL, Buitelaar J, Bulayeva K, Bustillo JR, Calhoun VD, Cannon DM, Cantor RM, Carless MA, Caseras X, Cavalleri GL, Chakravarty MM, Chang KD, Ching CR, Christoforou A, Cichon S, Clark VP, Conrod P, Coppola G, Crespo-Facorro B, Curran JE, Czisch M, Deary IJ, de Geus EJ, den Braber A, Delvecchio G, Depondt C, de Haan L, de Zubicaray GI, Dima D, Dimitrova R, Djurovic S, Dong H, Donohoe G, Duggirala R, Dyer TD, Ehrlich S, Ekman CJ, Elvsåshagen T, Emsell L, Erk S, Espeseth T, Fagerness J, Fears S, Fedko I, Fernández G, Fisher SE, Foroud T, Fox PT, Francks C, Frangou S, Frey EM, Frodl T, Frouin V, Garavan H, Giddaluru S, Glahn DC, Godlewska B, Goldstein RZ, Gollub RL, Grabe HJ, Grimm O, Gruber O, Guadalupe T, Gur RE, Gur RC, Göring HH, Hagenaars S, Hajek T, Hall GB, Hall J, Hardy J, Hartman CA, Hass J, Hatton SN, Haukvik UK, Hegenscheid K, Heinz A, Hickie IB, Ho BC, Hoehn D, Hoekstra PJ, Hollinshead M, Holmes AJ, Homuth G, Hoogman M, Hong LE, Hosten N, Hottenga JJ, Hulshoff Pol HE, Hwang KS, Jack CR, Jenkinson M, Johnston C, Jönsson EG, Kahn RS, Kasperaviciute D, Kelly S, Kim S, Kochunov P, Koenders L, Krämer B, Kwok JB, Lagopoulos J, Laje G, Landen M, Landman BA, Lauriello J, Lawrie SM, Lee PH, Le Hellard S, Lemaître H, Leonardo CD, Li CS, Liberg B, Liewald DC, Liu X, Lopez LM, Loth E, Lourdusamy A, Luciano M, Macciardi F, Machielsen MW, Macqueen GM, Malt UF, Mandl R, Manoach DS, Martinot JL, Matarin M, Mather KA, Mattheisen M, Mattingsdal M, Meyer-Lindenberg A, McDonald C, McIntosh AM, McMahon FJ, McMahon KL, Meisenzahl E, Melle I, Milaneschi Y, Mohnke S, Montgomery GW, Morris DW, Moses EK, Mueller BA, Muñoz Maniega S, Mühleisen TW, Müller-Myhsok B, Mwangi B, Nauck M, Nho K, Nichols TE, Nilsson LG, Nugent AC, Nyberg L, Olvera RL, Oosterlaan J, Ophoff RA, Pandolfo M, Papalampropoulou-Tsiridou M, Papmeyer M, Paus T, Pausova Z, Pearlson GD, Penninx BW, Peterson CP, Pfennig A, Phillips M, Pike GB, Poline JB, Potkin SG, Pütz B, Ramasamy A, Rasmussen J, Rietschel M, Rijpkema M, Risacher SL, Roffman JL, Roiz-Santiañez R, Romanczuk-Seiferth N, Rose EJ, Royle NA, Rujescu D, Ryten M, Sachdev PS, Salami A, Satterthwaite TD, Savitz J, Saykin AJ, Scanlon C, Schmaal L, Schnack HG, Schork AJ, Schulz SC, Schür R, Seidman L, Shen L, Shoemaker JM, Simmons A, Sisodiya SM, Smith C, Smoller JW, Soares JC, Sponheim SR, Sprooten E, Starr JM, Steen VM, Strakowski S, Strike L, Sussmann J, Sämann PG, Teumer A, Toga AW, Tordesillas-Gutierrez D, Trabzuni D, Trost S, Turner J, Van den Heuvel M, van der Wee NJ, van Eijk K, van Erp TG, van Haren NE, van 't Ent D, van Tol MJ, Valdés Hernández MC, Veltman DJ, Versace A,

Völzke H, Walker R, Walter H, Wang L, Wardlaw JM, Weale ME, Weiner MW, Wen W, Westlye LT, Whalley HC, Whelan CD, White T, Winkler AM, Wittfeld K, Woldehawariat G, Wolf C, Zilles D, Zwiers MP, Thalamuthu A, Schofield PR, Freimer NB, Lawrence NS, Drevets W. The ENIGMA Consortium: large-scale collaborative analyses of neuroimaging and genetic data. *Brain Imaging Behav* 2014; **8**: 153-182 [PMID: 24399358 DOI: 10.1007/s11682-013-9269-5]

P- Reviewer: Chen JX **S- Editor**: Song XX **L- Editor**: A
E- Editor: Wu HL

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i2.334

World J Gastrointest Pharmacol Ther 2016 May 6; 7(2): 334-342
ISSN 2150-5349 (online)

SYSTEMATIC REVIEWS

Review of efficacy and safety of laxatives use in geriatrics

Manhal Izzy, Anju Malieckal, Erin Little, Sury Anand

Manhal Izzy, Division of Gastroenterology and Liver Diseases, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY 10467, United States

Manhal Izzy, Anju Malieckal, Erin Little, Sury Anand, Division of Gastroenterology, the Brooklyn Hospital Center, Brooklyn, NY 11205, United States

Anju Malieckal, Division of Gastroenterology, NYU-Lutheran Medical Center, Brooklyn, NY 11220, United States

Author contributions: Izzy M and Malieckal A contributed equally to this work; Izzy M and Malieckal A designed the research; Izzy M and Little E did the data collection and analyzed the data; Izzy M, Malieckal A and Little E wrote the paper; Anand S reviewed the results, revised the paper, and granted the final approval.

Conflict-of-interest statement: The above-mentioned authors of this manuscript hereby declare that they do not have any conflict-of-interest (including but not limited to commercial, personal, political, intellectual, or religious interests) related to the work submitted herein.

Data sharing statement: Non applicable for this type of studies (minireview without statistical analysis).

Correspondence to: Manhal Izzy, MD, Division of Gastroenterology and Liver Diseases, Montefiore Medical Center, Albert Einstein College of Medicine, 111 East 210th Street, Bronx, NY 10467, United States. mizzy@montefiore.org
Telephone: +1-718-9204154
Fax: +1-917-6720198

Received: June 29, 2015
Peer-review started: July 2, 2015
First decision: September 17, 2015
Revised: November 29, 2015
Accepted: December 17, 2015
Article in press: December 18, 2015
Published online: May 6, 2016

Abstract

AIM: To study the efficacy and safety of pharmacological treatment of constipation in geriatrics.

METHODS: PubMed, MEDLINE, google scholar, and Ovid were searched to identify human studies performed on the use of laxatives in elderly with constipation, which were conducted between January 1990 and January 2013 using the specified keywords. Controlled studies that enrolled geriatric patients with a diagnosis of constipation and addressed the efficacy and/or the safety of pharmacological treatments were included. Studies were excluded from this review if they were non-controlled trials, case series, or case reports.

RESULTS: Out of twenty three studies we initially retrieved in our search, only nine studies met the eligibility criteria of being controlled trials within geriatrics. The laxatives examined in the nine studies were senna, lactulose, sorbital, polyethylene glycol (PEG), lubiprostone, linaclotide, and prucalopride. In those studies, senna combinations had a higher efficacy than sorbitol or lactulose as well as, a very good adverse effect profile. PEG was also shown to be safe and effective in geriatric population. Furthermore, it has been shown that PEG is as safe in geriatrics as in general population. New agents like lubiprostone and prucalopride show promising results but the data about these agents in geriatrics are still limited which warrant further investigation.

CONCLUSION: Senna combinations and PEG appear to have a more favorable profile over the other traditionally used laxatives in elderly patients with constipation.

Key words: Chronic constipation; Laxatives; Elderly; Lubiprostone; Linaclotide; Prucalopride

Core tip: Laxatives are among the most commonly prescribed medications for elderly patients, however, data about safety and efficacy of laxatives in this patient population are limited. We show in this paper, based on reviewing geriatric studies, that senna combinations and polyethylene glycol appear to have better outcomes in this population than other classic laxatives. We also discuss here the promising results of the new agents, lubiprostone, linaclotide, and prucalopride, which can be helpful in treating geriatric populations in the near future.

Izzy M, Malieckal A, Little E, Anand S. Review of efficacy and safety of laxatives use in geriatrics. *World J Gastrointest Pharmacol Ther* 2016; 7(2): 334-342 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i2/334.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i2.334

INTRODUCTION

A common complaint amongst the elderly is constipation. The prevalence of constipation increases with age and some statistics estimate that around 50% of the population of adults who are 80 years old and greater will suffer from this condition at some point in time[1]. Recent estimates from the United States Census Bureau showed that the population aged 65 and greater will rise to an estimated 88.5 million in 2050 making this a growing health care concern[2]. Interestingly, the increased prevalence of constipation as patients advance in age is more pronounced amongst male than female patients[3]. The consequences of constipation are important as they do not only negatively impact quality of life but also impact the cost of care. Therefore, it is important that health care practitioners have a well-rounded understanding of the efficacy of these medications and their safety while trying to combat this growing issue in the geriatric population, defined as greater than 65 years. Chronic constipation is routinely defined as no more than three spontaneous bowel movements a week with one or more of the following symptoms for at least twelve weeks during the past year: (1) straining in greater than one-fourth of defecations; (2) lumpy or hard stool in more than one-fourth of defecations; (3) sensation of incomplete evacuation in more than one-fourth of defecations; or (4) no loose or watery bowel movements, Bristol stool form scale score 6-7[4]. Chronic constipation should be distinguished from irritable bowel syndrome-constipation which is characterized by recurrent abdominal discomfort with two or more of the following: (1) improvement with defecation; (2) onset associated with a change in frequency of stool; and (3) onset associated with a change in stool form[5]. Furthermore, there are many conditions, both physiological and iatrogenic which may contribute to the increased prevalence in the geriatric population. Commonly used medications such as antihypertensive, diuretics, pain medications and iron supplements can cause constipation[6]. Possible psychosocial and behavioral factors may also contribute to the elderly developing constipation such as dehydration, decreased mobility and inadequate caloric intake. Anorectal sensation changes may also participate when patients ignore the call to defecate, which can lead to fecal retention. Suppression of rectal sensation will lead to only large stools being perceived and can eventually lead to difficulty defecating. Elderly patients possibly have physiological changes such as failure of recto-anal coordination or pelvic floor dysfunction, which also impact their ability to defecate[7]. In one study, age related neurodegenerative changes in the enteric nervous system were observed. There was shown to be a loss of 37% of enteric neurons in the geriatric subjects when compared to the age group of 20-35 years old. However, it is important to note that these studies do not find whether the difference in quantity of neurons is due to aging or caused by changes in behavior or the chronic use of laxatives in constipated patients[8]. Management strategies differ depending on the etiology, however this article will focus on the pharmacological treatments available for management of chronic idiopathic constipation. The oral pharmacological agents used for treatment of constipation are historically classified into bulking agents, osmotic or secretive agents, and stool softeners. Recently, chloride channel blockers and selective serotonin receptor agonists, represented by lubiprostone and prucalopride, respectively, were approved for treatment of specific cases of constipation. This review will investigate both the efficacy and safety of the aforementioned medications based on trials that included elderly patients with chronic constipation.

MATERIALS AND METHODS

A systematic review of the published literature that discussed the clinical effectiveness and safety of laxatives in the management of chronic constipation in the elderly was conducted. The searched databases included PubMed, MEDLINE, Ovid, and google scholar for published literature between January 1990 and January 2013.

The following keywords were used in the search process: Constipation, chronic constipation, elderly, geriatrics, laxatives, bulking agents, senna, lactulose, sorbital, polyethylene glycol (PEG), lubiprostone, linaclotide, and prucalopride. The following filters were applied: English language, human studies, and research support (United States, Non United States, governmental and nongovernmental). Case reports

Table 1 Controlled trials that studied use of oral laxatives in elderly

Ref.	Agent	Efficacy	Safety
Kinnunen *et al*[12]	Bulk + senna *vs* lactulose (20 mL bulk + senna, 30 mL lactulose)	Bulk with senna greater than lactulose	No drug related side effects
Passmore *et al*[13]	Senna + fibre *vs* lactulose (10 mL senna + fibre, 15 mL lactulose)	Senna - fibre greater than lactulose	No difference (most common urgency and flatulence)
Lederle *et al*[16]	Sorbitol *vs* lactulose (0 to 60 mL)	Lactulose greater than sorbital	Increase nausea in lactulose
DiPalma *et al*[17]	PEG *vs* placebo (17 g PEG)	PEG greater than placebo	Increased gastrointestinal complaints
Seinelä *et al*[18]	Isotonic PEG *vs* hypotonic PEG (12 g isotonic and hypotonic PEG)	Same for hypotonic and isotonic PEG	Hyponatremia in hypotonic PEG (Not clinically significant)
Ueno *et al*[20,21]	Lubiprostone *vs* placebo (24 mcg bid lubiprostone)	Lubiprostone greater than placebo	Increase nausea in placebo and general population
Muller-Lissner *et al*[25]	Prucalopride *vs* placebo (0.5, 1, 2 mg prucalopride)	Prucalopride greater than placebo	Increased diarrhea with dosage of Prucalopride
Camilleri *et al*[26]	Prucalopride *vs* placebo (1, 2, or 4 mg prucalopride)	Efficacy not studied	Increased diarrhea with increase dosage of prucalopride
Lembo *et al*[24]	Linaclotide *vs* placebo (75, 150, 300, 600 mcg)	Linaclotide greater than placebo	Increased GI adverse effects with increasing Linaclotide dosage

PEG: Polyethylene glycol; GI: Gastrointestinal.

have been excluded from this review. The retrieved studies were screened for inclusion and exclusion criteria. Controlled studies that investigated the use of laxatives in geriatrics with chronic constipation were included. Exclusion criteria were non controlled studies, or case series. Trials on the use of the aforementioned agents in irritable bowel syndrome patients were not included.

RESULTS

A total of 23 articles were found and manually reviewed by a team of two researchers. Out of 23 studies retrieved by the search, nine met the eligibility criteria of being controlled trials with a geriatric population or subpopulation who were diagnosed with chronic constipation and therefore included in our review. The laxatives examined in the nine studies were senna, lactulose, sorbital, PEG, lubiprostone, linaclotide, and prucalopride. The studies included in the article are summarized in Table 1.

DISCUSSION

Stimulant laxatives: Senna

Classification: An anthracine glycoside, senna is manufactured from either the Cassia acutifolia or C. angustifolia plant. The dried leaflets or legumes are hydrolyzed in the colon by bacteria into anthraquiones. These free anthraquiones alter the electrolyte transportation of the colon increasing the intraluminal fluids[9,10] as well as acting as irritants on the mucosa. The result is an increase in peristalsis producing mass peristalsis stimulation in the colon which leads to defecation (Figure 1)[11].

Clinical efficacy and safety: In two studies, a senna combination laxative was compared to the commonly used laxative, lactulose, in the treatment of constipation in geriatric patients. In the first trial, thirty patients aged 65-94 years participated in the open, randomized and controlled cross over study. One week run in without laxatives, called a wash out, was followed up with a 5 wk period of a daily dose of 20 mL of bulk laxative with senna (plantago ovata, isphagula, senna pods; Agiolax) or 30 mL lactulose. This 5 wk period was followed with a week's wash out then followed with another 5 wk period with cross over medications. The results showed that bowel habits were more frequent when treated with the bulk containing senna laxative. The bulk + senna had 4.5 bowel movements per week in both 5 wk periods compared to lactulose which had 2.2 and 1.9 movements. In terms of safety, all side effects were noted on a questionnaire while blood count and serum concentrations of calcium, magnesium, potassium, sodium, albumin and creatinine were measured and analyzed by *t* test. There were no changes in laboratory measurements or complications which could be considered drug related or statistically significant in either group. Therefore, both were considered safe to use in geriatric patients[12].

In a second trial comparing senna with lactulose, Passmore *et al*[13], used a senna fibre combination (ispaghula 54%-2%, senna 12%-4%; 10 mL Manevac) or lactulose (15 mL twice daily) with matching placebo for two 14 d periods. There was a 3 to 5 d wash out period before as well as in between treatments. This trial had 77 elderly subjects with a history of constipation. Efficacy wise, results showed that mean daily bowel frequency was greater with the senna-fibre combination than lactulose. Senna-fibre had daily frequency of 0-8, with a 95%CI of 0-7 to 0-9 while lactulose had a frequency of 0-6, 0-5 to 0-7 and a $P < 0.001$. As with the previous trial, safety showed no significant statistical difference between the two treatments. Most common adverse effects in both treatments were an increased in

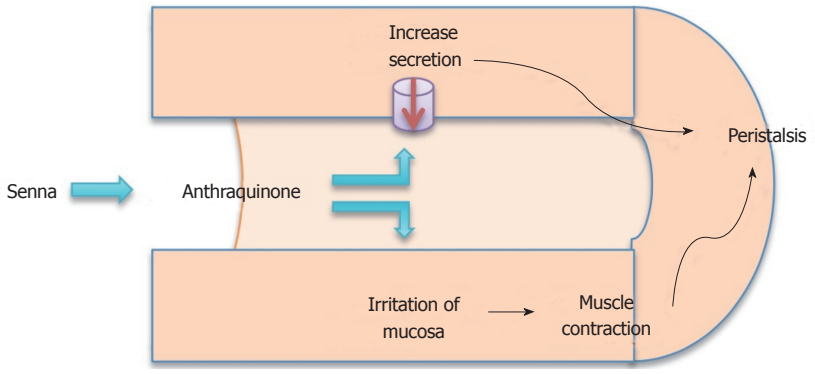

Figure 1 Mechanism of action of senna.

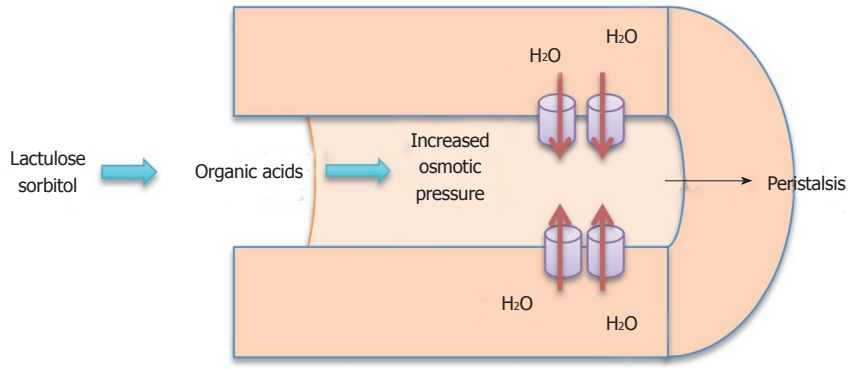

Figure 2 Mechanism of action of osmotic laxatives.

urgency and flatulence[13].

Osmotic laxatives: Lactulose, sorbitol and PEG

Classification: Lactulose and sorbitol are both non-absorbable disaccharides which pass unchanged into the colon where they are metabolized by bacteria into formic, acetic and lactic acids. The organic acids produced increase intraluminal fluid in the colon (Figure 2)[7]. PEG is an osmotic laxative that is minimally absorbed in the colonic tract. PEG softens stool and increases stool volume which lead to increased peristalsis[14]. PEG is often used in bowel preparations for colonoscopy in the elderly, as well as in treatment of constipation[15].

Clinical efficacy and safety: Lactulose was compared to sorbitol in 30 patients aged 65 to 86 in one trial which was randomized, double blind, cross over trial conducted by Lederle *et al*[16]. Patients were given either lactulose or 70% sorbitol for 4 wk after a 2-wk washout period and crossed over to take another 4 wk period of the other medication. The results showed an average number of bowel movements per week of 6.71 with sorbitol and 7.02 for patients on lactulose with a 95%CI of -0.43 to 1.06. Common side effects of osmotic laxatives are bloating, flatulence and diarrhea. In this trial, adverse symptoms were recorded by participants in a daily diary. The only difference between the two treatments was nausea. The score for nausea was significantly high in lactulose treatment than in sorbitol with a P value of < 0.05. However, overall the most common adverse effect reported was flatulence with 23 participants suffering from it at some point during the trial. Overall, sorbitol and lactulose had no difference in effect but sorbitol appears to be not only safer to use in the elderly but is also more cost effective[16].

This trial by DiPalma *et al*[17] was designed using PEG to evaluate its safety and efficacy compared to a placebo (maltodextrin) over a six-month period. A total of 304 patients were enrolled in the double-blind, placebo-controlled, parallel, multicenter study to receive PEG laxative as a single daily dose of 17 g or placebo for 6 mo. In the trial, there were 75 subjects older than 65 years. A baseline for constipation status was established during a 14-d observation period. Success was defined as relief of criteria for constipation in 50% or more of their weeks of treatment. This long term trial showed that use of PEG was better at achieving success in comparison to the placebo at the 6 mo mark in both the total subject population (52.0% of PEG and 11% of placebo subjects; $P < 0.001$) and the geriatric subpopulation (61% of PEG treatment weeks *vs* 22% of the placebo weeks; $P < 0.001$). Throughout the trial there were no statistically significant differences in adverse effects except in gastrointestinal issues (40% *vs* 25%, $P = 0.015$), which included nausea, diarrhea and flatulence but were mild and self-limiting. There were also no clinically significant laboratory changes

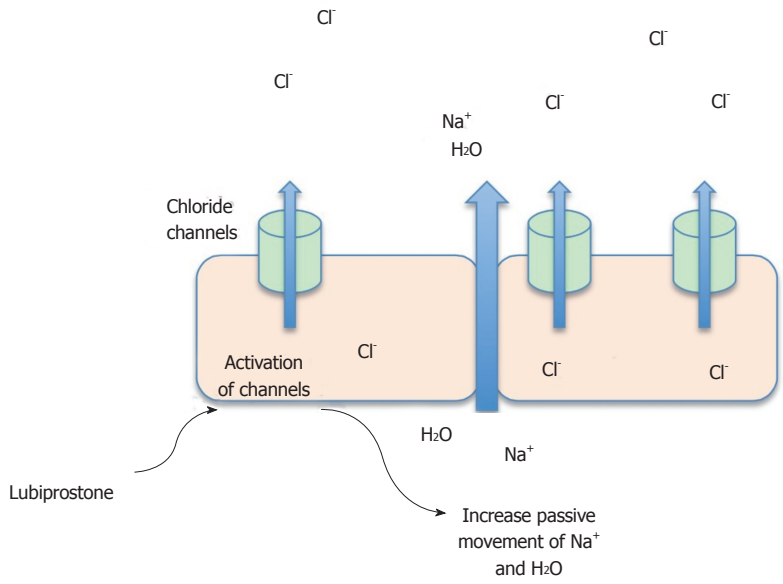

Figure 3 Mechanism of action of lubiprostone.

observed during the trial. Similar results were observed for the elderly subpopulation[17].

In a trial completed by Seinelä *et al*[18], the use of PEG with and without electrolytes was compared in terms of both efficacy and safety. This trial focused on the geriatric population with 62 participants receiving isotonic PEG for one week before patients were randomly assigned to either the hypotonic PEG or isotonic PEG group for the next 4 wk. At the end of 4 wk, the results showed a mean weekly stool frequency of 8.5 in the hypotonic and 8.4 in the isotonic PEG groups. The mean stool frequency ratio was calculated to be 0.90 with a 95%CI (0.74-1.10). Therefore, both isotonic and hypotonic PEG can be considered equal in efficacy. While there was no difference between groups in terms of straining or gastrointestinal complaints, plasma sodium levels were statistically significantly lower in the hypotonic PEG group (137.7 mmol/L *vs* 138.9 mmol/L, $P = 0.012$). However there are no clinical differences detected between testing groups and no intervention was needed[18].

Prosecretory agents: Chloride channel activator (lubiprostone)

Classification: Lubiprostone is a bicyclic fatty acid compound classified as a prostone. It is derived from a prostaglandin E1 metabolite. It acts by inducing secretion of both electrolytes and fluid through the activation of type 2 chloride channels in the small intestine. It also appears to reduce gastric emptying and increase gastric volume during fasting time (Figure 3)[19].

Clinical efficacy and safety: Two abstracts were presented by Ueno *et al*[20], which looked at the safety and efficacy of lubiprostone in the elderly. The first abstract was sub-analysis from a number of controlled trials that included a subgroup of elderly patients, 57 patients aged > 65 years who were randomized to lubiprostone 48 mcg/d or placebo for 4 wk. Spontaneous bowel movements significantly improved amongst the lubiprostone elderly group compared to the placebo elderly group ($P \leqslant 0.0286$). Increase in frequency of weekly bowel movements ranged from 4.6 to 5.4 bowel movements per week for elderly lubiprostone subjects compared to 1.29 to 2.27 bowel movements for elderly placebo subjects. Also, it is important to note was that fewer adverse effects were reported in the lubiprostone group *vs* subjects treated with the placebo (46% *vs* 61%, *P* not reported)[20]. The second abstract was pooled analysis of elderly population of open labeled trials, which included 163 elderly participants (> 65 years) and 715 non-elderly subjects. Fewer elderly patients reported adverse effects at 74.2% *vs* 80.1% in the non-elderly subjects. The most common reported side effect was nausea throughout the trial. Improvement in constipation severity and abdominal bloating or discomfort were significantly better in patients who received lubiprostone compared to placebo group of both elderly and non elderly patients[21]. Of note, another abstract presented by the same group about the safety of lubiprostone in general, regardless of the elderly status, showed that the side effects encountered with this medication which are mainly nausea, headache, and diarrhea are generally mild to moderate in severity, intermittent, and limited in duration[22].

Prosecretory agents: Guanylate cyclase C agonist (linaclotide)

Classification: Linaclotide is a 14 amino acid peptide that acts as a guanylate cyclase C (GC-C) agonist. Linaclotide binds to GC-C receptor on the surface on intestinal enterocytes which increases guanosine mono-

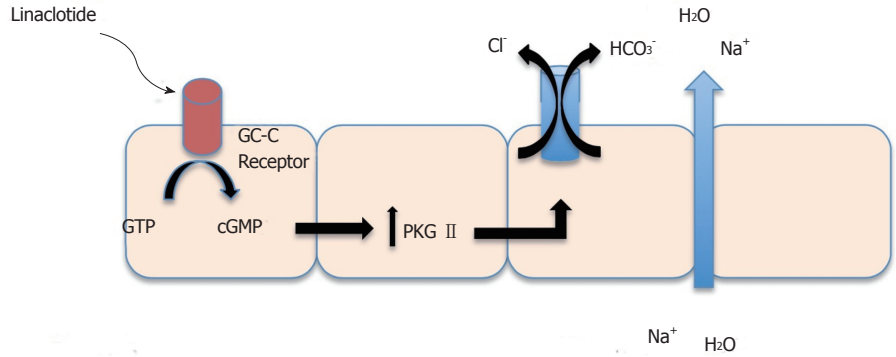

Figure 4 Mechanism of action of linaclotide.

phosphate triggering a signal transduction cascade resulting in the activation of cystic fibrosis transmembrane conductance regulator (Figure 4). This activation will result in chloride and fluid secretion into the lumen of the intestines as well as acceleration of intestinal transit[23].

Clinical efficacy and safety: One study for linaclotide which looked at a geriatric subgroup was completed by Lembo *et al*[24]. A total of 310 patients with a mean age of 47.3 years participated in the study with a subpopulation of 30 geriatric patients. The patients were randomly assigned to receive 75, 150, 300, or 600 g oral linaclotide or placebo once daily for 4 wk. The efficacy of linaclotide generally improved with increasing doses from 75 to 600 μg per day. Spontaneous bowel movement frequency in the 4-wk treatment period showed a linear dose-response with increases of 2.6, 3.3, 3.6, and 4.3 for linaclotide doses of 75, 150, 300, and 600 μg, respectively, compared to 1.5 for placebo with a *P* valve of less than 0.05 for each pairwise comparison. There was also a change in stool consistency. There was a 0.50 mean change for patients that received the placebo in comparison to 1.35, 1.57, 1.68 and 2.00 for linaclotide doses of 75, 150, 300 and 600 μg ($P \leqslant 0.0005$ for each dose of linaclotide). Linaclotide appears to be equally effective in the geriatric subpopulation as the general study population. Linaclotide was overall well-tolerated in this study population. Adverse effects occurred in 33.8% of patients receiving linaclotide while only 31.9% of patients receiving a placebo reported an adverse effect. Most of the adverse effects in this study were related to the GI tract. Diarrhea, mild to moderate in severity was the most commonly reported effect which is an expected result of linaclotide's pharmacology. The rate of adverse effects is slightly greater in patients receiving 600 μg linaclotide (38.1%) compared with the other linaclotide groups (29.0% to 35.0%)[24].

Selective 5HT$_4$ receptor agonist: Prucalopride

Classification: Prucalopride, a selective 5-HT$_4$ receptor agonist has strong enterokinetic activity. It is believed that patients with constipation have decreased frequency and duration of the giant migrating contractions in their colons. These high amplitude contractions stimulate the urge to defecate. Control of these contractions is suggested to involve serotonin release and its action on 5-HT$_4$ receptors. Therefore, it is believed that selective stimulation of these receptors would elicit strong enterokinetic activity in the colon and help restore the physiologic colonic motility (Figure 5)[25].

Clinical efficacy and safety: In a study with patients aged > 65 years, a 2 wk wash out period was followed by 4 wk of either 1, 2, 4 mg prucalopride or placebo daily with no change in diet or lifestyle. Efficacy was measured through patient's global assessment. It was found that patients on prucalopride in 1 or 4 mg doses reported a mean improvement in severity of constipation significantly higher that what was reported in the placebo group. At the end of the 4th week, 42% of patients receiving 1 mg, 24% of those receiving 2 mg, and 39% of those receiving 4 mg of prucalopride considered the treatment either moderately effective or extremely effective. However, only 16% of the placebo group believed their treatment was successful ($P < 0.001$ for 1 mg prucalopride *vs* placebo, $P < 0.05$ for both 2 and 4 mg prucalopride *vs* placebo)[25]. A second study looked at prucalopride in 89 elderly patients using dosages of 0.5, 1, and 2 mg *vs* placebo in order to compare adverse effects. This trial focused only on the safety of prucalopride in the geriatric population considering the notorious safety profile of other less selective serotonergic prokinetic agents such as tegaserod and cisapride. These drugs have been associated with serious cardiovascular side effects. Tegaserod was withdrawn for the United States market due to the number of patients that reported serious side effects, mainly ischemic cardiovascular events. Therefore the question was asked if prucalopride would also have adverse cardiovascular effects especially in a high-risk population like the elderly. This trial recorded not only reported adverse effects by the participants but also laboratory studies and cardiovascular parameters such as vital signs, EKGs, and Holter monitors. There were no clinically relevant or dose-related effects measured in laboratory. Similarly, no changes were noted in vital

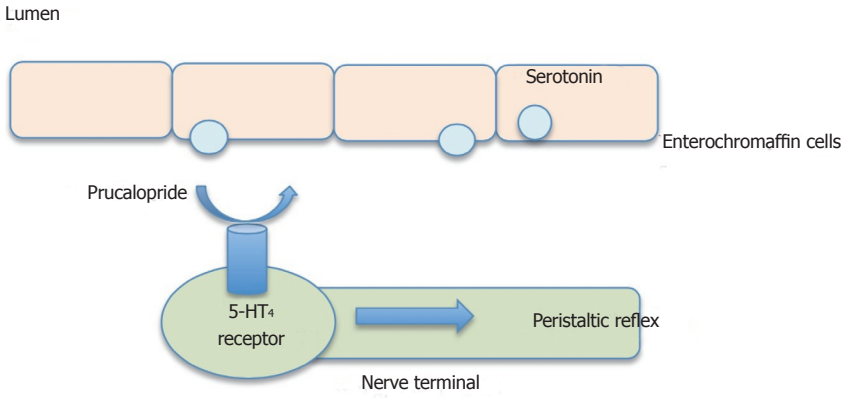

Figure 5 Mechanism of action of prucalopride.

signs between prucalopride and the placebo. Extensive ECG and Holter monitoring confirmed that prucalopride does not cause the induction of ventricular arrhythmias, QT prolongation or torsade de pointes. The majority of adverse effects were gastrointestinal related, diarrhea and abdominal pain being the most common. Diarrhea increased with the dose of prucalopride with none being reported in the placebo group[26].

Emerging treatments (pending trials in geriatrics)

Chenodeoxycholic acid (CDCA) is one of the major bile acids in the human biliary system. A small percentage of bile acids are not absorbed in the terminal ileum, rather they move into the proximal colon where they undergo modification by colonic bacteria to form secondary bile acids which induce colonic secretion[27]. Furthermore, there is evidence that bile acids are able to modify colonic motility separately from its secretory effects[28]. When used at pharmacological dosages, CDCA helps to reduce cholesterol saturation in bile thereby leading to the eventual dissolving of cholesterol gallstones. One of the most common side effects of using CDCA is diarrhea[29]. In one study using high doses of CDCA (750-1000 mg/d) in order to observe bile-lipid composition and common side effects, 40% of patients had diarrhea[30]. A more recent study conducted by Rao *et al*[31], showed accelerated colonic transit and improved bowel function in 36 females with Irritable Bowel Syndrome - constipation subtype[31]. However, larger studies with a geriatric population will need to be completed to determine whether these benefits can be replicated and maintained over a longer time period.

Elobixiblat (A3309) is an oral agent that decreases the reabsorption of bile acids in the terminal ileum by inhibiting bile acid transporters thereby increasing the concentration of bile acids that enter the colon. Studies of A3309 have shown acceleration in colon transit with relief of constipation related symptoms in patients[32]. One study of 36 women with functional chronic constipation in a double blind placebo controlled study focused on colonic transit at 24 then 48 h compared to placebo after 14 d of treatment. At both dosages of 15 and 20 mg, colonic transit was accelerated compared to the placebo. Improvement in stool consistency and straining by participants was also reported[33].

Colchicine is an anti-inflammatory medication used in the treatment of gout. It works by inhibiting micro-tubule assembly in white blood cells but has been shown to cause diarrhea when taken in higher dose. It is believed that colchicine increases the production of prostaglandins, increases gastrointestinal motility and secretion as well as decreasing the absorption of water and electrolytes in the intestine. One study done as a double-blind, placebo-controlled trial used participants diagnosed with slow transit constipation. The trial used low dose colchicine (1 mg daily) in an effort to improve symptoms and increase the number of spontaneous bowel movements. In this trial, patients using colchicine showed improvement in bloating and abdominal pain. They reported increased number of bowel movements. Eventually all participants were placed on open label colchicine for one month after the duration of the study was completed due to the considerable beneficial effects[34].

Conclusion

As the population in the Unites States continues to age, constipation will increase in prevalence, having an impact on the functional status and quality of life for many patients[6]. Traditionally, the first line pharmacological agents used to be lactulose, sorbitol and senna[8]; however, in studies that involved geriatric populations, senna combinations have shown greater efficacy and a more favorable side effect profile. Sorbitol had roughly the same efficacy as lactulose but had a better side effect and cost profile, which makes it an attractive alternative to using lactulose. PEG, another osmotic laxative, was shown to be effective in geriatric populations in comparison to general adult populations. Interestingly, geriatrics patients did not have more side effects than the general adult participants. Trials showed that lubiprostone has good outcomes in geriatric population in terms of both efficacy and safety. Similarly, prucalopride shows a great potential. Negative cardiovascular interactions were the primary concern with prucalopride. However, prucalopride did

not produce adverse cardiovascular effects throughout the trial. Both lubiprostone and prucalopride need further studies to determine their efficacy in geriatrics compared to common treatments such as senna and PEG but they remain a potential option in the treatment of constipation. Though studies of linaclotide have shown significant improvement in constipation, clinical trials for linaclotide with a larger geriatric population are needed to determine real life applicability in the elderly. Emerging treatments using CDCA, A3309 and colchicine for constipation show promise for the future. However, studies comparing these medications to older treatments are necessary to evaluate efficacy and whether the benefits can be reproduced for long-term use in the geriatric population.

COMMENTS

Background

Constipation in the elderly is a growing health care concern in the United States. It has a remarkable impact on their functional status and quality of life. As physicians treat elderly patients with this condition, it is important to know the efficacy and safety of the drugs they choose.

Research frontiers

The current literature lacks a review of the efficacy and safety of different classes of laxatives in the treatment of chronic constipation among elderly populations.

Innovations and breakthroughs

This review suggests that, in geriatric populations, senna combinations and polyethylene glycol are more efficacious than other traditionally-used laxatives including but not limited to lactulose and sorbitol. Lubiprostone, prucalopride, and linaclotide have been showing promising results but further geriatric studies are warranted.

Applications

The authors suggest prescribing senna combinations and/or polyethelene glycol as the first line for the treatment of chronic constipation in geriatrics. Routine use of the new, potentially effective medications (*i.e.*, Lubiprostone, Prucalopride, and Linaclotide) is pending further studies in this patient population.

Peer-review

The review is a good starting point.

REFERENCES

1 **Gandell D**, Straus SE, Bundookwala M, Tsui V, Alibhai SM. Treatment of constipation in older people. *CMAJ* 2013; **185**: 663-670 [PMID: 23359042 DOI: 10.1503/cmaj.120819]
2 Department of Health & Human Services: Adminstration on Aging (5/8/2013). [accessed 2013 Sept 20]. Available from: URL: http://www.aoa.gov/Aging_Statistics/future_growth/future_growth.aspx
3 **Werth BL**, Williams KA, Pont LG. A longitudinal study of constipation and laxative use in a community-dwelling elderly population. *Arch Gerontol Geriatr* 2015; **60**: 418-424 [PMID: 25736738 DOI: 10.1016/j.archger.2015.02.004]
4 **Bharucha AE**, Dorn SD, Lembo A, Pressman A. American Gastroenterological Association medical position statement on constipation. *Gastroenterology* 2013; **144**: 211-217 [PMID: 23261064 DOI: 10.1053/j.gastro.2012.10.029]
5 **Drossman DA**. The functional gastrointestinal disorders and the Rome III process. *Gastroenterology* 2006; **130**: 1377-1390 [PMID: 16678553]
6 **Rao SS**, Go JT. Update on the management of constipation in the elderly: new treatment options. *Clin Interv Aging* 2010; **5**: 163-171 [PMID: 20711435]
7 **Crane SJ**, Talley NJ. Chronic gastrointestinal symptoms in the elderly. *Clin Geriatr Med* 2007; **23**: 721-734, v [PMID: 17923334]
8 **Bouras EP**, Tangalos EG. Chronic constipation in the elderly. *Gastroenterol Clin North Am* 2009; **38**: 463-480 [PMID: 19699408 DOI: 10.1016/j.gtc.2009.06.001]
9 **Mascolo N**, Meli R, Autore G, Capasso F. Senna still causes laxation in rats maintained on a diet deficient in essential fatty acids. *J Pharm Pharmacol* 1988; **40**: 882-884 [PMID: 2907584]
10 **Dufour P**, Gendre P. Ultrastructure of mouse intestinal mucosa and changes observed after long term anthraquinone administration. *Gut* 1984; **25**: 1358-1363 [PMID: 6510768]
11 **Frexinos J**, Staumont G, Fioramonti J, Bueno L. Effects of sennosides on colonic myoelectrical activity in man. *Dig Dis Sci* 1989; **34**: 214-219 [PMID: 2914540]
12 **Kinnunen O**, Winblad I, Koistinen P, Salokannel J. Safety and efficacy of a bulk laxative containing senna versus lactulose in the treatment of chronic constipation in geriatric patients. *Pharmacology* 1993; **47** Suppl 1: 253-255 [PMID: 8234438]
13 **Passmore AP**, Wilson-Davies K, Stoker C, Scott ME. Chronic constipation in long stay elderly patients: a comparison of lactulose and a senna-fibre combination. *BMJ* 1993; **307**: 769-771 [PMID: 8219947]
14 **Corazziari E**, Badiali D, Bazzocchi G, Bassotti G, Roselli P, Mastropaolo G, Lucà MG, Galeazzi R, Peruzzi E. Long term efficacy, safety, and tolerabilitity of low daily doses of isosmotic polyethylene glycol electrolyte balanced solution (PMF-100) in the treatment of functional chronic constipation. *Gut* 2000; **46**: 522-526 [PMID: 10716682]
15 **Lashner BA**, Winans CS, Blackstone MO. Randomized clinical trial of two colonoscopy preparation methods for elderly patients. *J Clin Gastroenterol* 1990; **12**: 405-408 [PMID: 2204651]
16 **Lederle FA**, Busch DL, Mattox KM, West MJ, Aske DM. Cost-effective treatment of constipation in the elderly: a randomized double-blind comparison of sorbitol and lactulose. *Am J Med* 1990; **89**: 597-601 [PMID: 2122724]
17 **Dipalma JA**, Cleveland MV, McGowan J, Herrera JL. A randomized, multicenter, placebo-controlled trial of polyethylene glycol laxative for chronic treatment of chronic constipation. *Am J Gastroenterol* 2007; **102**: 1436-1441 [PMID: 17403074]
18 **Seinelä L**, Sairanen U, Laine T, Kurl S, Pettersson T, Happonen P. Comparison of polyethylene glycol with and without electrolytes in the treatment of constipation in elderly institutionalized patients: a randomized, double-blind, parallel-group study. *Drugs Aging* 2009; **26**: 703-713 [PMID: 19685935 DOI: 10.2165/11316470-000000000-00000]
19 **Lacy BE**, Levy LC. Lubiprostone: a novel treatment for chronic constipation. *Clin Interv Aging* 2008; **3**: 357-364 [PMID: 18686757]
20 **Ueno R**, Joswick TR, Wahle A, Zhu Y, Holland PC. Efficacy and safety of lubiprostone for the treatment of chronic constipation in elderly vs. non-elderly subjects. *Gastroenterology* 2006; **130** (Suppl 2): A189. Abstract S1262
21 **Ueno R**, Panas R, Wahle A, Zhu Y, Holland PC. Long-term safety and efficacy of lubiprostone for the treatment of chronic constipation in elderly subjects. *Gastroenterology* 2006; **130** (Suppl 2): A-188. Abstract S1260
22 **Ueno R**, Wahle A, Rivera E. Pooled analysis of the most frequent adverse events associated with the use of lubiprostone. *Am J Gastroenterol* 2006; **101** (Suppl 2): S489. Abstract 1264
23 **Schulz S**, Lopez MJ, Kuhn M, Garbers DL. Disruption of the guanylyl cyclase-C gene leads to a paradoxical phenotype of viable but heat-stable enterotoxin-resistant mice. *J Clin Invest* 1997; **100**: 1590-1595 [PMID: 9294128]
24 **Lembo AJ**, Kurtz CB, Macdougall JE, Lavins BJ, Currie MG, Fitch DA, Jeglinski BI, Johnston JM. Efficacy of linaclotide for patients with chronic constipation. *Gastroenterology* 2010; **138**: 886-895.e1 [PMID: 20045700 DOI: 10.1053/j.gastro.2009.12.050]
25 **Müller-Lissner S**, Rykx A, Kerstens R, Vandeplassche L. A double-blind, placebo-controlled study of prucalopride in elderly patients with

chronic constipation. *Neurogastroenterol Motil* 2010; **22**: 991-98, e255 [PMID: 20529205 DOI: 10.1111/j.1365-2982.2010.01533.x]
26 **Camilleri M**, Beyens G, Kerstens R, Robinson P, Vandeplassche L. Safety assessment of prucalopride in elderly patients with constipation: a double-blind, placebo-controlled study. *Neurogastroenterol Motil* 2009; **21**: 1256-e117 [PMID: 19751247 DOI: 10.1111/j.1365-2982.2009.01398.x]
27 **Mekjian HS**, Phillips SF, Hofmann AF. Colonic secretion of water and electrolytes induced by bile acids: perfusion studies in man. *J Clin Invest* 1971; **50**: 1569-1577 [PMID: 4938344]
28 **Bajor A**, Gillberg PG, Abrahamsson H. Bile acids: short and long term effects in the intestine. *Scand J Gastroenterol* 2010; **45**: 645-664 [PMID: 20334475 DOI: 10.3109/00365521003702734]
29 **Iser JH**, Sali A. Chenodeoxycholic acid: a review of its pharmacological properties and therapeutic use. *Drugs* 1981; **21**: 90-119 [PMID: 7009140]
30 **Mok HY**, Bell GD, Dowling RH. Effect of different doses of chenodeoxycholic acid on bile-lipid composition and on frequency of side-effects in patients with gallstones. *Lancet* 1974; **2**: 253-257 [PMID: 4135688]
31 **Rao AS**, Wong BS, Camilleri M, Odunsi-Shiyanbade ST, McKinzie S, Ryks M, Burton D, Carlson P, Lamsam J, Singh R, Zinsmeister AR. Chenodeoxycholate in females with irritable bowel syndrome-constipation: a pharmacodynamic and pharmacogenetic analysis. *Gastroenterology* 2010; **139**: 1549-158, 1558.e1 [PMID: 20691689 DOI: 10.1053/j.gastro.2010.07.052]
32 **Wong BS**, Camilleri M. Elobixibat for the treatment of constipation. *Expert Opin Investig Drugs* 2013; **22**: 277-284 [PMID: 23215781 DOI: 10.1517/13543784.2013.753056]
33 **Wong BS**, Camilleri M, McKinzie S, Burton D, Graffner H, Zinsmeister AR. Effects of A3309, an ileal bile acid transporter inhibitor, on colonic transit and symptoms in females with functional constipation. *Am J Gastroenterol* 2011; **106**: 2154-2164 [PMID: 21876564 DOI: 10.1038/ajg.2011.285]
34 **Taghavi SA**, Shabani S, Mehramiri A, Eshraghian A, Kazemi SM, Moeini M, Hosseini-Asl SM, Saberifiroozi M, Alizade-Naeeni M, Mostaghni AA. Colchicine is effective for short-term treatment of slow transit constipation: a double-blind placebo-controlled clinical trial. *Int J Colorectal Dis* 2010; **25**: 389-394 [PMID: 19705134 DOI: 10.1007/s00384-009-0794-z]

P- Reviewer: Carroccio A, Shailubhai K **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Wu HL

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

ISSN 2150-5349 (online)

World Journal of *Gastrointestinal Pharmacology and Therapeutics*

World J Gastrointest Pharmacol Ther 2016 August 6; 7(3): 343-468

Published by Baishideng Publishing Group Inc

WJGPT

World Journal of Gastrointestinal Pharmacology and Therapeutics

A peer-reviewed, online, open-access journal of gastrointestinal pharmacology and therapeutics

Editorial Board

2016-2019

The *World Journal of Gastrointestinal Pharmacology and Therapeutics* Editorial Board consists of 394 members, representing a team of worldwide experts in surgery research. They are from 45 countries, including Argentina (3), Australia (12), Austria (3), Belarus (1), Belgium (4), Brazil (10), Canada (10), China (39), Czech Repoublic (1), Egypt (3), Estonia (1), Finland (1), France (6), Germany (19), Greece (7), Hungary (4), India (17), Iran (5), Ireland (1), Israel (5), Italy (32), Japan (34), Lebanon (1), Lithuania (3), Mexico (2), Netherlands (10), New Zealand (2), Norway (2), Pakistan (1), Philippines (1), Poland (3), Portugal (2), Romania (1), Russia (1), Saudi Arabia (3), Singapore (3), Slovenia (1), South Africa (1), South Korea (14), Spain (13), Sweden (3), Thailand (4), Turkey (6), United Kingdom (19) and United States (80).

Duo Li, *Hangzhou*
Yu-Yuan Li, *Guangzhou*
Xiong Ma, *Shanghai*
Qin Pan, *Shanghai*
Guo-Ping Sun, *Hefei*
Xue-Ying Sun, *Harbin*
Ming-Fu Wang, *Hong Kong*
Che-Yuen Justin Wu, *Hong Kong*
De Xiang Xu, *Hefei*
Ruian Xu, *Xiamen*
Ming-Xian Yan, *Jinan*
Yong-Feng Yang, *Nanjing*
Thomas Yau, *Hong Kong*
Win-Nei Yeo, *Hong Kong*
Long Yu, *Guangzhou*
Jian-Ping Yuan, *Guangzhou*
Man-Fung Yuen, *Hong Kong*
Liqun Zhang, *Beijing*
Min-Sheng Zhu, *Nanjing*

Czech Republic

Rene Kizek, *Brno*

Denmark

Rene Kizek, *Brno*

Egypt

Omar ME Abdel-Salam, *Cairo*
Ahmed Osman Abdel-Zaher, *Assiut*
Osama Ahmed Badary, *Cairo*

Estonia

Riin Tamm, *Tartu*

Finland

Riitta Korpela, *Helsinki*

France

Tarik Asselah, *Clichy*
Ferrand Audrey, *Toulouse*
Frederic Batteux, *Paris*
Thierry Capiod, *Paris*
Frederic Lagarce, *Angers*
Hang Thi Thu Nguyen, *Clermont-Ferrand*

Germany

Susanne Beckebaum, *Essen*
Jürgen Borlak, *Hannover*
Güralp Onur Ceyhan, *Munich*
Walee Chamulitrat, *Heidelberg*
Anton Gillessen, *Munster*
Dirk Heitzmann, *Muenster*
Jens Michael Heyn, *München*
Joachim Labenz, *Siegen*
Thomas Müller, *Berlin*
Belal Naser, *Salzgitter*
Beate Niesler, *Heidelberg*
Matthias Ocker, *Marburg*
Andreas Marc Palmer, *Konstanz*
Dirk Rades, *Luebeck*
Fuat Hakan Saner, *Essen*
Manfred V Singer, *Mannheim*
Konrad Ludwig Streetz, *Aachen*
Frank Tacke, *Aachen*
Gerhard Treiber, *Balingen*

Greece

Moses S Elisaf, *Ioannina*
Anastasios Koulaouzidis, *Greek*
Ioannis E Koutroubakis, *Heraklion*
Spilios Manolakopoulos, *Athens*
Konstantinos Christou Mountzouris, *Athens*
George V Papatheodoridis, *Athens*
Konstantinos Tziomalos, *Thessaloniki*

Hungary

Zsolt Barta, *Debrecen*
László Czakó, *Szeged*
Bela Molnar, *Budapest*
Gyula Mózsik, *Pecs*

India

Anil Kumar Agarwal, *New Delhi*
Sandip Basu, *Bombay*
Chiranjib Chakraborty, *Vellore*
Rukhsana Chowdhury, *Kolkata*
C M Habibullah, *Hyderabad*
Mohandas K Mallath, *Mumbai*
Balraj Mittal, *Lucknow*
Rama Devi Mittal, *Lucknow*
Asish K Mukhopadhyay, *Kolkata*
Lekha Saha, *Chandigarh*
Darisetty Santosh, *Hyderabad*
Shiv Kumar Sarin, *New Delhi*
Jayshri Ankur Shah, *Mumbai*
Sonu Sundd Singh, *Gurgaon*
Sikta Swarnakar, *Calcutta*
Rakesh Kumar Tandon, *New Delhi*
Asna Urooj, *Mysore*

Iran

Seyed Mohsen Dehghani, *Shiraz*
Ahmad Reza Dehpour, *Tehran*
Sara Farhang, *Tabriz*
Parisa Hasanein, *Hamadan*
Amir Mohammad Mortazavian, *Tehran*

Ireland

Zaid S Heetun, *Dublin*

Israel

Nimer Assy, *Safed*
Simon Bar-Meir, *Tel-Hashomer*
Rami Eliakim, *Haifa*
Tiberiu Hershcovici, *Jerusalem*
Haim Shmuel Odes, *Beer Sheva*

Italy

Giovanni C Actis, *Turin*
Bruno Annibale, *Roma*
Leonardo Baiocchi, *Rome*
Gabrio Bassotti, *San Sisto*
Francesca Borrelli, *Napoli*
Giuseppe Brisinda, *Rome*
Renzo Caprilli, *Rome*
Mauro Antonio Maria Carai, *Cagliari*
Renato Caviglia, *Rome*
Carolina Ciacci, *Naples*
Mario Cottone, *Trabucco*
Roberto De Giorgio, *Bologna*
Luca Elli, *Milano*
Alessandro Granito, *Bologna*
Francesco William Guglielmi, *Bari*
Mario Guslandi, *Milano*
Pietro Invernizzi, *Monza*
Mariano Malaguarnera, *Catania*
Massimo C Mauri, *Milan*
Massimo Montalto, *Rome*
Giovanni Monteleone, *Rome*
Gerardo Nardone, *Naples*
Fabio Pace, *Seriate*
Raffaele Pezzilli, *Bologna*
Rita Rezzani, *Brescia*
Carmelo Scarpignato, *Parma*
Generoso Uomo, *Napoli*
Paolo Usai-Satta, *Cagliari*
Maurizio Vecchi, *Milan*
Massimiliano Veroux, *Catania*

Japan

Akira Andoh, *Otsu*
Yuichiro Eguchi, *Saga*
Munechika Enjoji, *Fukuoka*
Norihiro Furusyo, *Fukuoka*
Naoki Hotta, *Nagoya*
Shigeo Ikegawa, *Higashi-Osaka*
Susumu Ito, *Okinawa*
Satoru Kakizaki, *Maebashi*
Terumi Kamisawa, *Tokyo*
Motoyori Kanazawa, *Miyagi*
Takuma Kato, *Mie*
Takashi Kawai, *Tokyo*
Shirao Kuniaki, *Oita*
Nobuyuki Matsuhashi, *Tokyo*
Tatsuya Matsura, *Yonago*
Teruo Murakami, *Hiroshima*
Yuji Naito, *Kyoto*
Katsuyuki Nakajima, *Maebashi Gunma*
Hiroshi Nakase, *Kyoto*
Nobuhiro Ohkohchi, *Tsukuba*
Shogo Ohkoshi, *Niigata*
Tomohiko Shimatani, *Kure*
Yasuhiko Sugawara, *Tokyo*
Yoshitaka Takuma, *Okayama*
Tatsuhiro Tsujimoto, *Nara*
Takato Ueno, *Fukuoka*
Kenji Watanabe, *Osaka*
Toshiaki Watanabe, *Tokyo*

Jiro Watari, *Nishinomiya*
Satoshi Yamagiwa, *Niigata*
Takayuki Yamamoto, *Mie*
Norimasa Yoshida, *Kyoto*
Hitoshi Yoshiji, *Kashihara*
Katsutoshi Yoshizato, *Higashihiroshima*

Lebanon

AIa Sharara, *Beirut*

Lithuania

Dalia Adukauskiene, *Kaunas*
Giedrius Barauskas, *Kaunas*
Laimas Jonaitis, *Kaunas*

Mexico

Pablo Muriel, *Mexico City*
Guillermo Benito Robles-Díaz, *Mexico City*

Netherlands

Judith Elisabeth Baars, *Rotterdam*
Albert J Bredenoord, *Nieuwegein*
Nanne KH De Boer, *Amsterdam*
Pieter Jan Floris De Jonge, *Rotterdam*
Wouter J de Jonge, *Amsterdam*
Mireille A Edens, *Groningen*
Chris JJ Mulder, *Amsterdam*
Paul E Sijens, *Groningen*
Vera Esther Valkhoff, *Rotterdam*

New Zealand

Momir M Mikov, *Dunedin*
Maxim Petrov, *Auckland*

Norway

Guanglin Cui, *Tromso*
Reidar Fossmark, *Trondheim*

Pakistan

Anwar Hassan Gilani, *Karachi*

Philippines

Mark Anthony Ayuyao De Lusong, *Quezon City*

Poland

Halina Cichoz-Lach, *Lublin*
Jaroslaw Czyz, *Cracow*
Julian Teodor Swierczynski, *Gdansk*

Portugal

Cristina Freire, *Hull*
Ana Isabel Lopes, *Lisbon*

Romania

Dan Lucian Dumitrascu, *Cluj*

Russia

Tatyana A Korolenko, *Novosibirsk*

Saudi Arabia

Mohammad S Khuroo, *Riyadh*
Moamen Salah Refat, *Taif*
Shahab Uddin, *Riyadh*

Singapore

Kok-Ann Gwee, *Singapore*
Khek-Yu Ho, *Singapore*
Kok-Yuen Ho, *Singapore*

Slovenia

Rok Orel, *Ljubljana*

South Africa

Christoffel Van Rensburg, *Cape Town*

South Korea

Ki Baik Hahm, *Inchon*
Seok Joo Han, *Seoul*
Jeong Won Jang, *Seoul*
Dong Joon Kim, *Gangwon-do*
Jae J Kim, *Seoul*
Kyoung Mee Kim, *Seoul*
Nayoung Kim, *Songnam*
Sung-Bae Kim, *Seoul*
Byung-Hoon Lee, *Seoul*
Kwan S Lee, *Seoul*
Sang-Han Lee, *Daegu*
Yun Jeong Lim, *Goyang*
Ji-Young Park, *Seoul*
Uy Dong Sohn, *Seoul*

Spain

Matias Antonio Avila, *Pamplona*
Luis Bujanda, *San Sebastián*
Maria Carmen Collado, *Valencia*
Conrado M Fernandez-Rodriguez, *Madrid*
ángel Lanas, *Zaragoza*
Juan-R Malagelada, *Barcelona*
Jose JG Marin, *Salamanca*
Antonio Ruiz Medina, *Jaén*
Maria J Monte, *Salamanca*
Miguel Munoz, *Seville*
Jesus Prieto, *Pamplona*
Victor Manuel Victor, *Valencia*
Maria D Yago, *Granada*

Sweden

Bodil Ohlsson, *Malmo*
Henrik Thorlacius, *Malmo*
Curt Tysk, *Orebro*

Thailand

Weekitt Kittisupamongkol, *Bangkok*
Abhasnee Sobhonslidsuk, *Bangkok*
Suporn Treepongkaruna, *Bangkok*
Sombat Treeprasertsuk, *Bangkok*

Turkey

Fusun Acarturk, *Etiler-Ankara*
Engin Altintas, *Mersin*
Güldeniz K Cakmak, *Kozlu Zonguldak*
Hayrullah Derici, *Balkesir*
Mukaddes Esrefoglu, *Istanbul*
Ilker Tasci, *Ankara*

United Kingdom

Nadeem Ahmad Afzal, *Hampshire*
Qasim Aziz, *London*
Hugh Barr, *Gloucester*
Ian Leonard Phillip Beales, *Norwich*
Barbara Braden, *Oxford*
Susan J Duthie, *Aberdeen*
Eyad Elkord, *Manchester*
Anton Vignaraj Emmanuel, *London*
Konstantinos C Fragkos, *London*
Nusrat Husain, *Manchester*
Jin-Yong Kang, *London*
Mariusz Madalinski, *Ipswich*
Srinivasan Madhusudan, *Nottingham*
Subramanian Mahadevan, *Birmingham*
John Francis Mayberry, *Leicester*
Chuka Uche Nwokolo, *Coventry*
Ajith Kumar Siriwardena, *Manchester*
Her-Hsin Tsai, *Hull*
Craig LC Williams, *Glasgow*

United States

Zubair H Aghai, *Camden*
Shrikant Anant, *Oklahoma*
Kondala R Atkuri, *Stanford*
Cheryl Hunt Baker, *Orlando*
James M Becker, *Boston*
Qiang Cai, *Atlanta*
Jian-De Chen, *Galveston*
Liang Cheng, *Indianapolis*

WJGPT

World Journal of Gastrointestinal Pharmacology and Therapeutics

Contents

Quarterly Volume 7 Number 3 August 6, 2016

TOPIC HIGHLIGHT

REVIEW

MINIREVIEWS

ORIGINAL ARTICLE

Basic Study

World Journal of Gastrointestinal Pharmacology and Therapeutics
Volume 7 Number 3 August 6, 2016

Contents

NAME OF JOURNAL
World Journal of Gastrointestinal Pharmacology and Therapeutics

ISSN
ISSN 2150-5349 (online)

LAUNCH DATE
May 6, 2010

FREQUENCY
Quarterly

World Journal of Gastrointestinal Pharmacology and Therapeutics
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Inc
8226 Regency Drive,
Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLICATION DATE
August 6, 2016

INSTRUCTIONS TO AUTHORS
http://www.wjgnet.com/bpg/gerinfo/204

ONLINE SUBMISSION
http://www.wjgnet.com/esps/

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i3.343

World J Gastrointest Pharmacol Ther 2016 August 6; 7(3): 343-352
ISSN 2150-5349 (online)

TOPIC HIGHLIGHT

2016 Colorectal Cancer: Global view

Therapeutic options for peritoneal metastasis arising from colorectal cancer

Gabriel Glockzin, Hans J Schlitt, Pompiliu Piso

Gabriel Glockzin, Department of Surgery, Staedtisches Klinikum Muenchen GmbH, Klinikum Bogenhausen, 81925 Munich, Germany

Hans J Schlitt, Department of Surgery, University Hospital Regensburg, 93053 Regensburg, Germany

Pompiliu Piso, Department of Surgery, Hospital of the Order of St. John of God, 93049 Regensburg, Germany

Author contributions: Glockzin G drafted the manuscript; Schlitt HJ and Piso P revised the manuscript; all authors read and approved the final manuscript.

Conflict-of-interest statement: All authors declare that they have no conflicts of interest.

Manuscript source: Invited manuscript

Correspondence to: Gabriel Glockzin, MD, FEBS, Department of Surgery, Staedtisches Klinikum Muenchen GmbH, Klinikum Bogenhausen, Englschalkinger Str. 77, 81925 Munich, Germany. gabriel.glockzin@klinikum-muenchen.de
Telephone: +49-89-92702011
Fax: +49-89-92702016

Received: March 29, 2016
Peer-review started: April 4, 2016
First decision: May 17, 2016
Revised: June 22, 2016
Accepted: July 11, 2016
Article in press: July 13, 2016
Published online: August 6, 2016

Abstract

Peritoneal metastasis is a common sign of advanced tumor stage, tumor progression or tumor recurrence in patients with colorectal cancer. Due to the improvement of systemic chemotherapy, the development of targeted therapy and the introduction of additive treatment options such as cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC), the therapeutic approach to peritoneal metastatic colorectal cancer (pmCRC) has changed over recent decades, and patient survival has improved. Moreover, in contrast to palliative systemic chemotherapy or best supportive care, the inclusion of CRS and HIPEC as inherent components of a multidisciplinary treatment regimen provides a therapeutic approach with curative intent. Although CRS and HIPEC are increasingly accepted as the standard of care for selected patients and have become part of numerous national and international guidelines, the individual role, optimal timing and ideal sequence of the different systemic, local and surgical treatment options remains a matter of debate. Ongoing and future randomized controlled clinical trials may help clarify the impact of the different components, allow for further improvement of patient selection and support the standardization of oncologic treatment regimens for pmCRC. The addition of further therapeutic options such as neoadjuvant intraperitoneal chemotherapy or pressurized intraperitoneal aerosol chemotherapy, should be investigated to optimize therapeutic regimens and further improve the oncological outcome.

Key words: Peritoneal metastasis; Colorectal cancer; Systemic chemotherapy; Intraperitoneal chemotherapy; Cytoreductive surgery

Core tip: Beyond diverse systemic, interventional and

surgical palliative treatment options for peritoneal metastasis arising from colorectal cancer, the combination of systemic chemotherapy, cytoreductive surgery and hyperthermic intraperitoneal chemotherapy provides a therapeutic approach with curative intent for selected patients. Nevertheless, the treatment regimens, the sequence of therapy and the impact of the different components of the multidisciplinary treatment concept on clinical and oncological outcomes remain a matter of debate. Moreover, the addition of further therapeutic options to the existing treatment regimens might allow for higher complete resection rates and improved survival rates.

Glockzin G, Schlitt HJ, Piso P. Therapeutic options for peritoneal metastasis arising from colorectal cancer. *World J Gastrointest Pharmacol Ther* 2016; 7(3): 343-352 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i3/343.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i3.343

INTRODUCTION

Colorectal cancer (CRC) remains one of the leading causes of cancer-related death worldwide[1]. Peritoneal metastasis (PM) is common in patients with advanced stage primary and recurrent colorectal cancer[2,3]. The natural course of this disease is associated with poor prognosis and led to a mean overall survival of 5.2 mo in the prospective European multicenter EVOCAPE I study (n = 118)[4]. A retrospective analysis of 3000 patients with pmCRC reported a median survival of 7 mo without specific treatment[5]. Although peritoneal metastases develop avascular tumor nodules within the abdominal cavity that often cannot be efficiently addressed by systemic chemotherapy[6], advances in the development of cytostatic agents, targeted therapy and combined treatment regimens has led to significant improvement in survival rates. Moreover, additive treatment options such as cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) might be performed with curative intent in selected patients[7]. Thus, pmCRC currently requires a multidisciplinary treatment approach that considers the available treatment options and modalities (Figure 1).

STAGING SYSTEMS FOR PM

The estimation of the extent of peritoneal tumor dissemination plays an important role in choosing therapeutic options for patients with pmCRC. Different staging systems allow for the standardization of PM classification and facilitate prognosis estimation. The most commonly used classification system for peritoneal tumor dissemination is the peritoneal cancer index (PCI). This numerical score combines the lesion size (LS) and tumor localization in 13 abdomino-pelvic regions including four small bowel regions (region 0-12) and ranges from 0 to 39[8]. The PCI was initially introduced for intraoperative determination of the extent of peritoneal carcinomatosis but the extent can also be determined by staging laparoscopy or diagnostic imaging. Elias *et al*[9] showed that the PCI is easy to use and reproducible with high inter-surgeon concordance. Although the PCI is underestimated by computed tomography compared to intraoperative findings, the clinical impact of the inaccuracies of CT-PCI is modest[10]. Thus, the (CT-)PCI is a helpful tool to determine and communicate the extent of peritoneal disease and to select patients for different therapeutic options. Moreover, the PCI correlates with overall and progression-free survival in patients with pm CRC[11-14]. Nevertheless, the predictive value is limited with respect to PM and does not include other prognostic factors. Therefore, prognostic scores for patients with pm CRC have been developed. The Peritoneal Surface Disease Severity Score (PSDSS) is based on the following three important prognostic indicators: (1) clinical symptoms; (2) PCI; and (3) tumor histopathology. The PSDSS ranges from 2 to 22 and divides patients into four prognostic groups (stage Ⅰ = PSDSS 2-4, stage Ⅱ = PSDSS 4-7, stage Ⅲ = PSDSS 8-10 and stage Ⅳ = PSDSS > 10)[15]. Several retrospective analyses show a high correlation between PSDSS and the survival of patients with pmCRC. The score might be helpful for determining survival probability and resectability of peritoneal disease in the context of therapeutic decision-making[16-18]. Another recently developed prognostic score for patients with pmCRC is the Colorectal Peritoneal Score (COREP). COREP includes signet cell histology, hemoglobin, white blood cell count and the value and status of serum tumor markers and ranges from 0 to 18. The cut-off value for the poor-prognosis group is COREP > 6. In the first published evaluation of 77 patients the predictive value of COREP for open/close-procedure, R1 resection and one-year survival was superior to that of PSDSS[19]. Based on the Japanese classification of pmCRC, which divides peritoneal tumor dissemination into four groups (P0: no PM, P1: local PM, P2: limited distant, PM and P3: extended distant PM)[20,21] Noura *et al*[22] proposed a new simple classification system that includes the colorectal liver metastases (CLM) status. Patients without CLM and local (P1) or limited distant PM (P2) are classified as Grade A and Grade B, respectively. Patients with extended distant PM and all patients with CLM have been defined as Grade C. Initial data shows significant stratification of the survival and R0 resection rates[22]. However, new scores considering different histological and clinical factors might be helpful for decision-making and allow for further improvement of the selection of appropriate therapeutic options within a multidisciplinary treatment approach.

SYSTEMIC CHEMOTHERAPY FOR pmCRC

Although there are multiple prospective randomized trials and retrospective analyses about systemic chemotherapy in patients with advanced stage and metastatic CRC, data regarding the subgroup of patients with pmCRC

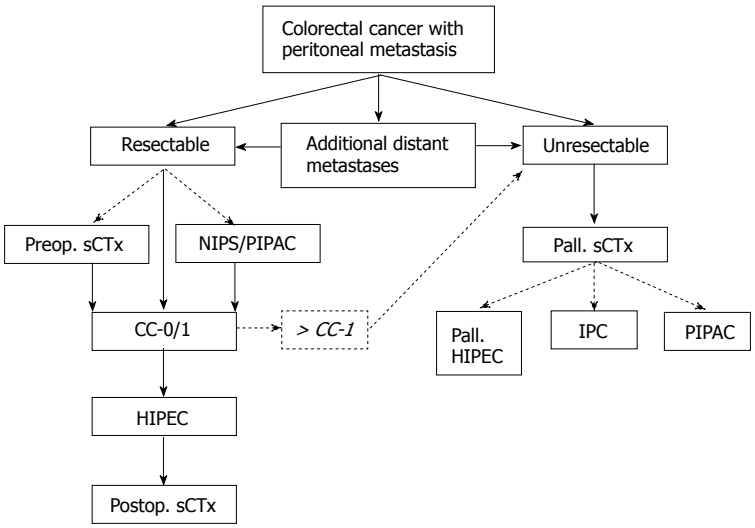

Figure 1 Proposed algorithm for treating peritoneal metastatic colorectal cancer. CC: Completeness of cytoreduction; CC-0/1: Complete macroscopic cytoreduction; IPC: Intraperitoneal chemotherapy; NIPS: Neoadjuvant intraperitoneal/systemic chemotherapy; PIPAC: Pressurized intraperitoneal aerosol chemotherapy; sCTx: Systemic chemotherapy; scattered lines indicate additional therapeutic options; HIPEC: Hyperthermic intraperitoneal chemotherapy.

are limited. Franko *et al*[23] analyzed 364 patients with PM out of 2095 patients enrolled in the two prospective randomized NCCTG phase Ⅲ trials N9741 and N9841 and showed a 30% relative reduction in overall survival in this subgroup. The 5-year OS rates were 4.1% and 6% and the median survival was 12.7 mo and 17.6 mo in the pmCRC and the non-pmCRC group, respectively. In this analysis infusional oxaliplatin-based chemotherapy was superior to irinotecan-based regimens irrespective of the PM status[23]. The subgroup analysis of patients with pmCRC enrolled in the prospective randomized CAIRO and CAIRO2 trials showed a significant impairment in the overall survival of these patients. Klaver *et al*[24] published a median OS of 10.4 and 17.3 mo in the CAIRO trial and 15.2 and 20.7 mo in the CAIRO2 trial. An Asian prospective single-arm phase Ⅱ study investigating FOLFOX-4 in patients with pmCRC reported median overall survival of 21.5 mo. The median time to progression was 4.4 mo[25]. Consistent with these reported survival rates, Elias *et al*[26] reported a median OS of 23.9 mo under modern multidrug systemic chemotherapy in 48 patients with pmCRC from the French registry.

Considering the promising results of the first line treatment of patients with metastatic colorectal cancer using systemic polychemotherapy plus targeted therapy with median OS ranging from 25 mo to 41.3 mo[27-30] these regimens have also been used for treating pmCRC. In a retrospective analysis of 65 consecutive patients with pmCRC, Adachi *et al*[31] reported an improvement in the survival rate in response to systemic chemotherapy after incomplete cytoreduction. The oxaliplatin-based regimen and addition of targeted therapy was superior to irinotecan-based chemotherapy[31]. Razenberg *et al*[32] analyzed 1235 patients treated with palliative systemic chemotherapy for pmCRC. In 436 patients (35%) bevacizumab has been added to the treatment regimen. The median OS was 7.5 mo *vs* 11 mo in the bevacizumab group[32]. In a population-based study patients with metachronous colorectal PM were analyzed with respect to their treatment as follows: 94 patients received palliative systemic chemotherapy, 36 patients had the addition of bevacizumab and 92 did not receive therapy and the median survival was 13 mo, 20.3 mo and 3.4 mo, respectively[33]. Comparable results are reported by van Oudheusden in 82 patients who underwent open/close procedures for unresectable colorectal PM. The median OS was 11.2 mo with palliative systemic chemotherapy and 2.7 mo with best supportive care[34].

These data demonstrate the efficacy of modern systemic chemotherapy regimens with or without targeted therapy in improving the survival of patients with unresectable pmCRC. Based on these findings systemic chemotherapy should be considered the standard of care in patients with unresectable pmCRC and should be the backbone of a multimodal treatment regimen in patients who qualify for a multidisciplinary therapeutic approach. In the absence of contraindications, infusional oxaliplatinbased regimens, such as FOLFOX with or without monoclonal antibodies like bevacizumab, cetuximab or panitumumab, might be preferred as first-line therapies for these patients. Moreover, based on the results of the RAISE trial, ramucirumab might also be considered for the second-line treatment of patients with pmCRC[35]. Nevertheless, reliable data for this subgroup are not available.

SURGERY FOR pmCRC

CRS

In contrast to palliative surgery, such as fecal diversion, intestinal bypass, primary tumor resection, *etc.*, CRS followed by HIPEC provides an additive treatment option for selected colorectal PM patients with a curative intent. Although disease recurrence is common[36], cure rates between 16% and 28% are reported after complete CRS and HIPEC[37,38]. The aim of surgical cytoreduction, which may consist of multiple peritonectomy procedures and visceral resections is the removal of all visible tumor deposits within the abdominal cavity[8,39,40]. Despite extensive and aggressive surgery, most patients return to baseline in terms of their quality of life within 6 mo

after surgery[41-43]. The success of surgery is classified according to the completeness of cytoreduction (CC) score[13,44]. Complete macroscopic cytoreduction (CC-0/1), defined as no visible tumor or single tumor nodules < 2.5 mm, is a precondition for the efficient application of HIPEC. Therefore, consistent preoperative patient selection is crucial for the efficacy of the multimodal treatment concept. A PCI > 20 might be considered a relative contraindication for CRS and HIPEC[45]. Da Silva *et al*[11] reported a median OS of 41 mo in patients with PCI < 20 and 16 mo in patients with PCI > 20 after complete macroscopic cytoreduction. Comparable results are published by Hompes *et al*[12] for patients with a PCI higher or lower than 15. A recently published analysis of 180 patients defined a cut-off PCI value of 17[14].

CRS in patients with additional CLM

There are limited published data regarding cytoreductive surgery in patients with additional resectable CLM. In a retrospective matched-pair analysis, hepatobiliary procedures during CRS and HIPEC did not lead to increased perioperative complication rates and/or overall mortality[46]. According to the Milan consensus statement of the Peritoneal Surface Malignancy Group International cytoreductive surgery (and HIPEC) should not be routinely recommended in patients with more than three peripheral resectable liver metastases[45]. However, two retrospective studies demonstrated median survival rates of approximately 36 mo after CRS, including mostly minor liver resections followed by HIPEC[47,48]. As expected, liver involvement is associated with decreased overall survival rates. Berger *et al*[49] reported a median overall survival of 45.1 mo in 108 patients with additional liver involvement and 73.5 mo in 166 patients with isolated PM after CRS and HIPEC. Nevertheless, patients with malignancies other than CRC were included in the analysis. There was no significant difference regarding the morbidity and mortality between the two groups[49]. Allard *et al*[50] reported a median survival of 42 mo in patients who underwent complete resection of CLM and unexpected limited CPM with a median PCI of 2. In a multivariate analysis Delhorme *et al*[51] identified the size of liver metastasis and grade Ⅱ/Ⅲ toxicity of preoperative chemotherapy as poor prognostic factors. Response to preoperative chemotherapy significantly increased overall survival. These data are supported by a recently published meta-analysis that identified concurrent CLM as an independent negative prognostic factor for overall survival in patients with pmCRC after CRS and HIPEC[52]. Noura *et al*[22] showed that the presence of CLM impairs survival and R0 resection rates. The 5-year overall survival rates of patients without CLM and local or limited distant PM were 25.6% and 12.0%, respectively. The 5-year survival rate of patients with extended distant PM and/or additional CLM was 5.6%. R0 resection rates were 65.9%, 44.6% and 8.1%[22]. However, the combination of extended liver surgery for CLM and extended cytoreductive surgery in patients with high PCI should be avoided because of the impaired clinical and oncological outcomes. Moreover, there are no reliable data on patients with pmCRC and additional isolated resectable lung metastases. Therefore, lung metastasis should be considered a contraindication of CRS and HIPEC.

HIPEC

The aim of intraoperative HIPEC is to consolidate complete surgical resection by destroying scattered (and residual) tumor cells within the abdominal cavity. In a prospective randomized phase Ⅲ trial comparing CRS and HIPEC plus systemic chemotherapy with 5-FU/FA to systemic chemotherapy with 5-FU/FA in selected patients with pmCRC there was a significant survival benefit for the treatment group. The median survival was 22 and 12.6 mo for the treatment and non-treatment groups. In the subgroup of patients with complete macroscopic cytoreduction (CC-0/1), the median survival reached 42.9 mo. The low survival rates might be explained by the use of 5-FU-based systemic chemotherapy in both groups in the pre-oxaliplatin era[53,54].

Elias *et al*[26] reported a median survival of 62.7 mo and a 5-year survival rate of 51% after complete macroscopic cytoreduction and bidirectional oxaliplatin-based HIPEC. All patients additionally received modern systemic chemotherapy[26]. A prospective phase Ⅱ study investigating complete macroscopic cytoreduction and bidirectional oxaliplatin-based HIPEC showed a 2-year overall survival rate of 88.7% and a median disease-free survival (DFS) of 19.8 mo[12]. Based on the promising results of the FOLFOXIRI protocol in the systemic treatment of mCRC, irinotecan has been added to the bidirectional oxaliplatin-based HIPEC regimen, leading to increased morbidity without improving the survival. Quenet *et al*[55] reported a median overall survival of 47 mo and a 5-year survival rate of 42.4%. Goéré *et al*[37] reported a cure rate, defined as the 5-year disease-free survival, of 16% after CRS and HIPEC in 107 patients with pmCRC. Another retrospective analysis of 342 patients with pmCRC from a prospective database showed a 10-year recurrence-free survival rate of 10% after CRS and HIPEC[56].

Although there are only few prospective RCTs, several studies and retrospective analyses show that the integration of CRS and HIPEC into a multidisciplinary treatment approach that includes systemic chemotherapy can improve the survival of selected patients with pmCRC[7,57]. Nevertheless, the exact role of the HIPEC procedure and components remains unclear. A comparative analysis published by Hompes *et al*[58] investigated different HIPEC regimens and their effects on patient survival. There was no statistically significant difference between bidirectional oxaliplatin-based HIPEC and MMC-based HIPEC after complete macroscopic cytoreduction. The median RFS was 12.2 mo in the oxaliplatin-group and 13.8 mo in the MMC group (P = 0.87). The median OS was 37.1 mo in the oxaliplatin group and 26.5 mo in the MMC

group (P = 0.45)[58]. A matched-pair analysis showed no significant differences in morbidity and mortality by HIPEC regimen. The grade 3/4 morbidity rates according to CTCAE were 42.5% in the OX group and 37.5% in the MMC group (P = 0.648) and the mortality rates of the OX and MMC groups were 2.5% and 0%, respectively[59]. Consistent with these findings the American Society of Peritoneal Surface Malignancies reported an OS of 32.7 mo in patients with MMC-based HIPEC and 31.4 mo for oxaliplatin-based HIPEC in 539 patients with pm CRC after complete macroscopic cytoreduction (P = 0.925). After stratification to PSDSS there was a statistically significant survival benefit for the MMC-subgroup with PSDSS Ⅰ/Ⅱ (P = 0.012)[60]. A retrospective analysis of a limited number of patients compared bidirectional oxaliplatin-based HIPEC to bidirectional irinotecan-based HIPEC. The 3-year survival rates were 65.0% in the OX group *vs* 41.7% in the IRI group (P = 0.295)[61].

In a recently published retrospective analysis of 50 consecutive patients with pmCRC, Désolneux *et al*[62] reported a median survival of 34.2 mo and a 5-year survival rate of 29.6% after complete macroscopic cytoreduction and systemic chemotherapy alone. These findings are supported by a retrospective Japanese multicenter database analysis of 564 patients who underwent surgery without HIPEC for pmCRC. In patients with R0 resection, the median overall survival was 30 mo and 5-year survival rate was 32.4%. The 5-year survival rate after R0 resection and adjuvant chemotherapy was 31.7% compared to 24.6% without adjuvant treatment. R0 resection and adjuvant chemotherapy were independent positive prognostic factors for survival[63]. This concept and the role of HIPEC is investigated by the French prospective randomized PRODIGE 7 trial that compares CRS and HIPEC plus systemic chemotherapy with CRS alone plus systemic chemotherapy. However, survival data are not yet available. Cashin *et al*[64] published survival data of a prematurely terminated prospective randomized trial evaluating CRS followed by normothermic intraperitoneal chemotherapy (IPC) with 5-FU *vs* CRS followed by systemic oxaliplatin-based chemotherapy. Both treatments were continued for 6 mo. The median overall survival times were 25 mo *vs* 18 mo (P = 0.04) and the 2-year survival rates were 54% *vs* 38% (P = 0.04)[64]. However, the optimal therapeutic regimen of IPC after complete CRS remains a matter of debate[65].

Prophylactic and palliative HIPEC

Another therapeutic concept that is evaluated by the ongoing French ProphyloCHIP trial is the prophylactic application of HIPEC in patients with CRC and high risk of developing PM, such as tumor perforation, isolated ovarian metastases or removal of localized PM during resection of primary tumor resection. The enrolled patients were randomized eight months after adjuvant chemotherapy to the control arm with follow-up or to the treatment arm with explorative laparotomy and prophylactic HIPEC (NCT01226394). The COLOPEC trial evaluates the effect of adjuvant HIPEC during or shortly after resection of primary CRC with a high risk of metachronous PM. A risk reduction from 25% to 10% and, therefore, improvement in the long-term survival is assumed[66].

HIPEC without cytoreductive surgery, also applied by the laparoscopic approach, might be considered in patients with unresectable PM (Figure 2) and symptomatic therapy for refractory malignant ascites. Several retrospective studies showed significant reduction of ascites production and efficient symptom control after HIPEC. Nevertheless, the number of reported patients and procedures is limited and data from prospective randomized trials are not available[67-69].

PERIOPERATIVE SYSTEMIC CHEMOTHERAPY

The importance of systemic chemotherapy in the context of CRS and HIPEC has been demonstrated. Postoperative systemic chemotherapy has been shown to be an independent positive prognostic marker in all registries and retrospective analyses[13,70]. In a recently published database analysis of 5516 patients with PM arising from colorectal adenocarcinoma, mucinous adenocarcinoma and signet ring cell carcinoma, Simkens *et al*[71] showed that systemic chemotherapy improved survival independent of the histological subtype. In contrast to these findings, a multicenter study, including 221 patients with pmCRC reported no significant difference in the OS after CRS and HIPEC between postoperative systemic chemotherapy and surveillance. The median OS was 43.3 mo. Nevertheless, during the first year the rates of progression and recurrence were significantly lower in the chemotherapy group[72]. However, the optimal sequence of the therapeutic modalities remains a matter of investigation. Elias *et al*[13] reported no significant impact on the prognosis of neoadjuvant systemic chemotherapy in patients undergoing CRS and HIPEC for pmCRC. Passot *et al*[73] showed an overall response rate of 36% and a disease progression rate of 21% in patients who received different regimens of modern neoadjuvant systemic chemotherapy before CRS and HIPEC. Interestingly, the response to neoadjuvant treatment was not a significant prognostic factor, therefore, it might not be considered a contraindication for CRS and HIPEC. The median survival of patients with disease progression was 31.4 mo[73]. Further analysis of different preoperative chemotherapy regimens consisting of 5-FU, oxaliplatin, irinotecan and/or monoclonal antibodies showed a 9.7% complete response rate, 20.2% major response and 70.1% rate of minor or no response. In the multivariate analysis the pathohistological response was an independent predictor of survival (P = 0.01)[74]. Devilee *et al*[75] compared patients with pmCRC who received neoadjuvant systemic chemotherapy before CRS and HIPEC with patients who were treated with adjuvant

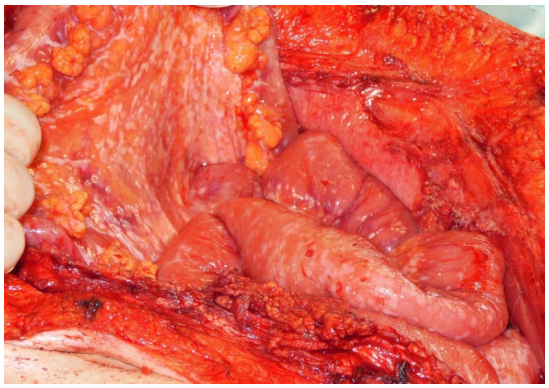

Figure 2 Diffuse peritoneal tumor dissemination.

systemic chemotherapy after CRS and HIPEC. All patients underwent complete or nearly complete macroscopic cytoreduction. The 3-year survival rates were 89% and 50% for the neoadjuvant and adjuvant groups. Although the PCI was lower and operation time was shorter for patients who received preoperative chemotherapy, neoadjuvant treatment was still independently associated with improved survival after correcting for other significant prognostic factors[75]. Kuijpers *et al*[76] analyzed a prospective database regarding the effect of systemic chemotherapy on survival of patients with lymph-node positive pm CRC undergoing CRS and HIPEC. There was a statistically significant increase in the median PFS (15 mo *vs* 4 mo, $P = 0.024$) and median OS (30 mo *vs* 14 mo, $P = 0.015$) in patients who received perioperative systemic chemotherapy. Interestingly, the timing of systemic chemotherapy had no influence on survival[76]. The prospective multicenter phase Ⅱ COMBATAC study evaluates CRS and bidirectional oxaliplatin-based HIPEC plus perioperative cetuximab-containing polychemotherapy[77]. The first safety data showed no increase in the morbidity or mortality when using the perioperative treatment approach[78]. There is another ongoing prospective phase Ⅱ study (BEV-IP) evaluating perioperative systemic chemotherapy plus bevacizumab in combination with CRS and oxaliplatin-based HIPEC[79]. However, survival data from both studies are not yet available.

IPC AND PRESSURIZED INTRAPERITONEAL AEROSOL CHEMOTHERAPY

Except for the case of early postoperative IPC (EPIC) instead of or in addition to HIPEC after cytoreductive surgery there are no reliable published data for normothermic IPC without cytoreductive surgery for local treatment of pmCRC. The concept of sequential intraperitoneal treatment, which could be applied over a peritoneal port system, has been demostrated for ovarian cancer[80]. Yonemura *et al*[81] developed a protocol consisting of neoadjuvant systemic and intraperitoneal chemotherapy (NIPS) for gastric cancer. Clinical trials are needed to evaluate the potential role of IPC in patients with pmCRC, especially in the neoadjuvant setting. Preoperative IPC or NIPS may allow for higher rates of CC-0/1 resection and may further improve the outcome after CRS and HIPEC. Moreover, sequential IPC with or without palliative systemic chemotherapy might improve response rates and local tumor control in patients with unresectable PM arising from CRC.

Pressurized intraperitoneal aerosol chemotherapy (PIPAC) is a new technique for the local application of cytostatics as aerosol under pressure that allows for improved drug distribution and tumor tissue penetration. The feasibility and safety of the procedure has been demosntrated[82,83]. Most data published for ovarian cancer show local anticancer activity after sequential application of PIPAC[84]. A recently published retrospective analysis of 48 applications of PIPAC given every six weeks in 17 patients with pretreated pmCRC reported a median OS of 15.7 mo. The overall response rate was 71%[85]. Quality of life analysis accessed by the EORTC-QLQ30 questionnaire in 48 patients with PM arising from different tumor entities (PCI: 16 ± 10) that received at least two PIPAC applications showed an impairment of the global physical score and pain score after the first treatment improved after the second PIPAC application. Gastrointestinal symptoms remained stable with PIPAC therapy[86]. Based on the promising preliminary data, PIPAC might become an additional therapeutic option for the palliative local treatment of pmCRC in the future. Moreover, it might be interesting as a neoadjuvant local treatment with or without the addition of systemic chemotherapy beyond CRS and HIPEC. Several prospective clinical trials evaluating this therapy approach are ongoing. The results may help to determine the role of PIPAC within a multidisciplinary treatment concept and allow for further improvement of patient selection.

CONCLUSION

The therapeutic approach to PM of colorectal cancer has changed in recent decades. There are multiple treatment options for patients with pmCRC that must be integrated in an individualized multidisciplinary treatment approach (Figure 1). Consistent diagnostics and patient selection are crucial to obtaining optimal oncologic outcome. Thus, the therapeutic approach should be discussed by an interdisciplinary tumor board, and, if necessary, patients should be referred to specialized treatment centers. In addition to multiple palliative treatment options, CRS and HIPEC provide an additive treatment modality with curative intent for selected patients with pmCRC. The integration of further treatment options such as repeated preoperative intraperitoneal chemotherapy or PIPAC in current treatment regimens should be discussed and evaluated in randomized controlled clinical trials. Prognostic factors, such as peritoneal tumor distribution, lymph node status, hematogenous metastasis, histology, tumor mutation status, tumor immunology, numerous

patient-related factors and the resection status must be considered during patient selection and should be further investigated. The development and clinical use of the prognostic scores may help tailor individual treatment regimens that consider all available therapeutic options. Further prospective randomized trials focussed on patients with pmCRC are highly recommended to optimize and standardize the multimodal treatment regimens.

REFERENCES

1 **Torre LA**, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015; **65**: 87-108 [PMID: 25651787 DOI: 10.3322/caac.21262]

2 **Russell AH**, Tong D, Dawson LE, Wisbeck W. Adenocarcinoma of the proximal colon. Sites of initial dissemination and patterns of recurrence following surgery alone. *Cancer* 1984; **53**: 360-367 [PMID: 6690020 DOI: 10.1002/1097-0142(19840115)53:2<360::AID-CNCR2820530232>3.0.CO;2-U]

3 **Carmignani CP**, Sugarbaker TA, Bromley CM, Sugarbaker PH. Intraperitoneal cancer dissemination: mechanisms of the patterns of spread. *Cancer Metastasis Rev* 2003; **22**: 465-472 [PMID: 12884919 DOI: 10.1023/A:1023791229361]

4 **Sadeghi B**, Arvieux C, Glehen O, Beaujard AC, Rivoire M, Baulieux J, Fontaumard E, Brachet A, Caillot JL, Faure JL, Porcheron J, Peix JL, François Y, Vignal J, Gilly FN. Peritoneal carcinomatosis from non-gynecologic malignancies: results of the EVOCAPE 1 multicentric prospective study. *Cancer* 2000; **88**: 358-363 [PMID: 10640968 DOI: 10.1002/(SICI)1097-0142(20000115)88:2<358::AID-CNCR16>3.0.CO;2-O]

5 **Jayne DG**, Fook S, Loi C, Seow-Choen F. Peritoneal carcinomatosis from colorectal cancer. *Br J Surg* 2002; **89**: 1545-1550 [PMID: 12445064 DOI: 10.1046/j.1365-2168.2002.02274.x]

6 **Stewart JH**, Shen P, Levine EA. Intraperitoneal hyperthermic chemotherapy for peritoneal surface malignancy: current status and future directions. *Ann Surg Oncol* 2005; **12**: 765-777 [PMID: 16132375 DOI: 10.1245/ASO.2005.12.001]

7 **Glockzin G**, Schlitt HJ, Piso P. Peritoneal carcinomatosis: patients selection, perioperative complications and quality of life related to cytoreductive surgery and hyperthermic intraperitoneal chemotherapy. *World J Surg Oncol* 2009; **7**: 5 [PMID: 19133112 DOI: 10.1186/1477-7819-7-5]

8 **Jacquet P**, Sugarbaker PH. Clinical research methodologies in diagnosis and staging of patients with peritoneal carcinomatosis. *Cancer Treat Res* 1996; **82**: 359-374 [PMID: 8849962 DOI: 10.1007/978-1-4613-1247-5_23]

9 **Elias D**, Souadka A, Fayard F, Mauguen A, Dumont F, Honore C, Goere D. Variation in the peritoneal cancer index scores between surgeons and according to when they are determined (before or after cytoreductive surgery). *Eur J Surg Oncol* 2012; **38**: 503-508 [PMID: 22281154 DOI: 10.1016/j.ejso.2012.01.001]

10 **Esquivel J**, Chua TC, Stojadinovic A, Melero JT, Levine EA, Gutman M, Howard R, Piso P, Nissan A, Gomez-Portilla A, Gonzalez-Bayon L, Gonzalez-Moreno S, Shen P, Stewart JH, Sugarbaker PH, Barone RM, Hoefer R, Morris DL, Sardi A, Sticca RP. Accuracy and clinical relevance of computed tomography scan interpretation of peritoneal cancer index in colorectal cancer peritoneal carcinomatosis: a multi-institutional study. *J Surg Oncol* 2010; **102**: 565-570 [PMID: 20976729 DOI: 10.1002/jso.21601]

11 **da Silva RG**, Sugarbaker PH. Analysis of prognostic factors in seventy patients having a complete cytoreduction plus perioperative intraperitoneal chemotherapy for carcinomatosis from colorectal cancer. *J Am Coll Surg* 2006; **203**: 878-886 [PMID: 17116556 DOI: 10.1016/j.jamcollsurg.2006.08.024]

12 **Hompes D**, D'Hoore A, Van Cutsem E, Fieuws S, Ceelen W, Peeters M, Van der Speeten K, Bertrand C, Legendre H, Kerger J. The treatment of peritoneal carcinomatosis of colorectal cancer with complete cytoreductive surgery and hyperthermic intraperitoneal peroperative chemotherapy (HIPEC) with oxaliplatin: a Belgian multicentre prospective phase II clinical study. *Ann Surg Oncol* 2012; **19**: 2186-2194 [PMID: 22395983 DOI: 10.1245/s10434-012-2264-z]

13 **Elias D**, Gilly F, Boutitie F, Quenet F, Bereder JM, Mansvelt B, Lorimier G, Dubè P, Glehen O. Peritoneal colorectal carcinomatosis treated with surgery and perioperative intraperitoneal chemotherapy: retrospective analysis of 523 patients from a multicentric French study. *J Clin Oncol* 2010; **28**: 63-68 [PMID: 19917863]

14 **Goéré D**, Souadka A, Faron M, Cloutier AS, Viana B, Honoré C, Dumont F, Elias D. Extent of colorectal peritoneal carcinomatosis: attempt to define a threshold above which HIPEC does not offer survival benefit: a comparative study. *Ann Surg Oncol* 2015; **22**: 2958-2964 [PMID: 25631064 DOI: 10.1245/s10434-015-4387-5]

15 **Pelz JO**, Chua TC, Esquivel J, Stojadinovic A, Doerfer J, Morris DL, Maeder U, Germer CT, Kerscher AG. Evaluation of best supportive care and systemic chemotherapy as treatment stratified according to the retrospective peritoneal surface disease severity score (PSDSS) for peritoneal carcinomatosis of colorectal origin. *BMC Cancer* 2010; **10**: 689 [PMID: 21176206 DOI: 10.1186/1471-2407-10-689]

16 **Esquivel J**, Garcia SS, Hicken W, Seibel J, Shekitka K, Trout R. Evaluation of a new staging classification and a Peritoneal Surface Disease Severity Score (PSDSS) in 229 patients with mucinous appendiceal neoplasms with or without peritoneal dissemination. *J Surg Oncol* 2014; **110**: 656-660 [PMID: 24898451 DOI: 10.1002/jso.23679]

17 **Esquivel J**, Lowy AM, Markman M, Chua T, Pelz J, Baratti D, Baumgartner JM, Berri R, Bretcha-Boix P, Deraco M, Flores-Ayala G, Glehen O, Gomez-Portilla A, González-Moreno S, Goodman M, Halkia E, Kusamura S, Moller M, Passot G, Pocard M, Salti G, Sardi A, Senthil M, Spilioitis J, Torres-Melero J, Turaga K, Trout R. The American Society of Peritoneal Surface Malignancies (ASPSM) Multiinstitution Evaluation of the Peritoneal Surface Disease Severity Score (PSDSS) in 1,013 Patients with Colorectal Cancer with Peritoneal Carcinomatosis. *Ann Surg Oncol* 2014; **21**: 4195-4201 [PMID: 24854493 DOI: 10.1245/s10434-014-3798-z]

18 **Yoon W**, Alame A, Berri R. Peritoneal Surface Disease Severity Score as a predictor of resectability in the treatment of peritoneal surface malignancies. *Am J Surg* 2014; **207**: 403-407 discussion 406-407 [PMID: 24444858 DOI: 10.1016/j.amjsurg.2013.09.021]

19 **Cashin PH**, Graf W, Nygren P, Mahteme H. Comparison of prognostic scores for patients with colorectal cancer peritoneal metastases treated with cytoreductive surgery and hyperthermic intraperitoneal chemotherapy. *Ann Surg Oncol* 2013; **20**: 4183-4189 [PMID: 23975316 DOI: 10.1245/s10434-013-3204-2]

20 **Rectum JSfCotCa**. Japanese classification of colorectal carcinoma. Tokyo: Kanehara & Co., Ltd., 2009

21 **Kobayashi H**, Enomoto M, Higuchi T, Uetake H, Iida S, Ishikawa T, Ishiguro M, Sugihara K. Validation and clinical use of the Japanese classification of colorectal carcinomatosis: benefit of surgical cytoreduction even without hyperthermic intraperitoneal chemotherapy. *Dig Surg* 2010; **27**: 473-480 [PMID: 21063124 DOI: 10.1159/000320460]

22 **Noura S**, Ohue M, Ito Y, Miyoshi N, Kobayashi H, Kotake K, Sugihara K. New Staging System for Colorectal Cancer Patients with Synchronous Peritoneal Metastasis in Accordance with the Japanese Classification of Colorectal Carcinoma: A Multi-Institutional Study. *Dig Surg* 2016; **33**: 66-73 [PMID: 26618448 DOI: 10.1159/000442027]

23 **Franko J**, Shi Q, Goldman CD, Pockaj BA, Nelson GD, Goldberg RM, Pitot HC, Grothey A, Alberts SR, Sargent DJ. Treatment of colorectal peritoneal carcinomatosis with systemic chemotherapy: a pooled analysis of north central cancer treatment group phase III trials N9741 and N9841. *J Clin Oncol* 2012; **30**: 263-267 [PMID: 22162570 DOI: 10.1200/JCO.2011.37.1039]

24 **Klaver YL**, Simkens LH, Lemmens VE, Koopman M, Teerenstra S, Bleichrodt RP, de Hingh IH, Punt CJ. Outcomes of colorectal cancer patients with peritoneal carcinomatosis treated with chemotherapy with and without targeted therapy. *Eur J Surg Oncol* 2012; **38**:

617-623 [PMID: 22572106 DOI: 10.1016/j.ejso.2012.03.008]
25 **Lee DH**, Oh SY, Lee YR, Huh SJ, Yoon HH, Kim SH, Lee S, Lee JH, Kim Y, Kim HJ, Kwon HC. A Phase II Study of Modified FOLFOX4 for Colorectal Cancer Patients with Peritoneal Carcinomatosis. *Cancer Res Treat* 2011; **43**: 225-230 [PMID: 22247707 DOI: 10.4143/crt.2011.43.4.225]
26 **Elias D**, Lefevre JH, Chevalier J, Brouquet A, Marchal F, Classe JM, Ferron G, Guilloit JM, Meeus P, Goéré D, Bonastre J. Complete cytoreductive surgery plus intraperitoneal chemohyperthermia with oxaliplatin for peritoneal carcinomatosis of colorectal origin. *J Clin Oncol* 2009; **27**: 681-685 [PMID: 19103728 DOI: 10.1200/JCO.2008.19.7160]
27 **Cremolini C**, Loupakis F, Antoniotti C, Lupi C, Sensi E, Lonardi S, Mezi S, Tomasello G, Ronzoni M, Zaniboni A, Tonini G, Carlomagno C, Allegrini G, Chiara S, D'Amico M, Granetto C, Cazzaniga M, Boni L, Fontanini G, Falcone A. FOLFOXIRI plus bevacizumab versus FOLFIRI plus bevacizumab as first-line treatment of patients with metastatic colorectal cancer: updated overall survival and molecular subgroup analyses of the open-label, phase 3 TRIBE study. *Lancet Oncol* 2015; **16**: 1306-1315 [PMID: 26338525 DOI: 10.1016/S1470-2045(15)00122-9]
28 **Heinemann V**, von Weikersthal LF, Decker T, Kiani A, Vehling-Kaiser U, Al-Batran SE, Heintges T, Lerchenmüller C, Kahl C, Seipelt G, Kullmann F, Stauch M, Scheithauer W, Hielscher J, Scholz M, Müller S, Link H, Niederle N, Rost A, Höffkes HG, Moehler M, Lindig RU, Modest DP, Rossius L, Kirchner T, Jung A, Stintzing S. FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab as first-line treatment for patients with metastatic colorectal cancer (FIRE-3): a randomised, open-label, phase 3 trial. *Lancet Oncol* 2014; **15**: 1065-1075 [PMID: 25088940 DOI: 10.1016/S1470-2045(14)70330-4]
29 **Schwartzberg LS**, Rivera F, Karthaus M, Fasola G, Canon JL, Hecht JR, Yu H, Oliner KS, Go WY. PEAK: a randomized, multicenter phase II study of panitumumab plus modified fluorouracil, leucovorin, and oxaliplatin (mFOLFOX6) or bevacizumab plus mFOLFOX6 in patients with previously untreated, unresectable, wild-type KRAS exon 2 metastatic colorectal cancer. *J Clin Oncol* 2014; **32**: 2240-2247 [PMID: 24687833 DOI: 10.1200/JCO.2013.53.2473]
30 **Venook AP**, Niedzwiecki D, Lenz HJ, Innocenti F, Mahoney MR, O'Neil BH, Shaw JE, Polite BN, Hochster HS, Atkins JN, Goldberg RM, Mayer RJ, Schilsky RL, Bertagnolli MM, Blanke CD, ALLIANCE, SWOG. CALGB/SWOG 80405: Phase III trial of irinotecan/5-FU/leucovorin (FOLFIRI) or oxaliplatin/5-FU/leucovorin (mFOLFOX6) with bevacizumab (BV) or cetuximab (CET) for patients (pts) with KRAS wild-type (wt) untreated metastatic adenocarcinoma of the colon or rectum (MCRC). *J Clin Oncol* 2014; **32**: LBA3
31 **Adachi T**, Hinoi T, Egi H, Shimomura M, Ohdan H. Oxaliplatin and molecular-targeted drug therapies improved the overall survival in colorectal cancer patients with synchronous peritoneal carcinomatosis undergoing incomplete cytoreductive surgery. *Surg Today* 2015; **45**: 986-992 [PMID: 25156007 DOI: 10.1007/s00595-014-1017-y]
32 **Razenberg LG**, van Gestel YR, Lemmens VE, de Hingh IH, Creemers GJ. Bevacizumab in Addition to Palliative Chemotherapy for Patients With Peritoneal Carcinomatosis of Colorectal Origin: A Nationwide Population-Based Study. *Clin Colorectal Cancer* 2016; **15**: e41-e46 [PMID: 26762572 DOI: 10.1016/j.clcc.2015.12.006]
33 **van Oudheusden TR**, Razenberg LG, van Gestel YR, Creemers GJ, Lemmens VE, de Hingh IH. Systemic treatment of patients with metachronous peritoneal carcinomatosis of colorectal origin. *Sci Rep* 2015; **5**: 18632 [PMID: 26686250 DOI: 10.1038/srep18632]
34 **van Oudheusden TR**, Braam HJ, Luyer MD, Wiezer MJ, van Ramshorst B, Nienhuijs SW, de Hingh IH. Peritoneal cancer patients not suitable for cytoreductive surgery and HIPEC during explorative surgery: risk factors, treatment options, and prognosis. *Ann Surg Oncol* 2015; **22**: 1236-1242 [PMID: 25319584 DOI: 10.1245/s10434-014-4148-x]
35 **Tabernero J**, Yoshino T, Cohn AL, Obermannova R, Bodoky G, Garcia-Carbonero R, Ciuleanu TE, Portnoy DC, Van Cutsem E, Grothey A, Prausová J, Garcia-Alfonso P, Yamazaki K, Clingan PR, Lonardi S, Kim TW, Simms L, Chang SC, Nasroulah F. Ramucirumab versus placebo in combination with second-line FOLFIRI in patients with metastatic colorectal carcinoma that progressed during or after first-line therapy with bevacizumab, oxaliplatin, and a fluoropyrimidine (RAISE): a randomised, double-blind, multicentre, phase 3 study. *Lancet Oncol* 2015; **16**: 499-508 [PMID: 25877855 DOI: 10.1016/S1470-2045(15)70127-0]
36 **van Oudheusden TR**, Nienhuijs SW, Luyer MD, Nieuwenhuijzen GA, Lemmens VE, Rutten HJ, de Hingh IH. Incidence and treatment of recurrent disease after cytoreductive surgery and intraperitoneal chemotherapy for peritoneally metastasized colorectal cancer: A systematic review. *Eur J Surg Oncol* 2015; **41**: 1269-1277 [PMID: 26175345 DOI: 10.1016/j.ejso.2015.05.018]
37 **Goéré D**, Malka D, Tzanis D, Gava V, Boige V, Eveno C, Maggiori L, Dumont F, Ducreux M, Elias D. Is there a possibility of a cure in patients with colorectal peritoneal carcinomatosis amenable to complete cytoreductive surgery and intraperitoneal chemotherapy? *Ann Surg* 2013; **257**: 1065-1071 [PMID: 23299520 DOI: 10.1097/SLA.0b013e31827e9289]
38 **Cashin PH**, Dranichnikov F, Mahteme H. Cytoreductive surgery and hyperthermic intra-peritoneal chemotherapy treatment of colorectal peritoneal metastases: cohort analysis of high volume disease and cure rate. *J Surg Oncol* 2014; **110**: 203-206 [PMID: 24846340 DOI: 10.1002/jso.23610]
39 **Sugarbaker PH**. Peritonectomy procedures. *Ann Surg* 1995; **221**: 29-42 [PMID: 7826158 DOI: 10.1097/00000658-199501000-00004]
40 **Moran BJ**, Mukherjee A, Sexton R. Operability and early outcome in 100 consecutive laparotomies for peritoneal malignancy. *Br J Surg* 2006; **93**: 100-104 [PMID: 16302177 DOI: 10.1002/bjs.5210]
41 **McQuellon RP**, Loggie BW, Fleming RA, Russell GB, Lehman AB, Rambo TD. Quality of life after intraperitoneal hyperthermic chemotherapy (IPHC) for peritoneal carcinomatosis. *Eur J Surg Oncol* 2001; **27**: 65-73 [PMID: 11237495 DOI: 10.1053/ejso.2000.1033]
42 **McQuellon RP**, Loggie BW, Lehman AB, Russell GB, Fleming RA, Shen P, Levine EA. Long-term survivorship and quality of life after cytoreductive surgery plus intraperitoneal hyperthermic chemotherapy for peritoneal carcinomatosis. *Ann Surg Oncol* 2003; **10**: 155-162 [PMID: 12620911 DOI: 10.1245/ASO.2003.03.067]
43 **Chia CS**, Tan GH, Lim C, Soo KC, Teo MC. Prospective Quality of Life Study for Colorectal Cancer Patients with Peritoneal Carcinomatosis Undergoing Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy. *Ann Surg Oncol* 2016; Epub ahead of print [PMID: 27016293 DOI: 10.1245/s10434-016-5203-6]
44 **Sugarbaker PH**, Jablonski KA. Prognostic features of 51 colorectal and 130 appendiceal cancer patients with peritoneal carcinomatosis treated by cytoreductive surgery and intraperitoneal chemotherapy. *Ann Surg* 1995; **221**: 124-132 [PMID: 7857141 DOI: 10.1097/00000658-199502000-00002]
45 **Esquivel J**, Sticca R, Sugarbaker P, Levine E, Yan TD, Alexander R, Baratti D, Bartlett D, Barone R, Barrios P, Bieligk S, Bretcha-Boix P, Chang CK, Chu F, Chu Q, Daniel S, de Bree E, Deraco M, Dominguez-Parra L, Elias D, Flynn R, Foster J, Garofalo A, Gilly FN, Glehen O, Gomez-Portilla A, Gonzalez-Bayon L, Gonzalez-Moreno S, Goodman M, Gushchin V, Hanna N, Hartmann J, Harrison L, Hoefer R, Kane J, Kecmanovic D, Kelley S, Kuhn J, Lamont J, Lange J, Li B, Loggie B, Mahteme H, Mann G, Martin R, Misih RA, Moran B, Morris D, Onate-Ocana L, Petrelli N, Philippe G, Pingpank J, Pitroff A, Piso P, Quinones M, Riley L, Rutstein L, Saha S, Alrawi S, Sardi A, Schneebaum S, Shen P, Shibata D, Spellman J, Stojadinovic A, Stewart J, Torres-Melero J, Tuttle T, Verwaal V, Villar J, Wilkinson N, Younan R, Zeh H, Zoetmulder F, Sebbag G. Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy in the management of peritoneal surface malignancies of colonic origin: a consensus statement. Society of Surgical Oncology. *Ann Surg Oncol* 2007; **14**: 128-133 [PMID: 17072675 DOI: 10.1245/s10434-006-9185-7]
46 **Glockzin G**, Renner P, Popp FC, Dahlke MH, von Breitenbuch

P, Schlitt HJ, Piso P. Hepatobiliary procedures in patients undergoing cytoreductive surgery and hyperthermic intraperitoneal chemotherapy. *Ann Surg Oncol* 2011; **18**: 1052-1059 [PMID: 21046262 DOI: 10.1245/s10434-010-1415-3]

47 **Kianmanesh R**, Scaringi S, Sabate JM, Castel B, Pons-Kerjean N, Coffin B, Hay JM, Flamant Y, Msika S. Iterative cytoreductive surgery associated with hyperthermic intraperitoneal chemotherapy for treatment of peritoneal carcinomatosis of colorectal origin with or without liver metastases. *Ann Surg* 2007; **245**: 597-603 [PMID: 17414609 DOI: 10.1097/01.sla.0000255561.87771.11]

48 **Chua TC**, Yan TD, Zhao J, Morris DL. Peritoneal carcinomatosis and liver metastases from colorectal cancer treated with cytoreductive surgery perioperative intraperitoneal chemotherapy and liver resection. *Eur J Surg Oncol* 2009; **35**: 1299-1305 [PMID: 19632081 DOI: 10.1016/j.ejso.2009.07.005]

49 **Berger Y**, Aycart S, Tabrizian P, Agmon Y, Mandeli J, Heskel M, Hiotis S, Sarpel U, Labow DM. Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy in patients with liver involvement. *J Surg Oncol* 2016; **113**: 432-437 [PMID: 26804119 DOI: 10.1002/jso.24153]

50 **Allard MA**, Adam R, Ruiz A, Vibert E, Paule B, Levi F, Sebagh M, Guettier C, Azoulay D, Castaing D. Is unexpected peritoneal carcinomatosis still a contraindication for resection of colorectal liver metastases? Combined resection of colorectal liver metastases with peritoneal deposits discovered intra-operatively. *Eur J Surg Oncol* 2013; **39**: 981-987 [PMID: 23845705]

51 **Delhorme JB**, Dupont-Kazma L, Addeo P, Lefebvre F, Triki E, Romain B, Meyer N, Bachellier P, Rohr S, Brigand C. Peritoneal carcinomatosis with synchronous liver metastases from colorectal cancer: Who will benefit from complete cytoreductive surgery? *Int J Surg* 2016; **25**: 98-105 [PMID: 26607853 DOI: 10.1016/j.ijsu.2015.11.025]

52 **Kwakman R**, Schrama AM, van Olmen JP, Otten RH, de Lange-de Klerk ES, de Cuba EM, Kazemier G, Te Velde EA. Clinicopathological Parameters in Patient Selection for Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy for Colorectal Cancer Metastases: A Meta-analysis. *Ann Surg* 2016; **263**: 1102-1111 [PMID: 26756756 DOI: 10.1097/SLA.0000000000001593]

53 **Verwaal VJ**, Bruin S, Boot H, van Slooten G, van Tinteren H. 8-year follow-up of randomized trial: cytoreduction and hyperthermic intraperitoneal chemotherapy versus systemic chemotherapy in patients with peritoneal carcinomatosis of colorectal cancer. *Ann Surg Oncol* 2008; **15**: 2426-2432 [PMID: 18521686 DOI: 10.1245/s10434-008-9966-2]

54 **Verwaal VJ**, van Ruth S, de Bree E, van Sloothen GW, van Tinteren H, Boot H, Zoetmulder FA. Randomized trial of cytoreduction and hyperthermic intraperitoneal chemotherapy versus systemic chemotherapy and palliative surgery in patients with peritoneal carcinomatosis of colorectal cancer. *J Clin Oncol* 2003; **21**: 3737-3743 [PMID: 14551293 DOI: 10.1200/JCO.2003.04.187]

55 **Quenet F**, Goéré D, Mehta SS, Roca L, Dumont F, Hessissen M, Saint-Aubert B, Elias D. Results of two bi-institutional prospective studies using intraperitoneal oxaliplatin with or without irinotecan during HIPEC after cytoreductive surgery for colorectal carcinomatosis. *Ann Surg* 2011; **254**: 294-301 [PMID: 21772129 DOI: 10.1097/SLA.0b013e3182263933]

56 **Passot G**, Vaudoyer D, Villeneuve L, Kepenekian V, Beaujard AC, Bakrin N, Cotte E, Gilly FN, Glehen O. What made hyperthermic intraperitoneal chemotherapy an effective curative treatment for peritoneal surface malignancy: A 25-year experience with 1,125 procedures. *J Surg Oncol* 2016; **113**: 796-803 [PMID: 27110915 DOI: 10.1002/jso.24248]

57 **Glehen O**, Gilly FN, Boutitie F, Bereder JM, Quenet F, Sideris L, Mansvelt B, Lorimier G, Msika S, Elias D. Toward curative treatment of peritoneal carcinomatosis from nonovarian origin by cytoreductive surgery combined with perioperative intraperitoneal chemotherapy: a multi-institutional study of 1,290 patients. *Cancer* 2010; **116**: 5608-5618 [PMID: 20737573 DOI: 10.1002/cncr.25356]

58 **Hompes D**, D'Hoore A, Wolthuis A, Fieuws S, Mirck B, Bruin S, Verwaal V. The use of Oxaliplatin or Mitomycin C in HIPEC treatment for peritoneal carcinomatosis from colorectal cancer: a comparative study. *J Surg Oncol* 2014; **109**: 527-532 [PMID: 24375059 DOI: 10.1002/jso.23546]

59 **Glockzin G**, von Breitenbuch P, Schlitt HJ, Piso P. Treatment-related morbidity and toxicity of CRS and oxaliplatin-based HIPEC compared to a mitomycin and doxorubicin-based HIPEC protocol in patients with peritoneal carcinomatosis: a matched-pair analysis. *J Surg Oncol* 2013; **107**: 574-578 [PMID: 22833286 DOI: 10.1002/jso.23228]

60 **Prada-Villaverde A**, Esquivel J, Lowy AM, Markman M, Chua T, Pelz J, Baratti D, Baumgartner JM, Berri R, Bretcha-Boix P, Deraco M, Flores-Ayala G, Glehen O, Gomez-Portilla A, González-Moreno S, Goodman M, Halkia E, Kusamura S, Moller M, Passot G, Pocard M, Salti G, Sardi A, Senthil M, Spiliotis J, Torres-Melero J, Turaga K, Trout R. The American Society of Peritoneal Surface Malignancies evaluation of HIPEC with Mitomycin C versus Oxaliplatin in 539 patients with colon cancer undergoing a complete cytoreductive surgery. *J Surg Oncol* 2014; **110**: 779-785 [PMID: 25088304 DOI: 10.1002/jso.23728]

61 **Glockzin G**, Gerken M, Lang SA, Klinkhammer-Schalke M, Piso P, Schlitt HJ. Oxaliplatin-based versus irinotecan-based hyperthermic intraperitoneal chemotherapy (HIPEC) in patients with peritoneal metastasis from appendiceal and colorectal cancer: a retrospective analysis. *BMC Cancer* 2014; **14**: 807 [PMID: 25369730 DOI: 10.1186/1471-2407-14-807]

62 **Désolneux G**, Mazière C, Vara J, Brouste V, Fonck M, Béchade D, Bécouarn Y, Evrard S. Cytoreductive surgery of colorectal peritoneal metastases: outcomes after complete cytoreductive surgery and systemic chemotherapy only. *PLoS One* 2015; **10**: e0122816 [PMID: 25825874 DOI: 10.1371/journal.pone.0122816]

63 **Kobayashi H**, Kotake K, Funahashi K, Hase K, Hirata K, Iiai T, Kameoka S, Kanemitsu Y, Maeda K, Murata A, Ohue M, Shirouzu K, Takahashi K, Watanabe T, Yano H, Yatsuoka T, Hashiguchi Y, Sugihara K. Clinical benefit of surgery for stage IV colorectal cancer with synchronous peritoneal metastasis. *J Gastroenterol* 2014; **49**: 646-654 [PMID: 23793379 DOI: 10.1007/s00535-013-0820-3]

64 **Cashin PH**, Mahteme H, Spång N, Syk I, Frödin JE, Torkzad M, Glimelius B, Graf W. Cytoreductive surgery and intraperitoneal chemotherapy versus systemic chemotherapy for colorectal peritoneal metastases: A randomised trial. *Eur J Cancer* 2016; **53**: 155-162 [PMID: 26751236 DOI: 10.1016/j.ejca.2015.09.017]

65 **Sugarbaker PH**, Van der Speeten K. Surgical technology and pharmacology of hyperthermic perioperative chemotherapy. *J Gastrointest Oncol* 2016; **7**: 29-44 [PMID: 26941982 DOI: 10.3978/j.issn.2078-6891.2015.105]

66 **Klaver CE**, Musters GD, Bemelman WA, Punt CJ, Verwaal VJ, Dijkgraaf MG, Aalbers AG, van der Bilt JD, Boerma D, Bremers AJ, Burger JW, Buskens CJ, Evers P, van Ginkel RJ, van Grevenstein WM, Hemmer PH, de Hingh IH, Lammers LA, van Leeuwen BL, Meijerink WJ, Nienhuijs SW, Pon J, Radema SA, van Ramshorst B, Snaebjornsson P, Tuynman JB, Te Velde EA, Wiezer MJ, de Wilt JH, Tanis PJ. Adjuvant hyperthermic intraperitoneal chemotherapy (HIPEC) in patients with colon cancer at high risk of peritoneal carcinomatosis; the COLOPEC randomized multicentre trial. *BMC Cancer* 2015; **15**: 428 [PMID: 26003804 DOI: 10.1186/s12885-015-1430-7]

67 **Garofalo A**, Valle M, Garcia J, Sugarbaker PH. Laparoscopic intraperitoneal hyperthermic chemotherapy for palliation of debilitating malignant ascites. *Eur J Surg Oncol* 2006; **32**: 682-685 [PMID: 16631341]

68 **Valle SJ**, Alzahrani NA, Alzahrani SE, Liauw W, Morris DL. Laparoscopic hyperthermic intraperitoneal chemotherapy (HIPEC) for refractory malignant ascites in patients unsuitable for cytoreductive surgery. *Int J Surg* 2015; **23**: 176-180 [PMID: 26475090 DOI: 10.1016/j.ijsu.2015.09.074]

69 **Cavazzoni E**, Bugiantella W, Graziosi L, Franceschini MS, Donini A. Malignant ascites: pathophysiology and treatment. *Int J Clin Oncol* 2013; **18**: 1-9 [PMID: 22460778 DOI: 10.1007/s10147-012-0396-6]

70 **Glehen O**, Cotte E, Schreiber V, Sayag-Beaujard AC, Vignal J, Gilly FN. Intraperitoneal chemohyperthermia and attempted cytoreductive

surgery in patients with peritoneal carcinomatosis of colorectal origin. *Br J Surg* 2004; **91**: 747-754 [PMID: 15164446]

71 **Simkens GA**, Razenberg LG, Lemmens VE, Rutten HJ, Creemers GJ, de Hingh IH. Histological subtype and systemic metastases strongly influence treatment and survival in patients with synchronous colorectal peritoneal metastases. *Eur J Surg Oncol* 2016; **42**: 794-800 [PMID: 27055946 DOI: 10.1016/j.ejso.2016.03.014]

72 **Maillet M**, Glehen O, Lambert J, Goere D, Pocard M, Msika S, Passot G, Elias D, Eveno C, Sabaté JM, Lourenco N, André T, Gornet JM. Early Postoperative Chemotherapy After Complete Cytoreduction and Hyperthermic Intraperitoneal Chemotherapy for Isolated Peritoneal Carcinomatosis of Colon Cancer: A Multicenter Study. *Ann Surg Oncol* 2016; **23**: 863-869 [PMID: 26480848 DOI: 10.1245/s10434-015-4914-4]

73 **Passot G**, Vaudoyer D, Cotte E, You B, Isaac S, Noël Gilly F, Mohamed F, Glehen O. Progression following neoadjuvant systemic chemotherapy may not be a contraindication to a curative approach for colorectal carcinomatosis. *Ann Surg* 2012; **256**: 125-129 [PMID: 22580942 DOI: 10.1097/SLA.0b013e318255486a]

74 **Passot G**, You B, Boschetti G, Fontaine J, Isaac S, Decullier E, Maurice C, Vaudoyer D, Gilly FN, Cotte E, Glehen O. Pathological response to neoadjuvant chemotherapy: a new prognosis tool for the curative management of peritoneal colorectal carcinomatosis. *Ann Surg Oncol* 2014; **21**: 2608-2614 [PMID: 24668148 DOI: 10.1245/s10434-014-3647-0]

75 **Devilee RA**, Simkens GA, van Oudheusden TR, Rutten HJ, Creemers GJ, Ten Tije AJ, de Hingh IH. Increased Survival of Patients with Synchronous Colorectal Peritoneal Metastases Receiving Preoperative Chemotherapy Before Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy. *Ann Surg Oncol* 2016; Epub ahead of print [PMID: 27044447 DOI: 10.1245/s10434-016-5214-3]

76 **Kuijpers AM**, Mehta AM, Boot H, van Leerdam ME, Hauptmann M, Aalbers AG, Verwaal VJ. Perioperative systemic chemotherapy in peritoneal carcinomatosis of lymph node positive colorectal cancer treated with cytoreductive surgery and hyperthermic intraperitoneal chemotherapy. *Ann Oncol* 2014; **25**: 864-869 [PMID: 24667719 DOI: 10.1093/annonc/mdu031]

77 **Glockzin G**, Rochon J, Arnold D, Lang SA, Klebl F, Zeman F, Koller M, Schlitt HJ, Piso P. A prospective multicenter phase II study evaluating multimodality treatment of patients with peritoneal carcinomatosis arising from appendiceal and colorectal cancer: the COMBATAC trial. *BMC Cancer* 2013; **13**: 67 [PMID: 23391248 DOI: 10.1186/1471-2407-13-67]

78 **Piso P**, Koller M, Arnold D, Schlitt HJ, Glockzin G. Multimodality treatment of colorectal peritoneal metastasis with perioperative systemic chemotherapy, cytoreductive surgery (CRS), and HIPEC: First safety results of the COMBATAC trial. *J Clin Oncol* 2014; **32**: LBA382

79 **Willaert W**, Van Der Speeten K, Liberale G, Ceelen W. BEV-IP: Perioperative chemotherapy with bevacizumab in patients undergoing cytoreduction and intraperitoneal chemoperfusion for colorectal carcinomatosis. *BMC Cancer* 2015; **15**: 980 [PMID: 26673788 DOI: 10.1186/s12885-015-1954-x]

80 **Jaaback K**, Johnson N, Lawrie TA. Intraperitoneal chemotherapy for the initial management of primary epithelial ovarian cancer. *Cochrane Database Syst Rev* 2016; **(1)**: CD005340 [PMID: 26755441 DOI: 10.1002/14651858.CD005340.pub4]

81 **Yonemura Y**, Elnemr A, Endou Y, Ishibashi H, Mizumoto A, Miura M, Li Y. Effects of neoadjuvant intraperitoneal/systemic chemotherapy (bidirectional chemotherapy) for the treatment of patients with peritoneal metastasis from gastric cancer. *Int J Surg Oncol* 2012; **2012**: 148420 [PMID: 22900159 DOI: 10.1155/2012/148420]

82 **Solass W**, Giger-Pabst U, Zieren J, Reymond MA. Pressurized intraperitoneal aerosol chemotherapy (PIPAC): occupational health and safety aspects. *Ann Surg Oncol* 2013; **20**: 3504-3511 [PMID: 23765417 DOI: 10.1245/s10434-013-3039-x]

83 **Robella M**, Vaira M, De Simone M. Safety and feasibility of pressurized intraperitoneal aerosol chemotherapy (PIPAC) associated with systemic chemotherapy: an innovative approach to treat peritoneal carcinomatosis. *World J Surg Oncol* 2016; **14**: 128 [PMID: 27125996 DOI: 10.1186/s12957-016-0892-7]

84 **Tempfer CB**, Solass W, Reymond MA. Pressurized intraperitoneal chemotherapy (PIPAC) in women with gynecologic malignancies: a review. *Wien Med Wochenschr* 2014; **164**: 519-528 [PMID: 25249025 DOI: 10.1007/s10354-014-0312-y]

85 **Demtröder C**, Solass W, Zieren J, Strumberg D, Giger-Pabst U, Reymond MA. Pressurized intraperitoneal aerosol chemotherapy with oxaliplatin in colorectal peritoneal metastasis. *Colorectal Dis* 2016; **18**: 364-371 [PMID: 26400556 DOI: 10.1111/codi.13130]

86 **Odendahl K**, Solass W, Demtröder C, Giger-Pabst U, Zieren J, Tempfer C, Reymond MA. Quality of life of patients with end-stage peritoneal metastasis treated with Pressurized IntraPeritoneal Aerosol Chemotherapy (PIPAC). *Eur J Surg Oncol* 2015; **41**: 1379-1385 [PMID: 26138283 DOI: 10.1016/j.ejso.2015.06.001]

P- Reviewer: Kulaylat MN, Wang GY **S- Editor**: Gong ZM
L- Editor: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i3.353

World J Gastrointest Pharmacol Ther 2016 August 6; 7(3): 353-360
ISSN 2150-5349 (online)

TOPIC HIGHLIGHT

2016 Inflammatory Bowel Disease: Global view

Overview of cytokines and nitric oxide involvement in immuno-pathogenesis of inflammatory bowel diseases

Imene Soufli, Ryma Toumi, Hayet Rafa, Chafia Touil-Boukoffa

Imene Soufli, Ryma Toumi, Hayet Rafa, Chafia Touil-Boukoffa, Department of Cellular and Molecular Biology, University of Sciences and Technology Houari Boumediene, 16111 Algiers, Algeria

Author contributions: Soufli I analyzed the literature, wrote, submitted and edited the manuscript; Toumi R and Touil-Boukoffa C analyzed the literature and wrote the manuscript; Rafa H analysed and wrote a part of the manuscript.

Conflict-of-interest statement: The authors have no conflict of interest to report.

Manuscript source: Invited manuscript

Correspondence to: Chafia Touil-Boukoffa, Professor, Department of Cellular and Molecular Biology, University of Sciences and Technology Houari Boumediene, El alia, Bab Ezzouar, BP 32, 16111 Algiers, Algeria. touilboukoffa@yahoo.fr
Telephone: +213-550-819857

Received: March 29, 2016
Peer-review started: April 5, 2016
First decision: May 23, 2016
Revised: June 10, 2016
Accepted: July 14, 2016
Article in press: July 18, 2016
Published online: August 6, 2016

Abstract

Inflammatory bowel diseases (IBDs), including Crohn's disease and ulcerative colitis are complex disorders with undetermined etiology. Several hypotheses suggest that IBDs result from an abnormal immune response against endogenous flora and luminal antigens in genetically susceptible individuals. The dysfunction of the mucosal immune response is implicated in the pathogenesis of IBD. The balance between pro-inflammatory cytokines [tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-8, and IL-17A], anti-inflammatory cytokines (IL-4 and IL-13), and immunoregulatory cytokines (IL-10 and transforming growth factors β) is disturbed. Moreover, evidence from animal and clinical studies demonstrate a positive correlation between an increased concentration of nitric oxide (NO) and the severity of the disease. Interestingly, proinflammatory cytokines are involved in the up-regulation of inducible oxide synthase (iNOS) expression in IBD. However, anti-inflammatory and immunoregulatory cytokines are responsible for the negative regulation of iNOS. A positive correlation between NO production and increased pro-inflammatory cytokine levels (TNF-α, IL-6, IL-17, IL-12, and interferon-γ) were reported in patients with IBD. This review focuses on the role of cytokines in intestinal inflammation and their relationship with NO in IBD.

Key words: Inflammatory bowel disease; Cytokines; Nitric oxide; Inducible nitric oxide synthase; Immunopathogenesis

Core tip: Inflammatory bowel diseases (IBDs), including Crohn's disease and ulcerative colitis are an immunolgically mediated disease with undetermined etiology. Evidence from animal and clinical studies demonstrate a positive correlation between an increased concentration of nitric oxide (NO) and the severity of the disease. Moreover, a positive correlation between NO production and increased pro-inflammatory cytokine levels [tumor necrosis factor-α, interleukin (IL)-6, IL-17, IL-12, and interferon-γ] were reported in patients with IBD. This review focuses on

the role of cytokines in intestinal inflammation and their relationship with NO in IBD.

Soufli I, Toumi R, Rafa H, Touil-Boukoffa C. Overview of cytokines and nitric oxide involvement in immuno-pathogenesis of inflammatory bowel diseases. *World J Gastrointest Pharmacol Ther* 2016; 7(3): 353-360 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i3/353.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i3.353

INTRODUCTION

Inflammatory bowel disease (IBD), represented primarily by ulcerative colitis (UC) and Crohn's disease (CD) is a multifactorial condition characterized by the chronic inflammation of the gastrointestinal tract. It is widely accepted that IBD results from an uncontrolled mucosal immune response to intestinal microflora in genetically susceptible hosts[1,2]. The mechanisms underlying the deregulated immune response in IBD continue to be extensively investigated to understand the etio-physiopathology of this disease further and to identify new therapeutic strategies. The inflamed intestine of patients with IBD is massively infiltrated by inflammatory cells that release a large number of pro-inflammatory mediators, such as cytokines and nitric oxide (NO)[3].

NO is a free radical which has several physiological and pathological functions. It is generated from the oxidation of the amino acid L-arginine by a family of enzymes called the nitric oxide synthases (NOS). Three distinct isoforms of NOS are known: (1) two isoforms constitutively expressed in neuronal (nNOS); and (2) endothelial (eNOS) tissues; as well as an inducible isoform (iNOS) expressed primarily by immune cells (*e.g.,* macrophages)[4,5]. The constitutively expressed isoforms release low levels of NO that exert physiological functions, whereas iNOS releases a high output of NO production under immunogenic and inflammatory stimuli[6,7].

iNOS is highly expressed upon activation of the transcription factor nuclear factor-kappa B (NF-κB) in response to many stimuli including tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), interleukin (IL)-6, interleukin-1alpha (IL-1α), lipopolysaccharide (LPS), bacterial and viral components[8,9]. The protective actions of inducible NO have clearly been demonstrated. Its actions include protection against pathogens, reduction of leukocyte adherence, inhibition of macrophage activation, and the inhibition of Th1 type cytokines[10]. Substantial evidence suggests that iNOS-induced NO exerts protective effects during acute experimental colitis[11]. However, in IBD, the high levels of NO released in the mucosa appear to be strongly implicated in the maintenance of the chronic inflammation. In this context, it has been shown that NO can cause tissue damage and an exacerbation of inflammation indirectly through the generation of peroxynitrite[6,12].

The dysregulated balance between pro- and anti-inflammatory cytokines, as well as the immuno-regulatory cytokines observed in IBD distinguish a distinct T cell profile in CD and UC. Classically, CD is described as Th1 type immune response characterized by the secretion of IFN-γ, IL-12, and TNF-α. In contrast, UC is viewed similar to an atypical Th2 type immune response which generates high levels of IL-5, IL-4, and IL-13[13,14]. In addition, several studies have shown the involvement of Th17 type cytokines (*i.e.,* IL-17, IL-23, IL-22, and IL-6) in the pathogenic process of both CD and UC[15,16]. Interestingly, Both Th1 and Th17 cytokines are involved in the up-regulation of iNOS expression in IBD. Indeed, a positive correlation between NO production and increased pro-inflammatory cytokine levels (*e.g.,* TNF-α, IL-6, IL-17 IL-12 and IFN-γ) have been reported in IBD plasma[16,17].

Considerable research has been conducted over the past year to better understand the pathogenesis of IBD, and has led to the development of novel therapeutic strategies based on targeting cytokines, their receptors, as well as the modulation of NO. The assessment of NO production in IBD might be a useful inflammatory marker to predict the stage and the progression of disease[18]. Unfortunately, some of the current strategies have shown limited efficacy. Hence, a better understanding of the underlying mechanisms of the inflammation and the immune response in IBD may give rise to new alternative, complementary therapeutic strategies.

This review will address the cytokine involvement and relationship with NO in the immuno-pathogenesis of IBD.

NO AND IBD

NO is a lipophilic free radical which plays a key role in regulating the homeostasis of many biological systems. It is synthesized by NOS which catalyzes the oxidation of the terminal nitrogen of the amino acid L-arginine and produces L-citrulline and NO. Three NOS isoforms have been identified and characterized in humans and mice; their nomenclature respects the chronological order in which they were purified: (1) the neuronal form (nNOS or NOS1); (2) the inducible form (iNOS or NOS2); and (3) the endothelial form (eNOS or NOS3). nNOS and eNOS are termed constitutive NOS (cNOS) as they are calcium-dependent, and are respectively expressed constitutively in neuronal and endothelial tissues[3,4,6]. The effects of NO differ depending on the rate, duration, place of production, and the nature of the target molecules. Under physiological conditions, cNOS generate low levels of NO which have direct regulatory effects (*e.g.,* neurotransmission and the regulation of blood vessels)[18,19]. In contrast, iNOS generates high levels of NO which mediates antimicrobial and antitumoral activities[19]. This isoform was first isolated in murine macrophages and was subsequently found in several other cell types, including epithelial cells, hepatocytes, endothelial cells, and fibroblasts. It is expressed after the

induction by immunologic and inflammatory stimuli[6,20-22]. However, when NO is produced in excess, it becomes noxious. It causes deleterious effects indirectly through the creation of reactive nitric oxygen species (RNOS), such as peroxynitrite anion (OONO-), the nitroxyl anion (NO-) and dioxide nitrogen (NO2), responsible for the oxidative stress[7,23]. Peroxynitrite, is a molecule with high oxidative potential that can trigger cytotoxic processes, such as lipid peroxidation and DNA damage leading to tissue damage and inflammation[24]. NO has been implicated as a pathogenic mediator in a variety of conditions, such as Alzheimer's disease, rheumatoid arthritis (RA), Behçet disease, multiple sclerosis (MS), Sjogren's syndrome, and IBD[25].

The deleterious role of NO in IBD was proposed after clinical studies reported the presence of a high levels of nitrite/nitrate in the plasma, urine, and the lumen of the colon[26-28]. Moreover, a correlation between the overexpression of iNOS, the increased concentration of NO, and the severity of diseases was shown[29]. In fact, increased levels of NO were found in the serum, stool, and urine of patients in the active phase of UC and CD compared to those in the inactive phase[26,29]. Our study[16,17] showed significantly higher serum levels of NO in CD patients compared to UC patients. However, data from previous studies reported no significant differences between these two categories of disease, whereas higher systemic levels of NO in UC compared to CD was found[16,17,26,29]. A significant difference was observed in the NO concentrations between the active and inactive phase of the disease. This observation suggests a possible use of serum NO levels for monitoring disease activity in both types of IBD[16,28,29].

While several studies conducted using animal models indicate the deleterious effect of NO, recent studies have shown that NO may also exert a protective effect against colitis[29-32]. One study conducted using a DSS-induced colitis model found that nitrite administration exerts both preventive and therapeutic effects in colonic inflammation[33]. More recently, iNOS deficiency enhanced the inflammation aggravation in an animal model of colitis through enhancing a Th17 differentiated subset[34].

CYTOKINES IMPLICATED IN IBD

The dysfunction of the mucosal immune response in IBD is characterized by abnormalities in both the innate and adaptive immune systems. The final common pathway of this dysregulated immune activation is an abundant infiltration of immune cells in the intestinal mucosa[15,35-39]. These cells were found to release excessive proinflammatory mediators that amplify the inflammatory cascade through the activation of mitogen-activated protein kinases (MAPK) and NF-κB. Several studies have reported evidence of the contribution of cytokines, adhesion molecules, reactive oxygen metabolites (ROMs), and NO in mucosal inflammation and injury in triggering IBDs[40,41]. Cytokines are small soluble peptides which are produced by diverse immune and non-immune cells. They exert their biological functions through specific receptors activating the janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling pathway that controls gene expression in target cells[42]. In IBDs, the balance between pro-inflammatory cytokines (*e.g.,* TNF-α, IL-1β, IL-8, and IL-17), anti-inflammatory cytokines (*e.g.,* IL-4 and IL-13), and immunoregulatory cytokines (*e.g.,* IL-10 and TGF α) is disrupted[43]. According to the cytokine environment found in IBDs patients, CD and UC were conventionally associated with a different $CD4^+$ helper T cell profile based on the Th2/Th1 paradigm. Thus, CD was described as Th1 type immune response promoted by the transcription factors STAT-4 and T-bet and characterized by the secretion of IFN-γ, IL-12, and TNF-α[13,44]. Indeed, the studies conducted by our group and other teams produced high levels of IL-12 and IFN-γ in CD patients with active disease[17,45]. IL-12 produced by macrophages/monocytes and dendritic cells plays a pivotal role in enhancing natural killer (NK) cell-mediated cytotoxicity. Moreover, it has been shown that both IL-12 and IL-18 induce a high levels of IFN-γ production leading to the reinforcement of the Th1 immune response[16,45-47]. In addition, TNF-α plays a pivotal role in the production of NO and enhances the production of metalloproteinases (MMP) leading to the loss of epithelial integrity[48,49]. In contrast, UC is viewed as a Th2 type immune response promoted by the expression of the transcription factors STAT-6 and Gata-3, as well as the secretion of IL-5, IL-4, and IL-13. Furthermore, Fuss *et al*[50] demonstrated that UC patients, unlike CD patients, have atypical natural killer T (NKT)-cells. These cells produce high levels of IL-13 and have cytotoxic activity toward epithelial cells. Similarly, studies using the experimental model of colitis induced by oxazolone have demonstrated that IL-13 produced by NKT cells is the driving cytokine of the disease. Indeed, IL-13 causes alterations of the epithelial barrier function by stimulating epithelial cell apoptosis and the downregulation of tight junction proteins.

Currently, the aforementioned classical concept of the pathogenesis of IBDs is reconsidered with the strong involvement of Th17 cells. This subset of $CD4^+$ T helper cells is promoted by the activation of the transcription factors STAT-3 and retinoid-related orphan receptor gamma (ROR-γt) and is characterized by the production of IL-17A, IL-17F, IL-22, IL-21, IL-6, and IL-26, as well as the chemokine CCL20[51,52]. Several pieces of evidence support the implication that Th17 cells in the intestinal mucosa provide protection against invading pathogens (*e.g., Candida* and *Salmonella*), through the chemotaxis of neutrophils and the stimulation of antimicrobial peptide production by epithelial cells[53]. However, both in CD and UC, high levels of Th17 cytokines have been found in the serum and inflamed mucosa. Increased IL-17A production can drive and aggravate the chronic inflammatory response[17,54,55]. More recently, another subset of Th17 cells, Th1/Th17cells producing both IFN-γ and IL-17 has been identified in the ileal form of active CD and experimental models of colitis[56-58]. In addition, it

has been reported that Th17 induces the production of a high levels of TNF-α, IL-1β, chemokines (IL-8), and matrix metalloproteinases (MMP) (*e.g.,* MMP-9). Moreover, the expression of the cytokine IL-23 and chemokine CCL20, a chemoattractant for Th17 cells expressing the receptor CCR6, is highly up-regulated in CD lesions. Additionally, IL-23 is a crucial effector cytokine necessary for the stabilization and expansion of Th17 cells. It enhances the expression of the master transcription factor (RORγt) following IL-6 and tumor growth factor-beta (TGF-β) stimulation[46]. Moreover, it plays an important role in the development and propagation of the inflammatory response in the gut by inhibiting the expression of the transcription factor Forkhead box P3 (Foxp3) and the development of T regulatory cells (Treg)[15,46].

The Th17/Treg balance plays an essential role in maintaining intestinal homeostasis. The immunoregulatory cytokine, TGF-β orchestrates the differentiation of Th17 and Treg cells in a dose-dependent manner. In the presence of high levels of IL-6 and inflammatory mediators, TGF-β promotes the differentiation of Th17 cells. Conversely, high levels of TGF-β and low levels of IL-6 and inflammatory mediators promote the development of inducible Foxp3+Treg cells (iTreg)[59-61]. Regarding the pro-inflammatory role of IL-6, elevated levels of this cytokine and its soluble receptor, sIL-6R were found in the colonic mucosa and sera of patients with IBD. Compelling evidence in human and in animal models has shown that IL-6 plays an important role in maintaining a chronic response by promoting the accumulation of T cells resistant to apoptosis. In addition, IL-6 induces the production of IFN-γ, TNF-α, and IL-1β, and increases the expression of adhesion proteins, such as intercellular adhesion molecule-1 (ICAM-1) protein which participates in the migration and activation of inflammatory cells to the intestine[62,63].

It is well established that ongoing inflammation in CD and UC is mediated by uncontrolled T cell responses. Altered Treg regulatory mechanisms have been documented in IBD. However, it remains unclear whether this defect is due to a numerical lack of Treg or a defective TGF-β and IL-10 immunoregulatory activity[64,65]. Interestingly, it has been shown that in the inflamed colon of CD patients, there is a common $CD4^+$T cell population which co-expresses both Foxp3 and RORγt. This resident Treg population exhibits plasticity towards Th17 in an inflammatory environment. The Treg/Th17 balance is tightly regulated by intestinal factors, such as endogenous microflora as well as the presence of retinoic acid. Indeed, it has been reported that the vitamin A metabolite, retinoic acid promotes Treg differentiation while inhibiting the formation of Th17 cells[66]. Thus, these data support the involvement of an altered intestinal microenvironment in the development of IBD and the rupture of gut homeostasis.

Other studies conducted on IBD experimental models reported the implication of other cytokines with an immunomodulatory role [*e.g.,* IL-25, thymic stromal lymphopoietin (TSLP), and IL-22], thereby paving the way for new therapeutic strategies in IBD[67-69].

CYTOKINE REGULATION OF NO IN IBD

The inflamed tissue of patients with active IBD is characterized by a massive infiltration of immune cells that release several pro-inflammatory mediators and produce high, *de novo* levels of NO. The expression of iNOS is highly regulated at both the transcriptional and post-transcriptional level by several pro-inflammatory cytokines and immunogenic stimuli (*e.g.,* LPS)[6,70].

In both patients and animal models of IBD, a positive correlation between the overproduction of pro-inflammatory cytokines (*e.g.,* IL-1β, TNF-α, IFN-γ) and an overexpression of iNOS was found. This expression was primarily detected in the lamina propria mononuclear cells and the colon epithelial cells of the inflamed mucosa[6,16,17,27,30,71,72]. Several studies conducted on a dextran sulfate sodium (DSS)-induced experimental model of colitis in BALB/c mice indicated that the neutralization of endogenous TNF-α and/or IFN-γ ameliorated the chronic colitis and concomitantly decreased the generation of NO. These data support the fact that IFN-γ and TNF-α are both involved in the exacerbation of DSS-induced colitis and may exert their detrimental role in the colonic mucosa partly through the induction of the high output of NO. These cytokines had an additive effect on the severity of histological damage and NO colonic levels. However, it seems that IFN-ϕ is the most potent inducer of iNOS in macrophages and epithelial cells than TNF-α, since its neutralization was more effective in attenuating the experimental colitis[30].

Moreover, our studies reported an up-regulation of iNOS expression in the inflamed colonic mucosa which correlates with high systemic levels of NO, IFN-γ, and IL-12. These observations suggest that IFN-γ and IL-12 may play a pivotal role in IBD pathogenesis through the NO pathway[16]. Human peripheral blood mononuclear cells (PBMC) from IBD patients were shown to produce elevated levels of NO compared to the controls. The proinflammatory cytokines: IFN-γ, IL-6, TNF-α, and IL-1β stimulate NO production *in vitro* in PBMCs from patients with CD and UC, suggesting that human PBMCs may constitute another cellular source of NO in IBD[16,17]. Interestingly, this work reported a positive correlation between Th17 cytokines including IL-6, IL-23, IL-17A, and NO production in the plasma of patients with IBD. Moreover, the mucosal alterations were strongly correlated with high NOS2 and pSTAT3 expression in the colonic mucosa of patients with active IBD. These observations suggest that IL-17 may be a potent inducer of iNOS expression in the inflamed mucosa of IBD patients leading to the exacerbation of the tissue damage. The mechanism by which IL-17 induces NO production is likely dependent on the expression of NF-κB. In this context, *in vitro* studies using osteoclast cells showed that IL-17 induced the high expression of the mRNA of the NF-κB isoform RelA et p50[73].

The negative regulation of iNOS could be achieved by Th2 derived cytokines (*e.g.,* IL-13, IL-4). The inhi-

bitory effect of this cytokine on iNOS protein and mRNA expression has been demonstrated in the HT-29 epithelial cell line induced by IL-1α/TNF-α/IFN-γ. Interestingly, at low levels and in the presence of TNF-α, these cytokines exert an inhibitory effect on iNOS expression and activation. While a high level of these cytokines could inhibit iNOS mRNA induction in the absence of TNF-α[74]. The mechanism of the inhibitory effect of IL-13 on iNOS expression in epithelial cells is dependent on the activation of the PtdIns 3-kinase pathway[75].

In the same way, it has been shown that the immunosuppressive cytokine IL-10 down-regulates iNOS expression depending on the cell type. Indeed, unlike IL-13, IL-10 had no effect on iNOS expression in colonic epithelial cells but was able to inhibit NO production in mouse activated macrophages[6,74]. Recently, it has been reported the inhibition of NO and reactive oxygen species (ROS) levels in a mouse carrying a selective deletion of IL-10Rα in macrophages, had less severe colitis than wild-type mice. These data suggest that the protective effect of IL-10 is mainly mediated through the down-regulation of NO and ROS production by macrophages[76].

Globally, these observations and others suggest that cytokines present in the mucosa of patients with IBD modulate the iNOS expression and activity in the colonic epithelium and could play a homeostatic or inflammatory role in gut inflammation through iNOS modulation.

Many teams have shown that NO can, in turn, modulate the immune response by suppressing IL-12 production from dendritic cells and macrophages. In this manner, NO may control the generation of the Th1 response[77]. More recently, a study reported that the expression of iNOS in macrophages and dendritic cells can modulate inflammatory cytokine expression including, TNF-α, IL-6, IL-12p70, and IL-23. Growing evidence supports this notion and suggests that NO may control T helper cell differentiation[34,78]. Indeed, works conducted in an experimental model of colitis showed that an iNOS deficiency aggravated inflammation repetition and increased the percentage of Th17 cells. However, an NO donor molecule suppressed the IL-17 production in T cell-deficient NOS cultures and reduced the percentage of IL-17-producing $CD4^+$ T cells. NO has been found to regulate IL-17 expression at the transcriptional level through the nitration of tyrosine residues in RORγt, inhibiting its binding to the promoter region of the *IL-17* gene[34].

CONCLUSION

Cytokines play a crucial role in the pathogenesis of CD and UC as they orchestrate many aspects of intestinal inflammation. A disturbed balance between proinflammatory and immunoregulatory cytokines has been reported in IBD. High levels of proinflammatory cytokines detected in the mucosa of patients with IBD induce a decrease in NO-derived iNOS production. A decrease in NO and iNOS activity has been closely associated with the initiation and maintenance of inflammation in human and experimental IBD. Evidence suggests that immunoregulatory and anti-inflammatory cytokines (*e.g.,* IL-10, IL-13, and TGF-β) modulate the pro-inflammatory cytokine-derived iNOS expression and activity in intestinal inflammation, thus contributing to the maintenance of homeostasis in gut inflammation. In this context, several studies suggest that pro-inflammatory cytokines might be an important target for the modulation of intestinal inflammation. Moreover, studies using experimental models of IBD have led to a better understanding of cytokine involvement in the pathogenesis of IBD and have opened new lines of research based on their therapeutic relevance. To date, anti-TNFα is one of the most effective cytokine-based therapies for IBD. Nevertheless, several data have shown that the existence of a network of cytokines with multi-layered responses are involved in the perpetuation of the diseases and tissue injury. Therefore, it becomes rational to consider the possibility of simultaneous neutralization of more than one cytokine to provide long-term control of inflammation.

REFERENCES

1 **Sartor RB**. Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. *Nat Clin Pract Gastroenterol Hepatol* 2006; **3**: 390-407 [PMID: 16819502 DOI: 10.1038/ncpgasthep0528]

2 **Xavier RJ**, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. *Nature* 2007; **448**: 427-434 [PMID: 17653185 DOI: 10.1038/nature06005]

3 **Abraham C**, Medzhitov R. Interactions between the host innate immune system and microbes in inflammatory bowel disease. *Gastroenterology* 2011; **140**: 1729-1737 [PMID: 21530739 DOI: 10.1053/j.gastro.2011.02.012]

4 **Knowles RG**, Moncada S. Nitric oxide synthases in mamals. *Biochem J* 1994; **298**: 249-258 [PMID: 7510950 DOI: 10.1042/bj2980249]

5 **Stuehr DJ,** Griffith OW. Mammalian nitric oxide synthases. *Adv Enzymol Relat Areas Mol Biol* 1992; **65**: 287-346 [PMID: 1373932 DOI: 10.1002/9780470123119.ch8]

6 **Kolios G**, Valatas V, Ward SG. Nitric oxide in inflammatory bowel disease: a universal messenger in an unsolved puzzle. *Immunology* 2004; **113**: 427-437 [PMID: 15554920 DOI: 10.1111/j.1365-2567.2004.01984.x]

7 **Dijkstra G**, Moshage H, van Dullemen HM, de Jager-Krikken A, Tiebosch AT, Kleibeuker JH, Jansen PL, van Goor H. Expression of nitric oxide synthases and formation of nitrotyrosine and reactive oxygen species in inflammatory bowel disease. *J Pathol* 1998; **186**: 416-421 [PMID: 10209492 DOI: 10.1002/(SICI)1096-9896(199812)186]

8 **Hibbs JB**, Taintor RR, Vavrin Z, Rachlin EM. Nitric oxide: a cytotoxic activated macrophage effector molecule. *Biochem Biophys Res Commun* 1988; **157**: 87-94 [PMID: 3196352 DOI: 10.1016/S0006-291X(88)80015-9]

9 **Xie QW,** Kashiwabara Y, Nathan C. Role of transcription factor NF-κB/Rel in induction of nitric oxide synthase. *J Biol Chem* 1994; **269**: 4705-4708 [PMID: 7508926]

10 **De Caterina R**, Libby P, Peng HB, Thannickal VJ, Rajavashisth TB, Gimbrone MA, Shin WS, Liao JK. Nitric oxide decreases cytokine-induced endothelial activation. Nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines. *J Clin Invest* 1995; **96**: 60-68 [PMID: 7542286 DOI: 10.1172/JCI118074]

11 **Krieglstein CF**, Cerwinka WH, Laroux FS, Salter JW, Russell JM,

Schuermann G, Grisham MB, Ross CR, Granger DN. Regulation of murine intestinal inflammation by reactive metabolites of oxygen and nitrogen: divergent roles of superoxide and nitric oxide. *J Exp Med* 2001; **194**: 1207-1218 [PMID: 11696587 DOI: 10.1084/jem.194.9.1207]

12 **Rachmilewitz D**, Stamler JS, Bachwich D, Karmeli F, Ackerman Z, Podolsky DK. Enhanced colonic nitric oxide generation and nitric oxide synthase activity in ulcerative colitis and Crohn's disease. *Gut* 1995; **36**: 718-723 [PMID: 7541008 DOI: 10.1136/gut.36.5.718]

13 **Parronchi P**, Romagnani P, Annunziato F, Sampognaro S, Becchio A, Giannarini L, Maggi E, Pupilli C, Tonelli F, Romagnani S. Type 1 T-helper cell predominance and interleukin-12 expression in the gut of patients with Crohn's disease. *Am J Pathol* 1997; **150**: 823-832 [PMID: 9060820]

14 **Brown SJ**, Mayer L. The immune response in inflammatory bowel disease. *Am J Gastroenterol* 2007; **102**: 2058-2069 [PMID: 17561966 DOI: 10.1111/j.1572-0241.2007.01343.x]

15 **Sarra M**, Pallone F, Macdonald TT, Monteleone G. IL-23/IL-17 axis in IBD. *Inflamm Bowel Dis* 2010; **16**: 1808-1813 [PMID: 20222127 DOI: 10.1002/ibd.21248]

16 **Rafa H**, Saoula H, Belkhelfa M, Medjeber O, Soufli I, Toumi R, de Launoit Y, Moralès O, Nakmouche M, Delhem N, Touil-Boukoffa C. IL-23/IL-17A axis correlates with the nitric oxide pathway in inflammatory bowel disease: immunomodulatory effect of retinoic acid. *J Interferon Cytokine Res* 2013; **33**: 355-368 [PMID: 23472658 DOI: 10.1089/jir.2012.0063]

17 **Rafa H**, Amri M, Saoula H, Belkhelfa M, Medjeber O, Boutaleb A, Aftis S, Nakmouche M, Touil-Boukoffa C. Involvement of interferon-γ in bowel disease pathogenesis by nitric oxide pathway: a study in Algerian patients. *J Interferon Cytokine Res* 2010; **30**: 691-697 [PMID: 20626296 DOI: 10.1089/jir.2010.0012]

18 **Calabrese V**, Mancuso C, Calvani M, Rizzarelli E, Butterfield DA, Stella AM. Nitric oxide in the central nervous system: neuroprotection versus neurotoxicity. *Nat Rev Neurosci* 2007; **8**: 766-775 [PMID: 17882254 DOI: 10.1038/nrn2214]

19 **Calver A**, Collier J, Vallance P. Nitric oxide and cardiovascular control. *Exp Physiol* 1993; **78**: 303-326 [DOI: 10.1113/expphysiol.1993.sp003687]

20 **Bogdan C.** Nitric oxide and the immune response. *Nat Immunol* 2001; **2**: 907-916 [DOI: 10.1038/ni1001-907]

21 **Bogdan C**, Röllinghoff M, Diefenbach A. The role of nitric oxide in innate immunity. *Immunol Rev* 2000; **173**: 17-26 [PMID: 10719664 DOI: 10.1034/j.1600-065X.2000.917307.x]

22 **Nathan C**, Xie QW. Nitric oxide synthases: roles, tolls, and controls. *Cell* 1994; **78**: 915-918 [PMID: 7522969 DOI: 10.1016/0092-8674(94)90266-6]

23 **Singer II**, Kawka DW, Scott S, Weidner JR, Mumford RA, Riehl TE, Stenson WF. Expression of inducible nitric oxide synthase and nitrotyrosine in colonic epithelium in inflammatory bowel disease. *Gastroenterology* 1996; **111**: 871-885 [PMID: 8831582 DOI: 10.1016/S0016-5085(96)70055-0]

24 **Beckman JS**, Koppenol WH. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *Am J Physiol* 1996; **271**: C1424-C1437 [PMID: 8944624]

25 **Predonzani A**, Calì B, Agnellini AH, Molon B. Spotlights on immunological effects of reactive nitrogen species: When inflammation says nitric oxide. *World J Exp Med* 2015; **5**: 64-76 [PMID: 25992321 DOI: 10.5493/wjem.v5.i2.64]

26 **Cross RK**, Wilson KT. Nitric oxide in inflammatory bowel disease. *Inflamm Bowel Dis* 2003; **9**: 179-189 [PMID: 12792224 DOI: 10.1097/00054725-200305000-00006]

27 **Kimura H**, Hokari R, Miura S, Shigematsu T, Hirokawa M, Akiba Y, Kurose I, Higuchi H, Fujimori H, Tsuzuki Y, Serizawa H, Ishii H. Increased expression of an inducible isoform of nitric oxide synthase and the formation of peroxynitrite in colonic mucosa of patients with active ulcerative colitis. *Gut* 1998; **42**: 180-187 [PMID: 9536941 DOI: 10.1136/gut.42.2.180]

28 **Rachmilewitz D**, Eliakim R, Ackerman Z, Karmeli F. Direct determination of colonic nitric oxide level--a sensitive marker of disease activity in ulcerative colitis. *Am J Gastroenterol* 1998; **93**: 409-412 [PMID: 9517649]

29 **Avdagić N**, Zaćiragić A, Babić N, Hukić M, Seremet M, Lepara O, Nakaš-Ićindić E. Nitric oxide as a potential biomarker in inflammatory bowel disease. *Bosn J Basic Med Sci* 2013; **13**: 5-9 [PMID: 23448603]

30 **Obermeier F**, Kojouharoff G, Hans W, Schölmerich J, Gross V, Falk W. Interferon-gamma (IFN-gamma)- and tumour necrosis factor (TNF)-induced nitric oxide as toxic effector molecule in chronic dextran sulphate sodium (DSS)-induced colitis in mice. *Clin Exp Immunol* 1999; **116**: 238-245 [PMID: 10337013 DOI: 10.1046/j.1365-2249.1999.00878.x]

31 **Abdelouhab K**, Rafa H, Toumi R, Bouaziz S, Medjeber O, Touil-Boukoffa C. Mucosal intestinal alteration in experimental colitis correlates with nitric oxide production by peritoneal macrophages: effect of probiotics and prebiotics. *Immunopharmacol Immunotoxicol* 2012; **34**: 590-597 [PMID: 22211319 DOI: 10.3109/08923973.2011.641971]

32 **Tun X**, Yasukawa K, Yamada K. Involvement of nitric oxide with activation of Toll-like receptor 4 signaling in mice with dextran sodium sulfate-induced colitis. *Free Radic Biol Med* 2014; **74**: 108-117 [PMID: 24992835 DOI: 10.1016/j.freeradbiomed.2014.06.020]

33 **Jädert C**, Phillipson M, Holm L, Lundberg JO, Borniquel S. Preventive and therapeutic effects of nitrite supplementation in experimental inflammatory bowel disease. *Redox Biol* 2014; **2**: 73-81 [PMID: 24494186 DOI: 10.1016/j.redox.2013.12.012]

34 **Jianjun Yang R**, Lu G, Shen Y, Peng L, Zhu C, Cui M, Wang W, Arnaboldi P, Tang M, Gupta M, Qi CF, Jayaraman P, Zhu H, Jiang B, Chen SH, He JC, Ting AT, Zhou MM, Kuchroo VK, Morse HC, Ozato K, Sikora AG, Xiong H. T cell-derived inducible nitric oxide synthase switches off Th17 cell differentiation. *J Exp Med* 2013; **210**: 1447-1462 [PMID: 23797094 DOI: 10.1084/jem.20122494]

35 **Fuss IJ**, Neurath M, Boirivant M, Klein JS, de la Motte C, Strong SA, Fiocchi C, Strober W. Disparate CD4+ lamina propria (LP) lymphokine secretion profiles in inflammatory bowel disease. Crohn's disease LP cells manifest increased secretion of IFN-gamma, whereas ulcerative colitis LP cells manifest increased secretion of IL-5. *J Immunol* 1996; **157**: 1261-1270 [PMID: 8757634]

36 **Xu XR**, Liu CQ, Feng BS, Liu ZJ. Dysregulation of mucosal immune response in pathogenesis of inflammatory bowel disease. *World J Gastroenterol* 2014; **20**: 3255-3264 [PMID: 24695798 DOI: 10.3748/wjg.v20.i12.3255]

37 **Yen D,** Cheung J, Scheerens H, Poulet F, McClanahan T, McKenzie B, Kleinschek MA, Owyang A, Mattson J, Blumenschein W, Murphy E, Sathe M, Cua DJ, Kastelein RA, Rennick D. IL-23 is essential for T cell-mediated colitis and promotes inflammation via IL-17 and IL-6. *J Clin Invest* 2006; **116**: 1310-1316 [PMID: 16670770 DOI: 10.1172/JCI21404]

38 **de Souza HS,** Fiocchi C. Immunopathogenesis of IBD: current state of the art. *Nat Rev Gastroenterol Hepatol* 2016; **13**: 13-27 [PMID: 26627550 DOI: 10.1038/nrgastro.2015.186]

39 **MacDonald TT**, Monteleone I, Fantini MC, Monteleone G. Regulation of homeostasis and inflammation in the intestine. *Gastroenterology* 2011; **140**: 1768-1775 [PMID: 21530743 DOI: 10.1053/j.gastro.2011.02.047]

40 **Rakoff-Nahoum S**, Medzhitov R. Innate immune recognition of the indigenous microbial flora. *Mucosal Immunol* 2008; **1** Suppl 1: S10-S14 [PMID: 19079220 DOI: 10.1038/mi.2008.49]

41 **Danese S**, Fiocchi C. Etiopathogenesis of inflammatory bowel diseases. *World J Gastroenterol* 2006; **12**: 4807-4812 [PMID: 16937461]

42 **Kiu H**, Nicholson SE. Biology and significance of the JAK/STAT signalling pathways. *Growth Factors* 2012; **30**: 88-106 [PMID: 22339650 DOI: 10.3109/08977194.2012.660936]

43 **Beck PL**, Wallace JL. Cytokines in inflammatory bowel disease. *Mediators Inflamm* 1997; **6**: 95-103 [PMID: 18472842 DOI: 10.1080/09629359791785]

44 **Szabo SJ**, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell* 2000; **100**: 655-669 [PMID: 10761931 DOI: 10.1016/

S0092-8674(00)80702-3]
45 **Matsuoka K**, Inoue N, Sato T, Okamoto S, Hisamatsu T, Kishi Y, Sakuraba A, Hitotsumatsu O, Ogata H, Koganei K, Fukushima T, Kanai T, Watanabe M, Ishii H, Hibi T. T-bet upregulation and subsequent interleukin 12 stimulation are essential for induction of Th1 mediated immunopathology in Crohn's disease. *Gut* 2004; **53**: 1303-1308 [PMID: 15306590 DOI: 10.1136/gut.2003.024190]
46 **McGovern D**, Powrie F. The IL23 axis plays a key role in the pathogenesis of IBD. *Gut* 2007; **56**: 1333-1336 [PMID: 17872562 DOI: 10.1136/gut.2006.115402]
47 **Monteleone G**, Trapasso F, Parrello T, Biancone L, Stella A, Iuliano R, Luzza F, Fusco A, Pallone F. Bioactive IL-18 expression is up-regulated in Crohn's disease. *J Immunol* 1999; **163**: 143-147 [PMID: 10384110]
48 **Bradley JR**. TNF-mediated inflammatory disease. *J Pathol* 2008; **214**: 149-160 [PMID: 18161752 DOI: 10.1002/path.2287]
49 **Begue B**, Wajant H, Bambou JC, Dubuquoy L, Siegmund D, Beaulieu JF, Canioni D, Berrebi D, Brousse N, Desreumaux P, Schmitz J, Lentze MJ, Goulet O, Cerf-Bensussan N, Ruemmele FM. Implication of TNF-related apoptosis-inducing ligand in inflammatory intestinal epithelial lesions. *Gastroenterology* 2006; **130**: 1962-1974 [PMID: 16762619 DOI: 10.1053/j.gastro.2006.03.022]
50 **Fuss IJ**, Heller F, Boirivant M, Leon F, Yoshida M, Fichtner-Feigl S, Yang Z, Exley M, Kitani A, Blumberg RS, Mannon P, Strober W. Nonclassical CD1d-restricted NK T cells that produce IL-13 characterize an atypical Th2 response in ulcerative colitis. *J Clin Invest* 2004; **113**: 1490-1497 [PMID: 15146247 DOI: 10.1172/JCI19836]
51 **Korn T**, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. *Annu Rev Immunol* 2009; **27**: 485-517 [PMID: 19132915 DOI: 10.1146/annurev.immunol.021908.132710]
52 **Monteleone I**, Sarra M, Pallone F, Monteleone G. Th17-related cytokines in inflammatory bowel diseases: friends or foes? *Curr Mol Med* 2012; **12**: 592-597 [PMID: 22515978 DOI: 10.2174/1566524128006200 66]
53 **McGeachy MJ**, McSorley SJ. Microbial-induced Th17: superhero or supervillain? *J Immunol* 2012; **189**: 3285-3291 [PMID: 22997231 DOI: 10.4049/jimmunol.1201834]
54 **Powrie F**. Gut reactions: immune pathways in the intestine in health and disease. *EMBO Mol Med* 2012; **4**: 71-74 [PMID: 22278905 DOI: 10.1002/emmm.201100197]
55 **Fujino S**, Andoh A, Bamba S, Ogawa A, Hata K, Araki Y, Bamba T, Fujiyama Y. Increased expression of interleukin 17 in inflammatory bowel disease. *Gut* 2003; **52**: 65-70 [PMID: 12477762 DOI: 10.1136/gut.52.1.65]
56 **Annunziato F**, Cosmi L, Santarlasci V, Maggi L, Liotta F, Mazzinghi B, Parente E, Filì L, Ferri S, Frosali F, Giudici F, Romagnani P, Parronchi P, Tonelli F, Maggi E, Romagnani S. Phenotypic and functional features of human Th17 cells. *J Exp Med* 2007; **204**: 1849-1861 [PMID: 17635957 DOI: 10.1084/jem.20070663]
57 **Brand S**. Crohn's disease: Th1, Th17 or both? The change of a paradigm: new immunological and genetic insights implicate Th17 cells in the pathogenesis of Crohn's disease. *Gut* 2009; **58**: 1152-1167 [PMID: 19592695 DOI: 10.1136/gut.2008.163667]
58 **Rovedatti L**, Kudo T, Biancheri P, Sarra M, Knowles CH, Rampton DS, Corazza GR, Monteleone G, Di Sabatino A, Macdonald TT. Differential regulation of interleukin 17 and interferon gamma production in inflammatory bowel disease. *Gut* 2009; **58**: 1629-1636 [PMID: 19740775 DOI: 10.1136/gut.2009.182170]
59 **Fehérvari Z**, Sakaguchi S. CD4+ Tregs and immune control. *J Clin Invest* 2004; **114**: 1209-1217 [PMID: 15520849 DOI: 10.1172/JCI200423395]
60 **Weaver CT**, Hatton RD. Interplay between the TH17 and TReg cell lineages: a (co-)evolutionary perspective. *Nat Rev Immunol* 2009; **9**: 883-889 [PMID: 19935807 DOI: 10.1038/nri2660]
61 **Fontenot JD**, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol* 2003; **4**: 330-336 [PMID: 12612578 DOI: 10.1038/ni904]
62 **Yamamoto M**, Yoshizaki K, Kishimoto T, Ito H. IL-6 is required for the development of Th1 cell-mediated murine colitis. *J Immunol* 2000; **164**: 4878-4882 [PMID: 10779797 DOI: 10.4049/jimmunol.164.9.4878]
63 **Atreya R**, Neurath MF. Involvement of IL-6 in the pathogenesis of inflammatory bowel disease and colon cancer. *Clin Rev Allergy Immunol* 2005; **28**: 187-196 [PMID: 16129903 DOI: 10.1385/CRIAI: 28: 3: 187]
64 **Asseman C**, Mauze S, Leach MW, Coffman RL, Powrie F. An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. *J Exp Med* 1999; **190**: 995-1004 [PMID: 10510089 DOI: 10.1084/jem.190.7.995]
65 **Buckner JH**. Mechanisms of impaired regulation by CD4(+) CD25(+)FOXP3(+) regulatory T cells in human autoimmune diseases. *Nat Rev Immunol* 2010; **10**: 849-859 [PMID: 21107346 DOI: 10.1038/nri2889]
66 **Benson MJ**, Pino-Lagos K, Rosemblatt M, Noelle RJ. All-trans retinoic acid mediates enhanced T reg cell growth, differentiation, and gut homing in the face of high levels of co-stimulation. *J Exp Med* 2007; **204**: 1765-1774 [PMID: 17620363 DOI: 10.1084/jem.20070719]
67 **Caruso R**, Sarra M, Stolfi C, Rizzo A, Fina D, Fantini MC, Pallone F, MacDonald TT, Monteleone G. Interleukin-25 inhibits interleukin-12 production and Th1 cell-driven inflammation in the gut. *Gastroenterology* 2009; **136**: 2270-2279 [PMID: 19505427 DOI: 10.1053/j.gastro.2009.02.049]
68 **Sugimoto K**, Ogawa A, Mizoguchi E, Shimomura Y, Andoh A, Bhan AK, Blumberg RS, Xavier RJ, Mizoguchi A. IL-22 ameliorates intestinal inflammation in a mouse model of ulcerative colitis. *J Clin Invest* 2008; **118**: 534-544 [PMID: 18172556 DOI: 10.1172/JCI33194]
69 **Rimoldi M**, Chieppa M, Salucci V, Avogadri F, Sonzogni A, Sampietro GM, Nespoli A, Viale G, Allavena P, Rescigno M. Intestinal immune homeostasis is regulated by the crosstalk between epithelial cells and dendritic cells. *Nat Immunol* 2005; **6**: 507-514 [PMID: 15821737 DOI: 10.1038/ni1192]
70 **Aktan F**. iNOS-mediated nitric oxide production and its regulation. *Life Sci* 2004; **75**: 639-653 [PMID: 15172174 DOI: 10.1016/j.lfs.2003.10.042]
71 **Soufli I**, Toumi R, Rafa H, Amri M, Labsi M, Khelifi L, Nicoletti F, Touil-Boukoffa C. Crude extract of hydatid laminated layer from Echinococcus granulosus cyst attenuates mucosal intestinal damage and inflammatory responses in Dextran Sulfate Sodium induced colitis in mice. *J Inflamm* (Lond) 2015; **12**: 19 [PMID: 25844068 DOI: 10.1186/s12950-015-0063-6]
72 **Toumi R**, Soufli I, Rafa H, Belkhelfa M, Biad A, Touil-Boukoffa C. Probiotic bacteria lactobacillus and bifidobacterium attenuate inflammation in dextran sulfate sodium-induced experimental colitis in mice. *Int J Immunopathol Pharmacol* 2014; **27**: 615-627 [PMID: 25572742]
73 **Van Bezooijen RL**, Papapoulos SE, Löwik CW. Effect of interleukin-17 on nitric oxide production and osteoclastic bone resorption: is there dependency on nuclear factor-kappaB and receptor activator of nuclear factor kappaB (RANK)/RANK ligand signaling? *Bone* 2001; **28**: 378-386 [PMID: 11336918 DOI: 10.1016/S8756-3282(00)00457-9]
74 **Linehan JD**, Kolios G, Valatas V, Robertson DA, Westwick J. Immunomodulatory cytokines suppress epithelial nitric oxide production in inflammatory bowel disease by acting on mononuclear cells. *Free Radic Biol Med* 2005; **39**: 1560-1569 [PMID: 16298681 DOI: 10.1016/j.freeradbiomed.2005.07.019]
75 **Wright K**, Ward SG, Kolios G, Westwick J. Activation of phosphatidylinositol 3-kinase by interleukin-13. An inhibitory signal for inducible nitric-oxide synthase expression in epithelial cell line HT-29. *J Biol Chem* 1997; **272**: 12626-12633 [PMID: 9139718 DOI: 10.1074/jbc.272.19.12626]
76 **Li B**, Alli R, Vogel P, Geiger TL. IL-10 modulates DSS-induced colitis through a macrophage-ROS-NO axis. *Mucosal Immunol* 2014; **7**: 869-878 [PMID: 24301657 DOI: 10.1038/mi.2013.103]
77 **Xiong H**, Zhu C, Li F, Hegazi R, He K, Babyatsky M, Bauer AJ, Plevy SE. Inhibition of interleukin-12 p40 transcription and NF-

kappaB activation by nitric oxide in murine macrophages and dendritic cells. *J Biol Chem* 2004; **279**: 10776-10783 [PMID: 14679201 DOI: 10.1074/jbc.M313416200]

78 **Niedbala W**, Cai B, Liew FY. Role of nitric oxide in the regulation of T cell functions. *Ann Rheum Dis* 2006; **65** Suppl 3: iii37-iii40 [PMID: 17038470 DOI: 10.1136/ard.2006.058446]

P- Reviewer: Liu MY, Rahimi R **S- Editor**: Qi Y **L- Editor**: A
E- Editor: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i3.361

World J Gastrointest Pharmacol Ther 2016 August 6; 7(3): 361-369
ISSN 2150-5349 (online)

TOPIC HIGHLIGHT

2016 Inflammatory Bowel Disease: Global view

Infertility in men with inflammatory bowel disease

Takeshi Shin, Hiroshi Okada

Takeshi Shin, Hiroshi Okada, Department of Urology, Dokkyo Medical University Koshigaya Hospital, Saitama 343-8555, Japan

Author contributions: Shin T designed the study, performed the literature review, and wrote the manuscript; Okada H collected data and analyzed them and performed critical revision of the manuscript for important intellectual content; all authors gave final approval of the version of the manuscript to be published.

Conflict-of-interest statement: The authors declare no conflict of interest related to this publication.

Manuscript source: Invited manuscript

Correspondence to: Takeshi Shin, MD, Assistant Professor, Department of Urology, Dokkyo Medical University Koshigaya Hospital, 2-1-50 Minamikoshigaya, Koshigaya City, Saitama 343-8555, Japan. shintakeshi@nifty.com
Telephone: +81-48-9651111
Fax: +81-46-9651111

Received: April 5, 2016
Peer-review started: April 5, 2016
First decision: June 6, 2016
Revised: June 19, 2016
Accepted: July 20, 2016
Article in press: July 22, 2016
Published online: August 6, 2016

Abstract

Inflammatory bowel disease (IBD) predominantly affects young adults. Fertility-related issues are therefore important in the management of patients with IBD. However, relatively modest attention has been paid to reproductive issues faced by men with IBD. To investigate the effects of IBD and its treatment on male fertility, we reviewed the current literature using a systematic search for published studies. A PubMed search were performed using the main search terms "IBD AND male infertility", "Crohn's disease AND male infertility", "ulcerative colitis AND male infertility". References in review articles were used if relevant. We noted that active inflammation, poor nutrition, alcohol use, smoking, medications, and surgery may cause infertility in men with IBD. In surgery such as proctocolectomy with ileal pouch-anal anastomosis, rectal incision seems to be associated with sexual dysfunction. Of the medications used for IBD, sulfasalazine reversibly reduces male fertility. No other medications appear to affect male fertility significantly, although small studies suggested some adverse effects. There are limited data on the effects of drugs for IBD on male fertility and pregnancy outcomes; however, patients should be informed of the possible effects of paternal drug exposure. This review provides information on fertility-related issues in men with IBD and discusses treatment options.

Key words: Crohn's disease; Infertility; Inflammatory bowel disease; Male; Ulcerative colitis

Core tip: In men with inflammatory bowel disease (IBD), factors such as surgery, medications, disease activity, and poor nutritional status are thought to contribute to infertility. Surgery with rectal incision is associated with sexual dysfunction (*e.g.*, erectile dysfunction, anejaculation, and retrograde ejaculation). Among medications, sulfasalazine causes reversible qualitative and quantitative semen abnormalities. No other medications seem to affect male fertility significantly. There are limited data on the effects of paternal exposure to IBD medications on pregnancy outcomes, but no significant increase in fetal risk has been noted except for thiopurines. Patients should be

appropriately informed of possible effects of paternal drug exposure.

Shin T, Okada H. Infertility in men with inflammatory bowel disease. *World J Gastrointest Pharmacol Ther* 2016; 7(3): 361-369 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i3/361.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i3.361

INTRODUCTION

Inflammatory bowel disease (IBD) such as Crohn's disease (CD) and ulcerative colitis (UC) is a chronic intestinal disorder usually diagnosed in early adulthood. The incidence of IBD has been found to be the highest between the second and fourth decade of life[1], and fertility-related issues are therefore important clinical considerations.

Infertility is defined as a disease of the reproductive system characterized by failure to achieve a clinical pregnancy after ⩾ 12 mo of regular unprotected sexual intercourse[2]. Much attention has been focused on issues related to fertility in women with IBD, but relatively little attention has been paid to the reproductive issues faced by men with IBD. Male infertility is thought to be more prevalent in IBD patients than in the general population[3]. From a case control study, Moody *et al*[4] showed that the number of children born to men with CD is significantly lower in comparison to men with UC and the general population, but found no difference in the number of children between men with UC and the general population. Notably, the fecundability of the three groups did not differ significantly[5], and the frequency of sexual intercourse was not significantly different between the patients with IBD and the matched controls[6]. Heetun *et al*[7] suggested that the smaller family size might be due to a fear of passing on the disease to offspring or a decision to limit family size rather than a physical effect of the disease. A recent systematic review of non-surgically treated men with CD revealed a 18%-50% reduction in fertility with no difference in reproductive capacity[8].

Even if overall IBD itself does not seem to affect fertility in men, medications used to treat the disease, surgery, and malnutrition resulting from IBD may cause male infertility, including sexual dysfunction. Table 1 shows the possible causes of infertility in men with IBD. This article summarizes sexual and reproductive issues associated with male IBD patients.

Table 1 Possible causes of infertility in men with inflammatory bowel disease

Causes of infertility in men with IBD	Ref.
Surgery	[17,19-25]
Medications	[4,5,7,15,16,28-32,42,43]
Active disease	[15,16,76]
Poor nutrition	[15,77]
Alcohol use	[15,81-83]
Tobacco use	[15,83,86,87]
Psychological factor	[7,88,89]

IBD: Inflammatory bowel disease.

SURGERY CAUSING MALE INFERTILITY

It is estimated that approximately 25%-35% of UC patients will ultimately require surgery for either a complication of the disease or inadequate control of symptoms, and 70%-90% of CD patients will need a surgical intervention at some point in the course of their disease[9-11]. Surgery is required in cases of failure of medical management, risk of malignancy, intestinal obstruction and toxic megacolon. Especially in patients with CD, complications such as perianal abscesses, fistulas, and stenosis can occur during the course of the disease, and surgery is often indicated in these cases[12,13]. Surgical treatment of perianal fistulas ranges from minimal surgery like seton and fistulotomy to definitive surgery with closure of the fistula tract or proctectomy and fecal diversion[13]. Currently, the most frequently performed surgical procedure for UC is restorative proctocolectomy with ileal pouch-anal anastomosis (IPAA), while intestinal resection is the most commonly performed surgical procedure for CD[14].

Proctocolectomy with IPAA seems to be associated with sexual dysfunction in men[15,16]. The sexual disturbances after proctocolectomy are usually due to damage to parasympathetic and sympathetic nerves during surgery, but sometimes due to anatomical alterations, fibrosis, or psychological factors[17].

Sexual dysfunction is one of the etiologies of male infertility, and it includes erectile dysfunction and ejaculatory dysfunction such as retrograde ejaculation and anejaculation (no ejaculation). A meta-analysis found that the pooled incidence of sexual dysfunction from 21 studies comprising 5112 patients was 3.6%[18], but this meta-analysis included both men and women. When focusing on only men, Berndtsson *et al*[19] found that 12% of male patients with UC had ejaculatory dysfunction after IPAA. In a retrospective study, Hueting *et al*[20] showed the incidence of erectile dysfunction or retrograde ejaculation in such patients to be 25.7%. A study of 122 men who underwent IPAA found that the prevalence of retrograde ejaculation increased from 1.6% preoperatively to 8.2% postoperatively, but the prevalence of erectile dysfunction was similar before and after IPAA[21]. On the other hand, a large study by Farouk *et al*[22] found sexual dysfunction in 1% of male patients (n = 762) at 1 year after IPAA, and in 2% (n = 215) at 12 years after IPAA. Table 2 shows an overview of past studies of sexual function after proctocolectomy in men[17,19-25]. Regarding the treatment of sexual dysfunction due to rectal excision, there has been one randomized placebo-controlled trial for sildenafil for erectile dysfunction[26]. This study showed a successful

Table 2 Studies of sexual function after proctocolectomy in men

Ref.	Year	No. of patients	Disease	Time since surgery	Sexual dysfunction after surgery	
					ED	EjD
Michelassi *et al*[23]	1993	24	UC	1.5 yr (median)	0%	19%
Damgaard *et al*[24]	1995	26	UC	2.8 yr (median)	3.80%	3.80%
Farouk *et al*[22]	2000	762	UC	1 yr	1%	
		215	UC	12 yr	2%	
Slors *et al*[17]	2000	40	Benign disease	2.8 yr (median)	10%	12.50%
Lindsey *et al*[25]	2001	156	CD, UC	6.2 yr (median)	14%	0%
Berndtsson *et al*[19]	2004	25	UC	1 yr	0%	12%
Hueting *et al*[20]	2004	35	CD, UC	3.5 yr (median)	25.70%	
Gorgun *et al*[21]	2005	122	CD, UC, others	3.6 yr (median)	12%	8.20%

CD: Crohn's disease; ED: Erectile dysfunction; EjD: Ejaculatory dysfunction; UC: Ulcerative colitis.

response in 79% of patients in the sildenafil-treated group. Given these data, Feagins *et al*[15] suggested that they could reassure male patients that the occurrence of postoperative sexual dysfunction after IPAA for IBD is low and, when it does occur, it can be successfully treated with sildenafil in most cases. However, sperm banking should be offered before surgery considering that some patients with erectile dysfunction after IPAA fail to respond to medications and some patients may develop ejaculatory dysfunction after surgery, although they are few in number.

There are other surgical options for IBD apart from IPAA, but the data on postoperative fertility (or sexual function) remain limited. One report by Hultén suggested that ileo-rectal anastomosis has the advantage of avoiding rectal dissection and the associated risks of sexual disturbance, but increases the risk of cancer in the rectal stump[27]. Good results in colectomy with ileo-rectal anastomosis require appropriate patient selection, good rectal distensibility criteria, and accurate endoscopic and histological surveillance for prompt treatment of any recurrence of pouchitis or onset of premalignant changes[27].

MEDICATIONS CAUSING MALE INFERTILITY

Table 3 summarizes the effect of IBD medications on male fertility and the partner's pregnancy outcomes. It also notes recommendations for discontinuation of medications before attempting to conceive.

Sulfasalazine and 5-aminosalicylates

Sulfasalazine and 5-aminosalicylates (5-ASAs) have been used for the initial treatment of IBD and for long-term maintenance of disease remission[28]. These drugs have anti-inflammatory activity.

Levi *et al*[29] first reported 4 cases of male infertility associated with sulfasalazine in 1979. In all 4 cases, discontinuation of sulfasalazine led to successful conception. Subsequent studies showed that this medication causes reversible non-dose-dependent quantitative and qualitative abnormalities of sperm in > 80% of men[28,30,31]. Birnie *et al*[32] examined 21 men with CD who received sulfasalazine and found that 18 of them had abnormal semen analysis results and 15 had oligozoospermia. Another study by Moody *et al*[4] showed that 25% of men with IBD had no children, compared with 15% of men in the general population. They also found that 60% of male IBD patients who had no children were taking sulfasalazine. Sulfasalazine is a molecule that has two components: 5-ASA and sulfapyridine. The sulfapyridine metabolite is thought to be responsible for adverse effects on sperm, causing impaired sperm maturation or oxidative stress production[33-36]. However, Wu *et al*[37] found no correlation between reactive oxygen species production and sperm density, sperm motility, or hamster oocyte penetration capacity. The adverse effects of sulfasalazine on sperm have been shown to be fully reversible after discontinuation[29,31,33,36,38]. Restoration of semen quality and fertility has also been shown after switching to a different 5-ASA compound without the sulfapyridine component, such as mesalazine (also called mesalamine)[39,40]. Zelissen *et al*[41] evaluated semen quality in 11 patients with IBD during sulfasalazine treatment and 4 mo after replacing sulfasalazine with an oral slow-release preparation of 5-ASA, and observed significant improvements in sperm count, morphology, and motility during 5-ASA treatment in comparison with sulfasalazine treatment. Notably, 3 pregnancies occurred during the study period.

On the other hand, there is a case report of mesalazine-induced oligozoospermia in a young man with UC. In that case, semen analysis results returned to near normal and pregnancy occurred after mesalazine treatment was stopped, but the patient's semen parameters worsened after resuming mesalazine[42]. Moreover, we have reported a retrospective study of the negative influence of mesalazine on fertility in men with IBD[43]. In this study, 7 of 1225 male subfertile patients had received mesalazine. In 6 of them, mesalazine was discontinued and sperm motility and total motile sperm count were significantly improved. After discontinuation of mesalazine, 4 of the 6 patients achieved pregnancy with their partners.

Table 3 Effects of medications used for inflammatory bowel disease on male fertility

	Infertility	Pregnancy complications	Recommendations
Sulfasalazine	Reversible	One study	Switch to a different 5-ASA
Mesalazine	One study	None reported	Discontinue only in stable disease
Corticosteroids	No	None reported	Only use short periods
Thiopurines	No	Controversial	No recommendation
Methotrexate	Unclear	None reported	Discontinue in the case of erectile dysfunction
Ciclosporine	No	None reported	No recommendation
Infliximab	Unclear	None reported	No recommendation

5-ASA: 5-Aminosalicylate.

However, mesalazine should be discontinued in only patients with stable disease, and it is possible that low IBD activity itself might have contributed to the improved semen analysis results in the patients who discontinued mesalazine.

With respect to pregnancy complications, Moody *et al*[4] suggested an increased risk of congenital malformations in children born to men on sulfasalazine, but a meta-analysis examining the risk of adverse pregnancy outcomes in women with IBD after exposure to 5-ASAs including sulfasalazine showed no significant increase in congenital abnormalities, stillbirths, spontaneous abortions, preterm deliveries, or low birth weight[44].

From the evidence accumulated to date, discontinuation of sulfasalazine is recommended for prospective fathers, but not discontinuation of 5-ASA compounds lacking the sulfapyridine moiety.

Corticosteroids

Corticosteroids are potent anti-inflammatory agents used for moderate to severe relapses of both CD and UC, but they have no role in maintenance therapy. Corticosteroids inhibit several inflammatory pathways by suppression of interleukin transcription; induction of I-kappa B, which stabilizes the nuclear factor kappa B complex; suppression of arachidonic acid metabolism; and stimulation of apoptosis of lymphocytes within the lamina propria of the gut[45].

Limited data are available on the effects of corticosteroids therapy on fertility for men with IBD. Lerman *et al*[46] found a reversible reduction in fertility in rats exposed to corticosteroids in spite of no changes in sperm count and motility. In a study of 5 endurance-trained men, Roberts *et al*[47] showed that an increase in endogenous steroids might be correlated with a subsequent decrease in sperm concentration 74 d later. In contrast, in a study of 70 men with CD and a group of age-matched controls, Burnell *et al*[48] found no correlation between male infertility and steroid use. In a study of IBD patients undergoing azathioprine (AZA) treatment, the additional administration of corticosteroids had no negative influence on seminogram findings[49]. Definite conclusions regarding the effects of corticosteroids on male fertility cannot be drawn at present because of insufficient data.

Thiopurines

AZA and its active metabolite 6-mercaptopurine (6-MP) are widely used as adjunctive therapy in IBD and as corticosteroid-sparing therapies although they are unapproved therapies for IBD[47].

In a study of 18 men with IBD who received AZA, no worsening of semen analysis results was found, and 6 of the men fathered children during the study period[49]. In a survey of 164 male renal transplant recipients, Xu *et al*[50] concluded that long-term treatment with cyclosporine, AZA, and corticosteroid had no obvious effect on fertility.

A study of male mice exposed to 6-MP showed no reduction in sperm quantity or quality, but a significantly increased incidence of abortion was noted. The authors suggested that this indicated occult sperm damage[51]. In a study of male patients with IBD who were treated with 6-MP, Rajapakse *et al*[52] revealed that the incidence of pregnancy-related complications was significantly increased when the father had used 6-MP within 3 mo of conception. Another study showed that paternal use of AZA or 6-MP before conception was associated with an increased, but not statistically significant, risk of congenital abnormalities[16,53]. Conversely, Francella *et al*[54] found no significant difference in pregnancy outcomes for both men and women taking 6-MP as compared with controls. Teruel *et al*[55] evaluated the outcomes of pregnancies in which the father was exposed to thiopurines at the time of conception, and found no significant difference in unsuccessful pregnancies, namely, spontaneous abortions, ectopic pregnancies, anembryonic pregnancies, or fetal deaths. They concluded that routine alteration of treatment regimens was not recommended for men taking thiopurines when attempting to conceive. According to a review by Akbari *et al*[56] concerning the effects of thiopurines on birth outcomes, thiopurine exposure in men with IBD at the time of conception was not associated with congenital abnormalities[28].

In summary, thiopurines do not appear to deteriorate semen quality. Some studies have suggested that paternal thiopurine treatment is associated with an increased risk of pregnancy complications, but in most past studies, paternal thiopurine exposure was not related to congenital

abnormalities. Regarding the use of thiopurines in male IBD patients who wish to conceive, Sands *et al*[28] proposed that health care providers should inform them that there is a possibility of an increased risk of congenital defects and pregnancy complications although fertility does not seem to be affected.

Methotrexate

Methotrexate (MTX) is positioned as a second-line immunosuppressive agent used in patients resistant or intolerant to AZA or 6-MP. Polyglutamated metabolites of MTX act through the inhibition of dihydrofolate reductase, and the inhibition of cytokine and eicosanoid synthesis are thought to play a role[45].

MTX is known to have teratogenic effects in women, and it is classified by the American Foods and Drug Administration under Pregnancy Category X, which means that it is contraindicated during pregnancy[7]. However, data are scarce on the effect of MTX on male fertility. Studies of animals exposed to MTX showed altered spermatogenesis, cytotoxicity, and degeneration of spermatocytes, Sertoli cells, and Leydig cells[15,28,57,58]. In 1980, Sussman *et al*[59] reported severe oligozoospermia after MTX administration but a return to normal sperm concentrations after discontinuation of MTX. The antifolate mechanism of MTX, which results in decreased DNA synthesis rates and subsequent inhibition of cellular proliferation, likely causes reversible oligozoospermia[28]. El-Beheiry *et al*[60] investigated the effects of MTX on fertility potential in 26 male psoriatic patients. They showed no abnormalities in semen analysis, testicular histology, or spermatogenic function observed using radioactive phosphorus, although a longer follow-up was required to rule out the possible teratogenic effects of the drug.

There have been no reports of MTX-induced adverse pregnancy outcomes in men exposed to the drug. Recently, Weber-Schoendorfer *et al*[61] performed a prospective observational cohort study involving 113 pregnancies where the father was treated with low-dose MTX around the time of conception. As compared with 412 pregnancies without MTX exposure, no increase was observed in the rate of major birth defects or the risk of spontaneous abortion. Further, gestational age at delivery and birth weights did not differ significantly between the groups. Given these results, they concluded that it seems reasonable not to postpone family planning in the case of unavoidable paternal MTX therapy.

However, the active metabolites of MTX could remain in cells or tissues for several months after discontinuation[16]. Furthermore, MTX seems to be associated with erectile dysfunction[62-64]. In most of the literature reviews, discontinuation of MTX was recommended at least 3-4 mo before a planned conception for men with IBD[7,15,16].

Ciclosporin (cyclosporine)/tacrolimus

Ciclosporin (CsA) is a calcineurin inhibitor used for treating severe IBD. It prevents clonal expansion of T cell subsets with a rapid onset of action. Tacrolimus is another calcineurin inhibitor, and is often preferred in transplant recipients[45]. CsA and tacrolimus differ in their chemical structure: Tacrolimus is a macrocyclic lactone, while CsA is a cyclic endecapeptide. However, they act in a similar manner as calcineurin inhibitors.

In a review, Sands *et al*[28] introduced one study using male mice exposed to CsA, and remarked on the presence of abnormal sperm, oligozoospermia, decreased motility, decreased testicular weight, and decreased testosterone concentrations. A study in rats found that CsA had a deleterious effect on spermiogenesis by directly impairing spermiogenic cell development and by impeding Sertoli cell function[65]. In humans, small studies have not found an association between CsA use and male fertility[16,66-68]. There have been no reports of adverse pregnancy outcomes in partners of men receiving CsA.

Monoclonal antibodies against tumor necrosis factor-alpha

Three biological agents are used for the treatment of IBD, namely, infliximab (IFX), adalimumab, and certolizumab. All agents are monoclonal antibodies against tumor necrosis factor-alpha (anti-TNF). IFX is a chimeric anti-TNF antibody, consisting of 75% human IgG and 25% murine component. Adalimumab and certolizumab are humanized anti-TNF antibodies. These agents are indicated in CD resistant to standard immunosuppression therapy. IFX is also indicated in UC and fistulating CD[45].

Few studies have examined the effects of anti-TNF on male fertility. IFX is the most studied of the three agents[28]. One animal study using analogous anti-TNF agents revealed no adverse effect on male fertility[7,69]. In a study of 10 men (8 with IBD, 2 with indeterminate colitis), Mahadevan *et al*[70] showed a significant increase in semen volume one week after IFX infusion and a trend toward decreased sperm motility. In contrast, in a study of 26 men with spondyloarthritis, Villiger *et al*[71] showed no statistically significant difference in sperm quality between healthy controls and patients treated with anti-TNF. They recommended the continuation of anti-TNF treatment when fatherhood was planned. Further, in a prospective study of 10 men with spondyloarthritis and 20 healthy male controls, Ramonda *et al*[72] found a statistically significant decrease in sperm aneuploidies and normal hormone levels after a 12-mo anti-TNF regimen and concluded that anti-TNF agents appeared to be safe for testicular function and male fertility.

Exposure to anti-TNF agents in men prior to a planned conception does not seem to cause embryo toxicity. One study that investigated medical records of men with ankylosing spondylitis reported that 4 patients had fathered 6 healthy children during IFX treatment[73]. A systematic review by Puchner *et al*[74] did not found any documentation of miscarriages or physical abnormities associated with anti-TNF treatment and paternity. Instead, an improvement in sperm motility and vitality during anti-TNF treatment was shown in that review. The

authors suggested that the improvement might be due to a decrease in disease activity.

OTHER FACTORS CAUSING MALE INFERTILITY

Disease activity

Active disease seems to affect male reproductive and sexual function[15,16]. The presence of pro-inflammatory cytokines, including TNF, in the male urogenital tract could lead to cytokine-mediated antifertility effects. Furthermore, inflammation is associated with elevated levels of reactive oxygen species and oxidative stress, both of which have a negative effect on male fertility[75]. Regarding sexual function, Timmer *et al*[76] showed that men with IBD in remission or with mild disease activity had similar rates of erectile dysfunction as compared with controls, whereas men with severe IBD activity had higher rates. Thus, control of IBD activity is recommended for men planning to conceive.

Nutrition

Poor nutritional status in men with IBD might cause infertility. El-Tawil suggested a possible relation between decreased testicular function and zinc deficiency, which has been found in up to 70% of patients with CD[77]. To date, no other studies have specifically addressed the contribution of nutritional status to male infertility in IBD, but Feagins *et al*[15] proposed that optimizing nutritional status is important for men with IBD who are attempting to father children.

Alcohol use

There are several studies that implicate a negative effect of alcohol consumption on the course of IBD[78]. Swanson *et al*[79] showed that alcohol resulted in exacerbation of gastrointestinal symptoms in patients with non-active UC and CD. Jowett *et al*[80] indicated that alcohol consumption increased the risk of disease exacerbation in patients with UC. Thus, alcohol use could activate the disease in the patients with IBD. Moreover, past studies implicated alcohol use in decreasing sperm quality and fertility in men[15,81-83]. Alcohol is considered as one of factors that might be contributing to male infertility in men with IBD.

Tobacco use

Smoking is the most researched environmental factor associated with IBD. It has been observed that smoking has a varying impact on CD and UC, contributing to an increased risk for individuals with CD and a protective role in individuals with UC[1]. The mechanism of these paradoxical effects of smoking on CD and UC is not well understood. It is hypothesized that nicotine and oxidative stress play some role[1,84].

Even if smoking protects against UC, smoking itself impairs fertilization capacity[83]. Tobacco combustion produces many chemical compounds with potential deleterious effects on male germ cells[85]. The toxins originating from cigarette smoke can decrease sperm mitochondrial activity and damage the chromatin structure in human sperm[83]. From a recent meta-analysis of 20 studies with 5865 participants, smoking was found to be a significant risk factor for decreased semen parameters in men[86]. Therefore, smoking cessation is expected to have a positive influence on semen quality and consequently male fertility.

Psychological factor

Past studies showed lower birth rates to men after IBD diagnosis than before diagnosis compared with controls[5,48]. These results meant that IBD men might consider voluntary childlessness apart from physiological factors that could reduce fertility[87]. This voluntary childlessness appears to result from concerns about adverse reproductive outcomes that may not be justified, or patients' fear of transmitting the disease[7,88]. In a questionnaire survey, Mountifield *et al*[88] concluded that patients require accurate counseling addressing fertility and pregnancy outcomes in IBD to assist in their decision making.

TREATMENT OF INFERTILITY IN MEN WITH IBD

Active inflammation, lifestyle factors (alcohol use, tobacco use), medications, poor nutritional status, and rectal incision seem to affect fertility in male IBD patients[15]. First of all, it is important to control IBD activity. If the patient shows poor nutritional status, optimizing their nutritional status is recommended. Tobacco cessation is strongly recommended when the patient is a smoker. If possible, discontinuation of medications associated with male infertility is recommended for prospective fathers. Table 3 shows the recommendations for each drug. In patients taking sulfasalazine, switching to a different 5-ASA is advised at least 4 mo prior to attempting to conceive[28]. In patients with stable IBD who are receiving mesalazine, discontinuation of the drug might restore fertility[43]. To avoid any potential adverse events, corticosteroids should be used for short periods to control active disease[28]. Although discontinuation of MTX is recommended 3-4 mo before attempting to conceive in most of the past reviews, there is insufficient evidence for males to support this recommendation. The risks of MTX discontinuation might outweigh the unsubstantiated hypothetical benefits. Discontinuation should be considered only in the case of erectile dysfunction. At present, there is insufficient evidence to recommend discontinuation of thiopurines, CsA, and anti-TNF agents such as IFX. Sperm banking should be offered to patients who plan to undergo proctocolectomy, because postoperative anejaculation, despite its low incidence, is a potential irreversible complication.

CONCLUSION

This review aimed to provide further insights into relationship between IBD and male fertility, a topic that has received relatively little attention in the literature. Rectal incision can potentially lead to sexual dysfunction after surgery, and sexual dysfunction may cause male infertility. Of the medications used for IBD, sulfasalazine causes reversible oligoasthenoteratozoospermia. No other medications seem to significantly affect fertility in men although small studies suggested some adverse effects. In the case of erectile dysfunction, discontinuation of MTX should be considered because MTX appears to be associated with erectile dysfunction. There are limited data about the effects of other drugs on male fertility and pregnancy outcomes; however, patients should be appropriately informed of the possible effects of paternal drug exposure. Considering that IBD predominantly affects young adults of reproductive age, gastroenterologists treating IBD patients should pay more attention to fertility-related issues. Sperm banking is an option for fertility preservation before surgery or initiation of a potentially gonadotoxic medication.

REFERENCES

1 **Ponder A**, Long MD. A clinical review of recent findings in the epidemiology of inflammatory bowel disease. *Clin Epidemiol* 2013; **5**: 237-247 [PMID: 23922506 DOI: 10.2147/CLEP.S33961]

2 **Zegers-Hochschild F**, Adamson GD, de Mouzon J, Ishihara O, Mansour R, Nygren K, Sullivan E, Vanderpoel S. International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology, 2009. *Fertil Steril* 2009; **92**: 1520-1524 [PMID: 19828144 DOI: 10.1016/j.fertnstert.2009.09.009]

3 **Rossato M**, Foresta C. Antisperm antibodies in inflammatory bowel disease. *Arch Intern Med* 2004; **164**: 2283 [PMID: 15534172]

4 **Moody GA**, Probert C, Jayanthi V, Mayberry JF. The effects of chronic ill health and treatment with sulphasalazine on fertility amongst men and women with inflammatory bowel disease in Leicestershire. *Int J Colorectal Dis* 1997; **12**: 220-224 [PMID: 9272451]

5 **Narendranathan M**, Sandler RS, Suchindran CM, Savitz DA. Male infertility in inflammatory bowel disease. *J Clin Gastroenterol* 1989; **11**: 403-406 [PMID: 2760429]

6 **Moody GA**, Mayberry JF. Perceived sexual dysfunction amongst patients with inflammatory bowel disease. *Digestion* 1993; **54**: 256-260 [PMID: 8243839]

7 **Heetun ZS**, Byrnes C, Neary P, O'Morain C. Review article: Reproduction in the patient with inflammatory bowel disease. *Aliment Pharmacol Ther* 2007; **26**: 513-533 [PMID: 17661756]

8 **Tavernier N**, Fumery M, Peyrin-Biroulet L, Colombel JF, Gower-Rousseau C. Systematic review: fertility in non-surgically treated inflammatory bowel disease. *Aliment Pharmacol Ther* 2013; **38**: 847-853 [PMID: 24004045 DOI: 10.1111/apt.12478]

9 **Wexner SD**, Rosen L, Lowry A, Roberts PL, Burnstein M, Hicks T, Kerner B, Oliver GC, Robertson HD, Robertson WG, Ross TM, Senatore PJ, Simmang C, Smith C, Vernava AM, Wong WD. Practice parameters for the treatment of mucosal ulcerative colitis--supporting documentation. The Standards Practice Task Force. The American Society of Colon and Rectal Surgeons. *Dis Colon Rectum* 1997; **40**: 1277-1285 [PMID: 9369100]

10 **Berg DF**, Bahadursingh AM, Kaminski DL, Longo WE. Acute surgical emergencies in inflammatory bowel disease. *Am J Surg* 2002; **184**: 45-51 [PMID: 12135718]

11 **Gardiner KR**, Dasari BV. Operative management of small bowel Crohn's disease. *Surg Clin North Am* 2007; **87**: 587-610 [PMID: 17560414]

12 **Klag T**, Goetz M, Stange EF, Wehkamp J. Medical Therapy of Perianal Crohn's Disease. *Viszeralmedizin* 2015; **31**: 265-272 [PMID: 26557835 DOI: 10.1159/000434664]

13 **Seifarth C**, Kreis ME, Gröne J. Indications and Specific Surgical Techniques in Crohn's Disease. *Viszeralmedizin* 2015; **31**: 273-279 [PMID: 26557836 DOI: 10.1159/000438955]

14 **Hwang JM**, Varma MG. Surgery for inflammatory bowel disease. *World J Gastroenterol* 2008; **14**: 2678-2690 [PMID: 18461653]

15 **Feagins LA**, Kane SV. Sexual and reproductive issues for men with inflammatory bowel disease. *Am J Gastroenterol* 2009; **104**: 768-773 [PMID: 19223893 DOI: 10.1038/ajg.2008.90]

16 **Palomba S**, Sereni G, Falbo A, Beltrami M, Lombardini S, Boni MC, Fornaciari G, Sassatelli R, La Sala GB. Inflammatory bowel diseases and human reproduction: a comprehensive evidence-based review. *World J Gastroenterol* 2014; **20**: 7123-7136 [PMID: 24966584 DOI: 10.3748/wjg.v20.i23.7123]

17 **Slors FJ**, van Zuijlen PP, van Dijk GJ. Sexual and bladder dysfunction after total mesorectal excision for benign diseases. *Scand J Gastroenterol Suppl* 2000; **(232)**: 48-51 [PMID: 11232492]

18 **Hueting WE**, Buskens E, van der Tweel I, Gooszen HG, van Laarhoven CJ. Results and complications after ileal pouch anal anastomosis: a meta-analysis of 43 observational studies comprising 9,317 patients. *Dig Surg* 2005; **22**: 69-79 [PMID: 15838175]

19 **Berndtsson I**, Oresland T, Hultén L. Sexuality in patients with ulcerative colitis before and after restorative proctocolectomy: a prospective study. *Scand J Gastroenterol* 2004; **39**: 374-379 [PMID: 15125470]

20 **Hueting WE**, Gooszen HG, van Laarhoven CJ. Sexual function and continence after ileo pouch anal anastomosis: a comparison between a meta-analysis and a questionnaire survey. *Int J Colorectal Dis* 2004; **19**: 215-218 [PMID: 14564464]

21 **Gorgun E**, Remzi FH, Montague DK, Connor JT, O'Brien K, Loparo B, Fazio VW. Male sexual function improves after ileal pouch anal anastomosis. *Colorectal Dis* 2005; **7**: 545-550 [PMID: 16232233]

22 **Farouk R**, Pemberton JH, Wolff BG, Dozois RR, Browning S, Larson D. Functional outcomes after ileal pouch-anal anastomosis for chronic ulcerative colitis. *Ann Surg* 2000; **231**: 919-926 [PMID: 10816636]

23 **Michelassi F**, Stella M, Block GE. Prospective assessment of functional results after ileal J pouch-anal restorative proctocolectomy. *Arch Surg* 1993; **128**: 889-894; discussion 894-895 [PMID: 8343061]

24 **Damgaard B**, Wettergren A, Kirkegaard P. Social and sexual function following ileal pouch-anal anastomosis. *Dis Colon Rectum* 1995; **38**: 286-289 [PMID: 7882794]

25 **Lindsey I**, George BD, Kettlewell MG, Mortensen NJ. Impotence after mesorectal and close rectal dissection for inflammatory bowel disease. *Dis Colon Rectum* 2001; **44**: 831-835 [PMID: 11391143]

26 **Lindsey I**, George B, Kettlewell M, Mortensen N. Randomized, double-blind, placebo-controlled trial of sildenafil (Viagra) for erectile dysfunction after rectal excision for cancer and inflammatory bowel disease. *Dis Colon Rectum* 2002; **45**: 727-732 [PMID: 12072621]

27 **Hultén L**. Proctocolectomy and ileostomy to pouch surgery for ulcerative colitis. *World J Surg* 1998; **22**: 335-341 [PMID: 9523513]

28 **Sands K**, Jansen R, Zaslau S, Greenwald D. Review article: the safety of therapeutic drugs in male inflammatory bowel disease patients wishing to conceive. *Aliment Pharmacol Ther* 2015; **41**: 821-834 [PMID: 25752753 DOI: 10.1111/apt.13142]

29 **Levi AJ**, Fisher AM, Hughes L, Hendry WF. Male infertility due to sulphasalazine. *Lancet* 1979; **2**: 276-278 [PMID: 88609]

30 **Taffet SL**, Das KM. Sulfasalazine. Adverse effects and desensitization. *Dig Dis Sci* 1983; **28**: 833-842 [PMID: 6136396]

31 **O'Moráin C**, Smethurst P, Doré CJ, Levi AJ. Reversible male infertility due to sulphasalazine: studies in man and rat. *Gut* 1984; **25**: 1078-1084 [PMID: 6148293]

32 **Birnie GG**, McLeod TI, Watkinson G. Incidence of sulphasalazine-induced male infertility. *Gut* 1981; **22**: 452-455 [PMID: 6114898]

33 **Toovey S**, Hudson E, Hendry WF, Levi AJ. Sulphasalazine and

male infertility: reversibility and possible mechanism. *Gut* 1981; **22**: 445-451 [PMID: 6114897]
34 **Fukushima T**, Hamada Y, Komiyama M, Matsuno Y, Mori C, Horii I. Early changes in sperm motility, acrosome reaction, and gene expression of reproductive organs in rats treated with sulfasalazine. *Reprod Toxicol* 2007; **23**: 153-157 [PMID: 17166698 DOI: 10.1016/j.reprotox.2006.10.003]
35 **Alonso V**, Linares V, Bellés M, Albina ML, Sirvent JJ, Domingo JL, Sánchez DJ. Sulfasalazine induced oxidative stress: a possible mechanism of male infertility. *Reprod Toxicol* 2009; **27**: 35-40 [PMID: 19028562 DOI: 10.1016/j.reprotox.2008.10.007]
36 **Linares V**, Alonso V, Domingo JL. Oxidative stress as a mechanism underlying sulfasalazine-induced toxicity. *Expert Opin Drug Saf* 2011; **10**: 253-263 [PMID: 21219240 DOI: 10.1517/14740338.2011.529898]
37 **Wu FC**, Aitken RJ, Ferguson A. Inflammatory bowel disease and male infertility: effects of sulfasalazine and 5-aminosalicylic acid on sperm-fertilizing capacity and reactive oxygen species generation. *Fertil Steril* 1989; **52**: 842-845 [PMID: 2572460]
38 **Cosentino MJ**, Chey WY, Takihara H, Cockett AT. The effects of sulfasalazine on human male fertility potential and seminal prostaglandins. *J Urol* 1984; **132**: 682-686 [PMID: 6147421]
39 **Riley SA**, Lecarpentier J, Mani V, Goodman MJ, Mandal BK, Turnberg LA. Sulphasalazine induced seminal abnormalities in ulcerative colitis: results of mesalazine substitution. *Gut* 1987; **28**: 1008-1012 [PMID: 2889648 DOI: 10.1136/]
40 **Chatzinoff M**, Guarino JM, Corson SL, Batzer FR, Friedman LS. Sulfasalazine-induced abnormal sperm penetration assay reversed on changing to 5-aminosalicylic acid enemas. *Dig Dis Sci* 1988; **33**: 108-110 [PMID: 2892654 DOI: 10.1007/BF01536639]
41 **Zelissen PM**, van Hattum J, Poen H, Scholten P, Gerritse R, te Velde ER. Influence of salazosulphapyridine and 5-aminosalicylic acid on seminal qualities and male sex hormones. *Scand J Gastroenterol* 1988; **23**: 1100-1104 [PMID: 2907823]
42 **Chermesh I**, Eliakim R. Mesalazine-induced reversible infertility in a young male. *Dig Liver Dis* 2004; **36**: 551-552 [PMID: 15334777]
43 **Shin T**, Kobori Y, Suzuki K, Iwahata T, Yagi H, Soh S, Arai G, Okada H. Inflammatory bowel disease in subfertile men and the effect of mesalazine on fertility. *Syst Biol Reprod Med* 2014; **60**: 373-376 [PMID: 25144125 DOI: 10.3109/19396368.2014.952391]
44 **Rahimi R**, Nikfar S, Rezaie A, Abdollahi M. Pregnancy outcome in women with inflammatory bowel disease following exposure to 5-aminosalicylic acid drugs: a meta-analysis. *Reprod Toxicol* 2008; **25**: 271-275 [PMID: 18242053 DOI: 10.1016/j.reprotox.2007.11.010]
45 **Mowat C**, Cole A, Windsor A, Ahmad T, Arnott I, Driscoll R, Mitton S, Orchard T, Rutter M, Younge L, Lees C, Ho GT, Satsangi J, Bloom S. Guidelines for the management of inflammatory bowel disease in adults. *Gut* 2011; **60**: 571-607 [PMID: 21464096 DOI: 10.1136/gut.2010.224154]
46 **Lerman SA**, Miller GK, Bohlman K, Albaladejo V, Léonard JF, Devas V, Clark RL. Effects of corticosterone on reproduction in male Sprague-Dawley rats. *Reprod Toxicol* 1997; **11**: 799-805 [PMID: 9407590]
47 **Roberts AC**, McClure RD, Weiner RI, Brooks GA. Overtraining affects male reproductive status. *Fertil Steril* 1993; **60**: 686-692 [PMID: 8405526]
48 **Burnell D**, Mayberry J, Calcraft BJ, Morris JS, Rhodes J. Male fertility in Crohn's disease. *Postgrad Med J* 1986; **62**: 269-272 [PMID: 2872665]
49 **Dejaco C**, Mittermaier C, Reinisch W, Gasche C, Waldhoer T, Strohmer H, Moser G. Azathioprine treatment and male fertility in inflammatory bowel disease. *Gastroenterology* 2001; **121**: 1048-1053 [PMID: 11677195]
50 **Xu L**, Han S, Liu Y, Wang H, Yang Y, Qiu F, Peng W, Tang L, Fu J, Zhu XF, Ding X, Zhu Y. The influence of immunosuppressants on the fertility of males who undergo renal transplantation and on the immune function of their offspring. *Transpl Immunol* 2009; **22**: 28-31 [PMID: 19818850 DOI: 10.1016/j.trim.2009.10.001]
51 **Ligumsky M**, Badaan S, Lewis H, Meirow D. Effects of 6-mercaptopurine treatment on sperm production and reproductive performance: a study in male mice. *Scand J Gastroenterol* 2005; **40**: 444-449 [PMID: 16028439]
52 **Rajapakse RO**, Korelitz BI, Zlatanic J, Baiocco PJ, Gleim GW. Outcome of pregnancies when fathers are treated with 6-mercaptopurine for inflammatory bowel disease. *Am J Gastroenterol* 2000; **95**: 684-688 [PMID: 10710057]
53 **Nørgård B**, Pedersen L, Jacobsen J, Rasmussen SN, Sørensen HT. The risk of congenital abnormalities in children fathered by men treated with azathioprine or mercaptopurine before conception. *Aliment Pharmacol Ther* 2004; **19**: 679-685 [PMID: 15023170 DOI: 10.1111/j.1365-2036.2004.01889.x]
54 **Francella A**, Dyan A, Bodian C, Rubin P, Chapman M, Present DH. The safety of 6-mercaptopurine for childbearing patients with inflammatory bowel disease: a retrospective cohort study. *Gastroenterology* 2003; **124**: 9-17 [PMID: 12512024 DOI: 10.1053/gast.2003.50014]
55 **Teruel C**, López-San Román A, Bermejo F, Taxonera C, Pérez-Calle JL, Gisbert JP, Martín-Arranz M, Ponferrada A, Van Domselaar M, Algaba A, Estellés J, López-Serrano P, Linares PM, Muriel A. Outcomes of pregnancies fathered by inflammatory bowel disease patients exposed to thiopurines. *Am J Gastroenterol* 2010; **105**: 2003-2008 [PMID: 20700117 DOI: 10.1038/ajg.2010.138]
56 **Akbari M**, Shah S, Velayos FS, Mahadevan U, Cheifetz AS. Systematic review and meta-analysis on the effects of thiopurines on birth outcomes from female and male patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2013; **19**: 15-22 [PMID: 22434610 DOI: 10.1002/ibd.22948]
57 **Saxena AK**, Dhungel S, Bhattacharya S, Jha CB, Srivastava AK. Effect of chronic low dose of methotrexate on cellular proliferation during spermatogenesis in rats. *Arch Androl* 2004; **50**: 33-35 [PMID: 14660169]
58 **Shrestha S**, Dhungel S, Saxena AK, Bhattacharya S, Maskey D. Effect of methotrexate (MTX) administration on spermatogenesis: an experimental on animal model. *Nepal Med Coll J* 2007; **9**: 230-233 [PMID: 18298010]
59 **Sussman A**, Leonard JM. Psoriasis, methotrexate, and oligospermia. *Arch Dermatol* 1980; **116**: 215-217 [PMID: 7356357]
60 **El-Beheiry A**, El-Mansy E, Kamel N, Salama N. Methotrexate and fertility in men. *Arch Androl* 1979; **3**: 177-179 [PMID: 518200]
61 **Weber-Schoendorfer C**, Hoeltzenbein M, Wacker E, Meister R, Schaefer C. No evidence for an increased risk of adverse pregnancy outcome after paternal low-dose methotrexate: an observational cohort study. *Rheumatology* (Oxford) 2014; **53**: 757-763 [PMID: 24369411 DOI: 10.1093/rheumatology/ket390]
62 **Blackburn WD**, Alarcón GS. Impotence in three rheumatoid arthritis patients treated with methotrexate. *Arthritis Rheum* 1989; **32**: 1341-1342 [PMID: 2803334 DOI: 10.1002/ anr.1780321029]
63 **Riba N**, Moreno F, Costa J, Olive A. [Appearance of impotence in relation to the use of methotrexate]. *Med Clin* (Barc) 1996; **106**: 558 [PMID: 8656751]
64 **Thomas E**, Koumouvi K, Blotman F. Impotence in a patient with rheumatoid arthritis treated with methotrexate. *J Rheumatol* 2000; **27**: 1821-1822 [PMID: 10914881]
65 **Masuda H**, Fujihira S, Ueno H, Kagawa M, Katsuoka Y, Mori H. Ultrastructural study on cytotoxic effects of cyclosporine A in spermiogenesis in rats. *Med Electron Microsc* 2003; **36**: 183-191 [PMID: 14505063 DOI: 10.1007/s00795-003-0213-4]
66 **Bouloux PM**, Wass JA, Parslow JM, Hendry WF, Besser GM. Effect of cyclosporin A in male autoimmune infertility. *Fertil Steril* 1986; **46**: 81-85 [PMID: 3720982]
67 **Sakamoto Y**, Matsumoto T, Kumazawa J. Cell-mediated autoimmune response to testis induced by bilateral testicular injury can be suppressed by cyclosporin A. *J Urol* 1998; **159**: 1735-1740 [PMID: 9554403 DOI: 10.1097/00005392-199805000-00103]
68 **Haberman J**, Karwa G, Greenstein SM, Soberman R, Glicklich D, Tellis V, Melman A. Male fertility in cyclosporine-treated renal transplant patients. *J Urol* 1991; **145**: 294-296 [PMID: 1988720]
69 **Treacy G**. Using an analogous monoclonal antibody to evaluate the reproductive and chronic toxicity potential for a humanized

anti-TNFalpha monoclonal antibody. *Hum Exp Toxicol* 2000; **19**: 226-228 [PMID: 10918512]
70 **Mahadevan U**, Terdiman JP, Aron J, Jacobsohn S, Turek P. Infliximab and semen quality in men with inflammatory bowel disease. *Inflamm Bowel Dis* 2005; **11**: 395-399 [PMID: 15803031 DOI: 10.1097/01.MIB.0000164023.10848.c4]
71 **Villiger PM**, Caliezi G, Cottin V, Förger F, Senn A, Østensen M. Effects of TNF antagonists on sperm characteristics in patients with spondyloarthritis. *Ann Rheum Dis* 2010; **69**: 1842-1844 [PMID: 20610443 DOI: 10.1136/ard.2009.127423]
72 **Ramonda R**, Foresta C, Ortolan A, Bertoldo A, Oliviero F, Lorenzin M, Pizzol D, Punzi L, Garolla A. Influence of tumor necrosis factor α inhibitors on testicular function and semen in spondyloarthritis patients. *Fertil Steril* 2014; **101**: 359-365 [PMID: 24332378 DOI: 10.1016/j.fertnstert.2013.10.048]
73 **Paschou S**, Voulgari PV, Vrabie IG, Saougou IG, Drosos AA. Fertility and reproduction in male patients with ankylosing spondylitis treated with infliximab. *J Rheumatol* 2009; **36**: 351-354 [PMID: 19040305 DOI: 10.3899/jrheum.080554]
74 **Puchner R**, Danninger K, Puchner A, Pieringer H. Impact of TNF-blocking agents on male sperm characteristics and pregnancy outcomes in fathers exposed to TNF-blocking agents at time of conception. *Clin Exp Rheumatol* 2012; **30**: 765-767 [PMID: 22935608]
75 **Sarkar O**, Bahrainwala J, Chandrasekaran S, Kothari S, Mathur PP, Agarwal A. Impact of inflammation on male fertility. *Front Biosci* (Elite Ed) 2011; **3**: 89-95 [PMID: 21196288 DOI: 10.2741/e223]
76 **Timmer A**, Bauer A, Dignass A, Rogler G. Sexual function in persons with inflammatory bowel disease: a survey with matched controls. *Clin Gastroenterol Hepatol* 2007; **5**: 87-94 [PMID: 17234557 DOI: 10.1016/j.cgh.2006.10.018]
77 **El-Tawil AM**. Zinc deficiency in men with Crohn's disease may contribute to poor sperm function and male infertility. *Andrologia* 2003; **35**: 337-341 [PMID: 15018135]
78 **Owczarek D**, Rodacki T, Domagała-Rodacka R, Cibor D, Mach T. Diet and nutritional factors in inflammatory bowel diseases. *World J Gastroenterol* 2016; **22**: 895-905 [PMID: 26811635 DOI: 10.3748/wjg.v22.i3.895]
79 **Swanson GR**, Sedghi S, Farhadi A, Keshavarzian A. Pattern of alcohol consumption and its effect on gastrointestinal symptoms in inflammatory bowel disease. *Alcohol* 2010; **44**: 223-228 [PMID: 20682190 DOI: 10.1016/j.alcohol.2009.10.019]
80 **Jowett SL**, Seal CJ, Pearce MS, Phillips E, Gregory W, Barton JR, Welfare MR. Influence of dietary factors on the clinical course of ulcerative colitis: a prospective cohort study. *Gut* 2004; **53**: 1479-1484 [PMID: 15361498]
81 **Kalyani R**, Basavaraj PB, Kumar ML. Factors influencing quality of semen: a two year prospective study. *Indian J Pathol Microbiol* 2007; **50**: 890-895 [PMID: 18306599]
82 **Emanuele MA**, Emanuele NV. Alcohol's effects on male reproduction. *Alcohol Health Res World* 1998; **22**: 195-201 [PMID: 15706796]
83 **Sharma R**, Biedenharn KR, Fedor JM, Agarwal A. Lifestyle factors and reproductive health: taking control of your fertility. *Reprod Biol Endocrinol* 2013; **11**: 66 [PMID: 23870423 DOI: 10.1186/1477-7827-11-66]
84 **Cosnes J**. Tobacco and IBD: relevance in the understanding of disease mechanisms and clinical practice. *Best Pract Res Clin Gastroenterol* 2004; **18**: 481-496 [PMID: 15157822]
85 **Zenzes MT**. Smoking and reproduction: gene damage to human gametes and embryos. *Hum Reprod Update* 2000; **6**: 122-131 [PMID: 10782570]
86 **Sharma R,** Harlev A, Agarwal A, Esteves SC. Cigarette Smoking and Semen Quality: A New Meta-analysis Examining the Effect of the 2010 World Health Organization Laboratory Methods for the Examination of Human Semen. *Eur Urol* 2016; Epub ahead of print [PMID: 27113031 DOI: 10.1016/j.eururo.2016.04.010]
87 **Andrews JM**, Mountifield RE, Van Langenberg DR, Bampton PA, Holtmann GJ. Un-promoted issues in inflammatory bowel disease: opportunities to optimize care. *Intern Med J* 2010; **40**: 173-182 [PMID: 19849744 DOI: 10.1111/j.1445-5994.2009.02110.x]
88 **Mountifield R**, Bampton P, Prosser R, Muller K, Andrews JM. Fear and fertility in inflammatory bowel disease: a mismatch of perception and reality affects family planning decisions. *Inflamm Bowel Dis* 2009; **15**: 720-725 [PMID: 19067431 DOI: 10.1002/ibd.20839]

P- Reviewer: Inoue T, Kellermayer R, Sinha R **S- Editor**: Qi Y
L- Editor: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i3.370

World J Gastrointest Pharmacol Ther 2016 August 6; 7(3): 370-386
ISSN 2150-5349 (online)

TOPIC HIGHLIGHT

2016 Pancreatic Cancer: Global view

Management of pain in chronic pancreatitis with emphasis on exogenous pancreatic enzymes

Paul M Hobbs, William G Johnson, David Y Graham

Paul M Hobbs, William G Johnson, David Y Graham, Department of Medicine, Michael E DeBakey Veterans Affairs Medical Center, Baylor College of Medicine, Houston, TX 77030, United States

Author contributions: Each of the authors have been involved equally in study design, data acquisition, analysis, interpretation and drafting the article; all have read and approved the final manuscript; each meets the criteria for authorship established by the International Committee of Medical Journal Editors and verify the validity of the results reported.

Conflict-of-interest statement: Dr. Graham is a paid consultant and has received research funding from RedHill Biopharma regarding novel *H. pylori* therapies and is a consultant to BioGaia regarding use of probiotics for *H. pylori* infections. He has nothing to declare in relation to this topic. Dr. Hobbs and Johnson also have nothing to declare.

Manuscript source: Invited manuscript

Correspondence to: David Y Graham, MD, Department of Medicine, Michael E DeBakey Veterans Affairs Medical Center, Baylor College of Medicine, RM 3A-318B (111D), 2002 Holcombe Boulevard, Houston, TX 77030, United States. dgraham@bcm.edu
Telephone: +1-713-7950232
Fax: +1-713-7954471

Received: March 25, 2016
Peer-review started: March 25, 2016
First decision: May 17, 2016
Revised: June 8, 2016
Accepted: July 14, 2016

Article in press: July 18, 2016
Published online: August 6, 2016

Abstract

One of the most challenging issues arising in patients with chronic pancreatitis is the management of abdominal pain. Many competing theories exist to explain pancreatic pain including ductal hypertension from strictures and stones, increased interstitial pressure from glandular fibrosis, pancreatic neuritis, and ischemia. This clinical problem is superimposed on a background of reduced enzyme secretion and altered feedback mechanisms. Throughout history, investigators have used these theories to devise methods to combat chronic pancreatic pain including: Lifestyle measures, antioxidants, analgesics, administration of exogenous pancreatic enzymes, endoscopic drainage procedures, and surgical drainage and resection procedures. While the value of each modality has been debated over the years, pancreatic enzyme therapy remains a viable option. Enzyme therapy restores active enzymes to the small bowel and targets the altered feedback mechanism that lead to increased pancreatic ductal and tissue pressures, ischemia, and pain. Here, we review the mechanisms and treatments for chronic pancreatic pain with a specific focus on pancreatic enzyme replacement therapy. We also discuss different approaches to overcoming a lack of clinical response update ideas for studies needed to improve the clinical use of pancreatic enzymes to ameliorate pancreatic pain.

Key words: Pancreatic enzyme replacement therapy; Chronic pancreatitis; Pancreatic insufficiency; Protease; Clinical trials; Trypsin; Fat malabsorption; Pain

Core tip: Pancreatic enzyme replacement therapy

has long been used as a non-invasive treatment for chronic pancreatic pain. Enzyme therapy aims to restore feedback inhibition of pancreatic secretion to lessen pain caused by pancreatic ductal hypertension, increased pancreatic interstitial pressure, and pancreatic ischemia. Although enzyme therapy may play a role the key is individualization of therapy based on disease etiology and severity. Here we review the literature regarding the efficacy of enzyme therapy and the evidence gathered for an entero-pancreatic feedback loop. We also describe alternative strategies for improving pain therapy including using uncoated enzymes with gastric acid suppression.

Hobbs PM, Johnson WG, Graham DY. Management of pain in chronic pancreatitis with emphasis on exogenous pancreatic enzymes. *World J Gastrointest Pharmacol Ther* 2016; 7(3): 370-386 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i3/370.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i3.370

INTRODUCTION

Although the pancreas was known to ancient Greeks, its role in health and disease remained obscure until recent times. One of the earliest cases of chronic calcific pancreatitis is described in *History of the Pancreas* by Howard and Hess[1] in which they relate that in 1678 de Graaf reported the case history of a patient seen by a Dr. Gajea. The patient, a nobleman, was "seized by vomiting and diarrhea because of an uncontrolled use of wine and seafood"[1]. At autopsy, seven or eight stones the size of a chick pea were found blocking the pancreatic duct[1]. Later, diabetes was recognized as a complication of chronic pancreatitis[2]. Despite a plethora of autopsy cases, case reports, and reviews[1,3], a clear understanding of the manifestations of chronic pancreatitis had to await medicine's advance to when surgeons could safely enter the abdomen as well as the development of laboratory testing and radiographic imaging.

Pancreatitis can be classified broadly as acute or chronic[4]. In acute pancreatitis, the glands undergo varying degrees of edema, inflammation, and possibly necrosis[4-6]. Although a majority of the glands may be injured, most recover[7]. Chronic pancreatitis is thought to be the end result of a long-term inflammatory process that results in both morphological and structural changes[7]. This has been proposed as a two-step process in which functional and structural impairment to pancreatic secretion eventually leads to activation of zymogens resulting in local destruction of glandular tissue eventuating in fibrosis[7]. This may also result in marked pancreatic structural alterations including formation of pseudocysts and ductal strictures and repeated cycles of increasing damage and inflammation ultimately resulting in both exocrine and endocrine insufficiency[4,6,7]. Ductal dilatation and intraductal calcifications are common in chronic pancreatitis[4-6] and such architectural changes allow one to reliably distinguish between acute and chronic pancreatitis. However, chronic pancreatitis can occur without gross changes and still be diagnosed based on the presence of abnormal structure and function[7].

Histology is the diagnostic gold standard for chronic pancreatitis, but pancreatic biopsy is potentially dangerous and not routinely performed[8]. Instead, there are a myriad of functional tests available such as the cholecystokinin-secretin test[8]. A comparison of the cholecystokinin-secretin test to pancreatic tissue biopsy reported a significant correlation between histology and peak bicarbonate concentration (sensitivity of 67% and specificity of 90%)[9]. In fact, the functional tests are even more sensitive than endoscopic retrograde cholangiopancreatography (ERCP)[9]. Overall, the secretin stimulation test is considered the most sensitive test for diagnosing chronic pancreatitis but is not widely available[7,10]. Imaging studies including radiographs, ultrasound, computed tomography, and magnetic resonance imaging identify abnormal pancreatic structure. ERCP and endoscopic ultrasound are the most widely used to diagnose chronic pancreatitis[8]. A number of classification schemes have been proposed such as the Cambridge and Rosemont Classifications. The Cambridge classification uses findings seen on ERCP, ultrasound, and CT[5], whereas the Rosemont classification diagnoses chronic pancreatitis based on major and minor features present on endoscopic ultrasound[11]. Chronic pancreatitis is also classified for therapeutic studies as large or small duct disease because the two variants differ in natural course and treatment responses[7]. For example, patients with small duct disease tend to have better pain response to pancreatic enzyme supplementation compared to those with large duct disease[7].

ETIOLOGY OF CHRONIC PANCREATITIS

Worldwide, alcohol use is the most common cause of chronic pancreatitis in adults and in most series accounts for approximately 70% of cases (Table 1). A wide variety of other etiologies (cystic fibrosis, hypertriglyceridemia, tumor, pancreatic resection, familial, congenital abnormalities, tropical, autoimmune, genetic) account for approximately 10%, and the remaining 20% are currently considered idiopathic[12]. The focus of this review is on the medical management of patients with chronic pancreatitis presenting with chronic abdominal pain.

PANCREATIC PAIN

Although the proportion of patients with chronic pancreatitis and pain is unclear, many, if not most, patients are originally identified because they seek medical help due to abdominal pain[13]. Other presentations include signs of endocrine or exocrine dysfunction without pain[14]. It has been estimated that overall 5% to 10% of patients with chronic pancreatitis, especially those with late-onset idiopathic disease, do not suffer from abdominal pain[13]. Episodic pain is a defining symptom of chronic

Table 1 Common causes of chronic pancreatitis

Toxic metabolic
Xenobiotics
Alcohol
Cigarette smoking[12]
Genetic mutations
CFTR mutation (Cystic Fibrosis Transmembrane Conductance Regulator) PRSS1 mutation (Protease, Serine 1)
SPINK1 mutation (Serine Peptidase Inhibitor, Kazal type 1)
CTRC (chymotrypsin C)
Chronic Obstruction of main pancreatic duct
Cancer
Post-duct destruction in severe attack
Recurrent acute pancreatitis
Autoimmune
Idiopathic
Early or late onset
Tropical

Table 2 Pattern of pancreatic pain

Episodic mild to moderate pain
Constant mild to moderate pain
Typically pain free between episodes of severe pain
Constant mild pain with episodes of severe pain
Constant pain

pancreatitis and is classically described as constant pain in the epigastric area with radiation to the back[13]. Painful episodes last roughly a week and are often accompanied by fatigue, nausea, vomiting, food avoidance, and weight loss[15]. Pain is typically worsened with food intake and may be ameliorated in part by leaning forward, sitting up, food avoidance, or use of heating pads to the back or abdomen[7,15]. The pain can be severe but varies widely among patients and even in the same individual[13]. This variation complicates interpretation of therapy during pain-free intervals between exacerbations[13]. A thorough history often reveals multiple similar prior episodes, alcohol abuse, and symptoms of weight loss, diarrhea and steatorrhea[7]. While alcohol use is often described as the most common trigger for symptoms (*e.g.*, pain occurring twelve to forty-eight hours after alcohol use)[16], many report no consistent association between alcohol use and pain[13,17-21]. Physical examination is typically negative with the exception that pain in the epigastric region may worsen with palpation.

Disease progression may be associated with a change in the characteristics of pain. Early in the disease, pain tends to be periodic which may then progress to constant debilitating pain[22]. Pain resolves in some patients as the glands are destroyed and the disease "burns out". However, this may require more than 18 years[13,19,20]. While it has been suggested that viable pancreatic tissue may be required for pancreatic pain[15], the natural course of pain in chronic pancreatitis is notoriously difficult to predict. For example, a longitudinal study with 113 patients noted that the pain decreased in 42%, did not change in 32%, and increased in 26% over a 4 year observation period[23]. In contrast, another study reported 85% of patients achieved pain relief at a median of 4.5 years[19]. Patients achieving pain relief were most often those with increased pancreatic calcifications and dysfunction[19].

A large multi-center study evaluated the frequency of different pain patterns among 540 patients with chronic pancreatitis[24]. Their characterization focused on frequency (intermittent *vs* constant) and severity (mild, moderate, or severe); only approximately 20% of patients were unable to self-characterize their pain pattern. Pain patterns (Table 2) were originally scored into one of 5 patterns based on the American Gastrointestinal Association's technical review[25]. The most common pain patterns were constant mild pain with episodic severe pain (56%) and typically pain free with episodic severe pain (31%). Overall, constant pain was more common than intermittent pain (52% *vs* 45%). Patients with intermittent pain tended to be older while those with constant pain were current smokers and had alcohol as the primary etiology of their chronic pancreatitis. As might be expected, those with constant pain and those with severe pain were more likely to be disabled, have poor quality of life, and to utilize health care resources. However, it has been estimated that 30% to 50% of patients with chronic pancreatitis will eventually become pain free[13].

Ammann *et al*[20] study of pain in chronic alcoholic pancreatitis provided data on 207 patients. None were addicted to narcotics or had an inflammatory mass as the potential cause of pain. Two pain patterns were common. In the first pattern, patients experienced short episodes of pain separated by pain-free periods lasting from months to years. Patients with the second pain pattern had persistent daily pain or clusters of severe pain typically occurring 2 or more days per week for at least 2 mo. Among those with intermittent pain and not requiring surgery, 50% had pain relief within 6 years increasing to more than 80% at 10 years. All of those with persistent pain underwent surgery because of the presence of a pseudocyst (most common), presumed high ductal pressure (large duct disease with or without ductal stones and minimal or no exocrine insufficiency), or biliary obstruction. Overall, the response to pain and the proportion developing pancreatic insufficiency in the two groups were similar. In the total series, the most common association with chronic pain was narcotic addiction, and these patients were few in number and were excluded. However, in many series the management of pain in patients with chronic pancreatitis is complicated by narcotic and alcohol dependencies[13].

PATHOGENESIS OF PANCREATIC PAIN

The pathogenesis of chronic pancreatic pain is poorly understood. In the 19th century, thought centered on ductal obstruction and the passage of a stone similar to what occurs with salivary gland or biliary stones,

Table 3 Mechanisms of pain in chronic pancreatitis

Increased intraductal pressure
Ductal obstruction from strictures/stones
Increased intrapancreatic pressure (compartment-like syndrome)
Fibrosis causing lack of distensibility
Neuropathic
Entrapment of nerves
Damage of nerves by enzymes
Increased nerve tissue
Pancreatic ischemia
Worsened during increased enzyme secretion

as well as pressure or other damage to the celiac axis (*e.g.,* neuralgia coeliaca)[3]. Currently, the major theories focus on increased pancreatic pressure (*e.g.,* intraductal pressure, pancreatic interstitial hypertension, or ischemia) and neurogenic causes (Table 3).

DUCTAL HYPERTENSION

Ductal hypertension is often considered "the most important cause of pain"[13] based on the concept that ductal strictures and calculi can cause ductal obstruction which leads to increased ductal pressure, and thus pain, during pancreatic secretion[7,26]. It has been suggested that one role of alcohol in pancreatitis is to promote stone formation in pancreatic secretions[27]. The presence of these stones promotes inflammation leading to scarring and strictures which then elevate intraluminal pressures[27]. Clinical studies have indeed confirmed elevated pancreatic ductal pressures in patients with chronic pancreatitis. For example, normal pancreatic ductal pressure ranges from 7 to 15 mmHg while ductal pressures ranging from 20 to 80 mmHg have been measured in patients with chronic pancreatitis[28-30]. A direct relationship between the reduction in ductal pressure and relief of pancreatic pain has also been reported[31]. For example, there are numerous studies demonstrating pain reduction or relief following decompression of a dilated duct or pseudocyst using drugs, endoscopic stents, by disintegration of pancreatic stones *via* extracorporeal shock waves, surgical drainage procedures, or pancreatic resections[28,32].

While clinical data suggests that pancreatic pain can be reduced by eliminating ductal strictures and obstructions, decreasing pancreatic secretion, or both, significant obstruction is not universally apparent in painful chronic pancreatitis[26] and ductal surgery does not uniformly relieve pain[16]. For example, the prevalence of major duct strictures was reported to be similar (*e.g.,* about 60%) in patients with painful and painless chronic pancreatitis[21]. However, ductal pressures were not measured[21,26].

INTERSTITIAL HYPERTENSION

A related theory focuses on increased pancreatic interstitial hypertension which has been reported to be higher in patients with painful chronic pancreatitis than in painless chronic pancreatitis (*e.g.,* a median of 7 mmHg *vs* 22.5 mmHg)[33]. In those patients with pain, drainage procedures involving the main duct or a communicating pseudocyst often result in both pain relief and a reduction in interstitial pressures to normal levels[33]. An extensive study of the relation of ductal and interstitial pressure in chronic pancreatitis was performed using a cat model[34]. Perfusion of the normal main duct at physiologic flow rates resulted in an increase in ductal pressures but no significant change in interstitial pressure. Perfusion following partial obstruction of the main pancreatic duct at the neck of the pancreas resulted in a further increase in ductal pressure but again without an increase in interstitial pressure. These data suggest that the normal pancreas has sufficient distensibility to dissipate the increase in ductal pressure. Following encasement of the pancreas in latex to decrease its ability to expand, perfusion of the pancreas resulted in significant increases in both ductal and interstitial pressures. Finally, to simulate chronic pancreatitis, the main pancreatic duct was obstructed for 5 wk resulting in histological changes similar to chronic pancreatitis in humans. Perfusion of the duct then resulted in an increase in both ductal and interstitial pressures leading the authors to conclude that the loss of distensibility in chronic pancreatitis likely results in a compartment-like syndrome in which secretion produces increased ductal and interstitial pressures both of which can be partially or completely relieved by pancreatic surgery[26]. They also showed reduced pancreatic blood flow in the cat model[34,35] suggesting possible pancreatic ischemia.

PANCREATIC ISCHEMIA

The ischemia hypothesis is based on the concept that increased interstitial pressures and surrounding fibrosis could increase vascular resistance leading to decreased perfusion of pancreatic tissues[34-38]. As noted above, in the cat chronic pancreatitis model, basal blood flow was reduced by 40% compared to the normal pancreas[34]. In addition, pancreatic secretagogues increased normal blood flow by 27% but decreased blood flow by 14% in animals with chronic pancreatitis. Decompression of the obstructed pancreatic duct resulted in both an increase in basal flow and return to the normal increase following stimulation of secretion consistent with the notion that parenchymal damage and pancreatic pain could be secondary to ischemia (*i.e.,* a compartment-like syndrome).

PANCREATIC NEURITIS

A final, but related, pain theory is based on the concept that altered pancreatic architecture results in inflammation of nerves and altered feedback mechanisms[13,39]. It has been proposed that chronic inflammation of peripancreatic nerves may increase nerve tissue through up-regulation of neuropeptides[28]. The fact that mean diameter of

peripancreatic nerves in chronic pancreatitis patients was significantly greater than controls led to the suggestion that the increased nerve diameter was caused by a fibrotic process that strangulated the nerves[13]. Microscopic analyses have also shown disruptions in perineural structure which could theoretically expose nerves to damaging inflammation, enzymes, and inflammatory cells[28]. Specifically, the number of eosinophils present in pancreatic perineural tissue was shown to correlate with pain and alcoholism scores[40]. The pancreas is highly innervated, and it has been suggested that pain reduction following surgical removal of the head of the pancreas is related to removal of the most highly innervated region[27,41]. The pain relief obtained by removal of inflammatory pancreatic masses is thought to possibly relate to removal of damaged nervous tissue[39]. In the last decade, research has focused on histologic and biochemical features in the involved pancreas, as well as changes in cortical reorganization and electroencephalographic findings and the similarities to patients with neuropathic pain (*e.g.,* reviewed in[39,42-44]). Similar findings have been described in humans and experimental animals with chronic pancreatitis. It is however important to note that the neurogenic theory cannot, by itself, explain pain relief in pancreatic "burn out" or after reduction of intraductal pressures through procedures[13].

TREATMENT OF PAIN IN CHRONIC PANCREATITIS

Pain in patients with chronic pancreatitis is often extremely difficult to manage in that patients frequently receive narcotics and a significant proportion of patients develop dependency on both narcotics and alcohol. Severe constant pain often indicates the presence of a complication such as a pancreatic pseudocyst and should prompt targeted investigations[45,46]. One of the first goals of the clinician is to ensure that the pain is related to chronic pancreatitis and not to some another condition[32]. Patients with chronic pancreatitis may also have malabsorption resulting in flooding of the colon with nutrients leading to meteorism or other symptoms of malabsorption[47-49]. One topic heading in Howard and Hess's *History of the Pancreas* is entitled "Treat the pain, not the disease"[1] (page 291), emphasizing that patients with chronic pancreatitis often have multiple overlapping issues and correct diagnoses and a multidisciplinary approach is essential for successful treatment[13]. Pain remains the most common primary indication for surgical or endoscopic intervention. Treatment failure or only partial success is common[13]. The focus of this paper is on medical treatment of pain in chronic pancreatitis. Nonetheless, medical, endoscopic, and surgical treatments may all be required for a successful outcome.

LIFESTYLE CHANGES

Cessation of alcohol

Although alcohol is involved in a large percentage of cases of painful chronic pancreatitis, it remains unclear why only a small percentage of those who abuse alcohol develop chronic pancreatitis. Chronic pancreatitis is more common among those who also smoke[12]. It is likely that there is a genetic predisposition that associates alcohol or alcohol and smoking with pancreatitis, but no single genetic association has yet been discovered[12]. Because alcohol-induced chronic pancreatitis is a progressive disease leading to structural and functional pancreatic changes, theoretically abstinence from alcohol could result in a reduction or elimination of pain, decrease the degree of pancreatic dysfunction, reduce mortality, and promote a return to normal activity[13]. It has repeatedly been suggested that cessation of alcohol improves the course of the disease[13,50]. For example, in one large study of the natural history of alcoholic chronic pancreatitis, 75% of patients continued to drink and in those patients the death rate and level of physical impairment were three times higher[19]. All agree that one focus should be on promoting cessation of alcohol and tobacco use. However, bouts of pain in alcohol-induced chronic pancreatitis still occur after the cessation of alcohol[18]. The benefits in terms of prevention of flares may in part depend on the stage of the disease in that alcohol, as a secretagogue, may have minimal effect on patients with little or no remaining exocrine function[13].

DIET

Patients and their families often inquire about diet therapy. It has been recommended that meals be low in fat and that large meals be avoided to possibly minimize hyperstimulation of the pancreas[13,27]. However, few of these dietary recommendations are evidence-based. Many patients with chronic pancreatitis will have clinical or subclinical deficiencies in vitamins and micronutrients[51,52]. Testing for retinol-binding protein, prealbumin, magnesium and transferrin has been recommended[8,51,53]. Because smoking is a risk factor for chronic pancreatitis, the formation of stones, and also calcifications, cigarette smoking should be strongly discouraged[15,54].

ANTIOXIDANTS

An intriguing aspect of dietary therapy in chronic pancreatitis is the emerging possible role of antioxidants. For example, Rose *et al*[55] reported deficiencies in selenium, vitamins A, C, and E, and riboflavin compared to healthy controls and patients with recurrent acute pancreatitis. Other studies have reported decreased intake of micronutrients in chronic pancreatitis patients[56]. These findings fueled the hypothesis that a reduction in these micronutrients could enhance oxidative stress and link to

the development of chronic pancreatitis[27,52]. Allopurinol can theoretically decrease toxic free radicals *via* its action on xanthine oxidase[27] leading to trials seeking to alleviate pancreatic pain with allopurinol. However, small studies reported no significant effects[57]. In contrast, a randomized trial of antioxidant supplementation with selenium, ascorbic acid, B-carotene, a-tocopherol, and methionine reported a significant reduction in the number of painful days per month[58]. A meta-analysis of antioxidants in chronic pancreatitis reported a small but significant reduction in visual analog scale pain scores (0.33 out of 10) along with an adverse effect rate of 16% of "mostly mild" symptoms[30]. Finally, a Cochrane review concluded that antioxidant therapy provides slight benefits and also reported adverse events in about 17%[59]. The role of antioxidant therapy in pain in chronic pancreatitis remains unclear and further investigation is warranted[60].

ENDOSCOPIC THERAPY

Endoscopic therapy has continued to play a role in the diagnosis and treatment of chronic pancreatic pain. Recent Cochrane reviews concluded that endoscopic therapy is not as effective as surgical intervention for pain relief, but endoscopy remains a viable option because of its availability and relative safety[59]. The Cochrane reviews were not able to clearly delineate differences between endoscopy and surgery regarding mortality and morbidity and recommended that options be presented to the patient and a joint decision be made[59]. Most endoscopic therapy is utilized for patients with intractable pain or nutritional deficiencies after more conservative therapy has failed[27]. Despite the lack of clear definitions of significant obstruction or methods to reliably identify patients amenable to endoscopic treatment, endoscopy has proven to be useful in relieving duct obstruction secondary to strictures, stones, or ampullary stenosis[27]. An alternative method is the combination of extracorporeal shock-wave lithotripsy followed by endoscopic removal of remaining debris and stones[27]. It has been reported that 80% of stones can be removed with approximately half of the patients reporting long-term pain relief[61]. A comparison of extracorporeal shock wave lithotripsy and extracorporeal shock wave lithotripsy plus endoscopic drainage in painful chronic pancreatitis found no significant difference after 2 years (*i.e.,* 38% of patients with extracorporeal shock wave lithotripsy alone reported pain relapse *vs* 45% of those with combined therapy)[62]. However, both groups experienced a significant reduction in pain episodes per year. Importantly, there was no placebo group and the cost of the combined treatment was three times greater[62].

Another alternative is placement of pancreatic ductal stents. In one study, 94% of 75 patients receiving pancreatic duct stents and dilation of duct strictures initially reported improved symptoms and, after a mean follow-up of three years, 53% remained symptom free[63]. Another study reported symptomatic improvement in 57% of 61 patients over a mean of 19 mo[64]. Anecdotally, pain relief appears to correlate with stone removal resulting in a decrease in main duct diameter[27]. Although stent placement is associated with stent migration, occlusion, aggravation of chronic pancreatitis and further duct changes, the availability and relatively low invasiveness compared to surgery makes endoscopic therapy a first-line consideration for treatment of ductal strictures and obstruction in the management of pancreatic pain[27].

SURGERY

Prior to the advent of endoscopic therapy for pancreatic ductal disease, the primary approach involved surgical interventions. A variety of surgical options were developed and the surgical approach continues to evolve. Nonetheless, no surgical intervention has proved to be one hundred percent effective. The role of surgical therapy is to deal with and prevent complications, as well as attempt to achieve pain control[61]. Indications for surgery include non-resolving ductal or common bile duct stenosis, intractable pain, internal pancreatic fistulas unresponsive to less invasive therapy, vascular erosions, or uncontrollable pancreatic pseudocysts[61]. Traditional surgical options for chronic pancreatitis can be divided into procedures that focus on resection of pancreatic tissue and procedures that focus on drainage of pancreatic ducts[61]. Resection-based procedures such as the Whipple operation, distal and total pancreatectomies, and the pylorus-preserving pancreatoduodenectomy were developed in part to relieve obstruction and because of the belief that chronic pancreatic pain also stemmed from perineural pancreatic inflammation. Drainage-based procedures such as the Frey procedure, Beger procedure, sphincterotomies, and pancreaticojejunostomies were designed to relieve ductal obstructions and ductal hypertension[61]. No gold standard exists, and the surgical procedures used to control pancreatic pain are individualized based on the anatomy, the condition of the patient, and the skill and experience of the surgeon.

In addition to more traditional options, pancreatic autotransplantation and a resurgence in neuroablation are emerging therapies. Endoscopic or even surgical neurolysis of the celiac ganglion remains an option in high-risk surgical patients or in patients who need additional therapy post-operatively[65]. Although less-invasive than traditional surgery, only 10% of neurolysis patients showed a benefit at 24 wk and two-thirds of patients required additional surgery[65]. Pancreatic autotransplantation can supplement resection-based surgery to preserve islet cell function and stave off endocrine insufficiency[65]. For example, the Mirkowitch technique uses a pellet of purified islet cells and segmental transplantation with resected pancreatic tissue that is implanted into the thigh[61].

There are significant limitations to surgical options for treating chronic pancreatic pain in that while pain relief and quality of life can be improved, exocrine and endocrine insufficiency frequently accompany respective options[66,67]. Patients undergoing surgery for chronic

pancreatitis have substantial hospital readmission rates. One recent study found that 31.5% of patients were readmitted in the first 30 d postoperatively and 42.3% were admitted in the first 90 d[68]. These substantial readmission rates are a significant problem especially since reimbursement rates are being more closely tied to outcomes such as rehospitalization. Factors that have been suggested to possibly help maximize surgical outcomes include early surgical intervention, alcohol cessation, retention of duodenal tissue, and concurrent medical therapy[68,69]. One reason given for poor pain control following surgical therapy is that some patients have altered central pain processing[70]. Methods are needed to be able to better select those patients who are destined to have a poor response as post-operative pain remains a significant problem.

The most recent Cochrane review of surgical intervention for obstructive chronic pancreatitis showed that early surgical intervention seemed, but was not definitely shown, to have potential benefits as compared to conservative therapy[59]. More importantly, the review concluded that surgical intervention produced better pain relief scores over a two and five year period (relative effect 1.62, 1.65) with a lower chance of resultant exocrine pancreatic insufficiency compared to endoscopic therapy[59].

PANCREATIC ENZYMES AND THE NEGATIVE FEEDBACK THEORY

The observation that pancreatic enzyme therapy in some patients with chronic pancreatitis results in a reduction in pain has lead to studies that attempt to understand the phenomenon and achieve more reliable results. The theories of ductal and interstitial hypertension and decreased distensibility of the damaged parenchyma note that pancreatic secretion is associated with a further increase in pressure and likely involved in the pathogenesis of pain. Surgical and endoscopic therapies are primarily aimed at altering pancreatic anatomy to facilitate passage of pancreatic juice. Theoretically, replacing endogenous secretion with exogenous pancreatic enzymes will reduce endogenous secretion in response to meals, blunt the increase in ductal and parenchymal pressure, and reduce pain.

NEGATIVE FEEDBACK INHIBITION OF PANCREATIC SECRETION

The normal human pancreas secretes continuously at a low rate. When food enters the duodenum, the hormones cholecystokinin (CCK) and secretin are secreted to deliver pancreatic enzymes (CCK) and bicarbonate (secretin) into the duodenum[32,71,72]. While much is known about the initiation of pancreatic enzyme release, less is known about how the process is stopped. However, there is evidence of negative feedback inhibition related to the presence of proteases in the duodenum. This was first shown in rats by Green and Lyman[73] and subsequently confirmed by a number of other investigators[74-77]. Feedback inhibition is known to occur in the rat, chicken[78] and pig[79]. In rats, one mediator of secretion is CCK[75]. For example, diversion of pancreaticobiliary secretions from the duodenal lumen resulted in a threefold increase in pancreaticobiliary protein secretion[75,80,81]. Pancreatic secretion was also associated with a significant rise of plasma CCK in diverted rats compared to basal levels (16 ± 4 pmol/L from 0.5 ± 0.8 pmol/L respectively)[75]. More specifically, perfusing the duodenum with pancreaticobiliary secretions or trypsin alone (*via* cannulation near the ampullary site) resulted in a decrease in pancreatic protein secretion and plasma CCK to near basal levels and essentially abolished the stimulatory effect of pancreaticobiliary secretion diversion on pancreatic secretion[75,77]. An alternate approach was to add a trypsin inhibitor to the pancreaticobiliary secretions to functionally remove trypsin which resulted in an increase in pancreaticobiliary protein output similar to pancreaticobiliary secretion diversion alone[75]. When the proteinase inhibitor, FOY-305, was given to rats by orogastric tube[76] there was a 15-fold increase in peak serum CCK levels and an increase in pancreatic protein and enzyme secretion[76].

In the rat, the negative feedback mechanism appears to be protease-specific as perfusing the duodenum with amylase does not affect protein output[75,82,83]. The role of CCK was confirmed by showing that the intravenous infusion of the CCK antagonist proglumide before and after pancreaticobiliary secretion diversion reduced protein outputs to near basal levels[75]. Discontinuation of the proglumide infusion removed the inhibition of pancreatic secretion[75]. The feedback mechanism appeared localized to the proximal intestine as ileal perfusion of trypsin did not affect pancreatic output[75]. Subsequent studies have been based on the hypothesis that the presence of trypsin in the duodenum down regulates CCK release resulting in a decrease in pancreatic protein output. The molecular mechanism of the interaction remains unclear. It has been suggested that a protease-sensitive mediator that controls CCK release is present in the duodenal mucosa, or alternatively, is secreted within the pancreatic juice[27,84,85]. Other data suggest that the feedback loop is not confined to the interactions between trypsin and CCK as neural pathways mediated by acetylcholine also appear to play a role[75]. For example, the intravenous infusion of acetylcholine, intraarterial infusion of tetrodotoxin, and intraluminal addition of lidocaine all abolished the rise in CCK and pancreatic output in pancreaticobiliary secretion diverted rats[75,86,87]. The mechanism for the cholinergic pathway remains unclear, but it has been suggested to possibly mediate secretion of the protease-sensitive proteins or be important to their action[75].

NEGATIVE FEEDBACK - EXPERIMENTAL STUDIES IN HUMANS

Owyang *et al*[88] attempted to demonstrate dose-de-

pendent pancreatic enzyme output suppression following intraduodenal infusion of proteases in healthy subjects. Exocrine pancreatic enzyme suppression required a minimum infusion of 0.5 mg/mL of trypsin, with maximal suppression with 1.0 mg/mL. Suppression was not seen with infusions of amylase and lipase. Suppression also correlated with a decline in CCK levels[88]. Interestingly, while a postprandial increase in plasma CCK was not seen in the presence of duodenal infusions of trypsin, a small increase in pancreatic enzyme secretion was observed. The authors hypothesized this was evidence of a separate pancreatic control mechanism, perhaps cholinergic[88]. A subsequent investigation examined the possibility of two distinct feedback mechanisms by stimulating duodenal volume and osmoreceptors by infusing normal saline at increasing rates and increasing osmolality[89]. They noted a dose-related increase in pancreatic output without an effect on plasma CCK levels[89]. Prior studies in rats had also shown a decrease in pancreatic output with anticholinergic agents, but plasma CCK was also affected[75]. The effect on pancreatic output was reversed by intraduodenal atropine but not by intraduodenal proteases[89]. However, the addition of a phenylalanine solution dramatically increased CCK levels and enzyme output. The effect was reduced with the intraduodenal infusion of proteases. The addition of both atropine and proteases completely abolished the pancreatic enzymatic response to intraduodenal phenylalanine[89].

While negative feedback mechanisms in humans have been clearly demonstrated, not all studies have been consistent[90], and many studies used super-physiologic amounts of trypsin[49,91]. The earliest example measured pancreatic secretory output after intraduodenal infusion in a man with carcinoma of the ampulla of Vater which completely blocked biliary and pancreatic secretions from the small intestine[92]. Pancreatic secretory output was measured *via* a percutaneous transhepatic cholangiography catheter. Intraduodenal infusion of the patient's pancreaticobiliary secretions reduced pancreatic secretions and the effect was reversed by a trypsin inhibitor (soy bean trypsin inhibitor which is relatively trypsin-specific)[92]. A similar experiment was done after pancreatoduodenectomy with similar results except that the proximal duodenum had been removed suggesting that the site for stimulation extends beyond the periampullary region[93].

The most detailed study infused an essential amino acid solution into the duodenum and compared pancreatic outputs in patients with differing severity of chronic pancreatitis and healthy controls[94]. The addition of trypsin, 10 mg/mL, resulted in an approximately 32% decrease in pancreatic secretions in patients with reduced pancreatic output and a 74% decrease in those with normal pancreatic secretion. No inhibition was seen in patients with low pancreatic bicarbonate secretion and steatorrhea[94]. Chronic pancreatic enzyme therapy was also associated with a 27% decrease in basal pancreatic secretion and a 46% decrease in amino acid stimulated secretion. A dose-response of trypsin inhibition of exocrine secretion was evaluated in one patient during amino acid infusion. The minimum concentration of trypsin required to inhibit pancreatic exocrine secretion was 0.9 mg/mL and maximum suppression required a trypsin concentration of at least 2.5 mg/mL. Perfusion experiments with amino acids plus trypsin and the relatively trypsin-specific inhibitor, ovomucoid, was still associated with an increase in chymotrypsin secretion. Chymotrypsin (10 mg/mL) also decreased amino acid-stimulated trypsin output whereas protease-free lipase and amylase did not, confirming that only trypsin, chymotrypsin, and pancreaticobiliary secretions suppress pancreatic enzyme secretion in humans. However, the effect was minimal to absent in patients with advanced pancreatic exocrine insufficiency[94]. In addition, patients with advanced insufficiency did not experience pain relief with enzyme supplementation[94].

Studies using pancreaticocystostomies following simultaneous kidney and segmental pancreatic transplantations have also demonstrated feedback inhibition[95,96]. For example, pancreatic exocrine secretions were collected from pancreaticocystostomies after administration of a Lundh test meal orally with or without addition of 6 pancrelipase capsules orally. Total amylase decreased by more than a third, and peak amylase fell 63% with supplemental enzymes. The pancrelipase capsules reduced amylase secretion 16% below basal secretion, and within 1.5 h two of the patients experienced cessation of all graft secretion[96]. Importantly, inhibition of pancreatic exocrine secretion occurred despite denervation of pancreatic tissue consistent with the presence of a hormonally mediated feedback mechanism.

Overall, the results in humans were consistent with the presence of several distinct feedback pathways, one being under hormonal control mediated by proteases (*e.g.,* trypsin/chymotrypsin)[31,97-99], and another by neural control mediated by acetylcholine[89]. However, not all studies have been positive. For example, intrajejunal infusion of normal saline, pancreaticobiliary secretions, and pancreaticobiliary secretions inactivated by heat into normal healthy humans found no significant difference suggesting the absence of a jejunal-pancreatic feedback mechanism[100]. However, this failure can likely be explained by the inhibitory effect being localized to the duodenum, which was not perfused. Studies that infused an active or an inactivated trypsin inhibitor (aprotinin, which is relatively trypsin-specific) into the duodenum of healthy subjects have also reported no significant difference in pancreatic output between the infusates[101,102]. However, as shown previously, in humans both trypsin and chymotrypsin are effective in activating the feedback pathway whereas in rodents the effect appears to be more specific to trypsin. Thus, the use of trypsin-specific inhibitors did not reduce the effect of chymotrypsin present.

USING ENZYMES FOR PAIN - CLINICAL TRIALS AND META ANALYSES

The use of pancreatic enzymes in the therapy of gastrointestinal disease has a long history[103,104]. As noted previously, some, but not all, patients with pancreatic pain respond[49]. The potential mechanisms include feedback inhibition of pancreatic secretion, improvement in digestion that reduces or eliminates symptoms attributable to malabsorption, or altered nutrient-microbiome interactions. As with any medical treatment, for effectiveness one first looks for the results of randomized placebo-controlled studies with well-matched and well-described patient populations and for head-to-head comparisons of different formulations. With pancreatic enzymes, the search leads to more disappointments than enlightenments. Investigators have generally studied what was readily available to them in terms of products and patients. Ideally, a study of pancreatic enzymes for feedback inhibition of enzyme secretion would utilize formulations that reliably produce high intraduodenal concentrations of trypsin and chymotrypsin. For maldigestion, one would choose a preparation that reliably delivered high concentrations of active lipase into the proximal intestine[105]. The choice of formulation has been complicated by the recent removal of traditional products and the substitution of products primarily available as enteric-coated enzymes that fail to reliably release their contents in the duodenum[105].

Despite these problems, it is worthwhile to review the available data which includes several meta-analyses such as one in 1997 by Brown *et al*[106] and another in 2010 by Shafiq *et al*[107]. Brown *et al*[106] included six randomized, placebo-controlled, double blind, prospective studies containing 189 patients with confirmed chronic pancreatitis[94,108-112]. The primary outcome measure was the percentage of patients preferring enzymes to placebo[106]. In only one study was there a greater than 50% preference for enzymes as compared to placebo (*i.e.,* 85%)[108]. Only that result was statistically significant[108] and the authors concluded that the available studies did not support the hypothesis that pancreatic enzyme supplementation was useful to treat abdominal pain associated with chronic pancreatitis[106]. However, it is important to note that the pancreatic enzyme products used differed among studies not only in formulation but also in dosage and timing[106]. The studies also differed in relation of method of diagnosing chronic pancreatitis, length of treatment, and scoring of pain, as well as etiology of pancreatitis, disease severity, and degree of exocrine dysfunction. Two of the studies used non-enteric-coated preparations[94,108] and four used enteric-coated formulations[109-112]. The study using non-enteric-coated enzymes was the only study that demonstrated a significant patient preference of enzymes over placebo[108]. The second meta-analysis set out to address the effect of enzymes on weight loss, steatorrhea, fecal fat, quality of life, and pain in patients with chronic pancreatitis. They also addressed the role of enteric-coated *vs* non-enteric-coated formulations and dosage schedules. They specifically reported on the frequency of abdominal pain, duration of pain episodes, intensity of pain, and analgesic use[107]. Ten studies were included with a total of 361 patients[94,108-116]. The analysis included five of the six studies in Brown *et al*[106] review. Heterogeneity and overall poor data continued to be a hindrance. There were many issues with regard to understanding the effect of pancreatic enzyme supplementation on pain intensity. Although five studies specifically addressed pain, only two studies[111,112] provided mean pain scores and standard deviations. However, the two studies used different pain scores (*i.e.,* 0 to 5 *vs* 0 to 3). Mössner *et al*[111] reported a nonsignificant improvement of pain with enteric-coated enzymes as compared with placebo (1.26 ± 0.8 *vs* 1.08 ± 0.8, respectively). Conversely, Larvin *et al*[112] reported a significant improvement with enteric-coated enzymes *vs* placebo (1.93 ± 1.04 *vs* 2.05 ± 0.8, respectively). The remaining 3 studies either did not report standard deviations or reported pain scores differently such as a mean, median, or sum. This mix of results precluded data pooling. Four studies examined the effect of enzymes on analgesic use but did not report standard deviations. Specifically, Isaakson *et al*[108] reported a small nonsignificant decrease in analgesic consumption (7.8 tablets with enzymes *vs* 8.9 with placebo) whereas Halgreen *et al*[110] reported a nonsignificant decrease in analgesic consumption scores with enzymes as compared to placebo in patients with steatorrhea (49 *vs* 58 respectively) and a nonsignificant increase in patients without steatorrhea (57 *vs* 48). Larvin *et al*[112] also reported a nonsignificant decrease in analgesic consumption, reported as mean daily analgesic use with enzymes as compared to placebo (*e.g.,* 45 mg *vs* 51 mg, respectively). Finally, Malesci *et al*[109] also reported a nonsignificant increase in median analgesic consumption score in enzymes *vs* placebo (12 with range of 0 to 34 *vs* 0 with range of 0 to 44, respectively). The frequency of abdominal pain and duration of pain episodes were not addressed in any of the included studies[107]. The meta-analysis also included one study of enzyme dosing schedules on effectiveness in improving malabsorption but not on reducing pain intensity, pain duration, or use of analgesics[115].

Only one study met the criteria for assessment of quality of life, perhaps an indirect measure of pain control[107]. The double-blind, two week study used the Clinical Global Impression of Disease Symptoms Scale to evaluate quality of life after only two weeks of enzyme supplementation or placebo[113]. The use of enzymes resulted in an improvement in quality of life which approached statistical significance ($P = 0.063$)[113]. The final study in the meta-analysis compared non-enteric-coated with enteric-coated enzymes and focused on changes in steatorrhea[114]. In that study, patients receiving uncoated enzymes plus cimetidine or uncoated

enzymes alone improved steatorrhea better than those receiving enteric-coated enzymes[114].

A recent review of clinical trials using enzymes for painful chronic pancreatitis[117] included three studies not previously discussed in the meta-analyses. One of them, a study by Czako *et al*[118], was a multi-center prospective observational study of pancreatic enzyme supplementation on quality of life and abdominal pain in 70 patients divided into supplemental enzyme naïve patients with a new diagnosis of chronic pancreatitis and patients previously diagnosed and treated with oral enzymes. Patients received enteric-coated microspheres with the dosage based on the severity of exocrine insufficiency[118] along with an H_2 receptor blocker. Thirty-five percent of patients in the new diagnosis group had severe degree pancreatic exocrine insufficiency compared to 64% in the previously diagnosed group. Analgesics were given if requested but the type, dosage, and frequency were not recorded and no control group was included. Outcome was assessed using the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 modified by adding a disease-specific symptom scale including questions about steatorrhea and abdominal pain[118]. The duration of the study was 4 wk. Overall, they reported a small but significant increase in mean body weight, decreases in defecations per week, and decreases in mean pain scores in both groups (pain score 47.1 to 35.9 in the mild steatorrhea group and 37.8 to 29.4 in the severe steatorrhea group)[118] as well as significant increases in global quality of life[118]. As promising as these results may seem in regards to improving symptoms of pancreatic exocrine insufficiency and relieving abdominal pain, no control group was included and the effect of analgesic use was not reported.

A recent study observed 294 patients with chronic pancreatitis and exocrine pancreatic insufficiency on pancreatic enzyme replacement for a year. The patients were divided into those currently taking enzymes and those with a new diagnosis of exocrine pancreatic insufficiency who were enzyme replacement naive[119]. Patients were given daily doses of an enteric-coated mini-microsphere preparation, Creon, and the presence of recurring pain and changes in quality of life were assessed. At the end of the study, a significant portion of patients reported a decrease in recurrent abdominal pain (66.3% with recurrent abdominal pain before treatment *vs* 34.3% after, $P < 0.001$)[119]. The percent decrease between cohorts was comparable. Similarly, after 12 mo of treatment, the mean total gastrointestinal quality of life index score improved significantly for the entire patient pool, as well as for individual cohorts[119]. Physical function and emotion subcategories also improved significantly[119]. However, despite the impressive results, the lack of a placebo makes it impossible to distinguish between the natural history of the disease and a specific effect of enzyme therapy. Actual dosages were not recorded which made analysis of optimal dosing impossible. The improvement in recurrent abdominal pain in the group previously treated with pancreatin could be related to improved compliance or a more effective treatment regimen. It would have been interesting to include a group randomized to continuing their previous regimen. Only 31% of the patient population had chronic pancreatitis due to alcohol abuse, which may represent a more difficult to treat group.

In conclusion, the heterogeneity in terms of patient characteristics (*e.g.,* presence, absence and severity of exocrine insufficiency, etiology of pancreatitis, reason for presentation, use of narcotics, formulation, dosage, and administration of enzymes in relation to meals, *etc.*) greatly affects the outcome of studies attempting to evaluate pain relief in chronic pancreatitis. Heterogeneity makes meta-analysis a very blunt instrument for evaluation of the effectiveness of therapy or for helping to decide which therapy is ideal for an individual patient. Clearly some patients respond. Current enteric-coated enzyme products are unlikely to be highly effective either in terms of providing sufficient intraduodenal trypsin activity to engage the feedback mechanism or to fully correct steatorrhea. Future studies should either focus on trying to understand why those patients respond or to carefully select parameters thought to be important, such as providing a critical amount of trypsin or chymotrypsin activity into the duodenum. One can reasonably conclude that patients with exocrine pancreatic insufficiency benefit from correction of malabsorption and the ensuing nutritional deficiencies as well as improvement of gastrointestinal symptoms including pain associated with malabsorption. Reviews of the issues with providing adequate delivery of pancreatic enzymes for treatment of malabsorption are recommended for those wanting additional details regarding use of pancreatic enzymes for malabsorption[105].

USE OF PANCREATIC ENZYMES IN CHRONIC PANCREATITIS

Administration of exogenous pancreatic enzymes has long been used as an adjuvant to the treatment of patients with pancreatic pain largely based on the premise that replacement of lost enzymes might rest the pancreas. The current rationale is that feedback inhibition of pancreatic secretion reduces CCK release and prevents pancreatic hyperstimulation and pain[27]. However, achieving this goal requires the ability to provide sufficient active trypsin/chymotrypsin to the proximal intestine. An alternative or complimentary use of enzymes in chronic pancreatitis is to treat overt or occult nutritional deficiencies. For example, low serum magnesium, hemoglobin, albumin, prealbumin, and retinol binding protein levels (a surrogate for fat soluble vitamins) along with a hemoglobin A1C above normal limits are all highly associated with exocrine pancreatic insufficiency[53]. Specifically, vitamin A (3%), D (53%), E (10%), and K (63%) deficiencies are often present in

patients without clinically apparent malabsorption[51,120]. The long term use of enzyme therapy for those with enzyme insufficiency is associated with improvements in stool frequency, fecal fat loss, stool consistency, and both clinician and patient assessment of symptoms[113,121]. However, past and current formulations of pancreatic enzymes are not ideal for achieving feedback inhibition or relief of exocrine pancreatic insufficiency[105,122].

The majority of currently available supplemental pancreatic enzymes are available as enteric-coated microspheres formulated as capsules or tablets. However, none of these preparations will reliably release their contents within the critical zone of the duodenum-proximal small bowel[105]. Uncoated enzymes are also available both from pharmaceutical companies and from health food stores[105,123-125]. Lipase is irreversibly inactivated when the pH falls below 4, whereas proteases are much more pH resistant and are more likely to survive transport through the stomach. However, they can both be destroyed by pepsin. The transplant studies used pancrelipase, specifically enteric-coated Pancrease[96]. Slaff *et al*[94] also clearly demonstrated feedback inhibition in 3 chronic pancreatitis patients without steatorrhea by using 30 d of non-coated Viokase, 8 tablets q.i.d. The high dose, currently available non-enteric enzyme, Viokace, (*i.e.,* with 20880 USP units of lipase) contains 78300 USP units of protease/tablet. If all the protease activity was from trypsin (which it is not) each tablet would contain only approximately 3 mg of trypsin. The dose-response experiments in man suggested at least 1 mg/mL was required for feedback inhibition. It would therefore be very unlikely that this minimum level would be achieved *in vivo* using Viokace even if all the protease activity survived transport through the stomach. Acid-stable proteases are available as over the counter medications, but to our knowledge the ability of the drug to initiate feedback regulation of pancreatic secretions or its resistance to acid-pepsin has not been tested in man. One such inexpensive, over the counter product, "Essential Enzymes 500", has been used successfully in irritable bowel syndrome. It contains 12 mg of acid stable proteases/capsule[124]. Studies are still needed using acid stable proteases for their ability to initiate feedback inhibition of pancreatic secretion.

GENERAL RECOMMENDATIONS FOR ENZYME USE AND TREATING CHRONIC PANCREATIC PAIN

Unfortunately, there are very little long-term data exploring the efficacy of treating chronic pancreatic pain with enzyme supplementation. One recent study included daily treatment with enteric-coated pancreatin for one year and noted a significantly positive impact on pain, quality of life, and emotional and physical well-being in both chronically treated and treatment naïve patients[119]. No placebo group was included which is important considering the high placebo effect reported in prior studies[32,109-111]. Another recent study compared pancreatin alone, pancreatin plus a proton pump inhibitor, or pancreatin plus a proton pump inhibitor and the NSAID aceclofenac[126]. All three regimens produced significant improvements in pain compared to no pretreatment, but the lack of a placebo questions whether the effect was due to the enzymes.

Evaluation of a new patient with suspected chronic pancreatitis requires careful consideration of multiple factors and includes a search for potentially correctable conditions (Table 3). One must also be aware of the possibility of an occult malignancy. It is important to attempt to identify and treat any nutritional deficiencies present and to strongly discourage alcohol use and smoking. This review focuses on pancreatic pain, a condition where treatment typically requires a variety of expertise often including experts in pain management. Severe pain will likely require narcotics which may eventuate in narcotic addiction. One should try to use non-narcotic drugs whenever possible (*e.g.,* nonsteroidal anti-inflammatory drugs, acetaminophen, and tramadol) and avoid opiates with a higher predilection to abuse such as *Dilaudid* (hydromorphone) and oxycodone.

Until recently the mainstay of chronic pancreatitis pain management has been opioid-based. However, as the risks of long-term opioid therapy have crystalized, clinicians are increasingly looking for alternatives. Prescription opioid overdoses have quadrupled in the last 15 years, and deaths from drug overdose are now more common than automobile collision fatalities[127]. In an effort to educate healthcare providers and curb these growing statistics, the United States Center for Disease Control issued a statement in March 2016 with guidelines regarding the prescription of opioids[127], which includes recommendations about the preferred use of non-opioid pharmacologic and non-pharmacologic therapy. They also recommend consistent reevaluation of risks and benefits of opioid therapy, using the lowest effective dosage, the avoidance of extended-release tablets and a warning against the use of opioids with benzodiazepine therapy. Clinicians providing care to chronic pancreatitis patients with high levels of pain may find these guidelines helpful in their efforts to help with pain control.

Opioid therapy can be quite effective for the short-term management of acute pain, but the long-term benefits of opioid therapy are murky as the majority of studies are of short duration[127]. Long-term comparative studies are rare and often show those who receive opioid therapy to have poorer function, are less likely to return to work, and are less likely to have good pain control[128,129]. Opioid therapy also affects smooth muscle tone leading to gastrointestinal motility disturbances and abdominal pain[130-134]. While morphine is effective in reducing pain in chronic pancreatitis, a double-blinded comparison with tramadol reported that patients with chronic pancreatitis preferred tramadol to morphine for anesthesia[130]. In addition, tramadol does not increase smooth muscle tone in the sphincter of Oddi[130].

The first choice for chronic pain should likely be non-steroidal anti-inflammatory drugs (NSAIDs) and/

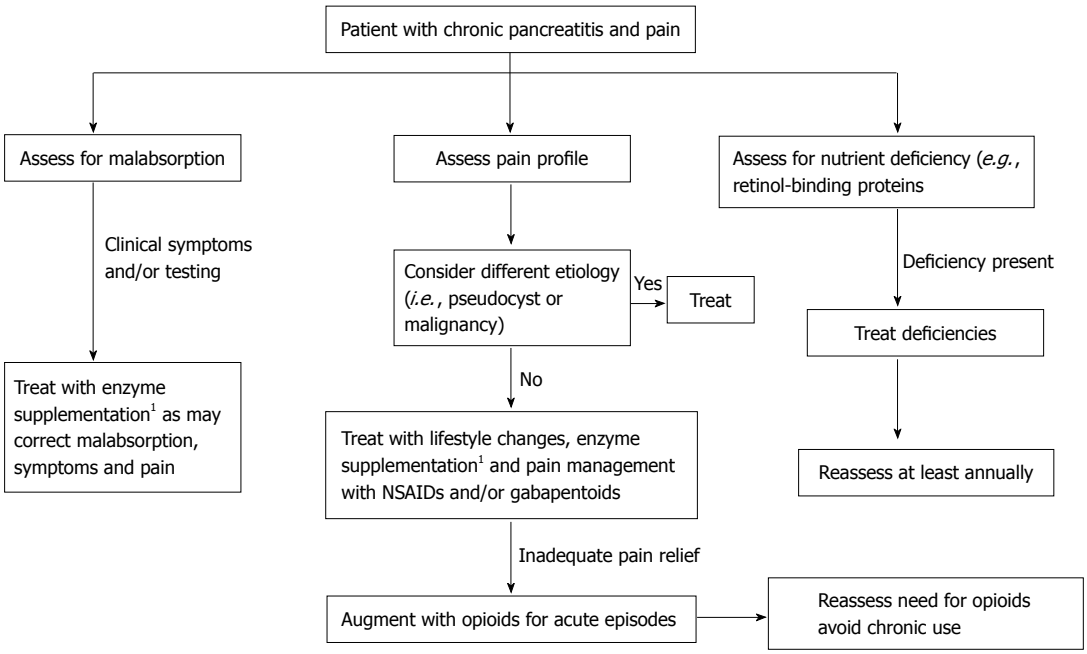

Figure 1 Flow chart demonstrating recommendations for using pancreatic enzyme replacement therapy in a patient with abdominal pain and chronic pancreatitis. [1]Start with non-enteric coated products such as Viokace along with a PPI. The figure suggests approaching the patient with a three-pronged method. First, one should assess the patient's pain profile and investigate whether the pain is from chronic pancreatitis alone or from other etiologies, *i.e.*, a developing pseudocyst or malignancy. Next, pain control should be attempted first with conservative measures such as lifestyle changes, enzyme supplementation, NSAIDs, and/or gabapentoids before moving to treat with opioids. If opioids are deemed appropriate for pain control, the decision should be consistently reassessed as to avoid dependency and addiction. Second, one should assess the patient for malabsorption, and if present, the patient should be treated with exogenous enzymes as that may improve absorption and pain symptoms. Lastly, the physician should assess the patient's nutritional status and correct deficiencies, if present. A non-enteric-coated enzyme such as Viokace along with a proton pump inhibitor is recommend for first-line enzymatic treatment. Alternatively, can use combination of non-enteric-coated and enteric-coated formulations. NSAIDs: Nonsteroidal antiinflammatory drugs; PPI: Proton pump inhibitors.

or gabapentoids to treat neuropathic pain. Generally, NSAIDs are used for analgesia and full anti-inflammatory doses are neither required nor indicated. Primarily analgesic NSAIDs include low dose naproxen, ibuprofen, nabumetome and etodolac[135]. Higher doses typically do not increase analgesia but increase risk of side effects. Co-therapy with a proton pump inhibitor should be considered. Gabapentoids such as pregabalin are often used as adjuvant therapy due to possible similarities between chronic pancreatic pain and neuropathic pain[136]. A study of pregabalin enrolled patients who were concurrently undergoing opioid therapy and reported success suggesting a role for pregabalin in chronic pancreatitis pain[136]. However, none of these approaches are without accompanying side effects and long-term studies are needed.

The natural history of pain in any particular patient is impossible to predict[23]. In general, those with constant pain have a worse prognosis than those with intermittent pain[24]. While pancreatic enzyme therapy is a mainstay in the therapy of exocrine pancreatic insufficiency, it can also be used in an attempt to produce feedback inhibition of enzyme secretion although this is likely only useful for those who retain exocrine function.

Prior studies have suggested that feedback inhibition was only effective in those without steatorrhea[94]. Indeed, longer term studies in pancreatic pain have confirmed that those with pain and pancreatic insufficiency generally are the most difficult to treat[17,94]. However, a reduction in malabsorption can also lead to reduced symptoms[47-49]. We recommend that all patients with chronic pancreatitis should be screened for nutritional deficiencies which includes measuring serum magnesium, hemoglobin, albumin, prealbumin, retinol binding protein levels, hemoglobin A1C, and body mass index. For those with low retinal binding protein, one should consider that fat-soluble vitamin deficiencies are likely present. For initial therapy for patients with pancreatic pain, we recommend that the focus be on correcting nutritional deficiencies and malabsorption. Treatment of fat malabsorption requires at least 20000-30000 USP units of lipase/meal[105]. One might start with the non-enteric-coated 10000 lipase unit Viokace formulation (*i.e.*, one tablet at the beginning of the meal or just before the meal, and 2 or 3 more tablets spread throughout the meal plus one per snack). The use of a proton pump inhibitor is recommended, possibly as a double dose such as 40 mg of esomeprazole twice a day, to reduce destruction of lipase during transit through the stomach. Potassium competing acid blockers should simplify therapy when they become available in that they provide reliable pH control. Alternatively, one could use a combination of non-enteric-coated and enteric-coated formulations[105]. Improved nutritional status should be assessed at least once a year and include measuring serum magnesium, hemoglobin, albumin, prealbumin, retinol binding protein levels (a surrogate for fat soluble

vitamins), and body mass index (Figure 1).

However, enzyme therapy is not without its pitfalls as properly mimicking the normal physiology of nutrient digestion and absorption is difficult[47,105]. Enteric-coated enzymes must mix properly in the stomach, not separate from the meal, and dissolve and remain active in the duodenum as to allow proper metabolism, digestion, and absorption for the completion of the feedback loop and resting of the pancreas. With current formulations, delivery of sufficient protease to the duodenum is impossible with enteric-coated products and difficult with non-enteric-coated ones.

CONCLUSION

Whether pancreatic enzyme administration for chronic pancreatic pain is effective remains a debated subject. Although reviews on the topic suggest there is little evidence for benefit, the conclusions are based on a potpourri of studies which vary dramatically in design and execution. Enzyme preparations also differ greatly in size, composition, and action but are generally treated as equal despite a lack of information and formal studies. A majority of experiments have used only enteric-coated preparations which have been shown to separate from meals and dissolve distal to the duodenum. Until formal studies including head to head comparisons are performed and characteristics such as mixing and dispersion properties are known, we are left without the crucial information needed. Uncoated enzymes, while largely being replaced by more modern preparations, have shown promise in treating pain and need to be explored further. Use of acid resistant proteases are needed as well as better strategies to overcome the gastric and intestinal pH barriers to maximize proper enzyme delivery to the duodenum for both uncoated and enteric-coated preparations. Future studies evaluating the use of enzymes with concurrent antacid and/or anti-secretory therapies, especially with potassium competing acid blockers, are needed. Furthermore, clinical trials will ideally include long-term treatment arms and large treatment groups to allow for more reliable data gathering. Patients should also be subcategorized based on etiology and severity in order to specifically study response to treatment. Most importantly, the complexity of data gathered here should serve to help individualize enzyme replacement therapy. For example, clinical trials could be done to confirm suggestions that patients with idiopathic chronic pancreatitis and mild to moderate exocrine insufficiency respond better to enzyme therapy than those of alcoholic origin and severe exocrine impairments. Pain scores must be standardized and validated. Exogenous enzyme therapy may decrease secretion, is noninvasive, has relatively no adverse effects, and improves malabsorption in those with exocrine insufficiency. There is little to be lost and potentially much to be gained by trying enzyme therapy, but more studies are needed before they can be used in evidence-based medicine.

ACKNOWLEDGMENTS

Dr. Graham is supported in part by Office of Research and Development Medical Research Service Department of Veterans Affairs, Public Health Service grant DK56338 which funds the Texas Medical Center Digestive Diseases Center. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the VA or NIH.

REFERENCES

1 **Howard JM**, Hess W. History of the pancreas: Mysteries of a hidden organ. New York: Kluwer Adademic/Plenum Publishers, 2002 [DOI: 10.1007/978-1-4615-0555-6]
2 **Cawley T.** A single case of diabetes, consisting entirely in the quality of the urine, with an inquiry into the different theories of that disease. *London Med J* 1788; **9**: 286-301
3 **Johnston GW**. Calculous and other affections of the pancreatic ducts. *Am J Med Sci* 1883; **86**: 404-429 [DOI: 10.1097/00000441-188310000-00005]
4 **Singer MV**, Gyr K, Sarles H. Revised classification of pancreatitis. Report of the Second International Symposium on the Classification of Pancreatitis in Marseille, France, March 28-30, 1984. *Gastroenterology* 1985; **89**: 683-685 [PMID: 4018507 DOI: 10.1016/0016-5085(85)90468-8]
5 **Sarner M**, Cotton PB. Classification of pancreatitis. *Gut* 1984; **25**: 756-759 [PMID: 6735257 DOI: 10.1136/gut.25.7.756]
6 **Steer ML**, Waxman I, Freedman S. Chronic pancreatitis. *N Engl J Med* 1995; **332**: 1482-1490 [PMID: 7739686 DOI: 10.1056/NEJM199506013322206]
7 **Gupta V**, Toskes PP. Diagnosis and management of chronic pancreatitis. *Postgrad Med J* 2005; **81**: 491-497 [PMID: 16085738 DOI: 10.1136/pgmj.2003.009761]
8 **Afghani E,** Sinha A, Singh VK. An overview of the diagnosis and management of nutrition in chronic pancreatitis. *Nutr Clin Pract* 2014; **29**: 295-311 [PMID: 24743046 DOI: 10.1177/0884533614529996]
9 **Hayakawa T**, Kondo T, Shibata T, Noda A, Suzuki T, Nakano S. Relationship between pancreatic exocrine function and histological changes in chronic pancreatitis. *Am J Gastroenterol* 1992; **87**: 1170-1174 [PMID: 1519575]
10 **Chowdhury RS**, Forsmark CE. Review article: Pancreatic function testing. *Aliment Pharmacol Ther* 2003; **17**: 733-750 [PMID: 12641496 DOI: 10.1046/j.1365-2036.2003.01495.x]
11 **Catalano MF**, Sahai A, Levy M, Romagnuolo J, Wiersema M, Brugge W, Freeman M, Yamao K, Canto M, Hernandez LV. EUS-based criteria for the diagnosis of chronic pancreatitis: the Rosemont classification. *Gastrointest Endosc* 2009; **69**: 1251-1261 [PMID: 19243769 DOI: 10.1016/j.gie.2008.07.043]
12 **Yadav D**, Hawes RH, Brand RE, Anderson MA, Money ME, Banks PA, Bishop MD, Baillie J, Sherman S, DiSario J, Burton FR, Gardner TB, Amann ST, Gelrud A, Lawrence C, Elinoff B, Greer JB, O'Connell M, Barmada MM, Slivka A, Whitcomb DC. Alcohol consumption, cigarette smoking, and the risk of recurrent acute and chronic pancreatitis. *Arch Intern Med* 2009; **169**: 1035-1045 [PMID: 19506173 DOI: 10.1001/archinternmed.2009.125]
13 **Pitchumoni CS**. Chronic pancreatitis: pathogenesis and management of pain. *J Clin Gastroenterol* 1998; **27**: 101-107 [PMID: 9754770 DOI: 10.1097/00004836-199809000-00002]
14 **Layer P**, Yamamoto H, Kalthoff L, Clain JE, Bakken LJ, DiMagno EP. The different courses of early- and late-onset idiopathic and alcoholic chronic pancreatitis. *Gastroenterology* 1994; **107**: 1481-1487 [PMID: 7926511 DOI: 10.1016/0016-5085(94)90553-3]
15 **Braganza JM**, Lee SH, McCloy RF, McMahon MJ. Chronic pancreatitis. *Lancet* 2011; **377**: 1184-1197 [PMID: 21397320 DOI: 10.1016/S0140-6736(10)61852-1]
16 **DiMagno EP,** Layer P, Clain JE. Chronic pancreatitis. In: V.L.W.Go,

editor. The Pancreas: Biology, Pathobiology, and Disease. 2nd ed. New York: Raven Press, 1993. 665-706
17 **Lankisch PG**, Löhr-Happe A, Otto J, Creutzfeldt W. Natural course in chronic pancreatitis. Pain, exocrine and endocrine pancreatic insufficiency and prognosis of the disease. *Digestion* 1993; **54**: 148-155 [PMID: 8359556 DOI: 10.1159/000201029]
18 **Pitchumoni CS**. Alcohol in chronic pancreatitis. Does it provoke the pain? *J Clin Gastroenterol* 1995; **20**: 4-5 [PMID: 7884176 DOI: 10.1097/00004836-199501000-00003]
19 **Ammann RW**, Akovbiantz A, Largiader F, Schueler G. Course and outcome of chronic pancreatitis. Longitudinal study of a mixed medical-surgical series of 245 patients. *Gastroenterology* 1984; **86**: 820-828 [PMID: 6706066]
20 **Ammann RW**, Muellhaupt B. The natural history of pain in alcoholic chronic pancreatitis. *Gastroenterology* 1999; **116**: 1132-1140 [PMID: 10220505 DOI: 10.1016/S0016-5085(99)70016-8]
21 **Girdwood AH**, Marks IN, Bornman PC, Kottler RE, Cohen M. Does progressive pancreatic insufficiency limit pain in calcific pancreatitis with duct stricture or continued alcohol insult? *J Clin Gastroenterol* 1981; **3**: 241-245 [PMID: 7288117 DOI: 10.1097/00004836-198109000-00007]
22 **Enweluzo C**, Tlhabano L. Pain management in chronic pancreatitis: taming the beast. *Clin Exp Gastroenterol* 2013; **6**: 167-171 [PMID: 24039444 DOI: 10.2147/CEG.S50827]
23 **Levrat M**, Descos L, Moulinier B, Pasquier J. [Long-course development of chronic pancreatitis (113 cases). II. Study of the course of chronic pancreatitis in operated patients]. *Arch Fr Mal App Dig* 1970; **59**: 305-314 [PMID: 5423785]
24 **Mullady DK**, Yadav D, Amann ST, O'Connell MR, Barmada MM, Elta GH, Scheiman JM, Wamsteker EJ, Chey WD, Korneffel ML, Weinman BM, Slivka A, Sherman S, Hawes RH, Brand RE, Burton FR, Lewis MD, Gardner TB, Gelrud A, DiSario J, Baillie J, Banks PA, Whitcomb DC, Anderson MA. Type of pain, pain-associated complications, quality of life, disability and resource utilisation in chronic pancreatitis: a prospective cohort study. *Gut* 2011; **60**: 77-84 [PMID: 21148579 DOI: 10.1136/gut.2010.213835]
25 **Warshaw AL**, Banks PA, Fernández-Del Castillo C. AGA technical review: treatment of pain in chronic pancreatitis. *Gastroenterology* 1998; **115**: 765-776 [PMID: 9721175 DOI: 10.1016/S0016-5085(98)70157-X]
26 **Karanjia ND**, Reber HA. The cause and management of the pain of chronic pancreatitis. *Gastroenterol Clin North Am* 1990; **19**: 895-904 [PMID: 2269524]
27 **Gachago C**, Draganov PV. Pain management in chronic pancreatitis. *World J Gastroenterol* 2008; **14**: 3137-3148 [PMID: 18506917 DOI: 10.3748/wjg.14.3137]
28 **Sakorafas GH**, Tsiotou AG, Peros G. Mechanisms and natural history of pain in chronic pancreatitis: a surgical perspective. *J Clin Gastroenterol* 2007; **41**: 689-699 [PMID: 17667054 DOI: 10.1097/MCG.0b013e3180301baf]
29 **Bradley EL**. Pancreatic duct pressure in chronic pancreatitis. *Am J Surg* 1982; **144**: 313-316 [PMID: 7114368 DOI: 10.1016/0002-9610(82)90008-3]
30 **Madsen P**, Winkler K. The intraductal pancreatic pressure in chronic obstructive pancreatitis. *Scand J Gastroenterol* 1982; **17**: 553-554 [PMID: 7134883 DOI: 10.3109/00365528209182248]
31 **Ebbehøj N**, Borly L, Bülow J, Rasmussen SG, Madsen P. Evaluation of pancreatic tissue fluid pressure and pain in chronic pancreatitis. A longitudinal study. *Scand J Gastroenterol* 1990; **25**: 462-466 [PMID: 2359973 DOI: 10.3109/00365529009095516]
32 **Mössner J**. Palliation of pain in chronic pancreatitis. Use of enzymes. *Surg Clin North Am* 1999; **79**: 861-872, xi [PMID: 10470332 DOI: 10.1016/S0039-6109(05)70048-7]
33 **Ebbehøj N**, Borly L, Bülow J, Rasmussen SG, Madsen P, Matzen P, Owre A. Pancreatic tissue fluid pressure in chronic pancreatitis. Relation to pain, morphology, and function. *Scand J Gastroenterol* 1990; **25**: 1046-1051 [PMID: 2263877 DOI: 10.3109/00365529008997633]
34 **Karanjia ND**, Singh SM, Widdison AL, Lutrin FJ, Reber HA. Pancreatic ductal and interstitial pressures in cats with chronic pancreatitis. *Dig Dis Sci* 1992; **37**: 268-273 [PMID: 1735346 DOI: 10.1007/BF01308182]
35 **Reber HA**, Karanjia ND, Alvarez C, Widdison AL, Leung FW, Ashley SW, Lutrin FJ. Pancreatic blood flow in cats with chronic pancreatitis. *Gastroenterology* 1992; **103**: 652-659 [PMID: 1634080 DOI: 10.1016/0016-5085(92)90861-R]
36 **Widdison AL**, Alvarez C, Schwarz M, Reber HA. The influence of ethanol on pancreatic blood flow in cats with chronic pancreatitis. *Surgery* 1992; **112**: 202-208 [PMID: 1641761]
37 **Widdison AL**, Karanjia ND, Reber HA. Pancreatic vascular regulation in chronic pancreatitis in cats. *Gut* 1995; **36**: 133-136 [PMID: 7890217 DOI: 10.1136/gut.36.1.133]
38 **Toyama MT**, Patel AG, Nguyen T, Ashley SW, Reber HA. Effect of ethanol on pancreatic interstitial pH and blood flow in cats with chronic pancreatitis. *Ann Surg* 1997; **225**: 223-228 [PMID: 9065300 DOI: 10.1097/00000658-199702000-00011]
39 **Fasanella KE**, Davis B, Lyons J, Chen Z, Lee KK, Slivka A, Whitcomb DC. Pain in chronic pancreatitis and pancreatic cancer. *Gastroenterol Clin North Am* 2007; **36**: 335-364, ix [PMID: 17533083 DOI: 10.1016/j.gtc.2007.03.011]
40 **Keith RG**, Keshavjee SH, Kerenyi NR. Neuropathology of chronic pancreatitis in humans. *Can J Surg* 1985; **28**: 207-211 [PMID: 3995416]
41 **Ahmed Ali U**, Pahlplatz JM, Nealon WH, van Goor H, Gooszen HG, Boermeester MA. Endoscopic or surgical intervention for painful obstructive chronic pancreatitis. *Cochrane Database Syst Rev* 2015; **(3):** CD007884 [PMID: 25790326 DOI: 10.1002/14651858.CD007884.pub3]
42 **Drewes AM**, Krarup AL, Detlefsen S, Malmstrøm ML, Dimcevski G, Funch-Jensen P. Pain in chronic pancreatitis: the role of neuropathic pain mechanisms. *Gut* 2008; **57**: 1616-1627 [PMID: 18566105 DOI: 10.1136/gut.2007.146621]
43 **Dimcevski G**, Staahl C, Andersen SD, Thorsgaard N, Funch-Jensen P, Arendt-Nielsen L, Drewes AM. Assessment of experimental pain from skin, muscle, and esophagus in patients with chronic pancreatitis. *Pancreas* 2007; **35**: 22-29 [PMID: 17575541 DOI: 10.1097/mpa.0b013e31805c1762]
44 **Dimcevski G**, Sami SA, Funch-Jensen P, Le Pera D, Valeriani M, Arendt-Nielsen L, Drewes AM. Pain in chronic pancreatitis: the role of reorganization in the central nervous system. *Gastroenterology* 2007; **132**: 1546-1556 [PMID: 17408654 DOI: 10.1053/j.gastro.2007.01.037]
45 **Andrén-Sandberg A**, Ansorge C, Eiriksson K, Glomsaker T, Maleckas A. Treatment of pancreatic pseudocysts. *Scand J Surg* 2005; **94**: 165-175 [PMID: 16111100 DOI: 10.1177/145749690509400214]
46 **Rosso E**, Alexakis N, Ghaneh P, Lombard M, Smart HL, Evans J, Neoptolemos JP. Pancreatic pseudocyst in chronic pancreatitis: endoscopic and surgical treatment. *Dig Surg* 2003; **20**: 397-406 [PMID: 12900529 DOI: 10.1159/000072706]
47 **Domínguez-Muñoz JE**. Pancreatic exocrine insufficiency: diagnosis and treatment. *J Gastroenterol Hepatol* 2011; **26** Suppl 2: 12-16 [PMID: 21323992 DOI: 10.1111/j.1440-1746.2010.06600.x]
48 **Whitcomb DC**, Lehman GA, Vasileva G, Malecka-Panas E, Gubergrits N, Shen Y, Sander-Struckmeier S, Caras S. Pancrelipase delayed-release capsules (CREON) for exocrine pancreatic insufficiency due to chronic pancreatitis or pancreatic surgery: A double-blind randomized trial. *Am J Gastroenterol* 2010; **105**: 2276-2286 [PMID: 20502447 DOI: 10.1038/ajg.2010.201]
49 **Mossner J**. Treatment of pain in chronic pancreatitis with pancreatic enzymes. Another point of view. In: Lankisch PG, editor. Pancreatic enzymes in health and disease. Berlin: Springer-Verlag, 1991: 103-112 [DOI: 10.1007/978-3-642-76097-6_12]
50 **Strum WB**. Abstinence in alcoholic chronic pancreatitis. Effect on pain and outcome. *J Clin Gastroenterol* 1995; **20**: 37-41 [PMID: 7884175 DOI: 10.1097/00004836-199501000-00010]
51 **Domínguez-Muñoz JE**, Iglesias-García J. Oral pancreatic enzyme substitution therapy in chronic pancreatitis: is clinical response an appropriate marker for evaluation of therapeutic efficacy? *JOP* 2010; **11**: 158-162 [PMID: 20208327]

52 **Bhardwaj P**, Thareja S, Prakash S, Saraya A. Micronutrient antioxidant intake in patients with chronic pancreatitis. *Trop Gastroenterol* 2004; **25**: 69-72 [PMID: 15471319]

53 **Lindkvist B**, Domínguez-Muñoz JE, Luaces-Regueira M, Castiñeiras-Alvariño M, Nieto-Garcia L, Iglesias-Garcia J. Serum nutritional markers for prediction of pancreatic exocrine insufficiency in chronic pancreatitis. *Pancreatology* 2012; **12**: 305-310 [PMID: 22898630 DOI: 10.1016/j.pan.2012.04.006]

54 **Cavallini G**, Talamini G, Vaona B, Bovo P, Filippini M, Rigo L, Angelini G, Vantini I, Riela A, Frulloni L. Effect of alcohol and smoking on pancreatic lithogenesis in the course of chronic pancreatitis. *Pancreas* 1994; **9**: 42-46 [PMID: 8108370 DOI: 10.1097/00006676-199401000-00006]

55 **Rose P**, Fraine E, Hunt LP, Acheson DW, Braganza JM. Dietary antioxidants and chronic pancreatitis. *Hum Nutr Clin Nutr* 1986; **40**: 151-164 [PMID: 3957720]

56 **Bowrey DJ**, Morris-Stiff GJ, Puntis MC. Selenium deficiency and chronic pancreatitis: disease mechanism and potential for therapy. *HPB Surg* 1999; **11**: 207-215; discussion 215-216 [PMID: 10468111]

57 **Banks PA**, Hughes M, Ferrante M, Noordhoek EC, Ramagopal V, Slivka A. Does allopurinol reduce pain of chronic pancreatitis? *Int J Pancreatol* 1997; **22**: 171-176 [PMID: 9444547 DOI: 10.1007/BF02788381]

58 **Bhardwaj P**, Garg PK, Maulik SK, Saraya A, Tandon RK, Acharya SK. A randomized controlled trial of antioxidant supplementation for pain relief in patients with chronic pancreatitis. *Gastroenterology* 2009; **136**: 149-159.e2 [PMID: 18952082 DOI: 10.1053/j.gastro.2008.09.028]

59 **Wolf G**, Müller GM, Kehrberg G. [The effect of lithium carbonate on the leukocyte count following ionizing radiation. 4. The effect of lithium carbonate on the activation of granulocytes]. *Radiobiol Radiother* (Berl) 1989; **30**: 546-552 [PMID: 2514441 DOI: 10.1002/14651858.CD008945.pub2]

60 **Morris-Stiff GJ**, Bowrey DJ, Oleesky D, Davies M, Clark GW, Puntis MC. The antioxidant profiles of patients with recurrent acute and chronic pancreatitis. *Am J Gastroenterol* 1999; **94**: 2135-2140 [PMID: 10445540 DOI: 10.1111/j.1572-0241.1999.01311.x]

61 **Delhaye M**, Vandermeeren A, Baize M, Cremer M. Extracorporeal shock-wave lithotripsy of pancreatic calculi. *Gastroenterology* 1992; **102**: 610-620 [PMID: 1732129 DOI: 10.1016/0016-5085(92)90110-K]

62 **Dumonceau JM**, Costamagna G, Tringali A, Vahedi K, Delhaye M, Hittelet A, Spera G, Giostra E, Mutignani M, De Maertelaer V, Devière J. Treatment for painful calcified chronic pancreatitis: extracorporeal shock wave lithotripsy versus endoscopic treatment: a randomised controlled trial. *Gut* 2007; **56**: 545-552 [PMID: 17047101 DOI: 10.1136/gut.2006.096883]

63 **Cremer M**, Devière J, Delhaye M, Baize M, Vandermeeren A. Stenting in severe chronic pancreatitis: results of medium-term follow-up in seventy-six patients. *Endoscopy* 1991; **23**: 171-176 [PMID: 1860448 DOI: 10.1055/s-2007-1010649]

64 **Grimm H**, Meyer WH, Nam VC, Soehendra N. New modalities for treating chronic pancreatitis. *Endoscopy* 1989; **21**: 70-74 [PMID: 2707174 DOI: 10.1055/s-2007-1012903]

65 **Perwaiz A**, Singh A, Chaudhary A. Surgery for chronic pancreatitis. *Indian J Surg* 2012; **74**: 47-54 [PMID: 23372307 DOI: 10.1007/s12262-011-0374-7]

66 **Klimenko AV**, Klimenko VN, Steshenko AA, Tumanskiĭ VA, Kovalenko IS. [Clinico-morphological substantations of parenchyma-preserving operations performance in surgery of chronic pancreatitis]. *Klin Khir* 2012; **(1)**: 14-18 [PMID: 22642081]

67 **Strobel O**, Büchler MW, Werner J. Surgical therapy of chronic pancreatitis: indications, techniques and results. *Int J Surg* 2009; **7**: 305-312 [PMID: 19501199 DOI: 10.1016/j.ijsu.2009.05.011]

68 **Fisher AV**, Sutton JM, Wilson GC, Hanseman DJ, Abbott DE, Smith MT, Schmulewitz N, Choe KA, Wang J, Sussman JJ, Ahmad SA. High readmission rates after surgery for chronic pancreatitis. *Surgery* 2014; **156**: 787-794 [PMID: 25239319 DOI: 10.1016/j.surg.2014.06.068]

69 **Yang CJ**, Bliss LA, Schapira EF, Freedman SD, Ng SC, Windsor JA, Tseng JF. Systematic review of early surgery for chronic pancreatitis: impact on pain, pancreatic function, and re-intervention. *J Gastrointest Surg* 2014; **18**: 1863-1869 [PMID: 24944153 DOI: 10.1007/s11605-014-2571-8]

70 **Bouwense SA**, Ahmed Ali U, ten Broek RP, Issa Y, van Eijck CH, Wilder-Smith OH, van Goor H. Altered central pain processing after pancreatic surgery for chronic pancreatitis. *Br J Surg* 2013; **100**: 1797-1804 [PMID: 24227367 DOI: 10.1002/bjs.9322]

71 **Fried M**, Schwizer W, Beglinger C, Keller U, Jansen JB, Lamers CB. Physiological role of cholecystokinin on postprandial insulin secretion and gastric meal emptying in man. Studies with the cholecystokinin receptor antagonist loxiglumide. *Diabetologia* 1991; **34**: 721-726 [PMID: 1959704 DOI: 10.1007/BF00401517]

72 **Keller J**, Layer P. Human pancreatic exocrine response to nutrients in health and disease. *Gut* 2005; **54** Suppl 6: vi1-28 [PMID: 15951527 DOI: 10.1136/gut.2005.065946]

73 **Green GM**, Lyman RL. Feedback regulation of pancreatic enzyme secretion as a mechanism for trypsin inhibitor-induced hypersecretion in rats. *Proc Soc Exp Biol Med* 1972; **140**: 6-12 [PMID: 5033119 DOI: 10.3181/00379727-140-36384]

74 **Ihse I**, Lilja P, Lundquist I. Trypsin as a regulator of pancreatic secretion in the rat. *Scand J Gastroenterol* 1979; **14**: 873-880 [PMID: 94175 DOI: 10.3109/00365527909181419]

75 **Louie DS**, May D, Miller P, Owyang C. Cholecystokinin mediates feedback regulation of pancreatic enzyme secretion in rats. *Am J Physiol* 1986; **250**: G252-G259 [PMID: 3953805]

76 **Rausch U**, Weidenbach H, Adler G, Kern HF. Stimulation of pancreatic secretory process in the rat by low-molecular weight proteinase inhibitor. II. Regulation of total protein and individual enzyme biosynthesis. *Cell Tissue Res* 1987; **249**: 63-67 [PMID: 2441868 DOI: 10.1007/BF00215419]

77 **Shiratori K**, Chen YF, Chey WY, Lee KY, Chang TM. Mechanism of increased exocrine pancreatic secretion in pancreatic juice-diverted rats. *Gastroenterology* 1986; **91**: 1171-1178 [PMID: 3758609]

78 **Chernick SS**, Lepkovsky S, Chaikoff IL. A dietary factor regulating the enzyme content of the pancreas; changes induced in size and proteolytic activity of the chick pancreas by the ingestion of raw soybean meal. *Am J Physiol* 1948; **155**: 33-41 [PMID: 18102665]

79 **Corring T.** Mechanism of the exocrine pancreatic secretion in the pig. Feed-back regulation. *Ann Biol Amin Biochim Biophys* 1973; **13**: 755-756 [DOI: 10.1051/rnd: 19730432]

80 **Louie DS**, Williams JA, Owyang C. Action of pancreatic polypeptide on rat pancreatic secretion: in vivo and in vitro. *Am J Physiol* 1985; **249**: G489-G495 [PMID: 2413769]

81 **Petersen H**, Grossman MI. Pancreatic exocrine secretion in anesthetized and conscious rats. *Am J Physiol* 1977; **233**: E530-E536 [PMID: 596448]

82 **Geratz JD**. Secretory stimulation of the rat pancreas by p-aminobenzamidine. *Am J Physiol* 1969; **216**: 812-817 [PMID: 5775880]

83 **Geratz JD**, Hurt JP. Regulation of pancreatic enzyme levels by trypsin inhibitors. *Am J Physiol* 1970; **219**: 705-711 [PMID: 5450875]

84 **Fukuoka S**, Kawajiri H, Fushiki T, Takahashi K, Iwai K. Localization of pancreatic enzyme secretion-stimulating activity and trypsin inhibitory activity in zymogen granule of the rat pancreas. *Biochim Biophys Acta* 1986; **884**: 18-24 [PMID: 2429706 DOI: 10.1016/0304-4165(86)90221-7]

85 **Lu L**, Louie D, Owyang C. A cholecystokinin releasing peptide mediates feedback regulation of pancreatic secretion. *Am J Physiol* 1989; **256**: G430-G435 [PMID: 2465698]

86 **Miyasaka K,** Green GM. Effect of atropine on rat basal pancreatic secretion during return or diversion of bile-pancreatic juice. *Proc Soc Exp Biol Med* 1983; **174**: 187-192 [PMID: 6634711 DOI: 10.3181/00379727-174-41723]

87 **Noda A**, Magee DF, Sarles H. The role of gastric secretion in post-diverted pancreatic hypersecretion in conscious rats. *J Physiol* 1982; **326**: 453-459 [PMID: 7108806 DOI: 10.1113/jphysiol.1982.sp014205]

88 **Owyang C**, Louie DS, Tatum D. Feedback regulation of pancreatic

enzyme secretion. Suppression of cholecystokinin release by trypsin. *J Clin Invest* 1986; **77**: 2042-2047 [PMID: 3711342 DOI: 10.1172/JCI112534]

89 **Owyang C**, May D, Louie DS. Trypsin suppression of pancreatic enzyme secretion. Differential effect on cholecystokinin release and the enteropancreatic reflex. *Gastroenterology* 1986; **91**: 637-643 [PMID: 3732765 DOI: 10.1016/0016-5085(86)90633-5]

90 **Ihse I**. Treatment of pain in chronic pancreatitis with pancreatic enzymes. In: Lankisch PG, editor. Pancreatic enzymes in health and disease. Berlin: Springer-Verlag, 1991: 89-94 [DOI: 10.1007/978-3-642-76097-6_10]

91 **Folsch UR**. Treatment of pain in chronic pancreatitis with pancreatic enzymes. The counterposition. In: Lankisch PG, editor. Pancreatic enzymes in health and disease. Berlin: Springer-Verlag, 1991: 97-102 [DOI: 10.1007/978-3-642-76097-6_11]

92 **Ihse I**, Lilja P, Lundquist I. Feedback regulation of pancreatic enzyme secretion by intestinal trypsin in man. *Digestion* 1977; **15**: 303-308 [PMID: 863133 DOI: 10.1159/000198016]

93 **Yasui A**, Nimura Y, Hayakawa N, Hayakawa T, Shibata T, Kondo T, Naruse S, Shionoya S. Feedback regulation of basal pancreatic secretion in humans. *Pancreas* 1988; **3**: 681-687 [PMID: 3222248 DOI: 10.1097/00006676-198812000-00009]

94 **Slaff J**, Jacobson D, Tillman CR, Curington C, Toskes P. Protease-specific suppression of pancreatic exocrine secretion. *Gastroenterology* 1984; **87**: 44-52 [PMID: 6202586 DOI: S0016508584001487]

95 **Burton FR**, Garvin PJ, Joshi SN. Human pancreatic graft fistula exocrine suppression by oral pancreatic enzymes. *Transplantation* 1989; **47**: 888-891 [PMID: 2655228 DOI: 10.1097/00007890-198905000-00028]

96 **Burton FR**, Burton MS, Garvin PJ, Joshi SN. Enteral pancreatic enzyme feedback inhibition of the exocrine secretion of the human transplanted pancreas. *Transplantation* 1992; **54**: 988-992 [PMID: 1281568 DOI: 10.1097/00007890-199212000-00009]

97 **Liener IE**, Goodale RL, Deshmukh A, Satterberg TL, Ward G, DiPietro CM, Bankey PE, Borner JW. Effect of a trypsin inhibitor from soybeans (Bowman-Birk) on the secretory activity of the human pancreas. *Gastroenterology* 1988; **94**: 419-427 [PMID: 2446949]

98 **Adler G**, Müllenhoff A, Koop I, Bozkurt T, Göke B, Beglinger C, Arnold R. Stimulation of pancreatic secretion in man by a protease inhibitor (camostate). *Eur J Clin Invest* 1988; **18**: 98-104 [PMID: 3130267 DOI: 10.1111/j.1365-2362.1988.tb01173.x]

99 **Adler G**, Müllenhoff A, Bozkurt T, Göke B, Koop I, Arnold R. Comparison of the effect of single and repeated administrations of a protease inhibitor (Camostate) on pancreatic secretion in man. *Scand J Gastroenterol* 1988; **23**: 158-162 [PMID: 3363288 DOI: 10.3109/00365528809103961]

100 **Krawisz BR**, Miller LJ, DiMagno EP, Go VL. In the absence of nutrients, pancreatic-biliary secretions in the jejunum do not exert feedback control of human pancreatic or gastric function. *J Lab Clin Med* 1980; **95**: 13-18 [PMID: 7350238]

101 **Hotz J**, Ho SB, Go VL, DiMagno EP. Short-term inhibition of duodenal tryptic activity does not affect human pancreatic, biliary, or gastric function. *J Lab Clin Med* 1983; **101**: 488-495 [PMID: 6186758]

102 **Dlugosz J**, Fölsch UR, Creutzfeldt W. Inhibition of intraduodenal trypsin does not stimulate exocrine pancreatic secretion in man. *Digestion* 1983; **26**: 197-204 [PMID: 6192029 DOI: 10.1159/000198890]

103 **Bastedo WA**. The use and utility of digestive enzymes in therapeutics: A summary of the replies to a questionnaire submitted to the members of the American Gastroenterological Association. *JAMA* 1925; **85**: 743-744 [DOI: 10.1001/jama.1925.26710100001009]

104 **Wright JS**. A guide to the organic drugs of the United State Pharmacopoeia 1890. Indianapolis: Eli Lilly and Company, 1895: 93

105 **Trang T**, Chan J, Graham DY. Pancreatic enzyme replacement therapy for pancreatic exocrine insufficiency in the 21(st) century. *World J Gastroenterol* 2014; **20**: 11467-11485 [PMID: 25206255 DOI: 10.3748/wjg.v20.i33.11467]

106 **Brown A**, Hughes M, Tenner S, Banks PA. Does pancreatic enzyme supplementation reduce pain in patients with chronic pancreatitis: a meta-analysis. *Am J Gastroenterol* 1997; **92**: 2032-2035 [PMID: 9362186]

107 **Shafiq N**, Rana S, Bhasin D, Pandhi P, Srivastava P, Sehmby SS, Kumar R, Malhotra S. Pancreatic enzymes for chronic pancreatitis. *Cochrane Database Syst Rev* 2009; **(4)**: CD006302 [PMID: 19821359 DOI: 10.1002/14651858.CD006302.pub2]

108 **Isaksson G**, Ihse I. Pain reduction by an oral pancreatic enzyme preparation in chronic pancreatitis. *Dig Dis Sci* 1983; **28**: 97-102 [PMID: 6825540 DOI: 10.1007/BF01315137]

109 **Malesci A**, Gaia E, Fioretta A, Bocchia P, Ciravegna G, Cantor P, Vantini I. No effect of long-term treatment with pancreatic extract on recurrent abdominal pain in patients with chronic pancreatitis. *Scand J Gastroenterol* 1995; **30**: 392-398 [PMID: 7610357 DOI: 10.3109/00365529509093296]

110 **Halgreen H**, Pedersen NT, Worning H. Symptomatic effect of pancreatic enzyme therapy in patients with chronic pancreatitis. *Scand J Gastroenterol* 1986; **21**: 104-108 [PMID: 3633631 DOI: 10.3109/00365528609034631]

111 **Mössner J**, Secknus R, Meyer J, Niederau C, Adler G. Treatment of pain with pancreatic extracts in chronic pancreatitis: results of a prospective placebo-controlled multicenter trial. *Digestion* 1992; **53**: 54-66 [PMID: 1289173 DOI: 10.1159/000200971]

112 **Larvin M**, McMahon MJ, Thomas WEG, Puntis MCA. Creon (enteric coated pancreatic microspheres) for the treatment of pain in chronic pancreatitis. A double blind randomized placebo controlled crossover study. *Gastroenterology* 1991; **100**: A283

113 **Safdi M**, Bekal PK, Martin S, Saeed ZA, Burton F, Toskes PP. The effects of oral pancreatic enzymes (Creon 10 capsule) on steatorrhea: a multicenter, placebo-controlled, parallel group trial in subjects with chronic pancreatitis. *Pancreas* 2006; **33**: 156-162 [PMID: 16868481 DOI: 10.1097/01.mpa.0000226884.32957.5e]

114 **Lankisch PG**, Lembcke B, Göke B, Creutzfeldt W. Therapy of pancreatogenic steatorrhoea: does acid protection of pancreatic enzymes offer any advantage? *Z Gastroenterol* 1986; **24**: 753-757 [PMID: 3548109]

115 **Domínguez-Muñoz JE**, Iglesias-García J, Iglesias-Rey M, Figueiras A, Vilariño-Insua M. Effect of the administration schedule on the therapeutic efficacy of oral pancreatic enzyme supplements in patients with exocrine pancreatic insufficiency: a randomized, three-way crossover study. *Aliment Pharmacol Ther* 2005; **21**: 993-1000 [PMID: 15813835 DOI: 10.1111/j.1365-2036.2005.02390.x]

116 **O'Keefe SJ**, Cariem AK, Levy M. The exacerbation of pancreatic endocrine dysfunction by potent pancreatic exocrine supplements in patients with chronic pancreatitis. *J Clin Gastroenterol* 2001; **32**: 319-323 [PMID: 11276275 DOI: 10.1097/00004836-200104000-00008]

117 **Winstead NS**, Wilcox CM. Clinical trials of pancreatic enzyme replacement for painful chronic pancreatitis--a review. *Pancreatology* 2009; **9**: 344-350 [PMID: 19451744 DOI: 10.1159/000212086]

118 **Czakó L**, Takács T, Hegyi P, Prónai L, Tulassay Z, Lakner L, Döbrönte Z, Boda K, Lonovics J. Quality of life assessment after pancreatic enzyme replacement therapy in chronic pancreatitis. *Can J Gastroenterol* 2003; **17**: 597-603 [PMID: 14571298 DOI: 10.1155/2003/515848]

119 **D'Haese JG**, Ceyhan GO, Demir IE, Layer P, Uhl W, Löhr M, Rychlik R, Pirilis K, Zöllner Y, Gradl B, Foerster D, Möbius J, Henniges F, Friess H. Pancreatic enzyme replacement therapy in patients with exocrine pancreatic insufficiency due to chronic pancreatitis: a 1-year disease management study on symptom control and quality of life. *Pancreas* 2014; **43**: 834-841 [PMID: 24717829 DOI: 10.1097/MPA.0000000000000131]

120 **Sikkens EC**, Cahen DL, Koch AD, Braat H, Poley JW, Kuipers EJ, Bruno MJ. The prevalence of fat-soluble vitamin deficiencies and a decreased bone mass in patients with chronic pancreatitis. *Pancreatology* 2013; **13**: 238-242 [PMID: 23719594 DOI: 10.1016/j.pan.2013.02.008]

121 **Gubergrits N**, Malecka-Panas E, Lehman GA, Vasileva G, Shen Y, Sander-Struckmeier S, Caras S, Whitcomb DC. A 6-month, open-label clinical trial of pancrelipase delayed-release capsules (Creon) in patients with exocrine pancreatic insufficiency due to

chronic pancreatitis or pancreatic surgery. *Aliment Pharmacol Ther* 2011; **33**: 1152-1161 [PMID: 21418260 DOI: 10.1111/j.1365-2036.2011.04631.x]

122 **Taylor JR**, Gardner TB, Waljee AK, Dimagno MJ, Schoenfeld PS. Systematic review: efficacy and safety of pancreatic enzyme supplements for exocrine pancreatic insufficiency. *Aliment Pharmacol Ther* 2010; **31**: 57-72 [PMID: 19804466 DOI: 10.1111/j.1365-2036.2009.04157.x]

123 **Money ME**, Hofmann AF, Hagey LR, Walkowiak J, Talley NJ. Treatment of irritable bowel syndrome-diarrhea with pancrealipase or colesevelam and association with steatorrhea. *Pancreas* 2009; **38**: 232-233 [PMID: 19238028 DOI: 10.1097/MPA.0b013e31817c1b36]

124 **Money ME**, Walkowiak J, Virgilio C, Talley NJ. Pilot study: a randomised, double blind, placebo controlled trial of pancrealipase for the treatment of postprandial irritable bowel syndrome-diarrhoea. *Frontline Gastroenterol* 2011; **2**: 48-56 [PMID: 22095308 DOI: 10.1136/fg.2010.002253]

125 **Money ME**, Camilleri M. Review: Management of postprandial diarrhea syndrome. *Am J Med* 2012; **125**: 538-544 [PMID: 22624684 DOI: 10.1016/j.amjmed.2011.11.006]

126 **Umnova L**, Orlikovs G, Voicehovska J, Voicehovskis V, Krustins E. Combined medical treatment of chronic pancreatitis. *Proc Latvian Acad Sci* 2016; **2015**: 20-26

127 **Frieden TR**, Houry D. Reducing the Risks of Relief--The CDC Opioid-Prescribing Guideline. *N Engl J Med* 2016; **374**: 1501-1504 [PMID: 26977701 DOI: 10.1056/NEJMp1515917]

128 **Deyo RA**, Von Korff M, Duhrkoop D. Opioids for low back pain. *BMJ* 2015; **350**: g6380 [PMID: 25561513 DOI: 10.1136/bmj.g6380]

129 **Braden JB**, Young A, Sullivan MD, Walitt B, Lacroix AZ, Martin L. Predictors of change in pain and physical functioning among post-menopausal women with recurrent pain conditions in the women's health initiative observational cohort. *J Pain* 2012; **13**: 64-72 [PMID: 22208802 DOI: 10.1016/j.jpain.2011.10.007]

130 **Wilder-Smith CH**, Hill L, Osler W, O'Keefe S. Effect of tramadol and morphine on pain and gastrointestinal motor function in patients with chronic pancreatitis. *Dig Dis Sci* 1999; **44**: 1107-1116 [PMID: 10389680 DOI: 10.1023/A: 1026607703352]

131 **Grunkemeier DM**, Cassara JE, Dalton CB, Drossman DA. The narcotic bowel syndrome: clinical features, pathophysiology, and management. *Clin Gastroenterol Hepatol* 2007; **5**: 1126-1139 [PMID: 17916540 DOI: 10.1016/j.cgh.2007.06.013]

132 **Tuteja AK**, Biskupiak J, Stoddard GJ, Lipman AG. Opioid-induced bowel disorders and narcotic bowel syndrome in patients with chronic non-cancer pain. *Neurogastroenterol Motil* 2010; **22**: 424-430, e96 [PMID: 20100280 DOI: 10.1111/j.1365-2982.2009.01458.x]

133 **Szigethy E**, Schwartz M, Drossman D. Narcotic bowel syndrome and opioid-induced constipation. *Curr Gastroenterol Rep* 2014; **16**: 410 [PMID: 25183577 DOI: 10.1007/s11894-014-0410-4]

134 **Drossman D**, Szigethy E. The narcotic bowel syndrome: a recent update. *Am J Gastroenterol Suppl* 2014; **2**: 22-30 [PMID: 25207609 DOI: 10.1038/ajgsup.2014.6]

135 **Graham DY**. Nonsteroidal anti-inflammatory drugs, Helicobacter pylori, and ulcers: where we stand. *Am J Gastroenterol* 1996; **91**: 2080-2086 [PMID: 8855725]

136 **Olesen SS**, Bouwense SA, Wilder-Smith OH, van Goor H, Drewes AM. Pregabalin reduces pain in patients with chronic pancreatitis in a randomized, controlled trial. *Gastroenterology* 2011; **141**: 536-543 [PMID: 21683078 DOI: 10.1053/j.gastro.2011.04.003]

P- Reviewer: Armellini E, Braden B, Zhang QS **S- Editor**: Qi Y
L- Editor: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i3.387

World J Gastrointest Pharmacol Ther 2016 August 6; 7(3): 387-396
ISSN 2150-5349 (online)

REVIEW

What is the best way to manage screening for infections and vaccination of inflammatory bowel disease patients?

Gianluca Andrisani, Alessandro Armuzzi, Manuela Marzo, Carla Felice, Daniela Pugliese, Alfredo Papa, Luisa Guidi

Gianluca Andrisani, Alessandro Armuzzi, Manuela Marzo, Carla Felice, Daniela Pugliese, Alfredo Papa, Luisa Guidi, IBD Unit, Complesso Integrato Columbus, Università Cattolica del Sacro Cuore, 00168 Rome, Italy

Author contributions: Andrisani G and Guidi L contributed to literature search and wrote the manuscript; Armuzzi A and Marzo M contributed to literature review and proof reading of the manuscript; Felice C, Pugliese D and Papa A performed proof reading of the manuscript.

Conflict-of-interest statement: All authors declare no conflict-of-interest related to this paper.

Manuscript source: Invited manuscript

Correspondence to: Luisa Guidi, MD, PhD, IBD Unit, Complesso Integrato Columbus, Università Cattolica del Sacro Cuore, via Moscati 31, 00168 Rome, Italy. guidi@rm.unicatt.it
Telephone: +39-6-3503310
Fax: +39-6-3054641

Received: May 14, 2015
Peer-review started: May 15, 2015
First decision: September 8, 2015
Revised: April 25, 2016
Accepted: June 14, 2016
Article in press: June 16, 2016
Published online: August 6, 2016

Abstract

The use of biological agents and immunomodulators for inflammatory bowel disease (IBD) is associated with an increased risk of opportunistic infections, in particular of viral or bacterial etiology. Despite the existence of international guidelines, many gastroenterologists have not adopted routine screening and vaccination in those patients with IBD, which are candidate for biologic therapy. Available strategies to screen, diagnose and prevent bacterial and viral infections in patients with IBD prior to start biological therapy are discussed in this review.

Key words: Inflammatory bowel disease; Opportunistic infections; Immunomodulators; Corticosteroids; Anti-tumor necrosis factor agents

Core tip: The increasing use of biologics as a mainstay of therapy in inflammatory bowel disease (IBD) is associated with an increased risk for a variety of infections, many of which are preventable by prior screening and vaccination. While immunocompetent IBD patients can be vaccinated using standard vaccination schedule, special guidelines need to be followed for IBD patients getting immunosuppressive therapy (IST). This article provides a review of the issues surrounding immunizations in the IBD patient and a practical guide for clinicians regarding the appropriate screening for infections and vaccinations to administer both before and during IST.

Andrisani G, Armuzzi A, Marzo M, Felice C, Pugliese D, Papa A, Guidi L. What is the best way to manage screening for infections and vaccination of inflammatory bowel disease patients? *World J Gastrointest Pharmacol Ther* 2016; 7(3): 387-396 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i3/387.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i3.387

INTRODUCTION

Biological agents have represented a breakthrough in the therapy of inflammatory bowel disease (IBD) in the last

20 years: Tumor necrosis factor alpha inhibitors (anti-TNF) and other monoclonal antibodies targeting interleukin 12 (IL-12), IL-23, and cellular adhesion molecule ligands a4 integrin and a4b7 integrin. The European Crohn's and Colitis Foundation (ECCO) outlines that IBD patients treated with corticosteroids (prednisone 20 mg/d equivalent for 2 wk or more), immunomodulators (6-mercaptopurine, Azathioprine, Methotrexate), and biological agents should be considered immunocompromised and at risk for opportunistic infections[1]. This has been confirmed by several studies, highlighting the increased incidence of severe infections in patients with IBD on biologics[2,3]. A pivotal study in the field[4] has evaluated the independent predisposing factors to severe infections with a case-control designed study. The results underlined how immunosuppressive therapy (steroids, thiopurines, and anti-TNF) were associated with an increased risk of severe infections (OR: 2.9, 95%CI: 1.5-5), and that the risk was greatly increased when two or more drugs were combined (OR: 14.5, 95%CI: 4.9-43). The TREAT Registry (Crohn's Therapy, Resource, Evaluation, and Assessment Tool) has individuated prednisone, infliximab, disease activity (moderate to severe), and narcotic analgesic treatment as independent factors associated with serious infections[3]. A recent Cochrane review, with a meta-analysis of randomized controlled trials, controlled clinical trials, and open-label extension studies of biologics for several indications, reported an OR of 1.28 (95%CI: 1.09-1.50) for serious infections for patients on any biologic[5]. However, a subgroup analysis of patients included in IBD trials did not show a significantly increased risk of infection (OR: 1.28, 95%CI: 0.67-2.44)[4]. In this review, we aim to outline the most relevant opportunistic infections in IBD with focus on the discussion of the screening and prevention strategies through vaccination or chemoprophylaxis in IBD patients prior to start biological therapy.

BACTERIAL DISEASES

Mycobacterium tuberculosis

The worldwide incidence of tuberculosis (TB) has been estimated by the World Health Organization in 9.6 million cases with 1.5 million deaths in 2014[6]. The risk of reactivation of latent TB (LTB) is 5-fold increased in the first 52 wk. after initiation of anti-TNF therapy[7-9].

TNF has a central role in the immune response to *Mycobacterium tuberculosis*. It is fundamental for macrophage activation and in the formation and maintenance of granuloma where mycobacteria are sequestrated[7]. This is a main reason why therapy with anti-TNF agents can reactivate latent TB. Generally these cases are extra pulmonary or disseminated TB[10]. The American College of Gastroenterology and the American Gastroenterological Association, as well as the ECCO recommend screening for LTB before starting biological therapy[11-14]. The most commonly employed screening tests are tuberculin skin test (TST), QuantiFERON TB-Gold (QFT-G) and chest radiography. *In vitro* assays based on interferon-gamma release (IGRA), such as the QFT-G and T-SPOT.TB, have been recently claimed to be more specific and sensitive than TST, particularly in the previously vaccinated and immunosuppressed population[9,15]. IGRAs employ antigens specific for *Mycobacterium tuberculosis*, not cross-reactive with Bacillus Calmette-Guérin (BCG). A meta-analysis[16] has calculated the specificity of QFT-G and TST for LTB screening. In subjects who had not been vaccinated with BCG, the specificity of QFT-G was 99% (95%CI: 98%-100%) and that of TST was 97% (95%CI: 95%-99%). However, in subjects vaccinated with BCG, the specificity of QFT-G was 96% (95%CI: 94%-98%) while that of TST was only 59% (95%CI: 46%-73%). A Swiss study has compared TST and QFT-G performances in IBD patients[17]. The studied population comprised 114 patients with Crohn's disease (CD), 44 with ulcerative colitis (UC), 10 with indeterminate colitis and 44 control subjects. In this study the prevalence of BCG vaccination was 71%, while 81% of the IBD patients were treated with immunosuppressive therapy (IST). Less patients treated with IST were TST positive compared to those not treated with IST (14% *vs* 34%, $P = 0.007$), while no difference was evident for the interferon-based test QFT-G (9% *vs* 6%). The correlation of TST and QFT-G in IBD patients was negative in this study (k = -0.0297, -0.0314 in vaccinated and -0.0538 in non-vaccinated patients). However the two tests showed a better agreement in control subjects (k = 0.13), and particularly in non-vaccinated controls (k = 0.62).

These results were confirmed by a study by Andrisani *et al*[18] performed on 92 Italian IBD patients who underwent infectious disease screening before starting therapy with anti-TNF (only one of them was vaccinated with BCG). A discordant result between QFT-G and TST was found in 10.8% IBD patients (k = 0.508). Patients treated with IST had higher degree of disagreement (14.3%, k = 0.39), while the patients not treated with IST had a 100% conformity of the two tests. A systematic review and meta-analysis has evaluated the findings of IGRA tests[19] in IBD patients. In the nine selected studies, different results were found for the agreement between skin test and the different IGRAs. TST and QFT-TB Gold/QFT-TB Gold In-Tube had a rate of uniformity of 85% (95%CI: 77-90), while the conformity of TST and T-SPOT.TB was 72% (95%CI: 64-78). A relevant problem in interpreting these results is the occurrence of indeterminate test. In this meta-analysis it was 5% (95%CI: 2-9) for all QFT-tests. IST therapy affected both QFT-G scores (OR: 0.37, 95%CI: 0.16-0.87) and TST outcomes (OR: 0.28, 95%CI: 0.10-0.80) in these studies ($P = 0.02$). Patients with LTB infection should be treated with a 9 mo. course of isoniazid. This prophylaxis should preferably be conducted in strict cooperation with infectious disease specialists and/or pneumologists. The usual isoniazid protocol is generally well tolerated. Although IBD patients may already be on pharmacological treatment, there is no evidence of an increased risk of liver toxicity related

to isoniazid[20]. Even if not formally assessed in clinical studies, there is general agreement that a minimum of 2 mo should be waited after start of chemoprophylaxis for LTB before anti-TNF therapy is initiated[7,15], if the clinical condition of the patient allow this delay. However, chemoprophylaxis does not guarantee that LTB will not reactivate during anti-TNF therapy: A reactivation rate of 19% has been described in a retrospective study, indicating that routine TB surveillance during and after anti-TNF drugs treatment must be performed[21].

Clostridium difficile

Clostridium difficile infection (CDI) manifests with laboratory signs and symptoms that may be confused with a relapse of inflammatory activity in an IBD patient[22]. For this reason, it is mandatory to perform specific diagnostic tests for CDI in IBD relapses characterized by profuse diarrhea, with or without the presence of blood, by signs of dehydration and leukocytosis. The most common tests employed for CDI diagnosis are enzyme-linked immunosorbent assay (ELISA) for toxin A and B[23] and polymerase chain reaction (PCR) assays (which have greater specificity and sensitivity). Although toxigenic culture can be considered as the "gold standard" technique for this diagnosis, it is infrequently performed[23]. According to the Infectious Disease Society of America (IDSA), a 2-step method should be used. As a first step, an ELISA for the Clostridium difficile common antigen, glutamate dehydrogenase is performed. If positive, the presence of pathogenic strains can be confirmed by other techniques as cell cytotoxicity assay or toxigenic culture[24]. Treatment includes initially oral metronidazole and oral vancomycin, or in severe cases simultaneous administration of intravenous metronidazole and oral vancomycin[25]. Fidaxomicin has been recently approved for CDI[26,27]. In recent years, a innovative methodology has demonstrated its efficacy for treatment of recurrent CDI: Fecal microbiota transplantation[28]. Although the donor selection criteria and the optimal condition for fecal instillation are still not clearly defined, the method is widely and successfully employed[29]. FMT has been employed also for IBD patients with CDI in a recent study[30] using standardized frozen preparation, showing efficacy in treating the infection.

Streptococcus pneumoniae

Pneumococcus may cause, besides lung infection, also invasive disease as bacteremia and meningitis. Immunocompromised hosts are at risk for these complications, and cases have been described in IBD patients treated with infliximab[31]. Vaccination is recommended for prevention of pneumococcal infections in special at risk populations. The main risk categories applicable to IBD patients are age 65 years and older, smoking and use of immunosuppressive agents. Two vaccines have been approved against pneumococcal infections: A 23-valent-polysaccharide vaccine (PPSV23) and a 13-valent conjugate vaccines (PCV13). The coverage of the two vaccines is only partly overlapping. The Advisory Committee on Immunization Practices (ACIP) guideline have been released with differential indications for different age and disease groups. In particular, ACIP suggests the following vaccination scheme for immunocompromised adults aged 19 years or older: If naïve to pneumococcal vaccine, they should receive first PCV13 and, at least 8 wk later, a shot of PPSV23[32]. Those subjects who had previously been vaccinated with PPSV23 should receive, at least one year later, an injection of PCV13[33]. While data concerning the need for revaccination with conjugated vaccine are scant, PPSV23 revaccination after 5 years is recommended for immunocompromised patients[34]. However, the response to *Streptococcus pneumoniae* vaccinations may be impaired in IBD patients treated with immunomodulators, particularly when they are used in combination[35,36]. For this reason, it would be advisable to perform vaccinations for pneumococcal infections before starting immunosuppressive drugs. Pneumococcal infections can usually be diagnosed by cultures or by search for urine antigens of *Streptococcus pneumoniae.* While pneumonia is generally treated with success with fluorquinolones, treatment of meningitis should rely on isolation of the organism and *in vitro* susceptibility testing[37].

VIRAL DISEASES

Hepatitis B virus

The prevalence of hepatitis B virus (HBV) in patients with IBD is similar to that of the general population[38]. The risk for hepatitis B reactivation has been clarified in a multicenter study[38] of 2076 Spanish IBD patients. This study has shown a lower prevalence of HBV antigens and/or antibodies than previously reported, and not different from control population. The HBV surface antigen (HBsAg) was present in no more than 1% of IBD patients, while the positivity rates for anti antibodies against the HBV core antigen (HBcAb) were 7.1% for CD and 8% for UC. A French study[39] showed similar results, with a prevalence of HBcAb of 2.78% in CD patients and of 1.59% in UC patients, not different from those detected in the control unselected population. Other studies[40-42] have shown in IBD patients a higher prevalence of HBV infection. Two Italian studies have reported somehow different results: Biancone *et al*[41] described a higher prevalence of HBcAb in CD and UC patients (10.9% and 11.5%, respectively), when compared to controls (5.1%, $P < 0.02$). Papa *et al*[43] reported that only one patient out of 301 (0.3%) was an HBsAg carrier, while 22 (7.3%) were anti-HBc positive.

TNF-α is important in regulating hepatitis B replication[44] and cases of reactivation of the virus under TNF inhibitors have been published[45,46]. All IBD patients should be tested for HBV infection (HBsAg, anti-HBs, anti-HBc) to assess infection or vaccination status. It is important to check also for anti core antibodies, as

they could represent the only positive test in particular situations, such as the case of immunosuppressed patients or hepatitis C virus (HCV)/human immunodeficiency virus (HIV) co-infections[47]. However, a low rate of false positivity has been described. In patients that show positive findings of HBV infection, the search of HBeAg, anti HBe, and HBV DNA should also be performed.

Cases of reactivation on Infliximab therapy have been described not only in hepatitis B surface antigen (HBsAg)-positive patients but also in HBsAg-negative/anti-HBC (hepatitis B core antigen)-positive patients[48]. Hepatitis B reactivation is associated with significant morbidity and mortality due to hepatic failure[49].

During anti-TNF therapy, "occult" HBV carriers (those who are anti-HBc+), need a frequent check of tests of liver function and of HBV markers: The appearance of HBV-DNA or HBsAg positivity indicates reactivation of the infection[1]. In chronic HBsAg-positive carriers, antiviral prophylaxis is recommended before administering immunosuppressive agents. If IST is anticipated to be conducted for a period of more than one year (as frequently happens in IBD), prophylaxis of HBV reactivation should be performed with nucleotide/nucleoside analogues rather than with lamivudine due to the lower incidence of mutations that generate resistance to the drug[1]. The American Association for the Study of Liver Diseases (AASLD)[50] and the European Association for the Study of the Liver recommend the early introduction of nucleoside/nucleotide analogues (NAs) for all HBsAg-positive patients requiring IST. Prophylaxis of HBV reactivation must be started at least one week before IST and it should last for 6 mo to 1 year after its accomplishment, because the reactivation of HBV may happen even after immunosuppression is withdrawn[50,51].

Patients with high levels of HBV DNA (> 2000 IU/mL) at baseline should carry on the antiviral therapy until the same end points as for non-immunosuppressed patients are reached.

All seronegative (negative or low-titer HBsAb) patients should be vaccinated at diagnosis; however, this occurs in less than half of the patients[52]. It is safe to administer the standard vaccination protocol to patients with IBD on immunosuppressive medications, but the response may be significantly reduced, and an intensified vaccination protocol may be required. Post-vaccination HBsAb titers should be monitored, and, if non-protective (< 10 mU/mL), a booster dose or revaccination should be administered[47]. HBV vaccination seems not to be very common in IBD patients, according to four studies exploring the topic. Positive anti-HBs and negative HBcAb, as indication of efficacious vaccination was detected in only 12%, 48.9%, 24% and 21.7% of the four patients cohorts from Spain, Italy, France and China, respectively[38,43,53,54]. Vaccination programs are significantly different across Europe for what concerns period of initiation of the programs age and target population (newborns, adolescent and pre-adolescent subjects, only for high-risk groups, *etc.*)[55]. For these reasons, it is recommended to determine of the infectious or vaccination status at the time of the first diagnosis of IBD. If possible, seronegative subjects (HBsAg, HBcAb and HBsAb negative) should be vaccinated as soon as possible in order to reduce future problems in management.

HCV

The prevalence of HCV in patients with IBD is similar to that of the general population[38]. There are no data to suggest that biologics are associated with reactivation or exacerbation of the course of HCV[56,57]. Anti-TNF medications are generally considered safe in patients with HCV[43,58]. The prevalence of HCV infections in IBD patients has been recently evaluated in studies performed in Italy[41,43], France[53], Spain[38], and China[54]. From these reports, the prevalence of hepatitis C infection in IBD patients seems to be not different from the general population. Biancone *et al*[41] reported that the prevalence of anti-HCV antibody positive individuals was 7.4% in CD patients, 0.6% in UC patients and 5.1% in the controls. The ECCO guideline[1] suggest to perform HCV screening before starting treatment with immunosuppressive drugs for IBD, although the positivity of HCV testing is not a contra-indication for IST. Testing should be performed by search for anti-HCV antibodies and, if antibodies are positive, by HCV-RNA. In case of positivity, these tests should be repeated periodically during immunosuppressive treatment. Prophylactic treatment is currently not available to prevent reactivation of HCV infection. Interferon, which was the milestone of antiviral therapy for HCV until the advent of direct-acting antiviral agents, is contraindicated in IBD forms that require IST.

Cytomegalovirus

Cytomegalovirus (CMV) infection or reactivation can occur in patients with immunosuppressive conditions. CMV may produce retinitis, pneumonia, encephalitis, and other invasive infections[59]. A number of studies have described an association between severe steroid-refractory IBD and CMV infection[60,61]. Colonic CMV disease was observed in steroid-refractory UC (active), with a prevalence of 32%[60] in a prospective case-control report. There is no CMV vaccine available. Histopathology combined with immunohistochemistry (IHC) is specific and sensitive for detecting CMV infection in tissue or biopsies. PCR for CMV DNA is commonly employed both in blood and in biopsies to confirm the diagnosis. Screening for CMV infection is not necessary before starting immunomodulator therapy[14]. When CMV is detected in the intestinal mucosa of patients with severe steroid-resistant colitis treated with immunomodulators therapy, antiviral therapy should be initiated. The discontinuation of immunomodulators should be considered until symptoms of colitis ameliorate or in case of systemic CMV disease.

Varicella zoster virus

Varicella zoster virus (VZV) can be associated with a significant morbidity and mortality in immunocompromised patients. VZV is an herpes viruses that persists after acute infection in a latent state in autonomic ganglia, dorsal nerve roots, and cranial nerves[62]. Later in life it can reactivate as zoster. In addition to clinical signs, that are generally typical, PCR for VZV or fluorescence testing can be performed on biological material such as vesicular fluid, sputum, and cerebrospinal fluid. A four-fold or greater rise in VZV antibody titer in acute and late serum specimens is diagnostic of VZV infection[63]. The increased risk of VZV reactivation is not specific only to biologics. In a recent large cohort study[64] including more than 33000 patients treated with anti-TNF medications and 27000 control individuals treated with non-biological anti-inflammatory medications for various indications (3850 patients with IBD), the risk of herpes zoster was similar in patients with IBD treated with anti-TNF agents and with thiopurines.

VZV-related complications can be easily prevented by vaccination. However, live vaccine for varicella must not be administered to patients on immunosuppressive therapies[65], including azathioprine, methotrexate, 6-mercaptopurine, and infliximab[66]. In this regard, it should be noted that Lu *et al*[67] have described good tolerance for VZV vaccine in six patients with IBD receiving immunosuppressive drugs (6-MP or infliximab). Prospective studies are needed to delineate the risks and benefits of live varicella vaccine in patients with IBD. Probably, the better behavior should be to test for VZV patients as early as possible after diagnosis and to vaccinate those previously unexposed before prescribing immunosuppressive treatments. Recently, the use of a zoster vaccine has been suggested for patients who are VZV positive and at risk of developing herpes zoster (*e.g.*, the elderly). Currently, guidelines suggest a lag time before the varicella and zoster vaccine and the start of immunosuppression of 14 d to 1 mo[68,69]. The vaccine should not be administered for at least 1 mo. after the cessation of immunosuppression[68,69]. A study of zoster vaccine given to patients on biologics has detected, however, no association with short-term increase in herpes zoster incidence. In the meantime, it was associated with a lower herpes zoster incidence at a follow-up of two years (6.7 *vs* 11.6 cases per 1000 person-years; $P < 0.001$)[70]. For those patients with IBD which are VZV seronegative and treated with immunosuppressive drugs, who experience exposure to subjects with active VZV infection, passive immunization with high-dose VZV IgG[69] should be considered.

HIV

All IBD patients undergoing IST should receive testing for HIV infection (by search of HIV p24 antigen and antibody, and, if acute infection is suspected, by PCR) to exclude unidentified infection. This should be done in order to avoid possible adverse outcomes of immunosuppressive drugs in HIV infected subjects[1]. Several case series and case reports describing patients who are infected with HIV and were treated with anti-TNF medications for various indications have been published and all the patients who were submitted to therapy had a satisfactory CD4 cells count, no co-infection, and low HIV viral load[71]. However, because there are limited data on the effect of treatment with HAART on the course of concomitant HIV and IBD, no recommendations are available[1]. Nevertheless, HIV infection is not to be considered a contra-indication to anti-TNF therapy.

Human papillomavirus

Human papillomavirus (HPV) is a sexually transmitted infection. It is a common infection and is the causative agent for cervical cancer and premalignant conditions[72,73]. The American College of Obstetricians and Gynecologists guideline requires to initiate screening for cervical cancer at 21 years of age, independently of the age of beginning of sexual activity[74]. There are some studies that have suggested how women with IBD could have a higher incidence of cervical dysplasia[75,76]. There is an increased incidence of HPV-associated warts or condylomata in patients taking immunosuppressants; however, no data suggesting a specific association with biologics are available[77]. Women affected by IBD should have cervical smears and HPV vaccination according to the general population guidelines[74]. The available vaccine is quadrivalent, and it is given as three doses during a period of 6 mo. The vaccine is indicated for women of the age of 9 to 26 years, both before and after initiation of sexual activity[75]. HPV vaccine is also recommended for young males, with vaccination at the age of 11 to 12 years, and catch-up for those aged 13 to 21 years. However, vaccination policies are diverse in different countries. Therapy of eventual abnormal findings at cervical smears includes colposcopic examination, and surgical excision.

Herpes simplex virus

In immunocompromised patients, herpes simplex virus (HSV) infection may cause severe disseminate infection of different organs (including encephalitis, meningitis, pneumonia, gastrointestinal infection, and hepatitis)[78,79]. Diagnosis of HSV infection is generally suspected based on clinical findings. It can be confirmed by cytology, by PCR, and by search for specific circulating immunoglobulin G (IgG) and IgM. IBD guidelines from the ECCO dissuade to start IST during when an HSV infection is ongoing[1]. Only those immunosuppressed patients who manifest recurrent infection from HSV type 1 or 2 should receive specific chemoprophylaxis[80].

Epstein-Barr virus

Epstein-Barr virus (EBV) is a common B-cell lymphotropic gamma-herpes virus infection in humans. Most of the severe EBV diseases, as hemophagocytic lymphohistiocytosis, occur when primary infection happens in immunosuppressed patients; for this reason it is

Table 1 Screening and vaccinations for inflammatory bowel disease patients prior to start immunosuppressive including anti-tumor necrosis factor therapy

Infection	Tests	Recommended screening	Vaccine
TB	LTB should be tested by a combination of patient history, chest X-ray, TST and QFT-G	Yes	Always contraindicated during immunosuppressive therapy and in children exposed in utero to anti-TNF, up to 6 mo of age, like any other live vaccine
Clostridium difficile	Enzyme immunoassay Against toxin A and B and PCR assays	Not necessary	Not available
S. Pneumonia	Culture of relevant clinical samples (blood, CSF, good respiratory sample), urine	Not necessary	Yes
HBV	Blood test for HBsAg, anti-HBsAb and HBcAb to determine HBV status. In patients with positive HBsAg, viremia HBV-DNA should also be quantified	Yes	Recommended standard or double dose schedule
HCV	HCV serology	Yes	Not available
CMV	CMV serology	No	Not available
HIV	Blood test for HIV serology	Yes	Not available
VZV	VZV serology	Yes	Vaccine available, vaccinate before starting immune suppressants
HPV	Cervical cytology	Yes	Recommended
HSV	HSV serology	Not necessary	Not available
EBV	EBV serology	Advisable	Not available
Influenza virus	clinical signs and laboratory evaluation	Not necessary	Recommended

TNF: Tumor necrosis factor; TB: Tuberculosis; LTB: Latent tuberculosis; TST: Tuberculin skin test; QFT-G: Quanti FERON TB-Gold; PCR: Polymerase chain reaction; CSF: Cerebrospinal fluid; HBV: Hepatitis B virus; HCV: Hepatitis C virus; CMV: Cytomegalovirus; HIV: Human immunodeficiency virus; VZV: Varicella zoster virus; HPV: Human papillomavirus; HSV: Herpes simplex virus; EBV: Epstein-Barr virus.

Table 2 Vaccination of inflammatory bowel disease patients on immunosuppressive therapy

Vaccine	Dose	Safety
Inactivated vaccines		
HAV	2 doses	Yes
HBV	3 doses	Yes
HAV and HBV	3 doses	Yes
HPV	3 doses	Yes
Influenza (trivalent)	Annually	Yes
Meningococcal	⩾ 1 dose	Yes
Pneumococcal	1 dose and 1 booster in 5 yr	yes
Tetanus and diphtheria	Every 10 yr	Yes
Live attenuated vaccines		
BCG	1 dose	Contraindicated
MMR	1 or 2 doses	Contraindicated
Varicella	2 doses	Contraindicated
Zoster	1 doses	Contraindicated

HAV: Hepatitis A virus; HBV: Hepatitis B virus; HPV: Human papillomavirus; BCG: Bacillus Calmette Guérin; MMR: Measles, mumps, and rubella.

advisable to test IBD patients for EBV serology before start biological or immunosuppressive therapy[81]. EBV-associated lymphomas have been described in patients with CD treated with 6-MP or azathioprine[82,83]. An observational cohort study was conducted in France, the CESAME (Cancers et Surrisque Associe aux Maladies inflammatoires intestinales En France) study. In this IBD cohort the incidence of lymphoproliferative diseases was evaluated according to the treatment with thiopurines during a period of 3 years. This research described how the risk of lymphoproliferative diseases is increased in thiopurine users with a hazard ratio of 5.28 (95%CI: 2.01-13.9, P = 0.001)[84]. Two types of thiopurine-induced lymphoma in IBD are EBV-related: the post-transplant-like lymphoma that develops in adult patients seropositive for EBV and a fatal early post-mononucleosis lymphoproliferation that may develop in young men (< 35 years) seronegative for EBV[85,86]. While antiviral drugs have no beneficial effect on EBV-induced B-cell proliferation, rituximab is the drug of choice for treating established B-cell lymphoma[87]. Screening for EBV infection before initiation of immunomodulator therapy should be considered. Anti-TNF monotherapy could be used in preference to thiopurines in EBV seronegative patients at the clinician's discretion[61]. No EBV vaccine is available.

Influenza virus

Influenza viruses A and B cause seasonal epidemics. In healthy subjects who are immunocompetent, influenza usually behaves as an acute, self-limiting illness of upper respiratory tract. Patients on IST, including patients with IBD on IST, are considered to be at high risk for complications: Viral and bacterial pneumonia, acute respiratory distress syndrome, encephalopathy, myocarditis, pericarditis, and myositis[1]. The diagnosis of influenza is made a combination of typical clinical signs and of laboratory tests. The gold standard for diagnosis is PCR testing from respiratory specimens[88]. The most effective way to prevent influenza and its complications is vaccination. The vaccine approved for use in individuals older than 6 mo of age, including immunosuppressed

patients is the injectable inactivated trivalent vaccine[89]. Vaccination against influenza with inactivated vaccines is recommended for (IBD) patients according to published guidelines both in the US and Europe. Some studies have suggested quantitatively reduced response to influenza vaccine in IBD patients on combined immunosuppression[90]. However, due to the lack of specific data, there is not a current recommendation for a repeated dose of vaccine or for checking serological response after vaccination in these patients[1]. Based on the currently available data, influenza vaccine is safe and well tolerated in IBD patients[91].

CONCLUSION

It is crucial that physicians involved in IBD care perform a careful investigation for infectious disease before starting immunomodulation. The development of new biological drugs and the increase in their use now and in the future involves a thorough selection of patients with IBD before starting therapy. A careful screening allows the doctor to avoid having to suspend a biological therapy due to the appearance of infections with the risk of reactivation of the underlying disease (Table 1). Although, it is necessary for the IBD community to obtain data on new biomarkers with predictive value on the development of opportunistic infections, in order to set up the necessary preventive measures and to chose the better therapeutic strategies for those high-risk patients. Particular attention must be paid to specific populations, like children and elderly patients, which might deserve peculiar clinical approaches to obtain the maximum clinical benefit and minimize the risks. Routine vaccination schedules are recommended for most IBD patients, following the standard guidelines applicable to general population. However, live vaccinations are contraindicated in immunocompromised patients (Table 2). Patients who are frequent travelers (both for job or recreation) particularly to geographic regions affected with endemic infections also warrant a specific consideration by the IBD specialist. A helpful aid for the clinician is the use of a specific checklist for infectious disease screening and vaccination[1]. A strict cooperation with infectious disease specialists is advisable for the correct prevention of opportunistic infections in IBD patients treated with biological therapies.

REFERENCES

1 **Rahier JF**, Ben-Horin S, Chowers Y, Conlon C, De Munter P, D'Haens G, Domènech E, Eliakim R, Eser A, Frater J, Gassull M, Giladi M, Kaser A, Lémann M, Moreels T, Moschen A, Pollok R, Reinisch W, Schunter M, Stange EF, Tilg H, Van Assche G, Viget N, Vucelic B, Walsh A, Weiss G, Yazdanpanah Y, Zabana Y, Travis SP, Colombel JF. European evidence-based Consensus on the prevention, diagnosis and management of opportunistic infections in inflammatory bowel disease. *J Crohns Colitis* 2009; **3**: 47-91 [PMID: 21172250 DOI: 10.1016/j.crohns.2009.02.010]

2 **Azie N**, Neofytos D, Pfaller M, Meier-Kriesche HU, Quan SP, Horn D. The PATH (Prospective Antifungal Therapy) Alliance® registry and invasive fungal infections: update 2012. *Diagn Microbiol Infect Dis* 2012; **73**: 293-300 [PMID: 22789847 DOI: 10.1016/j.diagmicrobio.2012.06.012]

3 **Lichtenstein GR**, Feagan BG, Cohen RD, Salzberg BA, Diamond RH, Price S, Langholff W, Londhe A, Sandborn WJ. Serious infection and mortality in patients with Crohn's disease: more than 5 years of follow-up in the TREAT™ registry. *Am J Gastroenterol* 2012; **107**: 1409-1422 [PMID: 22890223 DOI: 10.1038/ajg.2012.218]

4 **Toruner M**, Loftus EV, Harmsen WS, Zinsmeister AR, Orenstein R, Sandborn WJ, Colombel JF, Egan LJ. Risk factors for opportunistic infections in patients with inflammatory bowel disease. *Gastroenterology* 2008; **134**: 929-936 [PMID: 18294633 DOI: 10.1053/j.gastro.2008.01.012]

5 **Singh JA**, Wells GA, Christensen R, Tanjong Ghogomu E, Maxwell L, Macdonald JK, Filippini G, Skoetz N, Francis D, Lopes LC, Guyatt GH, Schmitt J, La Mantia L, Weberschock T, Roos JF, Siebert H, Hershan S, Lunn MP, Tugwell P, Buchbinder R. Adverse effects of biologics: a network meta-analysis and Cochrane overview. *Cochrane Database Syst Rev* 2011; **16**: CD008794 [PMID: 21328309 DOI: 10.1002/14651858.CD008794.pub2]

6 **WHO.** Global Tuberculosis report 2015. Available from: URL: http://www.who.int/**tb**/publications/global_report/en/

7 **Afif W**, Loftus EV. Safety profile of IBD therapeutics: infectious risks. *Med Clin North Am* 2010; **94**: 115-133 [PMID: 19944801 DOI: 10.1016/j.mcna.2009.08.016]

8 **Gómez-Reino JJ**, Carmona L, Valverde VR, Mola EM, Montero MD. Treatment of rheumatoid arthritis with tumor necrosis factor inhibitors may predispose to significant increase in tuberculosis risk: a multicenter active-surveillance report. *Arthritis Rheum* 2003; **48**: 2122-2127 [PMID: 12905464 DOI: 10.1002/art.11137]

9 **Qumseya BJ**, Ananthakrishnan AN, Skaros S, Bonner M, Issa M, Zadvornova Y, Naik A, Perera L, Binion DG. QuantiFERON TB gold testing for tuberculosis screening in an inflammatory bowel disease cohort in the United States. *Inflamm Bowel Dis* 2011; **17**: 77-83 [PMID: 20848501 DOI: 10.1002/ibd.21329]

10 **Raval A**, Akhavan-Toyserkani G, Brinker A, Avigan M. Brief communication: characteristics of spontaneous cases of tuberculosis associated with infliximab. *Ann Intern Med* 2007; **147**: 699-702 [PMID: 18025446 DOI: 10.7326/0003-4819-147-10-200711200-00006]

11 **Vaughn BP**, Doherty GA, Gautam S, Moss AC, Cheifetz AS. Screening for tuberculosis and hepatitis B prior to the initiation of anti-tumor necrosis therapy. *Inflamm Bowel Dis* 2012; **18**: 1057-1063 [PMID: 21953829 DOI: 10.1002/ibd.21824]

12 **Lichtenstein GR**, Abreu MT, Cohen R, Tremaine W. American Gastroenterological Association Institute technical review on corticosteroids, immunomodulators, and infliximab in inflammatory bowel disease. *Gastroenterology* 2006; **130**: 940-987 [PMID: 16530532 DOI: 10.1053/j.gastro.2006.01.048]

13 **Kornbluth A**, Sachar DB. Ulcerative colitis practice guidelines in adults: American College Of Gastroenterology, Practice Parameters Committee. *Am J Gastroenterol* 2010; **105**: 501-523; quiz 524 [PMID: 20068560 DOI: 10.1038/ajg.2009.727]

14 **Rahier JF**, Magro F, Abreu C, Armuzzi A, Ben-Horin S, Chowers Y, Cottone M, de Ridder L, Doherty G, Ehehalt R, Esteve M, Katsanos K, Lees CW, Macmahon E, Moreels T, Reinisch W, Tilg H, Tremblay L, Veereman-Wauters G, Viget N, Yazdanpanah Y, Eliakim R, Colombel JF. Second European evidence-based consensus on the prevention, diagnosis and management of opportunistic infections in inflammatory bowel disease. *J Crohns Colitis* 2014; **8**: 443-468 [PMID: 24613021 DOI: 10.1016/j.crohns.2013.12.013]

15 **Theis VS**, Rhodes JM. Review article: minimizing tuberculosis during anti-tumour necrosis factor-alpha treatment of inflammatory bowel disease. *Aliment Pharmacol Ther* 2008; **27**: 19-30 [PMID: 17944997 DOI: 10.1111/j.1365-2036.2007.03553.x]

16 **Pai M**, Zwerling A, Menzies D. Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update. *Ann Intern Med* 2008; **149**: 177-184 [PMID: 18593687 DOI: 10.7326/0003-4819-149-3-200808050-00241]

17 **Schoepfer AM**, Flogerzi B, Fallegger S, Schaffer T, Mueller S, Nicod L, Seibold F. Comparison of interferon-gamma release assay versus tuberculin skin test for tuberculosis screening in inflammatory bowel disease. *Am J Gastroenterol* 2008; **103**: 2799-2806 [PMID:

18684188 DOI: 10.1111/j.1572-0241.2008.02050.x]
18 **Andrisani G**, Armuzzi A, Papa A, Marzo M, Felice C, Pugliese D, De Vitis I, Rapaccini GL, Guidi L. Comparison of Quantiferon-TB Gold versus tuberculin skin test for tuberculosis screening in inflammatory bowel disease patients. *J Gastrointestin Liver Dis* 2013; **22**: 21-25 [PMID: 23539386]
19 **Shahidi N**, Fu YT, Qian H, Bressler B. Performance of interferon-gamma release assays in patients with inflammatory bowel disease: a systematic review and meta-analysis. *Inflamm Bowel Dis* 2012; **18**: 2034-2042 [PMID: 22294550 DOI: 10.1002/ibd.22901]
20 **Zabana Y**, Domènech E, San Román AL, Beltrán B, Cabriada JL, Saro C, Araméndiz R, Ginard D, Hinojosa J, Gisbert JP, Mañosa M, Cabré E, Gassull MA. Tuberculous chemoprophylaxis requirements and safety in inflammatory bowel disease patients prior to anti-TNF therapy. *Inflamm Bowel Dis* 2008; **14**: 1387-1391 [PMID: 18452206 DOI: 10.1002/ibd.20496]
21 **Sichletidis L**, Settas L, Spyratos D, Chloros D, Patakas D. Tuberculosis in patients receiving anti-TNF agents despite chemoprophylaxis. *Int J Tuberc Lung Dis* 2006; **10**: 1127-1132 [PMID: 17044206]
22 **Ananthakrishnan AN**, Guzman-Perez R, Gainer V, Cai T, Churchill S, Kohane I, Plenge RM, Murphy S. Predictors of severe outcomes associated with Clostridium difficile infection in patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2012; **35**: 789-795 [PMID: 22360370 DOI: 10.1111/j.1365-2036.2012.05022.x]
23 **Ananthakrishnan AN**. Detecting and treating Clostridium difficile infections in patients with inflammatory bowel disease. *Gastroenterol Clin North Am* 2012; **41**: 339-353 [PMID: 22500522 DOI: 10.1016/j.gtc.2012.01.003]
24 **Cohen SH**, Gerding DN, Johnson S, Kelly CP, Loo VG, McDonald LC, Pepin J, Wilcox MH. Clinical practice guidelines for Clostridium difficile infection in adults: 2010 update by the society for healthcare epidemiology of America (SHEA) and the infectious diseases society of America (IDSA). *Infect Control Hosp Epidemiol* 2010; **31**: 431-455 [PMID: 20307191 DOI: 10.1086/651706]
25 **Sinh P**, Barrett TA, Yun L. Clostridium difficile Infection and Inflammatory Bowel Disease: A Review. *Gastroenterol Res Pract* 2011; **2011**: 136064 [PMID: 21915178 DOI: 10.1155/2011/136064]
26 **Crook DW**, Walker AS, Kean Y, Weiss K, Cornely OA, Miller MA, Esposito R, Louie TJ, Stoesser NE, Young BC, Angus BJ, Gorbach SL, Peto TE. Fidaxomicin versus vancomycin for Clostridium difficile infection: meta-analysis of pivotal randomized controlled trials. *Clin Infect Dis* 2012; **55** Suppl 2: S93-103 [PMID: 22752871 DOI: 10.1093/cid/cis499]
27 **Louie TJ**, Miller MA, Mullane KM, Weiss K, Lentnek A, Golan Y, Gorbach S, Sears P, Shue YK. Fidaxomicin versus vancomycin for Clostridium difficile infection. *N Engl J Med* 2011; **364**: 422-431 [PMID: 21288078 DOI: 10.1056/NEJMoa0910812]
28 **van Nood E**, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, Visser CE, Kuijper EJ, Bartelsman JF, Tijssen JG, Speelman P, Dijkgraaf MG, Keller JJ. Duodenal infusion of donor feces for recurrent Clostridium difficile. *N Engl J Med* 2013; **368**: 407-415 [PMID: 23323867 DOI: 10.1056/NEJMoa1205037]
29 **Kelly CR**, Kahn S, Kashyap P, Laine L, Rubin D, Atreja A, Moore T, Wu G. Update on Fecal Microbiota Transplantation 2015: Indications, Methodologies, Mechanisms, and Outlook. *Gastroenterology* 2015; **149**: 223-237 [PMID: 25982290 DOI: 10.1053/j.gastro.2015.05.008]
30 **Hamilton MJ**, Weingarden AR, Sadowsky MJ, Khoruts A. Standardized frozen preparation for transplantation of fecal microbiota for recurrent Clostridium difficile infection. *Am J Gastroenterol* 2012; **107**: 761-767 [PMID: 22290405 DOI: 10.1038/ajg.2011.482]
31 **Farah R**, Lisitsin S, Shay M. Bacterial meningitis associated with infliximab. *Pharm World Sci* 2006; **28**: 123-125 [PMID: 17004022 DOI: 10.1007/s11096-006-9022-x]
32 **Pilishvili T,** Bennett NM Pneumococcal disease prevention among adults: Strategies for the use of pneumococcal vaccines. *Vaccine* 2015; **33** (S4): D 60-65 [PMID: 26116257 DOI: 10.1016/j.vaccine.2015.05.102]
33 **Centers for Disease Control and Prevention**. Use of 13-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine for adults with immunocompromising conditions: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2012; **61**: 816-819 [PMID: 23051612]
34 **Mandell LA**, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, Dean NC, Dowell SF, File TM, Musher DM, Niederman MS, Torres A, Whitney CG. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. *Clin Infect Dis* 2007; **44** Suppl 2: S27-S72 [PMID: 17278083 DOI: 10.1086/511159]
35 **Fiorino G**, Peyrin-Biroulet L, Naccarato P, Szabò H, Sociale OR, Vetrano S, Fries W, Montanelli A, Repici A, Malesci A, Danese S. Effects of immunosuppression on immune response to pneumococcal vaccine in inflammatory bowel disease: a prospective study. *Inflamm Bowel Dis* 2012; **18**: 1042-1047 [PMID: 21674732 DOI: 10.1002/ibd.21800]
36 **Kapetanovic MC**, Saxne T, Sjöholm A, Truedsson L, Jönsson G, Geborek P. Influence of methotrexate, TNF blockers and prednisolone on antibody responses to pneumococcal polysaccharide vaccine in patients with rheumatoid arthritis. *Rheumatology* (Oxford) 2006; **45**: 106-111 [PMID: 16287919 DOI: 10.1093/rheumatology/kei193]
37 **Hameed N**, Tunkel AR. Treatment of Drug-resistant Pneumococcal Meningitis. *Curr Infect Dis Rep* 2010; **12**: 274-281 [PMID: 21308542 DOI: 10.1007/s11908-010-0110-7]
38 **Loras C**, Saro C, Gonzalez-Huix F, Mínguez M, Merino O, Gisbert JP, Barrio J, Bernal A, Gutiérrez A, Piqueras M, Calvet X, Andreu M, Abad A, Ginard D, Bujanda L, Panés J, Torres M, Fernández-Bañares F, Viver JM, Esteve M. Prevalence and factors related to hepatitis B and C in inflammatory bowel disease patients in Spain: a nationwide, multicenter study. *Am J Gastroenterol* 2009; **104**: 57-63 [PMID: 19098850 DOI: 10.1038/ajg.2008.4]
39 **Chevaux JB**, Bigard MA, Bensenane M, Oussalah A, Jarlot S, Belle A, Nani A, Bronowicki JP, Peyrin-Biroulet L. Inflammatory bowel disease and hepatitis B and C. *Gastroenterol Clin Biol* 2009; **33**: 1082-1093 [PMID: 19896313]
40 **Tolentino YF**, Fogaca HS, Zaltman C, Ximenes LL, Coelho HS. Hepatitis B virus prevalence and transmission risk factors in inflammatory bowel disease patients at Clementino Fraga Filho university hospital. *World J Gastroenterol* 2008; **14**: 3201-3206 [PMID: 18506926 DOI: 10.3748/wjg.14.3201]
41 **Biancone L**, Pavia M, Del Vecchio Blanco G, D'Incà R, Castiglione F, De Nigris F, Doldo P, Cosco F, Vavassori P, Bresci GP, Arrigoni A, Cadau G, Monteleone I, Rispo A, Fries W, Mallardi B, Sturniolo GC, Pallone F. Hepatitis B and C virus infection in Crohn's disease. *Inflamm Bowel Dis* 2001; **7**: 287-294 [PMID: 11720317]
42 **Longo F**, Hebuterne X, Tran A, Staccini P, Hastier P, Schneider S, Benzaken S, Tirtaine C, Rampal P. [Prevalence of hepatitis C in patients with chronic inflammatory bowel disease in the region of Nice and evaluation of risk factors]. *Gastroenterol Clin Biol* 2000; **24**: 77-81 [PMID: 10679588]
43 **Papa A**, Felice C, Marzo M, Andrisani G, Armuzzi A, Covino M, Mocci G, Pugliese D, De Vitis I, Gasbarrini A, Rapaccini GL, Guidi L. Prevalence and natural history of hepatitis B and C infections in a large population of IBD patients treated with anti-tumor necrosis factor-α agents. *J Crohns Colitis* 2013; **7**: 113-119 [PMID: 22464811 DOI: 10.1016/j.crohns.2012.03.001]
44 **Herbein G**, O'Brien WA. Tumor necrosis factor (TNF)-alpha and TNF receptors in viral pathogenesis. *Proc Soc Exp Biol Med* 2000; **223**: 241-257 [PMID: 10719836]
45 **Cottone M**, Kohn A, Daperno M, Armuzzi A, Guidi L, D'Inca R, Bossa F, Angelucci E, Biancone L, Gionchetti P, Ardizzone S, Papi C, Fries W, Danese S, Riegler G, Cappello M, Castiglione F, Annese V, Orlando A. Advanced age is an independent risk factor for severe infections and mortality in patients given anti-tumor necrosis factor therapy for inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2011; **9**: 30-35 [PMID: 20951835 DOI: 10.1016/j.cgh.2010.09.026]
46 **Gisbert JP**, Chaparro M, Esteve M. Review article: prevention

and management of hepatitis B and C infection in patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2011; **33**: 619-633 [PMID: 21416659 DOI: 10.1111/j.1365-2036.2010.04570.x]

47 **Gandhi RT**, Wurcel A, Lee H, McGovern B, Boczanowski M, Gerwin R, Corcoran CP, Szczepiorkowski Z, Toner S, Cohen DE, Sax PE, Ukomadu C. Isolated antibody to hepatitis B core antigen in human immunodeficiency virus type-1-infected individuals. *Clin Infect Dis* 2003; **36**: 1602-1605 [PMID: 12802762]

48 **Madonia S**, Orlando A, Scimeca D, Olivo M, Rossi F, Cottone M. Occult hepatitis B and infliximab-induced HBV reactivation. *Inflamm Bowel Dis* 2007; **13**: 508-509 [PMID: 17206687 DOI: 10.1002/ibd.20035]

49 **Loras C**, Gisbert JP, Mínguez M, Merino O, Bujanda L, Saro C, Domenech E, Barrio J, Andreu M, Ordás I, Vida L, Bastida G, González-Huix F, Piqueras M, Ginard D, Calvet X, Gutiérrez A, Abad A, Torres M, Panés J, Chaparro M, Pascual I, Rodriguez-Carballeira M, Fernández-Bañares F, Viver JM, Esteve M. Liver dysfunction related to hepatitis B and C in patients with inflammatory bowel disease treated with immunosuppressive therapy. *Gut* 2010; **59**: 1340-1346 [PMID: 20577000 DOI: 10.1136/gut.2010.208413]

50 **Lok AS**, McMahon BJ. Chronic hepatitis B: update 2009. *Hepatology* 2009; **50**: 661-662 [PMID: 19714720 DOI: 10.1002/hep.23190]

51 **Hou JK**, Velayos F, Terrault N, Mahadevan U. Viral hepatitis and inflammatory bowel disease. *Inflamm Bowel Dis* 2010; **16**: 925-932 [PMID: 20480515 DOI: 10.1002/ibd.21284]

52 **Melmed GY**, Ippoliti AF, Papadakis KA, Tran TT, Birt JL, Lee SK, Frenck RW, Targan SR, Vasiliauskas EA. Patients with inflammatory bowel disease are at risk for vaccine-preventable illnesses. *Am J Gastroenterol* 2006; **101**: 1834-1840 [PMID: 16817843 DOI: 10.1111/j.1572-0241.2006.00646.x]

53 **Chevaux JB**, Nani A, Oussalah A, Venard V, Bensenane M, Belle A, Gueant JL, Bigard MA, Bronowicki JP, Peyrin-Biroulet L. Prevalence of hepatitis B and C and risk factors for nonvaccination in inflammatory bowel disease patients in Northeast France. *Inflamm Bowel Dis* 2010; **16**: 916-924 [PMID: 19885908 DOI: 10.1002/ibd.21147]

54 **Huang ML**, Xu XT, Shen J, Qiao YQ, Dai ZH, Ran ZH. Prevalence and factors related to hepatitis B and C infection in inflammatory bowel disease patients in China: a retrospective study. *J Crohns Colitis* 2014; **8**: 282-287 [PMID: 24067604 DOI: 10.1016/j.crohns.2013.08.017]

55 **Nardone A**, Anastassopoulou CG, Theeten H, Kriz B, Davidkin I, Thierfelder W, O'Flanagan D, Bruzzone B, Mossong J, Boot HJ, Butur D, Slacikova M, Panait ML, Hellenbrand W, DE Melker H, Sobotová Z, Icardi G, Andrews N, Pebody RG, VAN Damme P, Kafatos G, Miller E, Hatzakis A. A comparison of hepatitis B seroepidemiology in ten European countries. *Epidemiol Infect* 2009; **137**: 961-969 [PMID: 19102797 DOI: 10.1017/S0950268808001672]

56 **Brunasso AM**, Puntoni M, Gulia A, Massone C. Safety of anti-tumour necrosis factor agents in patients with chronic hepatitis C infection: a systematic review. *Rheumatology* (Oxford) 2011; **50**: 1700-1711 [PMID: 21690185 DOI: 10.1093/rheumatology/ker190]

57 **Vauloup C**, Krzysiek R, Greangeot-Keros L, Wendling D, Goupille P, Brault R, Brousse C, Mariette X, Emilie D. Effects of tumor necrosis factor antagonist treatment on hepatitis C-related immunological abnormalities. *Eur Cytokine Netw* 2006; **17**: 290-293 [PMID: 17353164]

58 **Viganò M**, Degasperi E, Aghemo A, Lampertico P, Colombo M. Anti-TNF drugs in patients with hepatitis B or C virus infection: safety and clinical management. *Expert Opin Biol Ther* 2012; **12**: 193-207 [PMID: 22188392 DOI: 10.1517/14712598.2012.646986]

59 **Matsuoka K**, Iwao Y, Mori T, Sakuraba A, Yajima T, Hisamatsu T, Okamoto S, Morohoshi Y, Izumiya M, Ichikawa H, Sato T, Inoue N, Ogata H, Hibi T. Cytomegalovirus is frequently reactivated and disappears without antiviral agents in ulcerative colitis patients. *Am J Gastroenterol* 2007; **102**: 331-337 [PMID: 17156136 DOI: 10.1111/j.1572-0241.2006.00989.x]

60 **Domènech E**, Vega R, Ojanguren I, Hernández A, Garcia-Planella E, Bernal I, Rosinach M, Boix J, Cabré E, Gassull MA. Cytomegalovirus infection in ulcerative colitis: a prospective, comparative study on prevalence and diagnostic strategy. *Inflamm Bowel Dis* 2008; **14**: 1373-1379 [PMID: 18452205 DOI: 10.1002/ibd.20498]

61 **Kambham N**, Vij R, Cartwright CA, Longacre T. Cytomegalovirus infection in steroid-refractory ulcerative colitis: a case-control study. *Am J Surg Pathol* 2004; **28**: 365-373 [PMID: 15104299 DOI: 10.1097/00000478-200403000-00009]

62 **Kennedy PG**. Varicella-zoster virus latency in human ganglia. *Rev Med Virol* 2002; **12**: 327-334 [PMID: 12211045 DOI: 10.1002/rmv.362]

63 **Ansari F**, Baker RD, Patel R, Baker SS. Varicella immunity in inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 2011; **53**: 386-388 [PMID: 21505365 DOI: 10.1097/mpg.0b013e31821e1917]

64 **Winthrop KL**, Baddley JW, Chen L, Liu L, Grijalva CG, Delzell E, Beukelman T, Patkar NM, Xie F, Saag KG, Herrinton LJ, Solomon DH, Lewis JD, Curtis JR. Association between the initiation of anti-tumor necrosis factor therapy and the risk of herpes zoster. *JAMA* 2013; **309**: 887-895 [PMID: 23462785 DOI: 10.1001/jama.2013.1099]

65 **Sands BE**, Cuffari C, Katz J, Kugathasan S, Onken J, Vitek C, Orenstein W. Guidelines for immunizations in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2004; **10**: 677-692 [PMID: 15472534 DOI: 10.1097/00054725-200409000-00028]

66 **Marin M**, Güris D, Chaves SS, Schmid S, Seward JF. Prevention of varicella: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2007; **56**: 1-40 [PMID: 17585291]

67 **Lu Y**, Bousvaros A. Varicella vaccination in children with inflammatory bowel disease receiving immunosuppressive therapy. *J Pediatr Gastroenterol Nutr* 2010; **50**: 562-565 [PMID: 20639716 DOI: 10.1097/MPG.0b013e3181bab351]

68 **Harpaz R**, Ortega-Sanchez IR, Seward JF. Prevention of herpes zoster: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2008; **57**: 1-30; quiz CE2-4 [PMID: 18528318]

69 **Kroger AT**, Atkinson WL, Marcuse EK, Pickering LK. General recommendations on immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2006; **55**: 1-48 [PMID: 17136024]

70 **Zhang J**, Xie F, Delzell E, Chen L, Winthrop KL, Lewis JD, Saag KG, Baddley JW, Curtis JR. Association between vaccination for herpes zoster and risk of herpes zoster infection among older patients with selected immune-mediated diseases. *JAMA* 2012; **308**: 43-49 [PMID: 22760290 DOI: 10.1001/jama.2012.7304]

71 **Cepeda EJ**, Williams FM, Ishimori ML, Weisman MH, Reveille JD. The use of anti-tumour necrosis factor therapy in HIV-positive individuals with rheumatic disease. *Ann Rheum Dis* 2008; **67**: 710-712 [PMID: 18079191 DOI: 10.1136/ard.2007.081513]

72 **Roset Bahmanyar E**, Paavonen J, Naud P, Salmerón J, Chow SN, Apter D, Kitchener H, Castellsagué X, Teixeira JC, Skinner SR, Jaisamrarn U, Limson GA, Garland SM, Szarewski A, Romanowski B, Aoki F, Schwarz TF, Poppe WA, De Carvalho NS, Harper DM, Bosch FX, Raillard A, Descamps D, Struyf F, Lehtinen M, Dubin G. Prevalence and risk factors for cervical HPV infection and abnormalities in young adult women at enrolment in the multinational PATRICIA trial. *Gynecol Oncol* 2012; **127**: 440-450 [PMID: 22940493 DOI: 10.1016/j.ygyno.2012.08.033]

73 **Walboomers JM**, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ, Muñoz N. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol* 1999; **189**: 12-19 [PMID: 10451482 DOI: 10.1002/(SICI)1096-9896(199909)189: 1<12: : AID-PATH431>3.0.CO; 2-F]

74 **Committee on Practice Bulletins-Gynecology**. ACOG Practice Bulletin Number 131: Screening for cervical cancer. *Obstet Gynecol* 2012; **120**: 1222-1238 [PMID: 23090560 DOI: 10.1097/

AOG.0b013e318277c92a]
75 **Kane S**, Khatibi B, Reddy D. Higher incidence of abnormal Pap smears in women with inflammatory bowel disease. *Am J Gastroenterol* 2008; **103**: 631-636 [PMID: 17941962 DOI: 10.1111/j.1572-0241.2007.01582.x]
76 **Bhatia J**, Bratcher J, Korelitz B, Vakher K, Mannor S, Shevchuk M, Panagopoulos G, Ofer A, Tamas E, Kotsali P, Vele O. Abnormalities of uterine cervix in women with inflammatory bowel disease. *World J Gastroenterol* 2006; **12**: 6167-6171 [PMID: 17036389]
77 **Seksik P**, Cosnes J, Sokol H, Nion-Larmurier I, Gendre JP, Beaugerie L. Incidence of benign upper respiratory tract infections, HSV and HPV cutaneous infections in inflammatory bowel disease patients treated with azathioprine. *Aliment Pharmacol Ther* 2009; **29**: 1106-1113 [PMID: 19222411 DOI: 10.1111/j.1365-2036.2009.03973.x]
78 **Schunter MO**, Walles T, Fritz P, Meyding-Lamadé U, Thon KP, Fellermann K, Stange EF, Lamadé W. Herpes simplex virus colitis complicating ulcerative colitis: A case report and brief review on superinfections. *J Crohns Colitis* 2007; **1**: 41-46 [PMID: 21172183 DOI: 10.1016/j.crohns.2007.06.004]
79 **François-Dufresne A**, Garbino J, Ricou B, Wunderli W. ARDS caused by herpes simplex virus pneumonia in a patient with Crohn's disease: a case report. *Intensive Care Med* 1997; **23**: 345-347 [PMID: 9083240 DOI: 10.1007/s001340050339]
80 **Ali T**, Yun L, Shapiro D, Madhoun MF, Bronze M. Viral infections in patients with inflammatory bowel disease on immunosuppressants. *Am J Med Sci* 2012; **343**: 227-232 [PMID: 22357111 DOI: 10.1097/MAJ.0b013e31821ff728]
81 **Weinstock DM**. Epstein-Barr virus, lymphoma risk and the potential role of HIV infection in IBD patients undergoing immunosuppression. *Dig Dis* 2010; **28**: 519-524 [PMID: 20926881 DOI: 10.1159/000320411]
82 **Larvol L**, Soule JC, Le Tourneau A. Reversible lymphoma in the setting of azathioprine therapy for Crohn's disease. *N Engl J Med* 1994; **331**: 883-884 [PMID: 8078549 DOI: 10.1056/NEJM199409293311321]
83 **Losco A**, Gianelli U, Cassani B, Baldini L, Conte D, Basilisco G. Epstein-Barr virus-associated lymphoma in Crohn's disease. *Inflamm Bowel Dis* 2004; **10**: 425-429 [PMID: 15475752 DOI: 10.1097/00054725-200407000-00015]
84 **Beaugerie L**, Brousse N, Bouvier AM, Colombel JF, Lémann M, Cosnes J, Hébuterne X, Cortot A, Bouhnik Y, Gendre JP, Simon T, Maynadié M, Hermine O, Faivre J, Carrat F. Lymphoproliferative disorders in patients receiving thiopurines for inflammatory bowel disease: a prospective observational cohort study. *Lancet* 2009; **374**: 1617-1625 [PMID: 19837455 DOI: 10.1016/S0140-6736(09)61302-7]
85 **Beaugerie L**. Lymphoma: the bête noire of the long-term use of thiopurines in adult and elderly patients with inflammatory bowel disease. *Gastroenterology* 2013; **145**: 927-930 [PMID: 24070724 DOI: 10.1053/j.gastro.2013.09.035]
86 **Weinstock DM**, Ambrossi GG, Brennan C, Kiehn TE, Jakubowski A. Preemptive diagnosis and treatment of Epstein-Barr virus-associated post transplant lymphoproliferative disorder after hematopoietic stem cell transplant: an approach in development. *Bone Marrow Transplant* 2006; **37**: 539-546 [PMID: 16462755 DOI: 10.1038/sj.bmt.1705289]
87 **Chartrand C**, Leeflang MM, Minion J, Brewer T, Pai M. Accuracy of rapid influenza diagnostic tests: a meta-analysis. *Ann Intern Med* 2012; **156**: 500-511 [PMID: 22371850 DOI: 10.7326/0003-4819-156-7-201204030-00403]
88 **deBruyn JC**, Hilsden R, Fonseca K, Russell ML, Kaplan GG, Vanderkooi O, Wrobel I. Immunogenicity and safety of influenza vaccination in children with inflammatory bowel disease. *Inflamm Bowel Dis* 2012; **18**: 25-33 [PMID: 21472826 DOI: 10.1002/ibd.21706]
89 **Fiore AE**, Uyeki TM, Broder K, Finelli L, Euler GL, Singleton JA, Iskander JK, Wortley PM, Shay DK, Bresee JS, Cox NJ. Prevention and control of influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2010. *MMWR Recomm Rep* 2010; **59**: 1-62 [PMID: 20689501]
90 **Andrisani G**, Frasca D, Romero M, Armuzzi A, Felice C, Marzo M, Pugliese D, Papa A, Mocci G, De Vitis I, Rapaccini GL, Blomberg BB, Guidi L. Immune response to influenza A/H1N1 vaccine in inflammatory bowel disease patients treated with anti TNF-α agents: effects of combined therapy with immunosuppressants. *J Crohns Colitis* 2013; **7**: 301-307 [PMID: 22673636 DOI: 10.1016/j.crohns.2012.05.011]
91 **Rahier JF**, Papay P, Salleron J, Sebastian S, Marzo M, Peyrin-Biroulet L, Garcia-Sanchez V, Fries W, van Asseldonk DP, Farkas K, de Boer NK, Sipponen T, Ellul P, Louis E, Peake ST, Kopylov U, Maul J, Makhoul B, Fiorino G, Yazdanpanah Y, Chaparro M. H1N1 vaccines in a large observational cohort of patients with inflammatory bowel disease treated with immunomodulators and biological therapy. *Gut* 2011; **60**: 456-462 [PMID: 21270121 DOI: 10.1136/gut.2010.233981]

P- Reviewer: Krawiec P, Lakatos PL, Sachar DB **S- Editor**: Kong JX
L- Editor: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i3.397

World J Gastrointest Pharmacol Ther 2016 August 6; 7(3): 397-405
ISSN 2150-5349 (online)

REVIEW

Intestinal neuronal dysplasia type B: A still little known diagnosis for organic causes of intestinal chronic constipation

Pedro Luiz Toledo de Arruda Lourenção, Simone Antunes Terra, Erika Veruska Paiva Ortolan, Maria Aparecida Marchesan Rodrigues

Pedro Luiz Toledo de Arruda Lourenção, Erika Veruska Paiva Ortolan, Department of Surgery and Orthopedics, Botucatu Medical School - Unesp, Botucatu, São Paulo 18618-687, Brazil

Simone Antunes Terra, Maria Aparecida Marchesan Rodrigues, Department of Pathology, Botucatu Medical School - Unesp, Botucatu, São Paulo 18618-687, Brazil

Author contributions: Toledo de Arruda Lourenção PL analysed and interpretated the data, drafted the initial manuscript and revised the article critically; Terra SA performed the collected the data and draft the initial manuscript; Ortolan EVP draft the initial manuscript; Rodrigues MAM analysed and interpreted the data, drafted the initial manuscript and revised critically.

Supported by São Paulo Research Foundation (FAPESP), No. 2014/042271-1.

Conflict-of-interest statement: Authors declare no conflict of interests for this article.

Manuscript source: Invited manuscript

Correspondence to: Pedro Luiz Toledo de Arruda Lourenção, Professor of Pediatric Surgery, Department of Surgery and Orthopedics, Botucatu Medical School - Unesp, Av. Prof. Montenegro, Distrito de Rubião Junior, s/n, Botucatu, São Paulo 18618-687, Brazil. lourencao@fmb.unesp.br
Telephone: +55-14-38801703
Fax: +55-14-38801669

Received: February 14, 2016
Peer-review started: February 15, 2016
First decision: March 31, 2016
Revised: April 14, 2016
Accepted: May 7, 2016
Article in press: May 9, 2016
Published online: August 6, 2016

Abstract

Intestinal neuronal dysplasia type B (IND-B) is a controversial entity among the gastrointestinal neuromuscular disorders. It may occur alone or associated with other neuropathies, such as Hirschsprung's disease (HD). Chronic constipation is the most common clinical manifestation of patients. IND-B primarily affects young children and mimics HD, but has its own histopathologic features characterized mainly by hyperplasia of the submucosal nerve plexus. Thus, IND-B should be included in the differential diagnoses of organic causes of constipation. In recent years, an increasing number of cases of IND-B in adults have also been described, some presenting severe constipation since childhood and others with the onset of symptoms at adulthood. Despite the intense scientific research in the last decades, there are still knowledge gaps regarding definition, pathogenesis, diagnostic criteria and therapeutic possibilities for IND-B. However, in medical practice, we continue to encounter patients with severe constipation or intestinal obstruction who undergo to diagnostic investigation for HD and their rectal biopsies present hyperganglionosis in the submucosal nerve plexus and other features, consistent with the diagnosis of IND-B. This review critically discusses aspects related to the disease definitions, pathophysiology and genetics, epidemiology distribution, clinical presentation, diagnostic criteria and therapeutic possibilities of this still little-known organic cause of intestinal chronic constipation.

Key words: Intestinal neuronal dysplasia type B; Hyperplasia of the submucosal nerve plexus; Intestinal chronic constipation; Gastrointestinal neuromuscular diseases; Dysganglionosis

Core tip: Intestinal neuronal dysplasia type B (IND-B) is a controversial entity among the gastrointestinal neuromuscular disorders. Chronic constipation is the most common clinical manifestation of patients. IND-B primarily affects young children and mimics Hirschsprung's disease, but has its own histopathologic features characterized mainly by hyperplasia of the submucosal nerve plexus. Despite the intense scientific research in the last decades, there are still knowledge gaps regarding IND-B. This review critically discusses aspects related to the disease definitions, pathophysiology and genetics, epidemiology distribution, clinical presentation, diagnostic criteria and therapeutic possibilities of this still little-known organic cause of intestinal chronic constipation.

Toledo de Arruda Lourenção PL, Terra SA, Ortolan EVP, Rodrigues MAM. Intestinal neuronal dysplasia type B: A still little known diagnosis for organic causes of intestinal chronic constipation. *World J Gastrointest Pharmacol Ther* 2016; 7(3): 397-405 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i3/397.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i3.397

INTRODUCTION

Intestinal neuronal dysplasia (IND) is a pathological condition that affects the intestinal submucosal nerve plexuses and may occur alone or associated with other neuropathies, such as Hirschsprung's disease (HD). IND belongs to the group of the gastrointestinal neuromuscular diseases and was most recently included in the classification for this heterogeneous group of complex changes in the enteric nervous system[1,2].

More than 40 years after its original description[3], the pathology of IND remains incompletely elucidated. Commonly, IND is associated with clinical symptoms of intestinal chronic constipation that affect children in their first years of life, similar to those of HD, but IND has its own histopathologic features characterized by hyperplasia of the submucosal nerve plexus[4].

Despite the intense scientific research performed in the last decades which includes more than 250 published scientific articles, there are still gaps in the knowledge on IND's definition, pathogenesis, diagnostic criteria and therapeutic possibilities[2,5,6].

HISTORICAL ASPECTS

The term IND was first used by Nezelof *et al*[7] in 1970 to describe three cases of congenital megacolon associated with hyperplasia of the myenteric nerve plexus. One year later, Meier-Ruge presented the first formal description of IND as a condition that is typically associated with low intestinal obstruction and that could resemble HD but with distinctive histopathological features, such as hyperplasia of the submucosal nerve plexuses and increased Acetylcholinesterase activity (AchE) in the parasympathetic nerve fibers in the lamina propria of the mucosa[3].

In 1977, Puri *et al*[8] described one case of IND associated with HD with rectosigmoid aganglionosis of the nerve plexus and IND in the descending and transverse colon.

In 1983, Fadda *et al*[9] proposed the classification of two clinical and histopathological subtypes of IND: IND type A (IND-A), an extremely rare form, characterized by congenital hypoplasia of the adrenergic enteric nervous system; and IND type B (IND-B), characterized by malformation of the cholinergic submucosal plexus, accounting for more than 95% of all cases.

In 1990, a consensus meeting in Frankfurt, Germany, defined the morphological criteria for the diagnosis of IND-B[10]. Since that time, these criteria have been widely used both in clinical practice, follow-up studies and genetic investigations[5,11-15]. Furthermore, in the 90s, new criteria were proposed that gave greater importance to the need for the identification of giant ganglia in the submucosa for the diagnosis of IND-B[16,17]. In the several published criteria, the giant ganglia are defined by the presence of a minimum number of ganglion cells ranging from 6 to more than 10 per ganglion[6,18-21].

Given this lack of diagnostic standardization, in 2004, Meier-Ruge *et al*[5,22] proposed quantitative criteria for the histopathologic diagnosis of IND-B. They defined IND-B by the presence of at least 20% giant nerve ganglia in the submucosa, with more than 8 ganglion cells each, based on the examination of a minimum of 25 submucosal ganglia. Additionally, they used a histochemical panel in frozen sections for the analyses of lactate dehydrogenase, succinyl dehydrogenase and nitric oxide synthase[5,22].

All of these changes in the proposed histopathological criteria for the diagnosis of IND-B have not only caused disparities in its definitions but also skepticism about its existence. The main unsolved problem highlighted in recent publications is if there is a causal relationship between the histological findings and clinical symptoms that would justify the characterization of IND-B as a specific entity[2,4,6,23]. Regarding this situation, the current opinions converge on the need for further research to elucidate the many uncertainties about the clinical and morphological characterization of IND-B[2,4,24].

CLASSIFICATION

Two forms of IND are recognized[9]. IND-A is extremely rare and occurs in less than 5% of all IND cases. Patients with IND-A typically present in the neonatal period with

symptoms may vary from acute intestinal obstruction to diarrhea with hemorrhagic stools. IND-A is characterized by hypoplasia or aplasia of the adrenergic enteric nervous system[6,25]. A moderate increase in the acetylcholinesterase activity of the parasympathetic nerves is the reason that such cases are termed IND. In 2005, Meier-Ruge and Bruder[26] considered IND-A to be as a necrotizing enterocolitis caused by immaturity of the sympathetic nervous system of the distal colon. The sympathetic innervation is decreased to different degrees in these patients. The absence of sympathetic synapses within the ganglia of the myenteric plexus and the resultant increase in parasympathetic tone are considered to be responsible for the focal colon spasms. Disorders of blood flow and decreased mucus production seem to be the major factors in the pathogenesis of necrotizing enterocolitis. In the majority of cases, the sympathetic innervation is normal by the eighth month of age. Cases that do not present with this development until 10 mo old may be related to sympathetic aplasia[26].

In contrast, IND-B represents more than 95% of all cases, which explains why this entity has been more frequently studied in the literature and why many authors consider IND to be a synonym for IND-B[2]. IND-B is characterized by hyperplasia of the parasympathetic submucosal plexuses. Typical histological features of IND-B include hyperganglionosis, giant ganglia, ectopic ganglion cells and increased AChE activity in the lamina propria and around the submucosal blood vessels. The changes associated with IND-B are more common in the distal colon; however, they can affect any segment of the enteric nervous system and occur in different age groups ranging from newborns to adults and alone or in combination with HD[5]. Subtype B can cause severe constipation in childhood, unresponsive to clinical management and can be associated with soiling and hemorrhagic stools, acute bowel obstruction or enterocolitis episodes. Occasionally, IND-B symptoms mimic those of HD, which is its main differential diagnosis. All IND cases associated with HD are of the B subtype[1,4,6,26].

EPIDEMIOLOGY

In 2007, Granero Cendón *et al*[27] estimated the incidence of IND-B as approximately 1 per 7500 newborns. However, the frequency of IND-B varies widely, and the reported rates range from 0.3% to 40% of all rectal suction biopsies[5,28-30]. This wide variation may be attributable to the lack of consensus on the diagnostic criteria[6,31]. There is also an irregular geographical distribution; the highest rates of diagnosis are in European countries, which can be explained by the fact that the majority of the published research comes from this continent[32].

The latest published series by Taguchi *et al*[33] (2014) involved a retrospective multicenter study of cases of IND-B in 167 centers in Japan from 2000 to 2009. These authors reported 13 cases based on standardized morphologic criteria from all of the included centers[33]. However, when the quantitative criteria of Meier-Ruge *et al*[5,22] were applied, only 4 of the 13 cases sustained the IND-B diagnosis.

IND proximal to a segment of aganglionosis is not uncommon and has been suggested to be a possible cause of persistent bowel problems after surgery for HD. This association may occur in 6% to 44% of HD patients[5,28,34,35].

GENETIC ASPECTS

Recent studies have addressed the role of genetic and molecular commands in the migration and development of the neuroenteric cells[36,37]. The proto-oncogene rearranged during transfection (RET) and RET protein act in the migration and proliferation of neuroblasts. Approximately 50% of patients with familial HD present RET proto-oncogenic mutations. This finding highlights the importance of this gene alteration in the pathogenesis of dysganglionosis[36]. Over 20 different mutations have been described in this proto-oncogene, and some of the polymorphisms are associated with particular phenotypes, such as the extension of the aganglionic segment in HD[37].

Similarly, the existence of a genetic component potentially responsible for IND-B has been investigated. The evidence for this component came from a study of monozygotic twins affected by the disease and reports of families in which several members had the histopathological diagnoses of IND-B over multiple generations[14,38]. Because IND-B and HD are derived from the enteric nervous system, changes often occur simultaneously in the same patient, and common molecular pathways are likely to be involved in the geneses of the two pathological conditions[39]. However, mutations in genes considered to be most relevant to HD, such as RET, glial cell line-derived neurotrophic factor (GDNF), and other selected genes in patients with IND-B, have not yet been identified in patients with IND-B[40-44]. Only some combinations of single nucleotide polymorphisms in the RET proto-oncogene have been identified in patients with IND-B[45].

IND-B has been described in some families with other associated congenital anomalies of the gastrointestinal tract, such as intestinal malrotation and multiple endocrine neoplasia type 2[15,30,46,47]. Recently, twins from a Turkish family who presented with IND-B associated with congenital short bowel syndrome were described[48], which raises the possibility that mutations in the Coxsackie- and adenovirus receptor-like membrane protein (CLMP) gene could be related to IND-B because CLMP is essential for intestinal development, and its expression is related to molecular junctional adhesion[46].

Different experimental studies in rats and mice have demonstrated that homozygous animals deficient in the *NCX/Hox11L.1* gene present with megacolon and hyperplasia of the myenteric nerve plexus[49-52].

However, Costa *et al*[14] (2000) and Fava *et al*[42] (2002) failed to demonstrate the presence of mutations or molecular defects in the Hox11L.1 coding region in humans with IND-B.

Another possible genetic mechanism is related to endothelin receptor B[31]. One of the endothelin receptors (END3) plays an important role in the development of the enteric nervous system of mice. Holland-Cunz *et al*[53] (2003) reported that mice presenting with a heterozygous deficiency in this receptor exhibit histopathological changes similar to IND-B, although they do not exhibit clinical signs of bowel dysmotility. These findings were also not reproducible in human research.

PATHOGENESIS

The pathogenesis is also a part of the array of uncertainties regarding IND-B. Several hypotheses have been discussed, although none are widely accepted[6,24].

The histopathological changes that characterize IND-B may come from a genetically primary change that directly influences the embryological development of tissues derived from the neural crest[6]. However, these findings have only been identified in experimental studies[14,49-52]. This hypothesis is supported by the association with other intestinal and extra-intestinal congenital anomalies[15,54,55].

Another research line conceives IND-B as an adaptive response of the enteric nervous system. IND-B has been considered to be secondary to acquired phenomena caused by congenital obstructions or inflammation occurred during pre-, peri- or post-natal periods in humans[12,13,18,56,57]. Morphological findings suggestive of IND-B have been observed in intestinal segments proximal to areas of intestinal atresia, rectal mucosal prolapse and ileostomy, intestinal intussusception, imperforate anus and necrotizing enterocolitis[56,58,59]. This secondary histopathologic response to a bowel obstruction has also been tested in experimental studies with conflicting results[60-62]. Pickard *et al*[60] (1981) observed ganglionic hyperplasia in the dilated segment of the proximal jejunum in an experimental model of intestinal atresia in sheep fetuses. The same results were not reproduced by Moore *et al*[61] (1993) in a model of partial colon obstruction in adult rats. These authors observed a decrease in the number of ganglion cells in the myenteric nerve plexuses of rats submitted to partial intestinal obstruction. This decrease was explained by an increase in colonic diameter secondary to bowel obstruction[61]. The most recent study on this subject was from Gálvez *et al*[62] (2004) who identified histopathological changes suggestive of IND-B in some adult rats in a model of chronic colonic obstruction.

An association between IND-B and HD has also been reported[8,35,63-65]. In such cases, the segments proximal to the aganglionic obstructed segment present histological characteristics of IND-B[6,54]. Thus, these morphological changes of the nerve plexuses of the proximal submucosa segment can be explained both by a primary embryonic modification of the enteric nervous system that could be considered a neurocristopathy that shares a common origin with HD and by a minor change in response to a distal intestinal obstruction[54,63-66].

There is also some evidence that the histopathological changes observed in IND-B can be part of the normal development of the enteric nervous system. As a patient gets older, there is an increase in the size of the ganglion cells and a decrease in their number in the submucosal nerve plexuses[5,22,67-69].

Another conflicting issue is related to whether a cause-effect relationship exists between the histopathological findings of IND-B and the clinical symptoms. In most cases, the diagnosis of IND-B is based on histopathological examinations of rectal biopsies from patients who presented severe constipation[6]. However, histopathological changes similar to those of IND-B have been found in the colon of 36 completely asymptomatic children[69]. Other studies have failed to demonstrate correlations between the histopathological findings, clinical symptoms, radiological and manometric changes[11,12,47,67]. These controversies support the authors who do not consider IND-B as a distinct entity but rather a histopathological alteration of the enteric nervous system that may or may not cause clinical manifestations[6,23,24,64].

CLINICAL PRESENTATION

Intestinal chronic constipation has been reported as the commonest clinical presentation in IND-B case series[6,57]. In addition to the decrease in bowel movement frequency, the presence of straining at stool, bulky and hardened stools, fecal overflow incontinence and rectal bleeding are usually present as signs and symptoms of chronic constipation[70,71]. Therefore, IND-B must be part of the differential diagnosis of possible organic causes for constipation in childhood[72].

In some cases, these symptoms may begin in the first years of life with delays in meconium passage, abdominal distension, vomiting and failure to thrive[73,74]. A portion of patients continue to exhibit symptoms throughout life and frequently present with severe constipation unresponsive to several treatment modalities[75-77]. These symptoms may improve after 4 years of age, which supports the hypothesis of maturation of the enteric nervous system early in life, since in these cases the histopathological findings of IND-B could disappear concurrently with the symptoms[5].

Severe symptoms, such as enterocolitis episodes, bowel obstruction, volvulus and intussusceptions are rare complications described in different age groups[78-81]. In recent years, an increasing number of cases of IND-B in adults have been described[82-86]. Some of these cases have exhibited symptoms of severe constipation since childhood[82,83], whereas others experienced the onset of symptoms at adulthood[84]. Some patients develop serious complications, such as chronic intestinal pseudo-obstruction, acute bowel obstruction or intestinal infarction[84-86]. The oldest reported patient received a

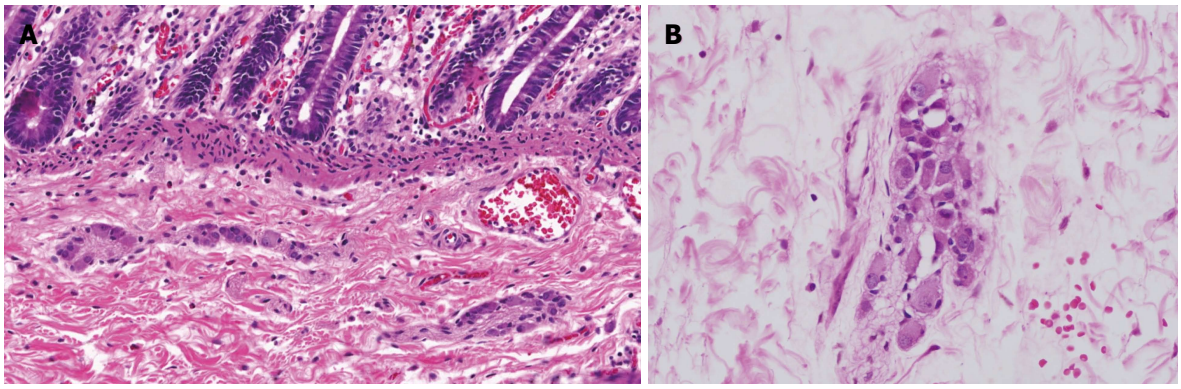

Figure 1 Histological findings of intestinal neuronal dysplasia. A: Giant ganglia in the submucous plexus with more than eight nerve cells (HE, 200 ×); B: High power view (HE, 400 ×).

confirmed diagnosis at 71 years of age[83].

DIAGNOSIS

The diagnostic workup used in patients with IND-B must be the same routinely performed during investigation for organic causes of intestinal constipation, particularly focused to exclude HD, which is the most prevalent intestinal dysganglionosis[6,57,72,87]. However, anorectal manometry and barium enema, which are established tests for HD screening, do not present specific results for IND-B[88,89]. Barium enema frequently demonstrate an increased caliber of the rectosigmoid, which is a nonspecific finding typical of patients with constipation, but also may demonstrate conical transition zone, similar to HD[31,89]. Anorectal manometry can reveal absence or presence of an anorectal inhibitory reflex, commonly with atypical morphology, which contributes little to the diagnostic investigation of IND-B[5,31,89].

Thus, the diagnosis of IND-B essentially relays on histopathological analyses of rectal biopsies[2,4]. The morphological criteria for its diagnosis have changed substantially over the years, leading to difficulties for clinical practice and comparisons between studies. Hyperplasia of the submucosal nerve plexuses is the morphological finding that defines IND-B but that is characterized in different manners according to the adopted criterion[5]. Some authors emphasize the need for the presence of a minimum number of ganglion cells per ganglion or a minimum number of ganglia with these characteristics among the analyzed ganglia for a diagnosis of plexuses hyperplasia[16,22,75,90] (Figure 1). Other morphological features, such as the presence of ectopic ganglion cells, increased acetylcholinesterase activity, ganglion cells with a "button" appearance and hypertrophy of the nerve trunks, are considered diagnostic criteria in some studies[9,10,16,68,79,91].

The criteria described by Meier-Ruge *et al*[22] (2004) and slightly altered by Meier-Rouge *et al*[5] (2006) suggest a quantitative analysis of the number of ganglion cells in the nervous submucosal plexuses and the identification of at least 20% giant ganglia with at least 8 neurons each, in 25 analyzed nerve ganglia. Frozen 15-μm-thick sections are mandatory and must be subjected to a panel of histochemical tests for lactate dehydrogenase, succinyl dehydrogenase and nitric oxide synthase, and the patient must be older than 1 year of age[5,22]. Although these criteria have been accepted by the scientific community, there are few reports of their use in large series of patients with IND-B[33]. The requirement for fresh frozen sections and the fact that the specific histochemical stainings are not available in most pediatric pathology laboratories are limitations that must be considered. Moreover, it is uncertain whether the numerical criteria applied in these analyses can be applied to 5-μm-thick histological sections embedded in paraffin for standard histological analyses with hematoxylin and eosin or immunohistochemical methods[2,5].

TREATMENT

Given the numerous uncertainties about the definition, pathogenesis and diagnosis of IND, the lack of consensus regarding its treatment is not surprising. Patients with IND have been subjected to different treatments modalities, that may vary from clinical management, to surgical procedures[57].

Clinical management includes dietary changes, laxatives and enemas[6,32]. Schimpl *et al*[32] (2004) reported satisfactory results in 80% of 105 patients treated with dietary changes, cisapride, laxatives and enemas, in a median follow up period of 7.2 years. Clinical management must follow the currently used guidelines for the treatment of intestinal chronic constipation in children, including fecal desimpaction and laxatives[72].

Although there is not a well-established role to surgical treatment as in Hirshcprung's disease, there are some reports of this modality of treatment in IND-B[83]. Surgical treatment can be performed through different techniques[11,46,63,92]. Schärli[11] (1992) reported favorable results with a posterior sphincteromyotomy in 13 patients, after a limited 6 mo follow-up period. Some case series with a small number of patients showed symptoms improvement after a temporary colostomy[6,46,63]. Several reports described a colonic resection in patients with

IND-B, commonly performed by an anal pull-through procedure. In most of the cases, there were improvement in the number of bowel movements and in the obstructive symptoms. However, the time of follow-up, the surgical techniques and the length of the resected bowel are quite variable[5,77,92].

The results obtained with these different types of treatment are very discordant. Long-term follow-up studies are lacking and the available studies involve limited numbers of patients[32,57,75]. Thus, the available data nowadays still remains too scarce to establish a therapeutic guideline for IND-B[32,57]. On the other hand, there is a real disease, with its own clinical manifestations and can not be classified only as an histopathological entity[75].

The several types of clinical manifestations directly influence in the treatment. Cases of mild intestinal constipation, without systemic complications or obstructive symptoms, tend to be treated with a conservative clinical management. Most of these cases may resolve spontaneously up to the age of 4 years, due to the maturation of the enteric nervous system[93]. On the other hand, IND-B may present with severe intestinal constipation, with infectious and obstructive symptoms, what require a more invasive treatment[77,91]. Therefore, there is a tendency to consider the conservative choice as a first line therapy in IND-B. The surgical treatment through intestinal resections should be reserved for the cases refractory to at least 6 mo of clinical management, or in the presence of obstructive complications[5,6,31,32,76].

CONCLUSION

IND-B can be considered as a pathological entity characterized by anomalies of the submucous plexus, with a considerable increase in the number of ganglion cells, commonly associated with different degrees of constipation in childhood. IND-B remains surrounded by controversies related to its definition, etiopathogenesis, diagnostic criteria and therapeutic possibilities. However, in medical practice, we continue to encounter children with severe constipation or intestinal obstruction who undergo to diagnostic investigation for HD and rectal biopsies show hyperplastic submucosal ganglia consistent with the diagnosis of IND-B.

In this context, it is of utmost importance to maintain our efforts to clarify the pathophysiology, diagnosis and treatment of this still little-known organic cause of intestinal chronic constipation.

REFERENCES

1 **Knowles CH**, De Giorgio R, Kapur RP, Bruder E, Farrugia G, Geboes K, Lindberg G, Martin JE, Meier-Ruge WA, Milla PJ, Smith VV, Vandervinden JM, Veress B, Wedel T. The London Classification of gastrointestinal neuromuscular pathology: report on behalf of the Gastro 2009 International Working Group. *Gut* 2010; **59**: 882-887 [PMID: 20581236 DOI: 10.1136/gut.2009.200444]

2 **Schäppi MG**, Staiano A, Milla PJ, Smith VV, Dias JA, Heuschkel R, Husby S, Mearin ML, Papadopoulou A, Ruemmele FM, Vandenplas Y, Koletzko S. A practical guide for the diagnosis of primary enteric nervous system disorders. *J Pediatr Gastroenterol Nutr* 2013; **57**: 677-686 [PMID: 24177787 DOI: 10.1097/MPG.0b013e3182a8bb50]

3 **Meier-Ruge W**. [Casuistic of colon disorder with symptoms of Hirschsprung's disease (author's transl)]. *Verh Dtsch Ges Pathol* 1971; **55**: 506-510 [PMID: 4130757]

4 **Friedmacher F**, Puri P. Classification and diagnostic criteria of variants of Hirschsprung's disease. *Pediatr Surg Int* 2013; **29**: 855-872 [PMID: 23943250 DOI: 10.1007/s00383-013-3351-3]

5 **Meier-Ruge WA**, Bruder E, Kapur RP. Intestinal neuronal dysplasia type B: one giant ganglion is not good enough. *Pediatr Dev Pathol* 2006; **9**: 444-452 [PMID: 17163795 DOI: 10.2350/06-06-0109.1]

6 **Holschneider AM**, Puri P, Homrighausen LH, Meier-Ruge W. Intestinal Neuronal Malformation (IND): Clinical Experience and Treatment. In: Holschneider AM, Puri P. Hirschsprung's disease and allied disorders. Berlin Heidelberg New York: Springer, 2008: 229-251 [DOI: 10.1007/978-3-540-33935-9_17]

7 **Nezelof C**, Guy-Grand D, Thomine E. [Megacolon with hyperplasia of the myenteric plexua. An anatomo-clinical entity, apropos of 3 cases]. *Presse Med* 1970; **78**: 1501-1506 [PMID: 5429378]

8 **Puri P**, Lake BD, Nixon HH, Mishalany H, Claireaux AE. Neuronal colonic dysplasia: an unusual association of Hirschsprung's disease. *J Pediatr Surg* 1977; **12**: 681-685 [PMID: 915657 DOI: 10.1016/0022-3468(77)90393-1]

9 **Fadda B**, Maier WA, Meier-Ruge W, Schärli A, Daum R. [Neuronal intestinal dysplasia. Critical 10-years' analysis of clinical and biopsy diagnosis]. *Z Kinderchir* 1983; **38**: 305-311 [PMID: 6649903 DOI: 10.1055/s-2008-1059994]

10 **Borchard F**, Meier-Ruge W, Wiebecke B, Briner J, Müntefering H, Födisch HF, Holschneider AM, Schmidt A, Enck P, Stolte M. [Disorders of the innervation of the large intestine--classification and diagnosis. Results of a consensus conference of the Society of Gastroenteropathology 1 December 1990 in Frankfurt/Main]. *Pathologe* 1991; **12**: 171-174 [PMID: 1876586]

11 **Schärli AF**. [Intestinal neuronal dysplasia]. *Cir Pediatr* 1992; **5**: 64-65 [PMID: 1503859 DOI: 10.1007/BF00180993]

12 **Koletzko S**, Ballauff A, Hadziselimovic F, Enck P. Is histological diagnosis of neuronal intestinal dysplasia related to clinical and manometric findings in constipated children? Results of a pilot study. *J Pediatr Gastroenterol Nutr* 1993; **17**: 59-65 [PMID: 8350212 DOI: 10.1097/00005176-199307000-00008]

13 **Sacher P**, Briner J, Hanimann B. Is neuronal intestinal dysplasia (NID) a primary disease or a secondary phenomenon? *Eur J Pediatr Surg* 1993; **3**: 228-230 [PMID: 8218074 DOI: 10.1055/s-2008-1063549]

14 **Costa M**, Fava M, Seri M, Cusano R, Sancandi M, Forabosco P, Lerone M, Martucciello G, Romeo G, Ceccherini I. Evaluation of the HOX11L1 gene as a candidate for congenital disorders of intestinal innervation. *J Med Genet* 2000; **37**: E9 [PMID: 10882761 DOI: 10.1136/jmg.37.7.e9]

15 **Martucciello G**, Torre M, Pini Prato A, Lerone M, Campus R, Leggio S, Jasonni V. Associated anomalies in intestinal neuronal dysplasia. *J Pediatr Surg* 2002; **37**: 219-223 [PMID: 11819202 DOI: 10.1053/jpsu.2002.30258]

16 **Meier-Ruge WA**, Brönnimann PB, Gambazzi F, Schmid PC, Schmidt CP, Stoss F. Histopathological criteria for intestinal neuronal dysplasia of the submucosal plexus (type B) *Virchows Arch* 1995; **426**: 549-556 [PMID: 7655734 DOI: 10.1007/BF00192108]

17 **Meier-Ruge WA**, Schmidt PC, Stoss FS. Intestinal neuronal dysplasia and its morphometric evidences. *Pediatr Surg Int* 1995: **10**: 447-453 [DOI: 10.1007/BF00176385]

18 **Schofield DE**, Yunis EJ. Intestinal neuronal dysplasia. *J Pediatr Gastroenterol Nutr* 1991; **12**: 182-189 [PMID: 2051270 DOI: 10.1097/00005176-199102000-00008]

19 **Moore SW**, Laing D, Kaschula RO, Cywes S. A histological grading system for the evaluation of co-existing NID with Hirschsprung's disease. *Eur J Pediatr Surg* 1994; **4**: 293-297 [PMID: 7857886 DOI: 10.1055/s-2008-1066120]

20 **Kobayashi H**, Hirakawa H, Puri P. What are the diagnostic criteria

for intestinal neuronal dysplasia? *Pediatr Surg Int* 1995; **10**: 459-464 [DOI: 10.1007/BF00176387]
21 **Mahesha V**, Saikia UN, Shubha AV, Rao KL. Intestinal neuronal dysplasia of the myenteric plexus--new entity in humans? *Eur J Pediatr Surg* 2008; **18**: 59-60 [PMID: 18302074 DOI: 10.1055/s-2008-1038324]
22 **Meier-Ruge WA**, Ammann K, Bruder E, Holschneider AM, Schärli AF, Schmittenbecher PP, Stoss F. Updated results on intestinal neuronal dysplasia (IND B). *Eur J Pediatr Surg* 2004; **14**: 384-391 [PMID: 15630639 DOI: 10.1055/s-2004-821120]
23 **de la Torre Mondragón L**, Reyes-Múgica M. R.I.P. for IND B. *Pediatr and Develop Pathol* 2006; **9**: 425-426 [DOI: 10.2350/06-08-0146.1]
24 **Martucciello G**, Pini Prato A, Puri P, Holschneider AM, Meier-Ruge W, Jasonni V, Tovar JA, Grosfeld JL. Controversies concerning diagnostic guidelines for anomalies of the enteric nervous system: a report from the fourth International Symposium on Hirschsprung's disease and related neurocristopathies. *J Pediatr Surg* 2005; **40**: 1527-1531 [PMID: 16226977 DOI: 10.1016/j.jpedsurg.2005.07.053]
25 **Aydın H**, Şenaylı A, Mehmet Kışlal F, Sarıcı D, Köseoğlu B, Güreşçi S. Coexistence of Anal Atresia, Anophthalmia and Intestinal Neuronal Dysplasia Type-A in a Newborn. *J Neonatal Surg* 2015; **4**: 44 [PMID: 26500854]
26 **Meier-Ruge WA**, Bruder E. Different colon diseases with chronic constipation. In: Meier-Ruge WA, Bruder E, editors. Histopathology of Chronic Constipation. Basel Switzerland: Karger, 2005: 3-32
27 **Granero Cendón R**, Millán López A, Moya Jiménez MJ, López Alonso M, De Agustín Asensio JC. [Intestinal neuronal dysplasia: association with digestive malformations]. *Cir Pediatr* 2007; **20**: 166-168 [PMID: 18018745]
28 **Meier-Ruge W**. Epidemiology of congenital innervation defects of the distal colon. *Virchows Arch A Pathol Anat Histopathol* 1992; **420**: 171-177 [PMID: 1549906 DOI: 10.1007/BF02358809]
29 **Milla PJ**, Smith VV. Intestinal neuronal dysplasia. *J Pediatr Gastroenterol Nutr* 1993; **17**: 356-357 [PMID: 8145087 DOI: 10.1097/00005176-199311000-00003]
30 **Martucciello G**, Caffarena PE, Lerone M, Mattioli G, Barabino A, Bisio G, Jasonni V. Neuronal intestinal dysplasia: clinical experience in Italian patients. *Eur J Pediatr Surg* 1994; **4**: 287-292 [PMID: 7857885 DOI: 10.1055/s-2008-1066119]
31 **Puri P**. Intestinal dysganglionosis and other disorders of intestinal motility. In: Coran AG, Adzick NS, Caldamone AA, Krummel TM, Laberge J, Shamberger RC, editors. Pediatric surgery. Philadelphia: Elsevier Inc, 2012: 1279-1287 [DOI: 10.1016/B978-0-323-07255-7.00102-1]
32 **Schimpl G**, Uray E, Ratschek M, Höllwarth ME. Constipation and intestinal neuronal dysplasia type B: a clinical follow-up study. *J Pediatr Gastroenterol Nutr* 2004; **38**: 308-311 [PMID: 15076632 DOI: 10.1097/00005176-200403000-00015]
33 **Taguchi T**, Kobayashi H, Kanamori Y, Segawa O, Yamataka A, Sugiyama M, Iwanaka T, Shimojima N, Kuroda T, Nakazawa A, Oda Y, Miyoshi K, Ieiri S. Isolated intestinal neuronal dysplasia Type B (IND-B) in Japan: results from a nationwide survey. *Pediatr Surg Int* 2014; **30**: 815-822 [PMID: 25052255 DOI: 10.1007/s00383-014-3542-6]
34 **Montedonico S**, Cáceres P, Muñoz N, Yáñez H, Ramírez R, Fadda B. Histochemical staining for intestinal dysganglionosis: over 30 years experience with more than 1,500 biopsies. *Pediatr Surg Int* 2011; **27**: 479-486 [PMID: 21327554 DOI: 10.1007/s00383-010-2849-1]
35 **Swaminathan M**, Oron AP, Chatterjee S, Piper H, Cope-Yokoyama S, Chakravarti A, Kapur RP. Intestinal Neuronal Dysplasia-Like Submucosal Ganglion Cell Hyperplasia at the Proximal Margins of Hirschsprung Disease Resections. *Pediatr Dev Pathol* 2015; **18**: 466-476 [PMID: 26699691 DOI: 10.2350/15-07-1675-OA.1]
36 **Amiel J**, Sproat-Emison E, Garcia-Barcelo M, Lantieri F, Burzynski G, Borrego S, Pelet A, Arnold S, Miao X, Griseri P, Brooks AS, Antinolo G, de Pontual L, Clement-Ziza M, Munnich A, Kashuk C, West K, Wong KK, Lyonnet S, Chakravarti A, Tam PK, Ceccherini I, Hofstra RM, Fernandez R. Hirschsprung disease, associated syndromes and genetics: a review. *J Med Genet* 2008; **45**: 1-14 [PMID: 17965226 DOI: 10.1136/jmg.2007.053959]
37 **Haricharan RN**, Georgeson KE. Hirschsprung disease. *Semin Pediatr Surg* 2008; **17**: 266-275 [PMID: 19019295 DOI: 10.1053/j.sempedsurg.2008.07.005]
38 **Kobayashi H**, Mahomed A, Puri P. Intestinal neuronal dysplasia in twins. *J Pediatr Gastroenterol Nutr* 1996; **22**: 398-401 [PMID: 8732905 DOI: 10.1097/00005176-199605000-00011]
39 **Sánchez-Mejías A**, Fernández RM, Antiñolo G, Borrego S. A new experimental approach is required in the molecular analysis of intestinal neuronal dysplasia type B patients. *Exp Ther Med* 2010; **1**: 999-1003 [PMID: 22993632 DOI: 10.3892/etm.2010.140]
40 **Barone V**, Weber D, Luo Y, Brancolini V, Devoto M, Romeo G. Exclusion of linkage between RET and neuronal intestinal dysplasia type B. *Am J Med Genet* 1996; **62**: 195-198 [PMID: 8882403 DOI: 10.1002/(SICI)1096-8628(19960315)62:2<195::AID-AJMG15>3.0.CO; 2-J]
41 **Gath R**, Goessling A, Keller KM, Koletzko S, Coerdt W, Müntefering H, Wirth S, Hofstra RM, Mulligan L, Eng C, von Deimling A. Analysis of the RET, GDNF, EDN3, and EDNRB genes in patients with intestinal neuronal dysplasia and Hirschsprung disease. *Gut* 2001; **48**: 671-675 [PMID: 11302967 DOI: 10.1136/gut.48.5.671]
42 **Fava M**, Borghini S, Cinti R, Cusano R, Seri M, Lerone M, De Giorgio R, Stanghellini V, Martucciello G, Ravazzolo R, Ceccherini I. HOX11L1: a promoter study to evaluate possible expression defects in intestinal motility disorders. *Int J Mol Med* 2002; **10**: 101-106 [PMID: 12060859 DOI: 10.3892/ijmm.10.1.101]
43 **Tou JF**, Li MJ, Guan T, Li JC, Zhu XK, Feng ZG. Mutation of RET proto-oncogene in Hirschsprung's disease and intestinal neuronal dysplasia. *World J Gastroenterol* 2006; **12**: 1136-1139 [PMID: 16534860 DOI: 10.3748/wjg.v12.i7.1136]
44 **Borghini S**, Duca MD, Pini Prato A, Lerone M, Martucciello G, Jasonni V, Ravazzolo R, Ceccherini I. Search for pathogenetic variants of the SPRY2 gene in intestinal innervation defects. *Intern Med J* 2009; **39**: 335-337 [PMID: 19545245 DOI: 10.1111/j.1445-5994.2009.01907.x]
45 **Fernández RM**, Sánchez-Mejías A, Ruiz-Ferrer MM, López-Alonso M, Antiñolo G, Borrego S. Is the RET proto-oncogene involved in the pathogenesis of intestinal neuronal dysplasia type B? *Mol Med Rep* 2009; **2**: 265-270 [PMID: 21475823 DOI: 10.3892/mmr_00000094]
46 **Rintala R**, Rapola J, Louhimo I. Neuronal intestinal dysplasia. *Prog Pediatr Surg* 1989; **24**: 186-192 [PMID: 2513603 DOI: 10.1007/978-3-642-74493-8_20]
47 **Ure BM**, Holschneider AM, Meier-Ruge W. Neuronal intestinal malformations: a retro- and prospective study on 203 patients. *Eur J Pediatr Surg* 1994; **4**: 279-286 [PMID: 7857884 DOI: 10.1055/s-2008-1066118]
48 **Van Der Werf CS**, Wabbersen TD, Hsiao NH, Paredes J, Etchevers HC, Kroisel PM, Tibboel D, Babarit C, Schreiber RA, Hoffenberg EJ, Vekemans M, Zeder SL, Ceccherini I, Lyonnet S, Ribeiro AS, Seruca R, Te Meerman GJ, van Ijzendoorn SC, Shepherd IT, Verheij JB, Hofstra RM. CLMP is required for intestinal development, and loss-of-function mutations cause congenital short-bowel syndrome. *Gastroenterology* 2012; **142**: 453-462.e3 [PMID: 22155368 DOI: 10.1053/j.gastro.2011.11.038]
49 **Hatano M**, Aoki T, Dezawa M, Yusa S, Iitsuka Y, Koseki H, Taniguchi M, Tokuhisa T. A novel pathogenesis of megacolon in Ncx/Hox11L.1 deficient mice. *J Clin Invest* 1997; **100**: 795-801 [PMID: 9259577 DOI: 10.1172/JCI119593]
50 **Shirasawa S**, Yunker AM, Roth KA, Brown GA, Horning S, Korsmeyer SJ. Enx (Hox11L1)-deficient mice develop myenteric neuronal hyperplasia and megacolon. *Nat Med* 1997; **3**: 646-650 [PMID: 9176491 DOI: 10.1038/nm0697-646]
51 **Yamataka A**, Hatano M, Kobayashi H, Wang K, Miyahara K, Sueyoshi N, Miyano T. Intestinal neuronal dysplasia-like pathology in Ncx/Hox11L.1 gene-deficient mice. *J Pediatr Surg* 2001; **36**: 1293-1296 [PMID: 11479879 DOI: 10.1053/jpsu.2001.25797]
52 **Yanai T**, Kobayashi H, Yamataka A, Lane GJ, Miyano T, Hayakawa T, Satoh K, Kase Y, Hatano M. Acetylcholine-related bowel dysmotility in homozygous mutant NCX/HOX11L.1-deficient

(NCX-/-) mice-evidence that acetylcholine is implicated in causing intestinal neuronal dysplasia. *J Pediatr Surg* 2004; **39**: 927-930 [PMID: 15185227 DOI: 10.1016/j.jpedsurg.2004.02.004]
53 **Holland-Cunz S**, Krammer HJ, Süss A, Tafazzoli K, Wedel T. Molecular genetics of colorectal motility disorders. *Eur J Pediatr Surg* 2003; **13**: 146-151 [PMID: 12939697 DOI: 10.1055/s-2003-41767]
54 **Berger S**, Ziebell P, OFFsler M, Hofmann-von Kap-herr S. Congenital malformations and perinatal morbidity associated with intestinal neuronal dysplasia. *Pediatr Surg Int* 1998; **13**: 474-479 [PMID: 9716673 DOI: 10.1016/S0022-3468(99)90801-1]
55 **Corduk N**, Koltuksuz U, Bir F, Karabul M, Herek O, Sarioglu-Buke A. Association of rare intestinal malformations: colonic atresia and intestinal neuronal dysplasia. *Adv Ther* 2007; **24**: 1254-1259 [PMID: 18165207 DOI: 10.1007/BF02877771]
56 **Holschneider AM**, Pfrommer W, Gerresheim B. Results in the treatment of anorectal malformations with special regard to the histology of the rectal pouch. *Eur J Pediatr Surg* 1994; **4**: 303-309 [PMID: 7857888 DOI: 10.1055/s-2008-1066122]
57 **Csury L**, Peña A. Intestinal neuronal dysplasia. *Pediatr Surg Int* 1995; **10**: 441-446 [DOI: 10.1007/BF00176384]
58 **Lumb PD**, Moore L. Are giant ganglia a reliable marker of intestinal neuronal dysplasia type B (IND B)? *Virchows Arch* 1998; **432**: 103-106 [PMID: 9504853 DOI: 10.1007/s004280050141]
59 **Zorenkov D**, Otto S, Böttner M, Hedderich J, Vollrath O, Ritz JP, Buhr H, Wedel T. Morphological alterations of the enteric nervous system in young male patients with rectal prolapse. *Int J Colorectal Dis* 2011; **26**: 1483-1491 [PMID: 21800050 DOI: 10.1007/s00384-011-1282-9]
60 **Pickard LR**, Santoro S, Wyllie RG, Haller JA. Histochemical studies of experimental fetal intestinal obstruction. *J Pediatr Surg* 1981; **16**: 256-260 [PMID: 6454775 DOI: 10.1016/S0022-3468(81)80675-6]
61 **Moore SW**, Laing D, Melis J, Cywes S. Secondary effects of prolonged intestinal obstruction on the enteric nervous system in the rat. *J Pediatr Surg* 1993; **28**: 1196-1199 [PMID: 7905922 DOI: 10.1016/0022-3468(93)90164-G]
62 **Gálvez Y**, Skába R, Vajtrová R, Frantlová A, Herget J. Evidence of secondary neuronal intestinal dysplasia in a rat model of chronic intestinal obstruction. *J Invest Surg* 2004; **17**: 31-39 [PMID: 14761826 DOI: 10.1080/08941930490269628]
63 **Briner J**, Oswald HW, Hirsig J, Lehner M. Neuronal intestinal dysplasia--clinical and histochemical findings and its association with Hirschsprung's disease. *Z Kinderchir* 1986; **41**: 282-286 [PMID: 3788295 DOI: 10.1055/s-2008-1043360]
64 **Berry CL**. Intestinal neuronal dysplasia: does it exist or has it been invented? *Virchows Arch A Pathol Anat Histopathol* 1993; **422**: 183-184 [PMID: 8493774 DOI: 10.1007/BF01621800]
65 **Schmittenbecher PP**, Sacher P, Cholewa D, Haberlik A, Menardi G, Moczulski J, Rumlova E, Schuppert W, Ure B. Hirschsprung's disease and intestinal neuronal dysplasia--a frequent association with implications for the postoperative course. *Pediatr Surg Int* 1999; **15**: 553-558 [PMID: 10631732 DOI: 10.1007/s003830050669]
66 **Kobayashi H**, Yamataka A, Lane GJ, Miyano T. Inflammatory changes secondary to postoperative complications of Hirschsprung's disease as a cause of histopathologic changes typical of intestinal neuronal dysplasia. *J Pediatr Surg* 2004; **39**: 152-156 [PMID: 14966730 DOI: 10.1016/j.jpedsurg.2003.10.008]
67 **Cord-Udy CL**, Smith VV, Ahmed S, Risdon RA, Milla PJ. An evaluation of the role of suction rectal biopsy in the diagnosis of intestinal neuronal dysplasia. *J Pediatr Gastroenterol Nutr* 1997; **24**: 1-6; discussion 7-8 [PMID: 9093979 DOI: 10.1097/00005176-199701000-00002]
68 **Koletzko S**, Jesch I, Faus-Kebetaler T, Briner J, Meier-Ruge W, Müntefering H, Coerdt W, Wessel L, Keller KM, Nützenadel W, Schmittenbecher P, Holschneider A, Sacher P. Rectal biopsy for diagnosis of intestinal neuronal dysplasia in children: a prospective multicentre study on interobserver variation and clinical outcome. *Gut* 1999; **44**: 853-861 [PMID: 10323889 DOI: 10.1136/gut.44.6.853]
69 **Coerdt W**, Michel JS, Rippin G, Kletzki S, Gerein V, Müntefering H, Arnemann J. Quantitative morphometric analysis of the submucous plexus in age-related control groups. *Virchows Arch* 2004; **444**: 239-246 [PMID: 14749927]
70 **Hyman PE**, Milla PJ, Benninga MA, Davidson GP, Fleisher DF, Taminiau J. Childhood functional gastrointestinal disorders: neonate/toddler. *Gastroenterology* 2006; **130**: 1519-1526 [PMID: 16678565 DOI: 10.1053/j.gastro.2005.11.065]
71 **Rasquin A**, Di Lorenzo C, Forbes D, Guiraldes E, Hyams JS, Staiano A, Walker LS. Childhood functional gastrointestinal disorders: child/adolescent. *Gastroenterology* 2006; **130**: 1527-1537 [PMID: 16678566 DOI: 10.1053/j.gastro.2005.08.063]
72 **Tabbers MM**, DiLorenzo C, Berger MY, Faure C, Langendam MW, Nurko S, Staiano A, Vandenplas Y, Benninga MA. Evaluation and treatment of functional constipation in infants and children: evidence-based recommendations from ESPGHAN and NASPGHAN. *J Pediatr Gastroenterol Nutr* 2014; **58**: 258-274 [PMID: 24345831 DOI: 10.1097/MPG.0000000000000266]
73 **Pini-Prato A**, Avanzini S, Gentilino V, Martucciello G, Mattioli G, Coccia C, Parodi S, Bisio GM, Jasonni V. Rectal suction biopsy in the workup of childhood chronic constipation: indications and diagnostic value. *Pediatr Surg Int* 2007; **23**: 117-122 [PMID: 17171377 DOI: 10.1007/s00383-006-1845-y]
74 **Hirayama Y**, Iinuma Y, Numano F, Masui D, Iida H, Komatsuzaki N, Nagayama Y, Naito S, Nitta K. Intestinal neuronal dysplasia-like histopathology in infancy. *Pediatr Int* 2015; **57**: 491-493 [PMID: 25711721 DOI: 10.1111/ped.12555]
75 **Schmittenbecher PP**, Glück M, Wiebecke B, Meier-Ruge W. Clinical long-term follow-up results in intestinal neuronal dysplasia (IND). *Eur J Pediatr Surg* 2000; **10**: 17-22 [PMID: 10770242 DOI: 10.1055/s-2008-1072317]
76 **Gillick J**, Tazawa H, Puri P. Intestinal neuronal dysplasia: results of treatment in 33 patients. *J Pediatr Surg* 2001; **36**: 777-779 [PMID: 11329588 DOI: 10.1053/jpsu.2001.22959]
77 **Mattioli G**, Castagnetti M, Martucciello G, Jasonni V. Results of a mechanical Duhamel pull-through for the treatment of Hirschsprung's disease and intestinal neuronal dysplasia. *J Pediatr Surg* 2004; **39**: 1349-1355 [PMID: 15359389 DOI: 10.1016/j.jpedsurg.2004.05.007]
78 **Sacher P**, Briner J, Stauffer UG. Unusual cases of neuronal intestinal dysplasia. *Pediatr Surg Int* 1991; **6**: 225-226 [DOI: 10.1007/BF00176076]
79 **Montedonico S**, Acevedo S, Fadda B. Clinical aspects of intestinal neuronal dysplasia. *J Pediatr Surg* 2002; **37**: 1772-1774 [PMID: 12483654 DOI: 10.1053/jpsu.2002.36720]
80 **Peng SS**, Lee WT, Hsui WM. An unusual cause of abdominal distension in a 4-year-old boy. Neuronal intestinal dysplasia. *Gastroenterology* 2011; **141**: e3-e4 [PMID: 21878329 DOI: 10.1053/j.gastro.2010.06.083]
81 **Jáquez-Quintana JO**, González-González JA, Arana-Guajardo AC, Larralde-Contreras L, Flores-Gutiérrez JP, Maldonado-Garza HJ. Sigmoid volvulus as a presentation of neuronal intestinal dysplasia type B in an adolescent. *Rev Esp Enferm Dig* 2013; **105**: 178-179 [PMID: 23735030 DOI: 10.4321/S1130-01082013000300014]
82 **Voderholzer WA**, Wiebecke B, Gerum M, Müller-Lissner SA. Dysplasia of the submucous nerve plexus in slow-transit constipation of adults. *Eur J Gastroenterol Hepatol* 2000; **12**: 755-759 [PMID: 10929902 DOI: 10.1097/00042737-200012070-00007]
83 **Vougas V**, Vardas K, Christou C, Papadimitriou G, Florou E, Magkou C, Karamanolis D, Manganas D, Drakopoulos S. Intestinal neuronal dysplasia type B in adults: a controversial entity. *Case Rep Gastroenterol* 2014; **8**: 7-12 [PMID: 24574943 DOI: 10.1159/000358045]
84 **Vijayaraghavan R**, Chandrashekar R, Melkote Jyotiprakash A, Kumar R, Rashmi MV, Shanmukhappa Belagavi C. Intestinal neuronal dysplasia (type B) causing fatal small bowel ischaemia in an adult: a case report. *Eur J Gastroenterol Hepatol* 2006; **18**: 773-776 [PMID: 16772835 DOI: 10.1097/01.meg.0000216933.14490.aa]
85 **López Sanclemente MC**, Castellvi J, Ortiz de Zárate L, Barrios P. Intestinal neuronal dysplasia in a patient with chronic colonic pseudo-obstruction. *Cir Esp* 2014; **92**: e59 [PMID: 25091184 DOI: 10.1016/j.ciresp.2014.06.008]
86 **Junquera Bañares S**, Oria Mundín E, Córdoba Iturriagagoitia A, Botella-Carretero JJ. [Chronic intestinal pseudo-obstruction due to

intestinal neuronal dysplasia type B (IND B), concerning one case]. *An Sist Sanit Navar* 2014; **37**: 157-164 [PMID: 24871124]

87 **Wu XJ**, Zhang HY, Li N, Yan MS, Wei J, Yu DH, Feng JX. A new diagnostic scoring system to differentiate Hirschsprung's disease from Hirschsprung's disease-allied disorders in patients with suspected intestinal dysganglionosis. *Int J Colorectal Dis* 2013; **28**: 689-696 [PMID: 23568714 DOI: 10.1007/s00384-013-1691-z]

88 **de Lorijn F**, Kremer LC, Reitsma JB, Benninga MA. Diagnostic tests in Hirschsprung disease: a systematic review. *J Pediatr Gastroenterol Nutr* 2006; **42**: 496-505 [PMID: 16707970 DOI: 10.1097/01.mpg.0000214164.90939.92]

89 **Gil-Vernet JM**, Broto J, Guillén G. [Hirschsprung-neurointestinal dyspasia: diferential diagnosis and reliability of diagnostic procedures]. *Cir Pediatr* 2006; **19**: 91-94 [PMID: 16846131]

90 **Meier-Ruge WA**. Histological diagnosis and differential diagnosis of Hirschsprung's disease. In: Holschneider AM, Puri P. Hirschsprung's disease and allied disorders. London: Harwood, 1999: 252-265

91 **Munakata K**, Morita K, Okabe I, Sueoka H. Clinical and histologic studies of neuronal intestinal dysplasia. *J Pediatr Surg* 1985; **20**: 231-235 [PMID: 3891955 DOI: 10.1016/S0022-3468(85)80109-3]

92 **Tang ST**, Yang Y, Wang GB, Tong QS, Mao YZ, Wang Y, Li SW, Ruan QL. Laparoscopic extensive colectomy with transanal Soave pull-through for intestinal neuronal dysplasia in 17 children. *World J Pediatr* 2010; **6**: 50-54 [PMID: 20143211 DOI: 10.1007/s12519-010-0006-5]

93 **Bruder E**, Meier-Ruge WA. [Intestinal neuronal dysplasia type B: how do we understand it today?]. *Pathologe* 2007; **28**: 137-142 [PMID: 17279410 DOI: 10.1007/s00292-007-0903-0]

P- Reviewer: El-Shabrawi MH, Li YY, Vermorken A **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i3.406

World J Gastrointest Pharmacol Ther 2016 August 6; 7(3): 406-411
ISSN 2150-5349 (online)

MINIREVIEWS

Clinical significance and management of Barrett's esophagus with epithelial changes indefinite for dysplasia

Prashanthi N Thota, Gaurav Kistangari, Ashwini K Esnakula, David Hernandez Gonzalo, Xiu-Li Liu

Prashanthi N Thota, Gaurav Kistangari, Department of Gastroenterology, Digestive Disease Institute, Cleveland Clinic, Cleveland, OH 44195, United States

Ashwini K Esnakula, David Hernandez Gonzalo, Xiu-Li Liu, Department of Pathology, Immunology and Laboratory Medicine, University of Florida College of Medicine, Gainesville, FL 32610-0275, United States

Author contributions: All authors have contributed to conception and design of the study, data acquisition and interpretation and drafting and critical revision of the manuscript; all authors approve the final manuscript.

Conflict-of-interest statement: No potential conflicts of interest. No financial support.

Manuscript source: Invited manuscript

Correspondence to: Xiu-Li Liu, MD, PhD, Department of Pathology, Immunology and Laboratory Medicine, University of Florida College of Medicine, P.O. Box 100275, 1600 SW Archer Road, Gainesville, FL 32610-0275, United States. xiuliliu@ufl.edu
Telephone: +1-352-6279257
Fax: +1-352-6279242

Received: April 18, 2016
Peer-review started: April 18, 2016
First decision: May 17, 2016
Revised: May 27, 2016
Accepted: June 27, 2016
Article in press: June 29, 2016
Published online: August 6, 2016

Abstract

Barrett's esophagus (BE) is defined as the extension of salmon-colored mucosa into the tubular esophagus ⩾ 1 cm proximal to the gastroesophageal junction with biopsy confirmation of intestinal metaplasia. Patients with BE are at increased risk of esophageal adenocarcinoma (EAC), and undergo endoscopic surveillance biopsies to detect dysplasia or early EAC. Dysplasia in BE is classified as no dysplasia, indefinite for dysplasia (IND), low grade dysplasia (LGD) or high grade dysplasia (HGD). Biopsies are diagnosed as IND when the epithelial abnormalities are not sufficient to diagnose dysplasia or the nature of the epithelial abnormalities is uncertain due to inflammation or technical issues. Specific diagnostic criteria for IND are not well established and its clinical significance and management has not been well studied. Previous studies have focused on HGD in BE and led to changes and improvement in the management of BE with HGD and early EAC. Only recently, IND and LGD in BE have become focus of intense study. This review summarizes the definition, neoplastic risk and clinical management of BE IND.

Key words: Barrett's esophagus; Dysplasia; Progression; Biomarkers; Esophageal adenocarcinoma; Indefinite for dysplasia

Core tip: Barrett's esophagus (BE) with indefinite for dysplasia (IND) is diagnosed when the epithelial abnormalities are not sufficient to diagnose dysplasia or the nature of the epithelial abnormalities is uncertain due to inflammation. The risk of prevalent neoplasia in BE with IND varies between 1.9% and 15%. The progression to advanced neoplasia reported varies from 0.43 to 1.2 cases per 100 person-years at risk. Predictors such as the length of BE segment, multi-focality of BE IND, age > 60

years, abnormal p53 expression, active inflammation, and abnormal DNA content as detected by flow cytometry may help in risk-stratifying this patient population.

Thota PN, Kistangari G, Esnakula AK, Gonzalo DH, Liu XL. Clinical significance and management of Barrett's esophagus with epithelial changes indefinite for dysplasia. *World J Gastrointest Pharmacol Ther* 2016; 7(3): 406-411 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i3/406.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i3.406

INTRODUCTION

Barrett's esophagus (BE) is a complication of chronic esophageal injury from gastroesophageal reflux disease (GERD) and develops when reflux damaged esophageal squamous cells are replaced by mucous-secreting columnar cells. A definitive diagnosis of BE is established by the extension of salmon-colored mucosa into the tubular esophagus $\geqslant$ 1 cm proximal to the gastroesophageal junction (GEJ) with esophageal biopsy showing intestinal metaplasia, defined by the presence of goblet cells[1]. Intestinal metaplasia in BE is a well-established marker of esophageal adenocarcinoma (EAC), and as such patients diagnosed with BE undergo regular endoscopic surveillance and biopsy to detect dysplasia or curable neoplasia. According to the published criteria by Reid *et al*[2] the biopsies are classified based on five-tiered histologic classification of dysplasia as negative for dysplasia, indefinite for dysplasia (IND), low-grade dysplasia (LGD), highgrade dysplasia (HGD) and intramucosal adenocarcinoma (IMAC).

Dysplasia remains the best available clinical marker for cancer risk. Published guidelines have recommended endoscopic surveillance and treatment strategies based on the grade of dysplasia. The management of LGD and HGD in BE has been reviewed extensively and discussed in many published guidelines. Many studies have focused on the high end of neoplasia in BE, HGD and IMAC, leading to a much improved and less invasive management[3-5]. However, there is a paucity of data to guide the management of BE patients with IND. Besides, due to lack of definitive criteria for diagnosis, and greatest inter-observer variability, and uncertain clinical significance, natural history of progression of BE with IND and management are not clear.

This paper discusses the current literature and examines available evidence for the histologic criteria for diagnosis, its clinical significance, prevalence and risk of progression to cancer, and also the clinicopathologic and biomarker predictors that are associated with dysplasia progression among patients diagnosed with BE with IND. PubMed search was performed for the term "Barrett's esophagus indefinite for dysplasia" as of November 1, 2015 and studies were reviewed for prevalence and incidence rates of HGD/EAC in BE IND as well as predictors for progression in IND. One study shared part of the same database and was excluded[6].

DIAGNOSIS OF BE WITH IND

The diagnosis "indefinite for dysplasia" is used when the biopsy findings are too marked for being negative, but not absolutely sufficient for the presence of dysplasia. The background regenerative changes may be related to inflammation or ulceration and may overlap with LGD that often makes it difficult to differentiate from true dysplasia. Less commonly technical factors related to biopsy specimen handling such as biopsy crushing artifact, thick tissue sectioning, marked thermal artifact and tangential embedding and sectioning also prevents accurate diagnosis of dysplasia and are categorized as BE with IND; In certain circumstances pathologists unaccustomed to certain types of fixatives, for example, Hollande's and Bouin fixatives that results in vesicular nucleus and prominent nucleolus, may overinterpret the changes as indicative of BE with IND[7]. Rarely, the diagnosis of IND may be due to the dysplasia like changes present only in the bases of the crypts, also called "basal crypt dysplasia-like atypia", where the surface epithelium may not be involved[8].

BE IND is diagnostically challenging and it is clear that its diagnostic reproducibility is poor[7,9,10]. Histologic criteria used to diagnose BE IND varied in different studies (Table 1) and even more so by pathologists in routine practice. For instance, the criteria for IND described by Reid *et al*[2] included moderate architectural distortion, nuclear abnormalities less marked than those seen in dysplasia, frequent dystrophic goblet cells, more extensive nuclear stratification, diminished or absent mucus production, increased cytoplasmic basophilia, and increased mitoses (Figure 1A). The diagnosis of IND should be limited to cases in which the changes are worrisome but not sufficient for the diagnosis of dysplasia (Figure 1B). Using similar criteria, other groups performed intraobserver and interobserver reproducibility studies and found that BE IND has significant interobserver variability[7,11]. In daily pathology practice, the BE IND category appears to expand, one such example being basal crypt dysplasia-like atypia. The concept of basal crypt dysplasia-like atypia remains controversial and is interpreted by some groups as IND while others believe that it truly represents dysplasia without surface involvement.

NEOPLASTIC RISK OF BE IND

Regardless of the definition, illustration, and intraobserver /interobserver variability, BE IND category is not uncommonly used in daily pathology practice. Several studies recently investigated the clinical significance of BE IND and the results are reviewed and summarized in Tables 2 and 3.

RISK OF PREVALENT NEOPLASIA IN BE IND

Only few studies investigated the risk of neoplasia in BE IND. Prevalent neoplasia risk, defined as LGD, HGD or EAC detected within 1 year of the diagnosis of BE IND,

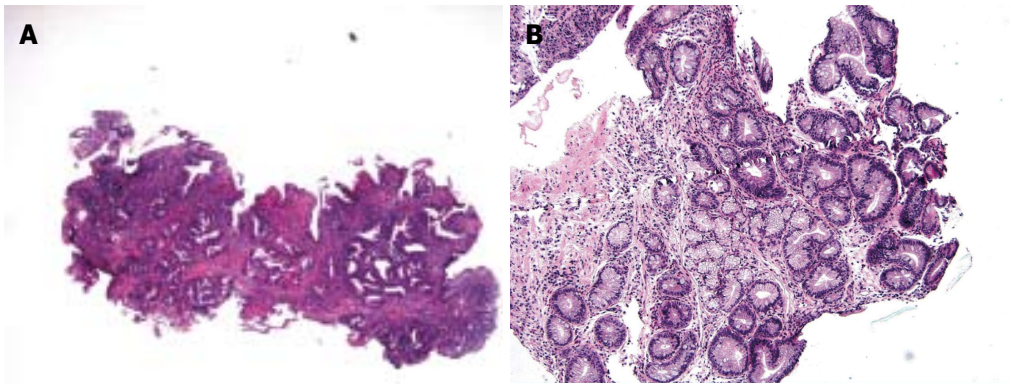

Figure 1 Examples of Barrett's esophagus with epithelial changes, indefinite for dysplasia. A: This esophageal biopsy shows inflamed BE with moderate architectural complexity, more extensive nuclear stratification, diminished or absent mucus production, increased cytoplasmic basophilia, resembling low-grade dysplasia, but there is presence of marked inflammation (HE stain, × 40). This biopsy is best interpreted as indefinite for dysplasia; B: This tangentially sectioned esophageal biopsy shows foci of glands with enlarged and hyperchromatic nuclei (HE stain, × 100). Because of the lack of surface epithelium as a result of tangential section, this biopsy is best interpreted as indefinite for dysplasia. BE: Barrett's esophagus.

Table 1 Histopathologic criteria for Barrett's esophagus with epithelial change indefinite for dysplasia

Ref.	Criteria
Reid *et al*[2], 1988; Montgomery *et al*[7], 2001	The architecture may be moderately distorted. Nuclear abnormalities are less marked than those seen in dysplasia. Other features that may lead to a diagnosis of IND include more numerous dystrophic goblet cells, more extensive nuclear stratification, diminished or absent mucus production, increased cytoplasmic basophilia, and increased mitoses
Sonwalkar *et al*[9], 2010	Preserved gland architecture, mild crypt distortion, minimal nuclear stratification and slight nuclear atypia or enlargement
Kestens *et al*[15], 2015	When a diagnosis of genuine dysplasia cannot be made. This is often due to the co-occurrence of inflammatory changes or when evaluation of surface maturation is not possible
Sinh *et al*[16], 2015	Cytologic changes similar to those seen in LGD but with surface maturation or presence of inflammation
Duits *et al*[13], 2015	Downgraded from BE LGD to BE IND by an expert pathology panel
Horvath *et al*[12], 2015	The presence of architectural and cytologic atypia in small and mal-oriented biopsy specimen or those with inflammation or ulceration exceeding those expected for reactive changes. In some cases, it is due to basal dysplasia with surface maturation

BE: Barrett's esophagus; BE IND: Barrett's esophagus with epithelial change indefinite for dysplasia; LGD: Low-grade dysplasia.

Table 2 Risk of Prevalent neoplasia in patients with Barrett's esophagus with epithelial change indefinite for dysplasia

Ref.	Number of cases	Prevalent LGD, *n* (%)	Prevalent HGD, *n* (%)	Prevalent adenocarcinoma *n* (%)	Prevalent advanced neoplasia
Montgomery *et al*[11], 2001	7	0 (0)	0 (0)	1 (15)	At least 1 (15)
Sonwalkar *et al*[9], 2010	41	At least 1 (2.4)	0 (0)	At least 1 (2.4)	At least 1 (2.4)
Choi *et al*[14], 2015	96	At least 14 (14.5)	Not known	Not known	At least 10 (10)
Horvath *et al*[12], 2015	107	7 (8.2)	2 (2.35)	2 (2.35)	4 (4.7)
Kestens *et al*[15], 2015	842	101 (12.1)	Not known	Not known	16 (1.9)
Sinh *et al*[16], 2015	83	Not known	0 (0)	0 (0)	0 (0)

LGD: Low-grade dysplasia; HGD: High-grade dysplasia.

was reported in 3 studies and ranged from 12.9% to 25%. Prevalence of advanced neoplasia, *i.e.*, detection of HGD or EAC within 1 year of the diagnosis of BE IND, varied between 1.9% and 15%[9,11,12,14,15]. When a 6-mo interval was used as a cut-off, the prevalence of LGD and advanced neoplasia in BE IND was at least 2.8%[9]. In one case, the mucosal ulceration was associated with EAC[11].

RISK OF INCIDENT NEOPLASIA IN BE IND

The incidence of neoplasia in BE IND is summarized in Table 3. The incidence of all neoplasia in BE-IND is reported to be 4.5 cases per 100 person-years at risk. The progression to advanced neoplasia was 0.43 to 1.2 cases per 100 person-years at risk. The progression to EAC varied between 0.18 to 1.10 cases per 100 person-years at risk. In a study of 82 patients with BE IND, the mean length of BE segment was 6 cm in progressors *vs* 3 cm in non progressors (P = 0.01). The length of BE segment (HR = 1.2, 1.03-1.3) and multi-focality of BE IND (HR = 2.9, 1.09-7.6) were significantly associated with a higher risk of progression[12]. One study examined the progression to advanced neoplasia

Table 3 Risk of Incident neoplasia in patients with Barrett's esophagus with epithelial change indefinite for dysplasia

Ref.	No. of cases	Follow up in months (range)	Incident LGD *n* (%)	Incident HGD *n* (%)	Incident adeno carcinoma *n* (%)	Incident advanced neoplasia (per 100 person-years	Risk factors for progression to advanced neoplasia
Duits *et al*[13], 2015	40	Median 31 (16-59)	0	1 (2.5)	0 (0)	0.9	Not done
Horvath *et al*[12], 2015	82	Mean 59 (13-182)	14 (8.3)	3 (2.3)	2 (2.3)	1.2	p53 expression in >5% nuclei
Kestens *et al*[15], 2015	631	Not known	No data	10 (1.6)	6 (1.0)	0.43	Older age
Sinh *et al*[16], 2015	83	Mean 68.4 (SD: 37.2)	No data	3 (3.6)	1 (1.2)	0.86	Not done for BE IND group
Sonwalkar *et al*[9], 2010	37	Median 38.7 (6-122)	3 (8.1)	0 (0)	3 (8.1)	Not done	Expression of AMACR in more than 1% of cells

BE IND: Barrett's esophagus with epithelial change indefinite for dysplasia; LGD: Low-grade dysplasia; HGD: High-grade dysplasia; SD: Standard deviation; AMACR: Alpha-methylacyl-CoA racemase.

Table 4 Guideline recommendations for the management of Barrett's esophagus with epithelial change indefinite for dysplasia

Guidelines	Diagnosis	Treatment and surveillance
ACG guidelines[1]		Acid suppressive medications for 3-6 mo A repeat endoscopy after optimization of should be performed If BE IND, surveillance in 12 mo
BSG guidelines[18]	Review by a second GI pathologist, and the reasons for use of the 'indefinite for dysplasia' category should be given in the histology report in order to aid patient management	Optimisation of antireflux medication Repeat endoscopy in 6 mo If no dysplasia is found, then the surveillance per non-dysplastic Barrett's oesophagus
ASGE[19]	Clarify presence and grade of dysplasia with expert GI pathologist	Increase antisecretory therapy to eliminate esophageal inflammation. Repeat EGD and biopsy to clarify dysplasia status
Australian Guidelines[20]	Confirm by a second pathologist, ideally an expert gastrointestinal pathologist.	Repeat endoscopy in 6 mo with Seattle protocol biopsies for suspected dysplasia (biopsy of any mucosal irregularity and quadrantic biopsies every 1 cm) on maximal acid suppression If repeat shows no dysplasia, then follow as per non-dysplastic protocol If repeat shows low-grade or high-grade dysplasia or adenocarcinoma, then follow protocols for these respective conditions If repeat again shows confirmed indefinite for dysplasia, then repeat endoscopy in 6 mo with Seattle protocol biopsies for suspected dysplasia

BE IND: Barrett's esophagus with epithelial change indefinite for dysplasia.

in a cohort of BE IND (n = 36) which was downgraded from an original diagnosis of BE LGD and reported an advanced neoplasia incidence of 0.9 cases per 100 person-years at risk, similar to a rate of 0.6 cases per 100 person-years at risk in patients with BE negative for dysplasia (n = 153)[13]. In contrast, BE LGD (n = 75) agreed upon by a panel of expert pathologists had an advanced neoplasia incidence of 9.1 cases per 100 person-years at risk[13]. Using 6-mo follow-up as a cutoff, Sonwalkar *et al*[9] (2010) reported that 8.1% of BE IND patients progressed to LGD and 8.1% BE IND progressed to EAC during a median follow up of 38.7 mo (range: 6-122). Interestingly, none of the 6 patients with BE IND progression had a consensus diagnosis of IND by all three reviewing pathologists.

Some studies did not distinguish between incident and prevalent dysplasia in BE IND. In a study by Montgomery *et al*[11] the neoplasia detection rate among patients with BE IND during a median follow-up of 36 mo was 18% where 4 of 22 patients developed carcinoma. In another study, Choi *et al*[14] reported 1-, 2-, and 3-year detection rates of HGD or EAC among patients with BE IND as 10%, 13% and 20%, respectively.

BIOMARKERS FOR RISK STRATIFICATION OF BE IND

Few studies evaluated the role of biomarkers to aid in predicting the progression of dysplasia and/or cancer. In a study of 96 BE IND patients, Choi *et al*[14] identified active inflammation (by histology) and DNA flow cytometric abnormalities (either aneuploidy and/or increased 4N fractions greater than 6% of the nuclei) as significant risk factors associated with subsequent detection of dysplasia or neoplasia (hazard ratio for the combiner marker was 18.8, $P < 0.0001$). Sonwalkar *et al*[9] reported that the expression of alpha-methylacyl-CoA racemase (AMACR) in more than 1% of cells correlated with progression in BE IND. However, this role of AMACR expression in risk stratifying BE IND was not seen in a subsequent study by Horvath *et al*[17] and they instead showed that high expression of p53 (defined as intense staining in > 5% nuclei), later associated with prevalent advanced neoplasia and progression to advanced neoplasia in BE IND.

Clinical management of BE IND

The diagnosis of BE IND is challenging due to varying definitions and inter and intraobserver variability. Therefore, all biopsies should be reviewed by a second pathologist preferably a gastrointestinal pathologist. The patients are treated with aggressive acid suppression. Then, a surveillance endoscopy is performed within 6-12 mo. The biopsy protocol consists of four quadrant biopsies every 1 cm interval. If nondysplastic BE is found, then surveillance interval can be lengthened beyond one year. If LGD or HGD are found, then endoscopic eradication therapy should be considered after confirmation of the diagnosis. The guidelines for management of BE IND are presented by major societies[1,18-20] and are summarized in Table 4.

CONCLUSION

In summary, the diagnosis of BE IND is difficult. Recent studies reveal that BE IND carries a significant risk of prevalent advanced neoplasia (at least 2.8%, 31 out of 1135 patients, ranging from 0% to 15%) (Table 2). In addition, the diagnosis of BE IND is associated with risk of progression to advanced neoplasia (0.43 to 1.2 cases person-years at risk) (Table 3). These figures are similar to the risk of LGD without histology review[16], but much lower than the progression risk in consensus diagnosis of LGD[13]. It is worth bearing in mind that 73% of cases with a diagnosis of BE LGD originally rendered by practicing pathologists were down-graded to BE IND or BE negative for dysplasia by an expert pathology panel[13]. Therefore, cases with initial impression of BE IND or LGD should be reviewed by additional GI pathologists to confirm the diagnosis. Patients with a confirmed diagnosis of BE IND should be placed on intensive acid suppressive therapy and have a surveillance endoscopy with four quadrant biopsies every 1 cm interval in BE segment within one year. BE IND patients with follow-up biopsies which are negative for dysplasia have low risk of neoplasia progression and may be reverted to routine surveillance. The length of BE, multi-focality of BE IND, older age (> 60 years old), abnormal p53 expression, active inflammation, and abnormal DNA content as detected by flow cytometry are useful to risk-stratify this patient population. The role of these predictors in clinical management of patients with BE IND requires further scrutiny.

REFERENCES

1 **Shaheen NJ**, Falk GW, Iyer PG, Gerson LB. ACG Clinical Guideline: Diagnosis and Management of Barrett's Esophagus. *Am J Gastroenterol* 2016; **111**: 30-50; quiz 51 [PMID: 26526079 DOI: 10.1038/ajg.2015.322]

2 **Reid BJ**, Haggitt RC, Rubin CE, Roth G, Surawicz CM, Van Belle G, Lewin K, Weinstein WM, Antonioli DA, Goldman H. Observer variation in the diagnosis of dysplasia in Barrett's esophagus. *Hum Pathol* 1988; **19**: 166-178 [PMID: 3343032]

3 **Nijhawan PK**, Wang KK. Endoscopic mucosal resection for lesions with endoscopic features suggestive of malignancy and high-grade dysplasia within Barrett's esophagus. *Gastrointest Endosc* 2000; **52**: 328-332 [PMID: 10968845]

4 **Li Z**, Rice TW, Liu X, Goldblum JR, Williams SJ, Rybicki LA, Murthy SC, Mason DP, Raymond DP, Blackstone EH. Intramucosal esophageal adenocarcinoma: primum non nocere. *J Thorac Cardiovasc Surg* 2013; **145**: 1519-1524 [PMID: 23158254 DOI: 10.1016/j.jtcvs.2012.10.027]

5 **Li ZG**, Zhu H, Shi H, Xie H, Goldblum JR, Thota PN, Liu X. Lymphovascular invasion and nodal metastasis in intramucosal adenocarcinoma of the esophagus and esophagogastric junction. *J Dig Dis* 2015; **16**: 197-204 [PMID: 25620066 DOI: 10.1111/1751-2980.12230]

6 **Thota PN**, Lee HJ, Goldblum JR, Liu X, Sanaka MR, Gohel T, Kanadiya M, Lopez R. Risk stratification of patients with barrett's esophagus and low-grade dysplasia or indefinite for dysplasia. *Clin Gastroenterol Hepatol* 2015; **13**: 459-465.e1 [PMID: 25102445 DOI: 10.1016/j.cgh.2014.07.049]

7 **Montgomery E**, Bronner MP, Goldblum JR, Greenson JK, Haber MM, Hart J, Lamps LW, Lauwers GY, Lazenby AJ, Lewin DN, Robert ME, Toledano AY, Shyr Y, Washington K. Reproducibility of the diagnosis of dysplasia in Barrett esophagus: a reaffirmation. *Hum Pathol* 2001; **32**: 368-378 [PMID: 11331953]

8 **Lomo LC**, Blount PL, Sanchez CA, Li X, Galipeau PC, Cowan DS, Ayub K, Rabinovitch PS, Reid BJ, Odze RD. Crypt dysplasia with surface maturation: a clinical, pathologic, and molecular study of a Barrett's esophagus cohort. *Am J Surg Pathol* 2006; **30**: 423-435

9 **Sonwalkar SA,** Rotimi O, Scott N, Verghese E, Dixon M, Axon AT, Everett SM. A study of indefinite for dysplasia in Barrett's oesophagus: reproducibility of diagnosis, clinical outcomes and predicting progression with AMACR (alpha-methylacyl-CoA-racemase). *Histopathology* 2010; **56**: 900-907 [PMID: 20636793 DOI: 10.1111/j.1365-2559.2010.03571.x]

10 **Coco DP**, Goldblum JR, Hornick JL, Lauwers GY, Montgomery E, Srivastava A, Wang H, Odze RD. Interobserver variability in the diagnosis of crypt dysplasia in Barrett esophagus. *Am J Surg Pathol* 2011; **35**: 45-54 [PMID: 21164286 DOI: 10.1097/PAS.0b013e3181ffdd14]

11 **Montgomery E**, Goldblum JR, Greenson JK, Haber MM, Lamps LW, Lauwers GY, Lazenby AJ, Lewin DN, Robert ME, Washington K, Zahurak ML, Hart J. Dysplasia as a predictive marker for invasive carcinoma in Barrett esophagus: a follow-up study based on 138 cases from a diagnostic variability study. *Hum Pathol* 2001; **32**: 379-388 [PMID: 11331954]

12 **Horvath B**, Singh P, Xie H, Thota PN, Allende DS, Pai RK, Patil DT, Plesec TP, Goldblum JR, Liu X. Risk for esophageal neoplasia in Barrett's esophagus patients with mucosal changes indefinite for dysplasia. *J Gastroenterol Hepatol* 2015; **30**: 262-267 [PMID: 25087917 DOI: 10.1111/jgh.12696]

13 **Duits LC**, Phoa KN, Curvers WL, Ten Kate FJ, Meijer GA, Seldenrijk CA, Offerhaus GJ, Visser M, Meijer SL, Krishnadath KK, Tijssen JG, Mallant-Hent RC, Bergman JJ. Barrett's oesophagus patients with low-grade dysplasia can be accurately risk-stratified after histological review by an expert pathology panel. *Gut* 2015; **64**: 700-706 [PMID: 25034523 DOI: 10.1136/gutjnl-2014-307278]

14 **Choi WT**, Emond MJ, Rabinovitch PS, Ahn J, Upton MP, Westerhoff M. "Indefinite for Dysplasia" in Barrett's Esophagus: Inflammation and DNA Content Abnormality are Significant Predictors of Early Detection of Neoplasia. *Clin Transl Gastroenterol* 2015; **6**: e81 [PMID: 25761942 DOI: 10.1038/ctg.2015.7]

16 **Sinh P**, Anaparthy R, Young PE, Gaddam S, Thota P, Balasubramanian G, Singh M, Higbee AD, Wani S, Gupta N, Rastogi A, Mathur SC, Bansal A, Horwhat JD, Cash BD, Falk GW, Lieberman DA, Vargo JJ, Sampliner RE, Sharma P. Clinical outcomes in patients with a diagnosis of "indefinite for dysplasia" in Barrett's esophagus: a multicenter cohort study. *Endoscopy* 2015; **47**: 669-674 [PMID: 25910065 DOI: 10.1055/s-0034-1391966]

15 **Kestens C**, Leenders M, Offerhaus GJ, van Baal JW, Siersema PD. Risk of neoplastic progression in Barrett's esophagus diagnosed as indefinite for dysplasia: a nationwide cohort study. *Endoscopy* 2015; **47**: 409-414 [PMID: 25521571 DOI: 10.1055/s-0034-1391091]

17 **Horvath B,** Singh P, Xie H, Thota PN, Sun X, Liu X. Expression of p53 predicts risk of prevalent and incident advanced neoplasia in

patients with Barrett's esophagus and epithelial changes indefinite for dysplasia. *Gastroenterol Rep* (Oxf) 2015; Epub ahead of print [PMID: 26486567 DOI: 10.1093/gastro/gov045]

18 **Fitzgerald RC**, di Pietro M, Ragunath K, Ang Y, Kang JY, Watson P, Trudgill N, Patel P, Kaye PV, Sanders S, O'Donovan M, Bird-Lieberman E, Bhandari P, Jankowski JA, Attwood S, Parsons SL, Loft D, Lagergren J, Moayyedi P, Lyratzopoulos G, de Caestecker J. British Society of Gastroenterology guidelines on the diagnosis and management of Barrett's oesophagus. *Gut* 2014; **63**: 7-42 [PMID: 24165758 DOI: 10.1136/gutjnl-2013-305372]

19 **Evans JA**, Early DS, Fukami N, Ben-Menachem T, Chandrasekhara V, Chathadi KV, Decker GA, Fanelli RD, Fisher DA, Foley KQ, Hwang JH, Jain R, Jue TL, Khan KM, Lightdale J, Malpas PM, Maple JT, Pasha SF, Saltzman JR, Sharaf RN, Shergill A, Dominitz JA, Cash BD. The role of endoscopy in Barrett's esophagus and other premalignant conditions of the esophagus. *Gastrointest Endosc* 2012; **76**: 1087-1094 [PMID: 23164510 DOI: 10.1016/j.gie.2012.08.004]

20 **Whiteman DC**, Appleyard M, Bahin FF, Bobryshev YV, Bourke MJ, Brown I, Chung A, Clouston A, Dickins E, Emery J, Eslick GD, Gordon LG, Grimpen F, Hebbard G, Holliday L, Hourigan LF, Kendall BJ, Lee EY, Levert-Mignon A, Lord RV, Lord SJ, Maule D, Moss A, Norton I, Olver I, Pavey D, Raftopoulos S, Rajendra S, Schoeman M, Singh R, Sitas F, Smithers BM, Taylor AC, Thomas ML, Thomson I, To H, von Dincklage J, Vuletich C, Watson DI, Yusoff IF. Australian clinical practice guidelines for the diagnosis and management of Barrett's esophagus and early esophageal adenocarcinoma. *J Gastroenterol Hepatol* 2015; **30**: 804-820 [PMID: 25612140 DOI: 10.1111/jgh.12913]

P- Reviewer: Elpek GO, Guo YM, Ono T **S- Editor**: Qi Y
L- Editor: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i3.412

World J Gastrointest Pharmacol Ther 2016 August 6; 7(3): 412-415
ISSN 2150-5349 (online)

MINIREVIEWS

Chinese *Helicobacter pylori* vaccine: Solution for an old challenge?

Amin Talebi Bezmin Abadi, Yeong Yeh Lee

Amin Talebi Bezmin Abadi, Department of Bacteriology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran 14115-111, Iran

Yeong Yeh Lee, Department of Medicine, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian 16150, Kelantan, Malaysia

Author contributions: Talebi Bezmin Abadi A wrote the primary draft; Lee YY provided new modification for new version and both authors agreed on final version of the manuscript.

Conflict-of-interest statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Manuscript source: Invited manuscript

Correspondence to: Amin Talebi Bezmin Abadi, PhD, Assistant Professor, Department of Bacteriology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran 14115-111, Iran. amin.talebi@modares.ac.ir
Telephone: +98-21-82884883
Fax: +98-21-82884883

Received: March 16, 2016
Peer-review started: March 18, 2016
First decision: April 5, 2016
Revised: April 9, 2016
Accepted: May 17, 2016
Article in press: May 27, 2016
Published online: August 6, 2016

Abstract

Helicobacter pylori (*H. pylori*) is an important cause for gastric cancer in high risk individuals. *H. pylori* colonizes more than 50% of the world's population and associated peptic ulcer disease and gastric malignancy have important public health implications. It has been classified as a class Ⅰ carcinogen in 1994 by the World Health Organization. Clinicians are often prompted to eliminate the infection the moment it is detected. This also, unfortunately, led to reckless use of antibiotics and reports of increasing resistance are now worldwide. Each year, many of people die from gastric cancer; thus application of effective vaccine can reduce this relatively high mortality worldwide. *H. pylori* can be eliminated by antibiotics but efficacy is sharply decreasing. Moreover, current therapy is also expensive and with side effects. Vaccine may be the best solution to the above problem but there are many challenges in producing such an effective therapeutic vaccine. Recently, the Chinese group published in Lancet, a single-center, randomized, phase Ⅲ study of an oral recombinant vaccine (Urease B subunit fused with heat-labile enterotoxin B derived from *Escherichia coli*) prescribed in the Chinese children (6-15 years) without a history of *H. pylori* infection. This review provides an insight into this new solution for an old challenge.

Key words: *Helicobacter pylori*; Resistance; Therapy; Vaccine; Antibiotics

Core tip: *Helicobacter pylori* (*H. pylori*) remains the most prevalent gastric infection. One of the main questionable aspects of *H. pylori* is its high resistance to most of prescribed antibiotics and lack of useful vaccines. Vaccine may be the best solution to the above problem but there are many challenges in producing such an effective

therapeutic vaccine. That will be ideal that Chinese vaccine removes the need for bicarbonate administration because of its adverse side effects. Taking together, it is the first time that such a protective *H. pylori* vaccine is introduced to the world for high risk individuals.

Talebi Bezmin Abadi A, Lee YY. Chinese *Helicobacter pylori* vaccine: Solution for an old challenge? *World J Gastrointest Pharmacol Ther* 2016; 7(3): 412-415 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i3/412.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i3.412

INTRODUCTION

It is time to stop Helicobacter pylori

Helicobacter pylori (*H. pylori*) infects over half of the world's population and associated peptic ulcer disease and gastric malignancy have important public health implications. Despite after two decades of antibiotics success, the primary problem still exists, and the reasons can be multifactorial[1-3]. Some *in-vivo* conditions favor the persistence of *H. pylori* in the stomach but others oppose, and the clinical outcomes can be dependent on a delicate balance between a harmless inflammation and a more severe kind[4]. Furthermore, we do not know the most effective eradication regime of *H. pylori*, as the following questions remained unsolved including the best duration of recommended regimens, best dosages and also the right combination of antibiotics[5-8]. Although *H. pylori* infection can be efficiently eradicated using antibiotics, at least, in some patients, there are now reports of antibiotic resistance worldwide (Table 1). Finding an effective vaccine is the answer if resistance continues to increase[9-12]. More than five international guidelines have been published that covers all aspects of *H. pylori* infection including diagnostic, treatment and also vaccine[13,14]. Following years of continuous clinical experiments and trials, the promising goal for an effective vaccine now seems feasible. In September 2015, a report published in *Lancet* by the Chinese group brings high hope on a highly effective vaccine that we have been waiting for[15]. If proven in further studies, then this groundbreaking finding will change management paradigm of *H. pylori* in the near future.

A *H. PYLORI* VACCINE THAT WORKS, FINALLY?

Effective vaccine should not just reduce the incidence but also global prevalence of *H. pylori*. Furthermore, to prove its efficacy we need a longer period of study observation and with a greater number of study participants to conclude its reliability before it can be recommended into any healthcare systems. There have been considerable interests to develop such an effective *H. pylori* vaccine for a long time but many obstacles had hampered the development[16]. Many of the *H. pylori* virulence factors and also secreted proteins such as urease were used as recombinant proteins to produce a protective vaccine, but because these factors only induced weak forms of immunity and also lack of safety, therefore many projects were abandoned[13,17]. Therapeutic vaccines should be able to administer to both *H. pylori* positive children but also adults; although there is a potential risk for developing gastritis in susceptible patients[18,19]. In these susceptible patients, however, we can still recommend therapeutic vaccine; since it can reduce: (1) risk of re-infection; and (2) decrease treatment duration. The disappointment in vaccine development may tip following the Chinese *H. pylori* vaccine published in Lancet[15]. This was a single-center, randomized trial and a phase Ⅲ study that examined an oral recombinant vaccine (based on Urease B subunit fused with heat-labile enterotoxin B derived from *Escherichia coli*) among the Chinese children (aged 6-15 years) without a prior history of *H. pylori* infection. In brief, after 12 mo of vaccination, 71% efficacy rate was observed, and this rate was around 55% after 3 years. Although seems effective in children, this study needs repeat among adults. Another limitation of this vaccine is that the authors found 20% of younger children were not protected from the infection. Notably, using better adjuvant in order to remove boosters for this vaccine may increase its popularity among clinicians for widespread prescription. Also it will be ideal that the Chinese vaccine removes the need for bicarbonate administration because of its adverse side effects. Taking together, it is the first time that such a protective *H. pylori* vaccine is introduced to the world for high risk individuals.

WHAT NOW AFTER THE CHINESE VACCINE?

While the published results for the Chinese vaccine seems promising, but there are still barriers before it gains wide acceptance. Besides the limitations mentioned in above section, the vaccine needs a proper Phase-Ⅲ clinical trials for other populations. Besides the Chinese vaccine, there are other novel developments in the pipeline. Currently, there is a lack in knowledge on exact molecular mechanisms that contributed to cellular immunity against *H. pylori*. The urease enzyme was the first recombinant protein used to provide an effective vaccine for *H. pylori* in animal models[20,21]. Recently, it has been established that regulatory T-cells are necessary to mount sufficient immune responses and this is important information for future development of a protective anti-*H. pylori* vaccine[22]. *H. pylori*-immunogenic antigens such as catalase, vacuolating cytotoxin (VacA), urease, cytotoxin-associated gene A (CagA), heat shock proteins and also neutrophil-activating protein (NAP) had been examined to see if they are potential candidate antigens for vaccine[23-27], but so far, the results have been inconclusive. Moreover, different mucosal routes such as

Table 1 Worldwide report of increasing *Helicobacter pylori* antibiotic resistance

Year	Eradication rate	Ref.	Antibiotics
2001	97%	Asaka *et al*[9]	Clarithromycin Amoxicillin
2014	61%	Chen *et al*[10]	Clarithromycin Amoxicillin
2014	55%	Kutluk *et al*[32]	Clarithromycin Amoxicillin
2013	76%	Sardarian *et al*[11]	Clarithromycin Amoxicillin tinidazole
2013	80%	Zullo *et al*[33]	Clarithromycin Amoxicillin tinidazole
2014	69%	Nishida *et al*[7]	Clarithromycin Amoxicillin
2011	87%	Greenberg *et al*[6]	Clarithromycin Amoxicillin
2014	98%	Sugimoto *et al*[12]	Metronidazole Clarithromycin
2013	38%	Nishizava *et al*[34]	Metronidazole Amoxicillin Clarithromycin

sublingual, rectal and intranasal were being evaluated but results were inconsistent[27-30]. Recently, Chen *et al*[31] examined *oipA* DNA construct carried by the bacterium, Salmonella typhimurium as a therapeutic vaccine. The authors concluded that *H. pylori* virulence factors including OipA and NAP may seem to be the better candidates to induce effective immunity, at least in the mouse models, and we shall await more results.

CONCLUSION

Due to the relatively high rate of antibiotic therapy failure in recent years, we have to investigate more about novel vaccines on *H. pylori*. At last, Chinese group proposed a useful formulation with less side effects which can inspire more hopes for clinicians to think actually about *H. pylori* mass eradication worldwide.

ACKNOWLEDGMENTS

I thank Dr. Ronald Gorham from University of California, Riverside, United States, for his critical reading of the manuscript. I thank the reviewers for their helpful comments on this manuscript. The contents of this review article are sole responsibility of the author and necessarily represent personal perspective.

REFERENCES

1 **Ferreira J**, Moss SF. Current Paradigm and Future Directions for Treatment of Helicobacter pylori Infection. *Curr Treat Options Gastroenterol* 2014; **12**: 373-384 [PMID: 25187235 DOI: 10.1007/s11938-014-0027-6]
2 **Ren L**, Lu H, Li HY, Zhu LY, Xu XQ, Gu LY, Ge ZZ, Li XB. New dual therapy for primary treatment of Helicobacter pylori infection: A prospective randomized study in Shanghai, China. *J Dig Dis* 2014; **15**: 622-627 [PMID: 25205201 DOI: 10.1111/1751-2980.12186]
3 **Talebi Bezmin Abadi A**. Vaccine against Helicobacter pylori: Inevitable approach. *World J Gastroenterol* 2016; **22**: 3150-3157 [PMID: 27003991 DOI: 10.3748/wjg.v22.i11.3150]
4 **Abadi ATB**, Ierardi E, Lee YY. Why do we still have Helicobacter Pylori in our Stomachs? *Malays J Med Sci* 2015; **22**: 70-75
5 **Başyiğit S**, Kefeli A, Yeniova AÖ, Uzman M, Nazlıgül Y, Aktaş B. Different treatment choice for first-line treatment of Helicobacter pylori in an area with a high antibiotic resistance. *Turk J Gastroenterol* 2014; **25** Suppl 1: 293-294 [PMID: 25910347 DOI: 10.5152/tjg.2014.6085]
6 **Greenberg ER**, Anderson GL, Morgan DR, Torres J, Chey WD, Bravo LE, Dominguez RL, Ferreccio C, Herrero R, Lazcano-Ponce EC, Meza-Montenegro MM, Peña R, Peña EM, Salazar-Martínez E, Correa P, Martínez ME, Valdivieso M, Goodman GE, Crowley JJ, Baker LH. 14-day triple, 5-day concomitant, and 10-day sequential therapies for Helicobacter pylori infection in seven Latin American sites: a randomised trial. *Lancet* 2011; **378**: 507-514 [PMID: 21777974 DOI: 10.1016/S0140-6736(11)60825-8]
7 **Nishida T**, Tsujii M, Tanimura H, Tsutsui S, Tsuji S, Takeda A, Inoue A, Fukui H, Yoshio T, Kishida O, Ogawa H, Oshita M, Kobayashi I, Zushi S, Ichiba M, Uenoyama N, Yasunaga Y, Ishihara R, Yura M, Komori M, Egawa S, Iijima H, Takehara T. Comparative study of esomeprazole and lansoprazole in triple therapy for eradication of Helicobacter pylori in Japan. *World J Gastroenterol* 2014; **20**: 4362-4369 [PMID: 24764674 DOI: 10.3748/wjg.v20.i15.4362]
8 **Mokhtare M**, Hosseini V, Tirgar Fakheri H, Maleki I, Taghvaei T, Valizadeh SM, Sardarian H, Agah S, Khalilian A. Comparison of quadruple and triple Furazolidone containing regimens on eradication of helicobacter pylori. *Med J Islam Repub Iran* 2015; **29**: 195 [PMID: 26157713]
9 **Asaka M**, Sugiyama T, Kato M, Satoh K, Kuwayama H, Fukuda Y, Fujioka T, Takemoto T, Kimura K, Shimoyama T, Shimizu K, Kobayashi S. A multicenter, double-blind study on triple therapy with lansoprazole, amoxicillin and clarithromycin for eradication of Helicobacter pylori in Japanese peptic ulcer patients. *Helicobacter* 2001; **6**: 254-261 [PMID: 11683930]
10 **Chen MC**, Lei WY, Lin JS, Yi CH, Wu DC, Hu CT. Levofloxacin-amoxicillin/clavulanate-rabeprazole versus a standard seven-day triple therapy for eradication of Helicobacter pylori infection. *Biomed Res Int* 2014; **2014**: 158520 [PMID: 24995271 DOI: 10.1155/2014/158520]
11 **Sardarian H**, Fakheri H, Hosseini V, Taghvaei T, Maleki I, Mokhtare M. Comparison of hybrid and sequential therapies for Helicobacter pylori eradication in Iran: a prospective randomized trial. *Helicobacter* 2013; **18**: 129-134 [PMID: 23121338 DOI: 10.1111/hel.12017]
12 **Sugimoto M**, Uotani T, Sahara S, Ichikawa H, Yamade M, Sugimoto K, Furuta T. Efficacy of tailored Helicobacter pylori eradication treatment based on clarithromycin susceptibility and maintenance of acid secretion. *Helicobacter* 2014; **19**: 312-318 [PMID: 24690010 DOI: 10.1111/hel.12128]
13 **Moise L**, Moss SF, De Groot AS. Moving Helicobacter pylori vaccine development forward with bioinformatics and immunomics. *Expert Rev Vaccines* 2012; **11**: 1031-1033 [PMID: 23151160 DOI: 10.1586/erv.12.80]
14 **Guo L**, Liu K, Zhao W, Li X, Li T, Tang F, Zhang R, Wu W, Xi T. Immunological features and efficacy of the reconstructed epitope vaccine CtUBE against Helicobacter pylori infection in BALB/c mice model. *Appl Microbiol Biotechnol* 2013; **97**: 2367-2378 [PMID: 23064457 DOI: 10.1007/s00253-012-4486-1]
15 **Zeng M**, Mao XH, Li JX, Tong WD, Wang B, Zhang YJ, Guo G, Zhao ZJ, Li L, Wu DL, Lu DS, Tan ZM, Liang HY, Wu C, Li DH, Luo P, Zeng H, Zhang WJ, Zhang JY, Guo BT, Zhu FC, Zou QM. Efficacy, safety, and immunogenicity of an oral recombinant Helicobacter pylori vaccine in children in China: a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2015; **386**: 1457-1464 [PMID: 26142048 DOI: 10.1016/S0140-6736(15)60310-5]
16 **Li WQ**, Ma JL, Zhang L, Brown LM, Li JY, Shen L, Pan KF, Liu

WD, Hu Y, Han ZX, Crystal-Mansour S, Pee D, Blot WJ, Fraumeni JF, You WC, Gail MH. Effects of Helicobacter pylori treatment on gastric cancer incidence and mortality in subgroups. *J Natl Cancer Inst* 2014; **106**: dju116 [PMID: 24925350 DOI: 10.1093/jnci/dju116]

17 **Baldari CT**, Lanzavecchia A, Telford JL. Immune subversion by Helicobacter pylori. *Trends Immunol* 2005; **26**: 199-207 [PMID: 15797510]

18 **Lv X**, Yang J, Song H, Li T, Guo L, Xing Y, Xi T. Therapeutic efficacy of the multi-epitope vaccine CTB-UE against Helicobacter pylori infection in a Mongolian gerbil model and its microRNA-155-associated immuno-protective mechanism. *Vaccine* 2014; **32**: 5343-5352 [PMID: 25093281 DOI: 10.1016/j.vaccine.2014.07.041]

19 **Rossi G**, Ruggiero P, Peppoloni S, Pancotto L, Fortuna D, Lauretti L, Volpini G, Mancianti S, Corazza M, Taccini E, Di Pisa F, Rappuoli R, Del Giudice G. Therapeutic vaccination against Helicobacter pylori in the beagle dog experimental model: safety, immunogenicity, and efficacy. *Infect Immun* 2004; **72**: 3252-3259 [PMID: 15155627 DOI: 10.1128/IAI.72.6.3252-3259.2004]

20 **Lee CK**, Weltzin R, Thomas WD, Kleanthous H, Ermak TH, Soman G, Hill JE, Ackerman SK, Monath TP. Oral immunization with recombinant Helicobacter pylori urease induces secretory IgA antibodies and protects mice from challenge with Helicobacter felis. *J Infect Dis* 1995; **172**: 161-172 [PMID: 7797906]

21 **Hatzifoti C**, Roussel Y, Harris AG, Wren BW, Morrow JW, Bajaj-Elliott M. Mucosal immunization with a urease B DNA vaccine induces innate and cellular immune responses against Helicobacter pylori. *Helicobacter* 2006; **11**: 113-122 [PMID: 16579841]

22 **Larussa T**, Leone I, Suraci E, Imeneo M, Luzza F. Helicobacter pylori and T Helper Cells: Mechanisms of Immune Escape and Tolerance. *J Immunol Res* 2015; **2015**: 981328 [PMID: 26525279 DOI: 10.1155/2015/981328]

23 **Kang QZ**, Duan GC, Fan QT, Xi YL. Fusion expression of Helicobacter pylori neutrophil-activating protein in E.coli. *World J Gastroenterol* 2005; **11**: 454-456 [PMID: 15637768]

24 **D'Elios MM**, Czinn SJ. Immunity, inflammation, and vaccines for Helicobacter pylori. *Helicobacter* 2014; **19** Suppl 1: 19-26 [PMID: 25167941 DOI: 10.1111/hel.12156]

25 **de Bernard M**, D'Elios MM. The immune modulating activity of the Helicobacter pylori HP-NAP: Friend or foe? *Toxicon* 2010; **56**: 1186-1192 [PMID: 19818802 DOI: 10.1016/j.toxicon.2009.09.020]

26 **Goh KL**, Chan WK, Shiota S, Yamaoka Y. Epidemiology of Helicobacter pylori infection and public health implications. *Helicobacter* 2011; **16** Suppl 1: 1-9 [PMID: 21896079 DOI: 10.1111/j.1523-5378.2011.00874.x]

27 **Malfertheiner P**, Schultze V, Rosenkranz B, Kaufmann SH, Ulrichs T, Novicki D, Norelli F, Contorni M, Peppoloni S, Berti D, Tornese D, Ganju J, Palla E, Rappuoli R, Scharschmidt BF, Del Giudice G. Safety and immunogenicity of an intramuscular Helicobacter pylori vaccine in noninfected volunteers: a phase I study. *Gastroenterology* 2008; **135**: 787-795 [PMID: 18619971 DOI: 10.1053/j.gastro.2008.05.054]

28 **Müller A**, Solnick JV. Inflammation, immunity, and vaccine development for Helicobacter pylori. *Helicobacter* 2011; **16** Suppl 1: 26-32 [PMID: 21896082 DOI: 10.1111/j.1523-5378.2011.00877.x]

29 **Ruggiero P**, Censini S. Helicobacter pylori: A Brief History of a Still Lacking Vaccine. *Diseases* 2014; **2**: 187-208

30 **Rupnow MF**, Chang AH, Shachter RD, Owens DK, Parsonnet J. Cost-effectiveness of a potential prophylactic Helicobacter pylori vaccine in the United States. *J Infect Dis* 2009; **200**: 1311-1317 [PMID: 19751153 DOI: 10.1086/605845]

31 **Chen J**, Lin M, Li N, Lin L, She F. Therapeutic vaccination with Salmonella-delivered codon-optimized outer inflammatory protein DNA vaccine enhances protection in Helicobacter pylori infected mice. *Vaccine* 2012; **30**: 5310-5315 [PMID: 22749593 DOI: 10.1016/j.vaccine.2012.06.052]

32 **Kutluk G**, Tutar E, Bayrak A, Volkan B, Akyon Y, Celikel C, Ertem D. Sequential therapy versus standard triple therapy for Helicobacter pylori eradication in children: any advantage in clarithromycin-resistant strains? *Eur J Gastroenterol Hepatol* 2014; **26**: 1202-1208 [PMID: 25171023 DOI: 10.1097/MEG.0000000000000190]

33 **Zullo A**, Hassan C, Ridola L, De Francesco V, Vaira D. Standard triple and sequential therapies for Helicobacter pylori eradication: an update. *Eur J Intern Med* 2013; **24**: 16-19 [PMID: 22877993 DOI: 10.1016/j.ejim.2012.07.006]

34 **Nishizawa T**, Maekawa T, Watanabe N, Harada N, Hosoda Y, Yoshinaga M, Yoshio T, Ohta H, Inoue S, Toyokawa T, Yamashita H, Saito H, Kuwai T, Katayama S, Masuda E, Miyabayashi H, Kimura T, Nishizawa Y, Takahashi M, Suzuki H. Clarithromycin Versus Metronidazole as First-line Helicobacter pylori Eradication: A Multicenter, Prospective, Randomized Controlled Study in Japan. *J Clin Gastroenterol* 2015; **49**: 468-471 [PMID: 24921211 DOI: 10.1097/MCG.0000000000000165]

P- Reviewer: Chmiela M, De Re V, Homan M, Velin D
S- Editor: Gong ZM **L- Editor**: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i3.416

World J Gastrointest Pharmacol Ther 2016 August 6; 7(3): 416-427
ISSN 2150-5349 (online)

ORIGINAL ARTICLE

Basic Study

Effects of aging on the architecture of the ileocecal junction in rats

Maria Cícera de Brito, Renato Paulo Chopard, Diego Pulzatto Cury, Ii Sei Watanabe, Cristina Eusébio Mendes, Patricia Castelucci

Maria Cícera de Brito, Diego Pulzatto Cury, Department of Surgery, Faculty of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo 05508-900, Brazil

Renato Paulo Chopard, Diego Pulzatto Cury, Ii Sei Watanabe, Cristina Eusébio Mendes, Patricia Castelucci, Department of Anatomy/Biomedical Science Institute, University of São Paulo, São Paulo 05508-900, Brazil

Author contributions: de Brito MC performed all of the experiments and analyzed the results for this work; Chopard RP planned experiments; Cury DP and Watanabe IS helped and analyzed the transmission and scan electron microscopy studies; Mendes CE helped edit the manuscript and figures; Castelucci P planned the immunohistochemistry study, wrote and edit the manuscript.

Institutional review board statement: This study approved in the meeting of day 2/19/2014 and agree with Ethical principles in animal research adopted by ethic committee in the use of animals of the school of veterinary medicine and animal science of University of São Paulo.

Conflict-of-interest statement: No potential conflicts of interest relevant to this article were reported.

Data sharing statement: No additional data are available.

Correspondence to: Dr. Patricia Castelucci, PhD, Associate Professor, Department of Anatomy/Biomedical Science Institute, University of São Paulo, R. Dr. Lineu Prestes 2415, São Paulo 05508-900, Brazil. pcastel@usp.br

Telephone: +55-11-30918463
Fax: +55-11-30917366

Received: January 3, 2016
Peer-review started: January 4, 2016
First decision: February 26, 2016
Revised: March 15, 2016
Accepted: April 5, 2016
Article in press: April 6, 2016
Published online: August 6, 2016

Abstract

AIM: To evaluate the structural organization of the elastic and collagen fibers in the region of the ileocecal transition in 30 young and old male Wistar rats.

METHODS: Histology, immunohistochemistry (IHC), transmission electron microscopy and scanning electron microscopy were employed in this study. The results demonstrated that there was a demarcation of the ileocecal region between the ileum and the cecum in both groups.

RESULTS: The connective tissue fibers had different distribution patterns in the two groups. IHC revealed the presence of nitric oxide synthase, enteric neurons and smooth muscle fibers in the ileocecal junctions (ICJs) of both groups. Compared to the young group, the elderly group exhibited an increase in collagen type Ⅰ fibers, a decrease in collagen type Ⅲ fibers, a decreased linear density of oxytalan elastic fibers, and a greater linear density of elaunin and mature elastic fibers.

CONCLUSION: The results revealed changes in the patterns of distribution of collagen and elastic fibers that may lead to a possible decrease in ICJ functionality.

Key words: Ileocecal junction; Elastic fibers; Collagen fibers; Aging; Rats

Core tip: The ileocolonic sphincter controls the forward and backward flow by integrating its motility with that of the distal ileum and proximal the ileocolonic sphincter controls the forward and backward flow by integrating its motility with that of the distal ileum and proxima. The ileocecal junction (ICJ) includes the muscle bundles in the terminal ileum, the intrinsic nerve plexus. The ICJ includes the muscle bundles in the terminal ileum, the intrinsic nerve plexus. Given the importance of knowing how the ICJ changes with age, the objective of this study was to characterize the morphological changes in the ICJ in rats aged 21 d and 2 years, using optical microscopy and electronic scanning and transmission methodologies. Additionally, the neurochemical characterization of the inhibitory neurons of the myenteric plexus, which are immunoreactive to the enzyme nitric oxide synthase, and the staining of the neuronal population were employed to identify immunoreactivity to HuC/D.

de Brito MC, Chopard RP, Cury DP, Watanabe IS, Mendes CE, Castelucci P. Effects of aging on the architecture of the ileocecal junction in rats. *World J Gastrointest Pharmacol Ther* 2016; 7(3): 416-427 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i3/416.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i3.416

INTRODUCTION

The ileocecal junction (ICJ) has two aspects: A wedge-shaped cavity that progressively narrows the orifice to form the ileum and is bordered by an upper lip and lower lip, joined by front and posterior commissures; and an invagination of the small intestine to the large intestine[1-4]. Morphological differences between species can be related to the type of digestion, either partial or total, in the cecum[5-8]. The smooth muscle cells of the ICJ maintain a high tone[9]. The ileocolonic sphincter controls the forward and backward flow by integrating its motility with that of the distal ileum and proximal[9]. The ICJ includes the muscle bundles in the terminal ileum, the intrinsic nerve plexus, and the presence or absence of the interstitial cells of Cajal (ICC). Many ICC associated with the myenteric plexus are observed in both the ileal and cecal sides of the valve[10]. The neuronal density is lower in the cecum and ileal papilla compared to the terminal ileum. ICC exist within the myenteric plexus of the ICJ, and their density is similar to the adjacent bowel[4]. Histochemistry for acetylcholinesterase (AchE) and NADPH-diaphorase (NADPH-d) histochemistry and immunohistochemistry for protein gene product 9.5 (PGP 9.5) and C-kit in the ICJ revealed two distinct coaxial myenteric plexuses, together with superficial and deep submucosal plexuses. The C-kit immunostaining showed a continuous myenteric ICC network within the ICV[6].

Additionally, the ICJ is accompanied by a framework of collagen and elastic fibers[1-3,11]. The elastic fibers function to maintaining the elasticity of tissues throughout life[12]. Changes to the collagen arrangement and its three-dimensional (3D) distribution may be related to the dissimilar biomechanical proprieties in the terminal ileum[11]. Changes have been observed in the composition and architecture of the connective tissue with aging, resulting in the loss of elasticity and extensibility of different tissues[12,13].

The loss of ICJ function is clinically important. The loss of ICJ function may cause fecal reflux, with the risk of bacterial colonization in the terminal ileum[14]. Given the importance of knowing how the ICJ changes with age, the objective of this study was to characterize the morphological changes in the ICJ in rats aged 21 d and 2 years, using optical microscopy and electronic scanning and transmission methodologies. Additionally, the neurochemical characterization of the inhibitory neurons of the myenteric plexus, which are immunoreactive to the enzyme nitric oxide synthase (NOS), and the staining of the neuronal population were employed to identify immunoreactivity to HuC/D.

MATERIALS AND METHODS

We used 30 male Wistar rats (Rattus norvegicus) from the Central Animal Facility of the Institute of Biomedical Sciences, University of São Paulo in this study. The animals were housed in polypropylene cages that could hold up to three animals. The temperature was controlled at 21 ℃ ± 1 ℃, and the humidity was approximately 60%. The animals were maintained on alternating cycles of 12 h of light and 12 h of dark, with a balanced diet and water provided *ad libitum*. All the procedures were approved by the Ethics Committee on Animal Experiments of the Faculty of Veterinary Medicine and Animal Science of the University of São Paulo. The animals were divided into two groups: (1) a young group, 21 d old (n = 15); and (2) an older group, 24 mo old (n = 15). Each group was analyzed using microscopy (n = 7), immunohistochemistry (IHC; n = 2), transmission electron microscopy (TEM; n = 2) and scanning electron microscopy (SEM; n = 4).

Histology methods

For light microscopy, the animals were euthanized with an overdose of xylazine (40 mg/kg) and ketamine (120 mg/kg) and then the ICJ was removed. The samples were fixed in 10% paraformaldehyde fixative for 24 h at room temperature before undergoing routine histological processing. Cuts were made in the longitudinal direction with a thickness of 5 μm using a Reichert Jung ultramicrotome. Samples were stained using hematoxylin and eosin (HE). Elastic fibers were revealed by staining with iron hematoxylin (Verhoeff), resulting in blue and

Table 1 Characteristics of the primary and secondary antibodies used in this study

Antigen	Host	Dilution	Source
Nitric oxide synthage	Sheep	1:1000	Chemicon
HuC/D	Mouse	1:100	Molecular probes
Anti-sheep IgG 488	Donkey	1:100	Molecular probes
Anti-mouse IgG 594	Donkey	1:200	Molecular probes

black tones[15-17]. Staining with resorcin-fuchsin (Weigert), with and without oxone, showed mature elastic fibers as pink, and elaunin and oxytalan were stained in shades of purple and black[16,17].

Picrosirius staining under polarized light allowed the observation of collagen birefringence, allowing the classification of the type or age of the collagen according to the color and light intensity of the refringence. Polarized light of picrosirius-stained samples evidenced yellow and red fibers (type Ⅰ collagen) and green fibers (type Ⅲ collagen). The stained slides were observed and digitized with a NIKON Eclipse E600 microscope and NIS-Elements AR software for documentation and further qualitative and quantitative analysis.

IHC fluorescence method

For IHC, the animals were euthanized with an overdose of xylazine (40 mg/kg) and ketamine (120 mg/kg). After collection, the samples were washed by immersion in phosphate-buffered saline solution (PBS; 1.15 mol/L NaCl and 0.01 mol/L sodium phosphate buffer, pH = 7.2), and then they were washed with PBS. After this procedure, the samples were sectioned by mesenteric margins and were subsequently fixed in wooden rafts with the mucosa facing down with the aid of pins. Subsequently, the sections were immersed in 4% paraformaldehyde fixative in 0.1 mol/L sodium phosphate buffer, pH 7.3 at 4 ℃ for 24 h. On the following day, the samples were removed from the fixative and washed in PBS three times with intervals of 10 min each. Then, some of the samples were stored in PBS containing sodium azide (0.1%) at 4 ℃ for preservation, and the others were transferred to PBS + 30% sucrose for 24 h at 4 ℃. The next day, an exchange of substances in 50:50 PBS + 30% sucrose + Optimum Cutting Temperature Tissue Tek, Elkhart (OCT) was performed, and the samples were stored overnight. After this period, the switch was made at 100%. The samples were then stored at -80 ℃ to maintain their conservation. After the completion of all the procedures mentioned above, the samples were fixed on metal bases (stubs) in 10 μm slices, and cuts were made with an 1850 Leica cryostat at -25 ℃. The sections were mounted on slides, which were stored at room temperature for 1 h and then immersed in 10% normal horse serum solution (NHS) and 1.5% Triton (Sigma) in PBS for 45 min at room temperature. Then, the samples were incubated with primary antibody (Table 1) for 48 h.

After 24 h, the samples were again subjected to washes with PBS (three times for 10 min each) and were further incubated with a secondary antibody (Table 1). The tissues were immersed in 2.6-diamino-2-phenylindole dihydrochloride (DAPI) for five minutes to stain the nuclei of all the cells. Subsequently, the tissues were washed in PBS (3 times for 5 min each). Then, the slides were covered with a glycerol coverslip buffered in 0.5 mol/L calcium carbonate buffer (pH 8.6). Observations were performed with a Nikon 80i fluorescence microscope using the Nis Elements program. Sample preparations were also analyzed with a confocal scanning microscope (Olympus Fluorview FV10SW Laser).

TEM method

After pre-anesthesia, the animals were perfused with a modified Karnovsky fixative solution containing 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 mol/L sodium phosphate buffer at pH 7.4[18]. After perfusion, samples were collected from the ICJ. The samples were post-fixed in 2.5% glutaraldehyde for 2 h at 4 ℃. They were then washed in sodium phosphate buffer and post-fixed in 1% osmium tetroxide for 2 h at 4 ℃. The samples were washed with brine, and then were immersed in 0.5% uranyl acetate overnight. On the following day, the samples were dehydrated in a series of increasing alcohol concentrations (from 2% to 70%) before baths in absolute alcohol and propylene oxide for 15 min each. The samples were then embedded in a mixture of Spurr® resin. The samples were placed in shallow molds of silicone, which were then placed in an oven at 60 ℃ for 48 h for polymerization of the resin. The blocks were then subjected to trimming, and thin sections from 0.5 μm to 1 μm were obtained with an ultra-microtome. The sections were then collected on glass slides, stained with toluidine blue and washed with 1% distilled water for observation by light microscopy for the delimitation of areas. Ultrathin sections approximately 70 nm thick were made and collected in 200 mesh copper screens. The sections were contrasted with 4% uranyl acetate solution for 10 min and were then washed with distilled water[18] and 4% lead citrate for 10 min[18]. All the cuts were made with an ultra-microtome (Leica Ultracut, Germany) in the multiuser laboratory of the Department of Biomedical Sciences Institute of Anatomy, University of São Paulo. The sections were analyzed by TEM (Fei Morgagni 265 and Jeol 1010 brand microscopes set to 80 kV).

SEM method

For SEM, the animals were anesthetized intraperitoneally with ketamine (60 mg/kg) and xylazine (20 mg/kg) and then were perfused with a fixative solution of modified Karnovsky containing 2.5% glutaraldehyde, 2% paraformaldehyde, and a 0.1 mol/L solution of sodium phosphate buffer at pH = 7.4[18]; approximately 60 mL of solution was injected through the left ventricle of the heart. After perfusion, samples from the ICJ were then removed and immersed in fixative for 48 h at 4 ℃. Then, the tissues were sectioned at the mesenteric margin.

Some of the samples were selected for SEM, and some were subjected to treatment with a 5% aqueous solution of sodium hydroxide (NaOH)[19,20], which was changed daily for 4 d; the samples were then washed in distilled water for 2 d at 4 ℃. After this step, all the samples (sectioned and macerated) were post-fixed in aqueous 1% osmium tetroxide for 2 h at 4 ℃ and dehydrated in a series of increasing alcohol concentrations and dried in a critical point apparatus (Balzers CPD-020) using liquid CO_2. After drying, the samples were mounted on aluminum metal bases and subjected to a metal cover with gold ions in the "sputtering" unit (Union Balzers SCD 040).

Quantitative analysis

Collagen fibers: The study of the collagen fiber system was performed by capturing random fields using the Imaging Software NIS program - Elements AR 3.1 for the observation of collagen types Ⅰ and Ⅲ. Six fields of each slide were captured with a × 20 lens. From these images, collagen fiber type Ⅰ (red) and type Ⅲ (green) densities were quantified with a software tool that recognizes color variations in an image using the color channels of red, green and blue (RGB). This software allows the identification of one color at a time, yielding the average color intensity of the given area and the area of each field equivalent to 274252.78 pixels[21].

Elastic fibers: The histomorphometrical examination was performed using linear density (Ld) estimation of the elastic system. The estimated elastic fiber length was derived from the formula L = 2Q × EV, where L = length of the elastic fiber structure per unit volume, Q = number of cross-sectioned elastic fibers in the plane of the section and EV = unit volume. The fiber length per unit volume is directly proportional to the number of fiber intersections within the unit section area[21-23]. The samples were analyzed with a 100X lens, immersion oil, and eye Kf 10 × 18 compensation, with 117 points integration, showing 13 parallel lines vertically and 9 horizontally. The total area was 117000 μm^2. The distance (L) between the points in this system was 10 μm according to the procedure reported[24]. Histological sections of the ICJ were observed and documented under a microscope (Nikon Eclipse E600/NIS - Elements).

Statistical analysis

A statistical analysis was performed by comparing the linear density of the collagen and elastic fibers of the young and elderly groups. The data were analyzed statistically using Student's *t*-test with a significance level of $P < 0.05$.

RESULTS

Histological analyses

The HE staining showed that smooth muscle cells were distributed in the mucosa and submucosa of the intestine in both groups, and both regions showed an arrangement in three different muscle layers: Two circular layers and one longitudinal muscle layer, with their cores in the central portion of the cells (Figure 1A-D). Spaces between smooth muscle cells were observed in the young group (Figure 1A, B); however, in the older group (Figure 1C, D), connective tissue fiber condensation between the smooth muscle cells was observed. The transition region had different cell characteristics. The ileum protruded into the cecum, while thickening occurred in the circular muscle layer. The cecum of both groups comprised a glandular epithelium without villi (Figure 1A-D).

Picrosirius staining under non-polarized light (Figure 1E and G) showed the general appearance of the ICJ. Picrosirius staining under polarized light showed the architecture of the Type Ⅰ and Type Ⅲ collagen fibers of the ICJ in both groups. These fibers were arranged around the smooth muscle cells and originated from both sides of the cecal ileum to form the transition surface. In addition, evaluating the structure of the connective framework revealed a clear predominance of type Ⅰ collagen fibers, which was characteristic of mature tissue in the elderly group (Figure 1H), that were shorter, thicker and more numerous. Note that the type Ⅰ collagen fibers in the young group (Figure 1F) were more elongated and thin and were less prevalent compared to the elderly group. Type Ⅲ collagen fibers were more numerous in the young group (Figure 1F) and were thinner compared to the older group (Figure 1H).

Elastic fibers: Weigert staining with previous oxidation showed oxytalan fibers (Figure 2A and B). In the young group (Figure 2A), these fibers were arranged in parallel, were thinner compared to the elderly group (Figure 2B), and were thicker and curved. Weigert staining (Figure 2C) also showed elaunin fibers. These fibers were arranged in parallel and were straight and slender in the young group (Figure 2C), which was different from the observations in the elderly group (Figure 2D) in which these fibers were more curved and thick. With Verhoeff staining (Figure 2E and F), it was possible to identify mature elastic fibers in both groups. These showed more slender and straight fibers in the young group (Figure 2E). However, in the elderly group (Figure 2F) these fibers were thicker, shorter and more crooked.

IHC: Immunoreactive neurons and fibers were identified by HuC/D (Figure 3A and D) and NOS (Figure 3B, E) in the young and elderly groups. The nuclei of the smooth muscle cells were identified by DAPI staining (Figure 3C and D). Figure 3C and F shows the triple labeling of immunoreactive neurons with NOS, HuC/D and DAPI. There was a homogeneous distribution of cytoplasmic immunoreactivity for HuC/D and NOS in the myenteric neurons of both groups.

TEM: In the young group (21-d-old), the observation of the mucosa of the ileocecal transition region revealed the presence of smooth muscle cells sectioned transversely

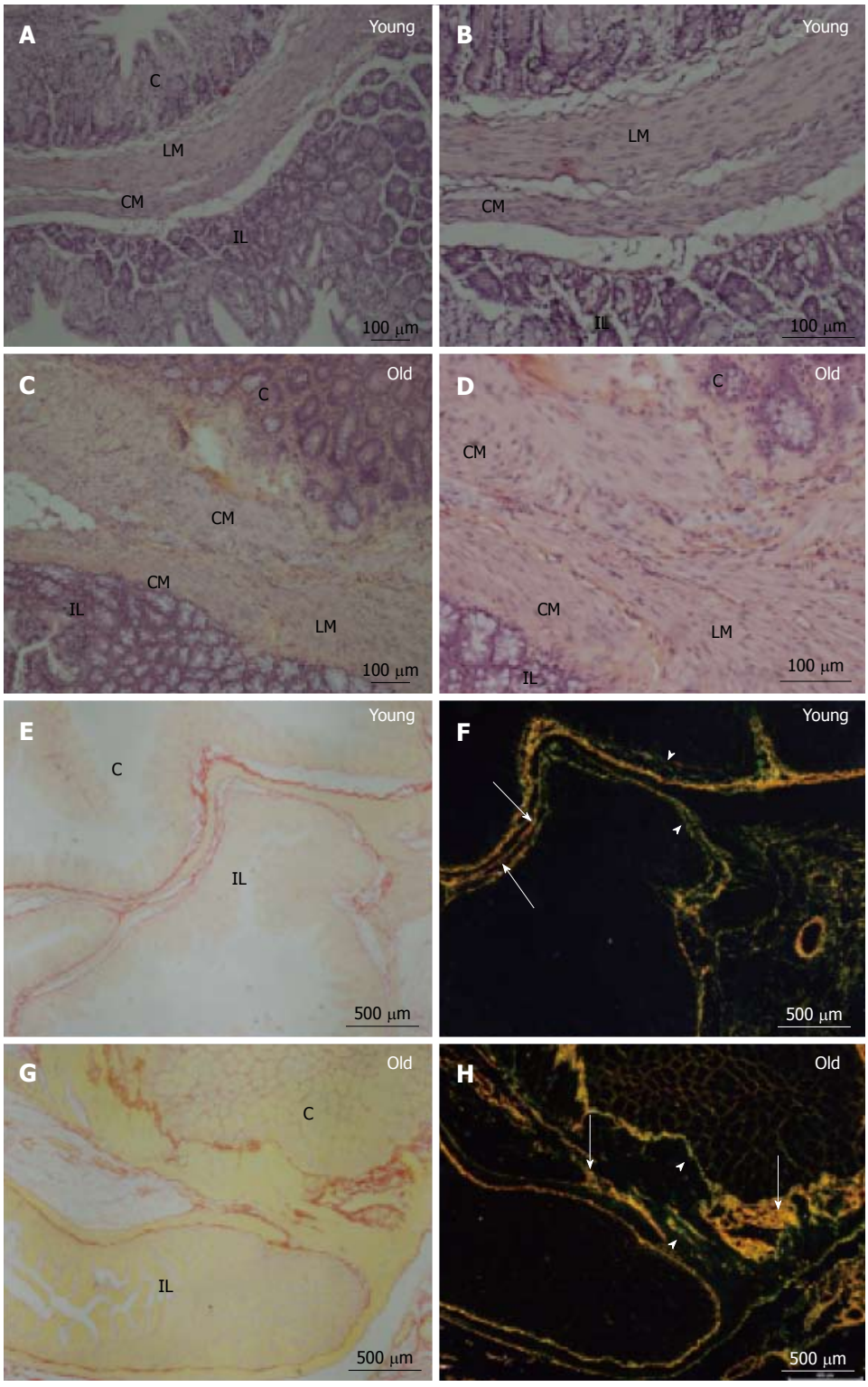

Figure 1 Histological sections of the ileocecal junctions in the young group and the elderly group. Hematoxylin-eosin staining (A-D) was performed to examine the ileum (IL), cecum (C), circular muscle layer (CM) and longitudinal muscle layer (ML). Picrosirius staining under non-polarized light (E and G) showed the transition region between the ileum (IL) and cecum (C). Picrosirius staining under polarized light (F and H) showed type I collagen fibers (yellow, orange and red) (arrows) and type III collagen fibers (green) (arrowhead).

(Figure 4A). The smooth muscle cells were surrounded by bundles of collagen fibers (Figure 4A and B). Between muscle cells, we observed numerous unmyelinated fibers (Figure 4B). The nerve fibers contained neurofilaments and mitochondria (Figure 4B). In the elderly group (24-mo-old), longitudinal sections were observed (Figure 4C). Between muscle fibers in the elderly group, there was a larger amount of collagen fibers forming the

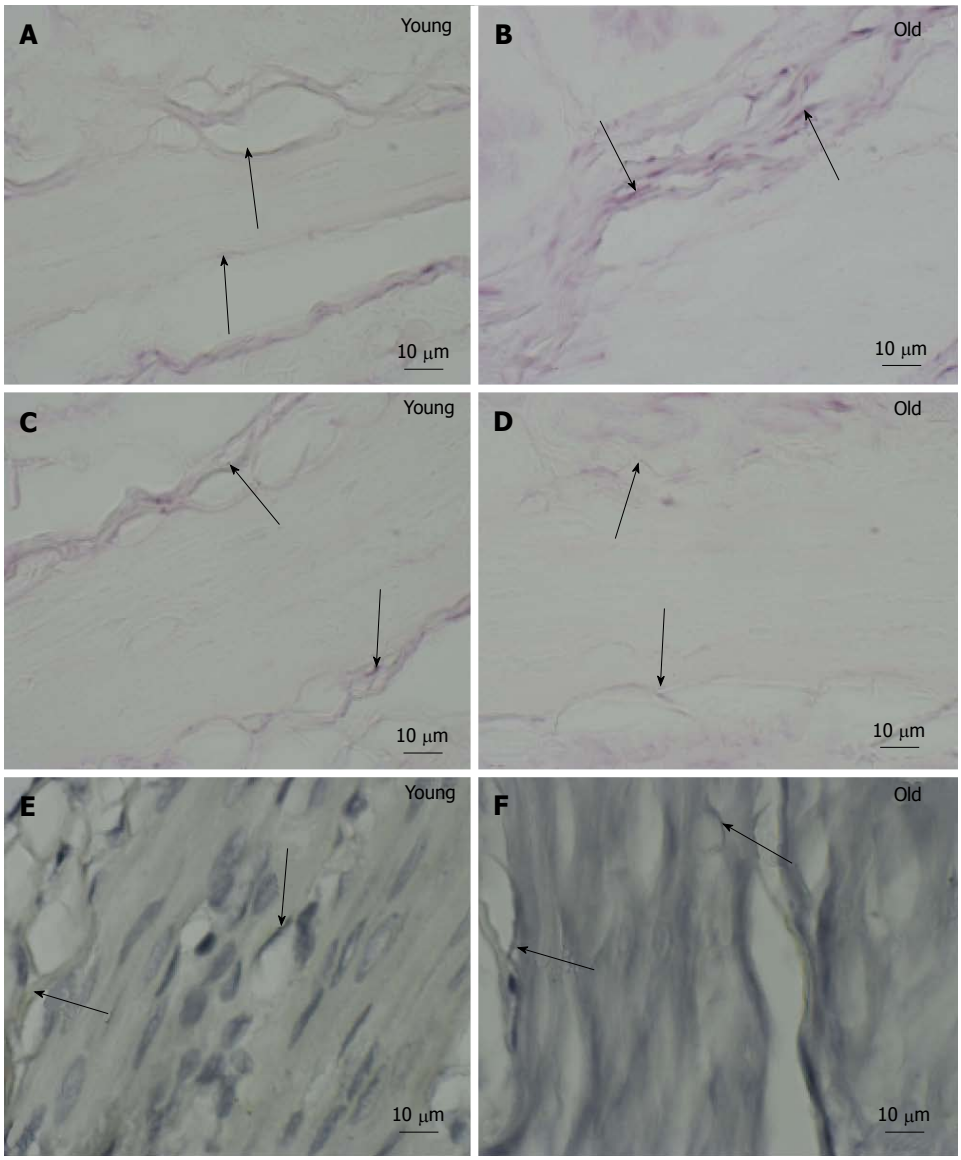

Figure 2 Histological sections of ileocecal junctions in the young group (A, C, E) and the elderly group (B, D, F) stained with Weigert with oxone (A, B), Weigert (C, D) and Verhoeff (E, F).

endomysium of the cells (Figure 4C). There were many unmyelinated nerve fibers of different diameters (Figure 4D).

SEM

SEM of ileocecal transition segment samples from the young and elderly groups revealed the transition region, with mucosal projections of the ileal and cecal regions (Figure 5). In the young group, the mucosal surface of the transition between the ileum and cecum showed a perfectly demarcated area where numerous elongated buds had formed on the microvilli in the ileum region (Figure 5A and B). After treatment with sodium hydroxide solution, the cell layers were completely removed and the ileocecal transition region was clearly identified, revealing numerous foramens in the cecum. There were foramens and laminar projections of collagen fibers in the ileum (Figure 5C and D); however, the cecal region had only foramens without collagen (Figure 5D).

In the elderly group, the line delimiting the two regions was visible as a surface containing a groove (Figure 5E). A characteristic, normal-looking mucosa forming the microvilli was observed (Figure 5F). After treatment with a sodium hydroxide solution, the epithelial-tissue interface regions of the ileum and cecum showed that the ileum region had numerous laminar projections of collagen fibers interspersed with an essentially circular foramen (Figure 5G). In the region of the cecum, numerous circular and elongated foramens in a three-dimensional arrangement were observed (Figure 5H).

Quantitative analysis

An analysis of light intensity after picrosirius staining under polarized light showed that the value for the average linear type Ⅰ collagen fibers in the young group

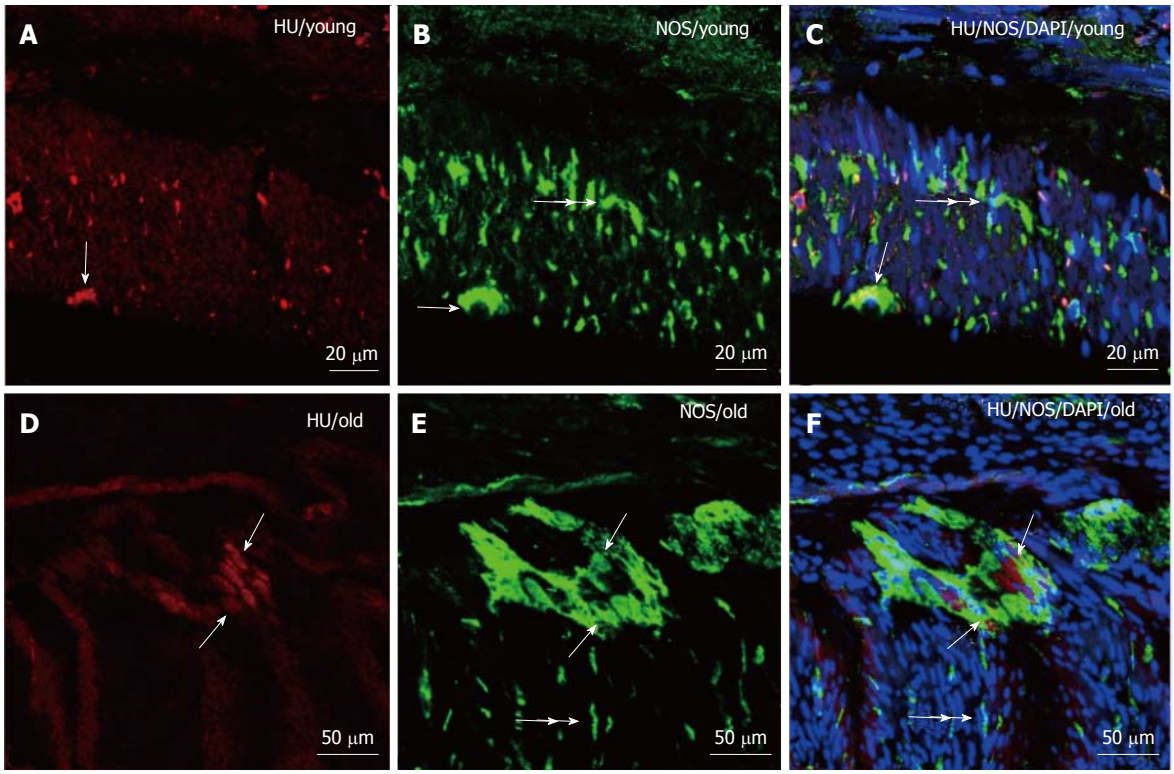

Figure 3 Immunohistochemistry mistry final. HuC/D staining in immunoreactive neurons (A, D), NOS staining in immunoreactive neurons and nerve fibers (B, E), and DAPI staining of the nuclei of immunoreactive cells (C, F) of the ileocecal junction in young rats (A-C) and elderly rats. The co-localization of HuC/D, NOS and DAPI (C, F). The arrows indicate immunoreactive neurons stained for HuC/D (A, D), NOS (B, E), and triple colocalization with HuC/D, NOS and DAPI (C, F). The double arrows indicate immunoreactive fibers stained for NOS (B, E). The double arrows show muscle fiber nuclei stained with DAPI (C, F). NOS: Nitric oxide synthage; DAPI: 4',6-diamidino-2-phenylindole.

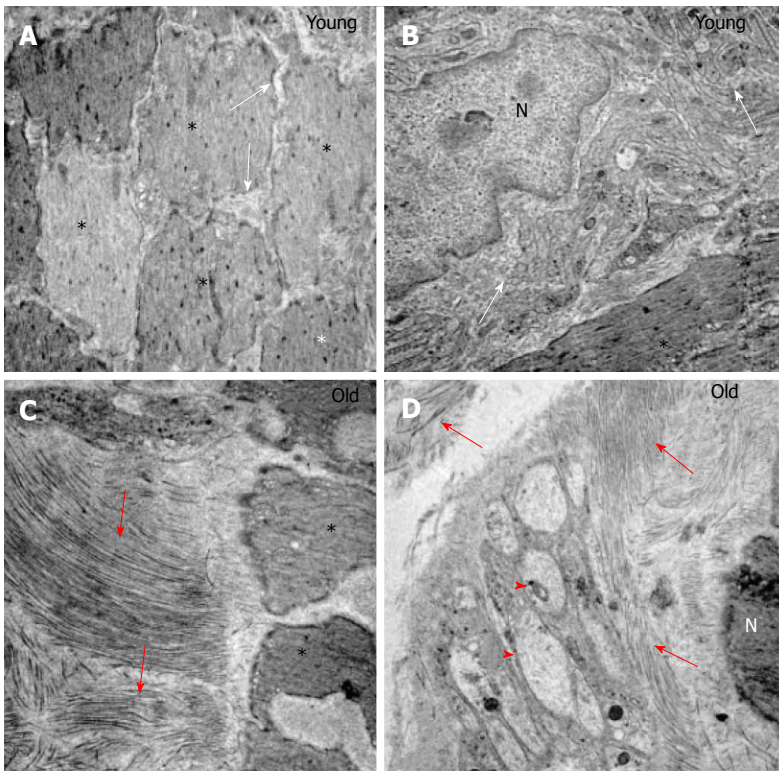

Figure 4 Transmission electron microscopy. A: A cross-section of the muscle layer showing the muscle fibers (*) and connective tissue (arrow) of the young group. Magnification: × 6000; B: A longitudinal section of the muscle layer showing muscle fibers (*), nuclei (N) and a set of unmyelinated fibers (arrows) of the young group Magnification: × 20000; C: A longitudinal section of the muscular layer showing collagen fibers arranged in different directions (arrows) and muscle fibers of the elderly group. Magnification: × 10000; D: Nucleus (N) and collagen fibers arranged in different directions (arrows) and synaptic vesicles (arrowhead) of the elderly group. Magnification: × 10000.

was 10842.7 ± 212.6 pixels. The elderly group had a mean linear value of 16465.4 ± 184.4 pixels, a significant increase of 51.9% ($P < 0.001$) compared to that in the young group (Figure 6A). The average linear value of type Ⅲ collagen fibers in the young group was 21706.4 ± 47.9 pixels. The elderly group showed a mean linear

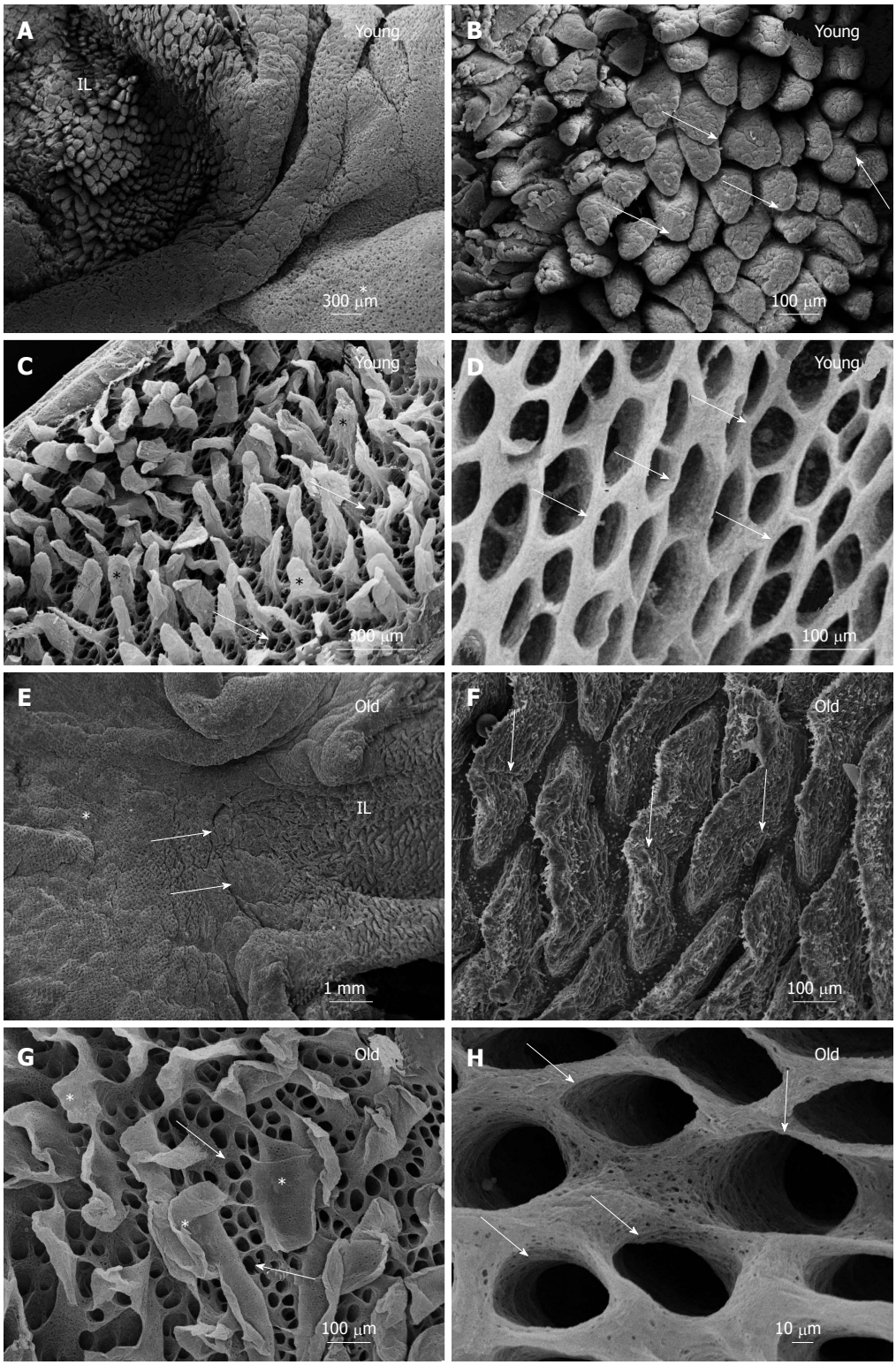

Figure 5 Normal mucosa of the rat ileocecal transition point of the young and elderly groups. A: Normal mucosa of the rat ileocecal transition point of the young showing the ileum (IL) and cecum (*); B: Villi with a normal appearance (arrows); C: Sample treated with NaOH solution. Image showing the connective tissue with different forms of blades (*) and numerous foramens (arrows) in a network of collagen fibers; D: Image showing the interphase cell surface tissue of the cecum, including the foramen; E: Ileum (IL), cecum (*) and ileocecal junction (arrows) of the elderly group; F: The highest increase in ileal villi (arrows); G: Sample treated with NaOH solution. Ileum tissue-shaped blade (*) and numerous foramens as a network of collagen fibrils (arrows). Image showing the foramen cecum surface (arrows).

value of 20876.9 ± 60.4 pixels, a significant decrease of 3.8% ($P < 0.001$) compared to the young group (Figure 6A).

The results for elaunin, which were obtained from samples stained with resorcin-fuchsin (Weigert), showed that the average linear density was 0.00692 ± 0.00015/

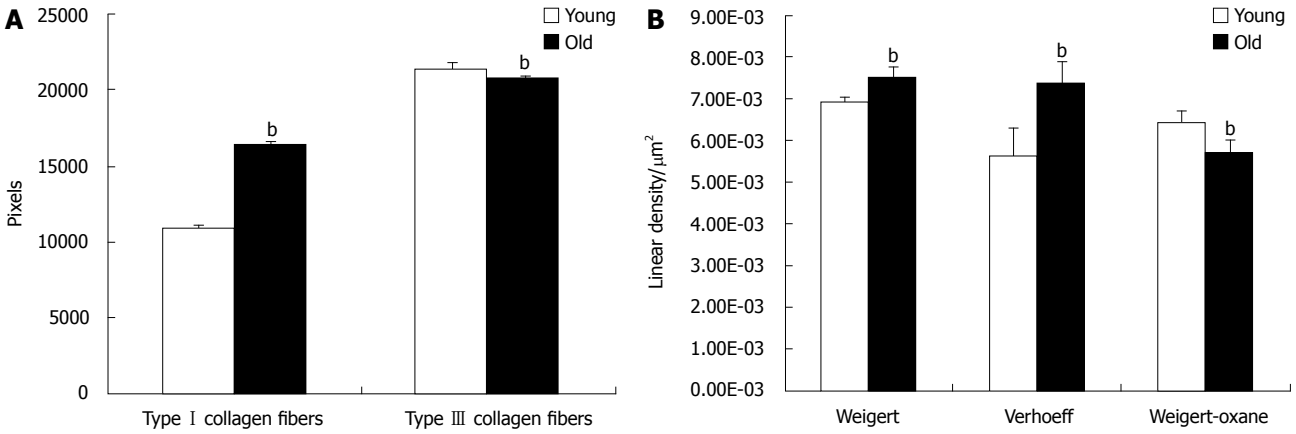

Figure 6 Quantitative analysis. Data related to the means ± SD of the mean linear (pixels) of type Ⅰ and type Ⅲ collagen (A) and data indicating the linear density (LD) expressed as the mean ± SD of oxytalan elastic fibers (Weigert) (μm^2), elaunin fibers (Verhoeff) (μm^2), and mature elastic fibers (Weigert-Oxane) (μm^2) (B) of the young and old groups. $^bP < 0.001$.

μm^2 in the young group and 0.0075 ± 0.00023/μm^2 in the elderly group, a significant increase of 8.7% in the elderly group compared to the young group ($P < 0.001$; Figure 6B). Regarding the mature elastic fibers that underwent Verhoeff staining, the mean linear density was 0.00564 ± 0.00067/μm^2 in the young group and 0.0074 ± 0.00049/μm^2 in the elderly group, a significant increase of 31.2% ($P < 0.001$) in the elderly group compared to the young group (Figure 6B). After oxytalan, resorcin, and fuchsin staining (Weigert) and after oxidation with a 1% aqueous solution of oxone, the linear density of the fibers in the young group was 0.0064 ± 0.0067 μm^2, and that in the elderly group was 0.00575 ± 0.00027/μm^2. There was a significant decrease of 10.3% in the elderly group compared to that in the young group ($P = 0.001$; Figure 6B).

DISCUSSION

This study examined and compared the ICJ structures of young and aged rats. Histological analysis revealed that connective tissue fibers and smooth muscle cells were present in the ICJs of both groups. The ileum protruded into the cecum, as reported[4], who used human ICJ samples and reported that the muscle layers of the ileum and large intestine extended into the ileal papilla. In this study, we observed a thickening and narrowing of the smooth muscle layer in both groups, as reported[1-4,10].

Histologically, the elderly group exhibited a condensation of the connective tissue between smooth muscle cells in the transition; however, the young group displayed noticeable gaps between the smooth muscle fibers, which likely indicate limited development of collagen fibers. Similar results were described in colons of humans at different ages[25]; their data indicated that the connective tissue was more slender in the young group but had a larger amount of collagen and elastic fibers around the myenteric plexus in older individuals. Our results demonstrated that smooth muscle cells of the ICJ were distributed with three distinct muscle layers, *i.e.,* two circular muscle layers and a longitudinal layer, as also observed[10]. In the transition region, there was a thickening of the circular muscle layer, as previously observed[4].

The present data revealed the presence of NOS in the ICJ. Furness[26] (2006) emphasized that NOS catalyzes the formation of the NO that is present in the myenteric plexus and neuronal processes in the gut, which acts to relax the muscle fiber function of the gastrointestinal musculature. Previous studies have shown the presence of NOS in the enteric ganglia and muscle fibers under various conditions, such as malnutrition and renutrition[27,28], ischemia and reperfusion[29-31] and obesity[32,33]. The enteric neurons of the ICJ stain positive for NOS, PGP 9.5, and c-kit[3,4,6,8]. Additionally, neuronal nitric oxide synthase (nNOS) is present in the myenteric, the submucosal ganglia and the IJC muscle layers in horse[8]. In our study, we noticed the presence of NOS in the muscle layers and neurons in the ICJs of both the young and elderly groups, but we did not observe a difference in the intensity of the stain for HuC/D or NOS between groups. Major losses of enteric neurons occur during aging; these losses have been described in both the myenteric and submucous plexus ganglia of all regions of the gut in several mammalian species[24,34]. Regarding significant changes in the number of myenteric neurons during aging in several species, including humans[35,36], previous studies have suggested the possible involvement of the regulation of gastrointestinal functions. Moreover, Hoyle and Saffrey[37] (2012) stressed that the thickening of the circular muscle layer is due to increased contractility during aging.

In our ultrastructural analysis, both groups had smooth muscle cells with elongated nuclei between these collagen fibers, forming an endomysium of smooth muscle cells. In the group of older animals, the collagen fibers were thicker. The mitochondria in both groups had different shapes and sizes. It should be noted that, in this study, the mitochondria were not quantified, but they appeared to be present in greater quantity in the elderly group. In agreement with observations obtained in this study,

the authors reported that in the submucosal plexus of the small intestine of rats at different ages, mitochondria were present in greater numbers in the elderly group and had a degenerative aspect[38]. In the process of aging, changes in cell morphology occur that include the presence of pleomorphic mitochondria[39].

The SEM results in both groups revealed a clear demarcation of the transition region, which was bounded by a line of flat connective tissue between the ileum and the cecum. In the ileum, numerous elongated buds were present, constituting microvilli. The villus morphology seemed to depend on age, exhibiting a sheet form, which has been predominantly reported in children. However, this form is present in adults as fingerlike projections[40]. Collagen fibers were present on both sides of the cecal ileum, forming the transition surface. In the cecal portion, low laminar-form microvilli were observed, as were numerous foramens of regular collagen fibers with interconnected formats.

Our results showed that the type Ⅰ collagen fibers in the elderly group were more bulky and appeared thicker; they were classified as mature collagen. These fibers were resistant to traction and tension, giving strength to the tissue. In contrast, in the young group, we noticed a predominance of type Ⅲ collagen fibers, which were thinner and were characterized as immature collagen, which produces flexibility in the tissue. In accordance with the results observed[38], the elderly group exhibited a replacement of type Ⅲ collagen with type Ⅰ collagen around the submucosal plexus of the jejunum and ileum compared to the young group. This finding suggests that changes in the distribution of collagen fibers could damage the function of the submucosal ganglia. Also, authors reported an increase in the number of collagen fibers in the aorta of aged mice[41]. In addition, authors showed that aging favored an increase in the diameter of collagen fibrils[42], in agreement with our findings. Additionally, both collagen and elastic system fibers were more numerous in the enteric ganglia from the old subjects[25]. Changes in the distribution pattern of collagen fibers in the ICJ can lead to intestinal disorders, such as decreased motility and changes in the retrograde return of feces, which consequently leads to the inflammation of the ileal mucosa. Therefore, the replacement of the collagen in the ICJ is not beneficial to the operating mechanism of the intestinal segment.

During aging, changes occur in the architecture of the collagen fibers, compromising the biomechanical and biochemical properties of tissues due to an accumulation of advanced glycation end-products (AGEs)[43]. The authors also suggested that aging structurally changes the collagen monomer, which greatly affects both the fibrillogenesis process and the architecture of the collagen fibers.

Additionally, our results reveal that elastic fibers were identified by staining with Weigert oxone, Weigert and Verhoeff, indicating that three types of the elastic system fibers were present along the smooth muscles of the ICJ. The linear density analysis revealed that oxytalan fibers were in greater quantity in the young group and were diminished in the elderly group. Similar results in the gastroduodenal junctions of young and old animals were reported[21]. In the present work, the linear density of elaunin fibers and elastic fibers was increased in the elderly group compared to the young group, and oxytalan fibers was decreased in the elderly group compared to the young group. Similar results were described by[44,45], who found that during aging, there was a decrease in oxytalan fibers and an increase mature elaunin and elastic fibers. Furthermore, elastin was thicker and more fragmented in older people, and there was a greater deposition of calcium in the amorphous material. In a study of aging cerebral meninges, reported decreases and increases in the oxytalan fiber contents of mature elaunin and elastic fibers, respectively[46]. In studies on the vas deferens reinforced these findings, stating that there was an increase in elastin during aging[47]. Moreover, study of the interspinous ligament during aging found the disappearance of oxytalan fibers[48], which is in agreement with our results. In addition to our study, authors suggested that aging is accompanied by a significant and progressive reduction in oxytalan fibers and significant increases in mature elaunin and elastic fibers in the interfoveolar ligament[44].

Elaunin fibers play an intermediary role between oxytalan fibers and mature elastic fibers, providing functional adaptation in different tissues. Based on the results, the function of the ICJ appears to change with aging, which is associated with changes in the patterns of distribution of collagen and elastic fibers, resulting in increased tensile strength and firmness, but decreased elasticity. With the decrease in the ICJ, oxytalan fibers can lose complacency, becoming less flexible, looser and less able to retreat. Moreover, increased amounts of mature elaunin and elastic fibers are present. We suggest that, with the gradual reduction of the elastic components and replacement of the collagen types in the fibers, ICJ function loss occurs due to the loss of elasticity and the resultant decreased distensibility.

Finally, we highlight the importance of the results of our morphoquantitative analysis of changes in the connective tissue of the ICJ in young and elderly groups, which revealed changes in the patterns of distribution of collagen and elastic fibers that may lead to a possible decrease in ICJ functionality, which may favor the occurrence of pathological processes. These results do not elucidate all the aspects of ICJ function; additional studies should be conducted in the future.

ACKNOWLEDGMENTS

We thank Rosana Prisco for performing the statistical analysis and Sonia Regina Yokomizo Marta Maria da Silva Righetti, Sebastião Aparecido Boleta and Kelly Patricia Nery Borges for technical assistance.

COMMENTS

Background

The ileocecal junction (ICJ) has two aspects: A wedge-shaped cavity that progressively narrows the orifice to form the ileum and is bordered by an upper lip and lower lip, joined by front and posterior commissures; and an invagination of the small intestine to the large intestine.

Research frontiers

The neurochemical characterization of the inhibitory neurons of the myenteric plexus, which are immunoreactive to the enzyme nitric oxide synthase, and the staining of the neuronal population were employed to identify immunoreactivity to HuC/D.

Innovations and breakthroughs

The authors highlight the importance of the results of our morphoquantitative analysis of changes in the connective tissue of the ICJ in young and elderly groups, which revealed changes in the patterns of distribution of collagen and elastic fibers that may lead to a possible decrease in ICJ functionality, which may favor the occurrence of pathological processes.

Peer-review

The aim of the paper was to analyze the structural organization of the elastic and collagen fibers in the region of the ileocecal transition in 30 young and old male Wistar rats by using different updating techniques. The study is original, interesting and well conducted.

REFERENCES

1 **Ferraz de Carvalho CA**, Rodrigues de Souza R, Henrique A, Henrique A, Nogueira de Lima MA. Functional anatomy of the tela submucosa of the valva ileocecalis in the adult man. *Anat Anz* 1987; **164**: 63-76 [PMID: 3662029]
2 **Shafik AA**, Shafik A, Asaad S, Wahdan M. A study of an anatomic-physiological cecocolonic sphincter in humans. *Clin Anat* 2010; **23**: 851-861 [PMID: 20641065 DOI: 10.1002/ca.21026]
3 **Shafik AA**, Ahmed IA, Shafik A, Wahdan M, Asaad S, El Neizamy E. Ileocecal junction: anatomic, histologic, radiologic and endoscopic studies with special reference to its antireflux mechanism. *Surg Radiol Anat* 2011; **33**: 249-256 [PMID: 21184079 DOI: 10.1007/s00276-010-0762-x]
4 **Pollard MF**, Thompson-Fawcett MW, Stringer MD. The human ileocaecal junction: anatomical evidence of a sphincter. *Surg Radiol Anat* 2012; **34**: 21-29 [PMID: 21863224 DOI: 10.1007/s00276-011-0865-z]
5 **Kumar D**, Phillips SF. The contribution of external ligamentous attachments to function of the ileocecal junction. *Dis Colon Rectum* 1987; **30**: 410-416 [PMID: 3595357 DOI: 10.1007/BF02556486]
6 **Cserni T**, Paran S, Kanyari Z, O'Donnell AM, Kutasy B, Nemeth N, Puri P. New insights into the neuromuscular anatomy of the ileocecal valve. *Anat Rec* (Hoboken) 2009; **292**: 254-261 [PMID: 19089903 DOI: 10.1002/ar.20839]
7 **Vidotti AP**, Didio LJA, Prado MIM. Caracterização morfológica da região de Transição entre o intestino delgado e o grosso no javali. *Brazilian J Veterin Res Animal Sci* 2007; **44**: 151-158
8 **Russo D**, Bombardi C, Grandis A, Furness JB, Spadari A, Bernardini C, Chiocchetti R. Sympathetic innervation of the ileocecal junction in horses. *J Comp Neurol* 2010; **518**: 4046-4066 [PMID: 20737599 DOI: 10.1002/cne.22443]
9 **Malbert CH**. The ileocolonic sphincter. *Neurogastroenterol Motil* 2005; **17** Suppl 1: 41-49 [PMID: 15836454 DOI: 10.1111/j.1365-2982.2005.00657.x]
10 **Miyamoto-Kikuta S**, Ezaki T, Komuro T. Distribution and morphological characteristics of the interstitial cells of Cajal in the ileocaecal junction of the guinea-pig. *Cell Tissue Res* 2009; **338**: 29-35 [PMID: 19823824 DOI: 10.1007/s00441-009-0854-2]
11 **Prado IM**, Di Dio LJ, Miranda-Neto MH, Molinari SL, Macchiarelli G. Three-dimensional distribution of the collagen fibers in the submucosa of the swine terminal ileum. *Ital J Anat Embryol* 2005; **110**: 77-86 [PMID: 16101024]
12 **Kielty CM**, Sherratt MJ, Shuttleworth CA. Elastic fibres. *J Cell Sci* 2002; **115**: 2817-2828 [PMID: 12082143]
13 **Cotta-Pereira G**, Rodrigo FG, David-Ferreira JF. The elastic system fibers. *Adv Exp Med Biol* 1977; **79**: 19-30 [PMID: 68662 DOI: 10.1007/978-1-4684-9093-0_3]
14 **Quigley EM**, Thompson JS. Effects of artificial ileocolonic sphincter on motility in intestinal remnant following subtotal small intestinal resection in the dog. *Dig Dis Sci* 1994; **39**: 1222-1229 [PMID: 8200254 DOI: 10.1007/BF02093787]
15 **Fullmer HM**, Lillie J. The oxytalan fiber: a previously undescribed connective tissue fiber. *Journal of Histochemistry and Cytochemistry* 1958; **6**: 425-430 [PMID: 13598878]
16 **Bittencourt-Sampaio S**, Cotta-Pereira G, Guerra-Rodrigo F. Oxytalan, elaunin and elastic fibers in the human skin. *J Invest Dermatol* 1971; **66**: 143-148 [PMID: 1249442]
17 **Cotta-Pereira G**, Rodrigo FG, David-Ferreira JF. The use of tannic acid-glutaraldehyde in the study of elastic and elastic-related fibers. *Stain Technol* 1976; **51**: 7-11 [PMID: 59416]
18 **Watanabe I**, Yamada E. The fine structure of lamellated nerve endings found in the rat gingiva. *Arch Histol Jpn* 1983; **46**: 173-182 [PMID: 6882151 DOI: 10.1679/aohc.46.173]
19 **Ohtani O**. Three-dimensional organization of the connective tissue fibers of the human pancreas: a scanning electron microscopic study of NaOH treated-tissues. *Arch Histol Jpn* 1987; **50**: 557-566 [PMID: 3326543 DOI: 10.1679/aohc.50.557]
20 **Kronka MC**, Watanabe I, Cavenaghi Pereira da Silva M, König Júnior B. Corrosion casts of young rabbit palatine mucosa angioarchitecture. *Ann Anat* 2000; **182**: 529-531 [PMID: 11125803 DOI: 10.1016/S0940-9602(00)80097-7]
21 **Brito MC**, Cury DP, Barbosa ACS, Silva P.M, Chopard RP. Morphological and morphometric study of collagen and elastic fibers of the gastroduodenal junction of adult and old Wistar rats. *J Morphol Sci* 2013; **30**: 281-288
22 **Chopard RP**, Lucas GA, Gerhard R, Lourenço MG. A histomorphological study in age related changes in the elastic fiber system of the basilar artery. *Ital J Anat Embryol* 1998; **103**: 157-175 [PMID: 9882958]
23 **Chopard RP**, Gerhard R. Histomorphometrical study of the elastic fiber system in the anterior cerebral artery of man. *Arq Neuropsiquiatr* 2000; **58**: 1040-1046 [PMID: 11105071 DOI: 10.1590/S0004-282X2000000600011]
24 **Niewoehner DE**, Kleinerman J. Morphometric study of elastic fibers in normal and emphysematous human lungs. *Am Rev Respir Dis* 1977; **115**: 15-21 [PMID: 835883]
25 **Gomes OA**, de Souza RR, Liberti EA. A preliminary investigation of the effects of aging on the nerve cell number in the myenteric ganglia of the human colon. *Gerontology* 1997; **43**: 210-217 [PMID: 9222749 DOI: 10.1159/000213852]
26 **Furness JB**. The enteric nervous system. Malden: Blackwell Publishing Inc., 2006
27 **Misawa R**, Girotti PA, Mizuno MS, Liberti EA, Furness JB, Castelucci P. Effects of protein deprivation and re-feeding on P2X2 receptors in enteric neurons. *World J Gastroenterol* 2010; **16**: 3651-3663 [PMID: 20677337 DOI: 10.3748/wjg.v16.i29.3651]
28 **Girotti PA**, Misawa R, Palombit K, Mendes CE, Bittencourt JC, Castelucci P. Differential effects of undernourishment on the differentiation and maturation of rat enteric neurons. *Cell Tissue Res* 2013; **353**: 367-380 [PMID: 23644765 DOI: 10.1007/s00441-013-1620-z]
29 **Paulino AS**, Palombit K, Cavriani G, Tavares-de-Lima W, Mizuno MS, Marosti AR, da Silva MV, Girotti PA, Liberti EA, Castelucci P. Effects of ischemia and reperfusion on P2X2 receptor expressing neurons of the rat ileum enteric nervous system. *Dig Dis Sci* 2011; **56**: 2262-2275 [PMID: 21409380 DOI: 10.1007/s10620-011-1588-z]
30 **Palombit K**, Mendes CE, Tavares-de-Lima W, Silveira MP, Castelucci P. Effects of ischemia and reperfusion on subpopulations of rat enteric neurons expressing the P2X7 receptor. *Dig Dis Sci* 2013; **58**: 3429-3439 [PMID: 23990036 DOI: 10.1007/s10620-013-2847-y]

31 **Marosti AR**, da Silva MV, Palombit K, Mendes CE, Tavares-de-Lima W, Castelucci P. Differential effects of intestinal ischemia and reperfusion in rat enteric neurons and glial cells expressing P2X2 receptors. *Histol Histopathol* 2015; **30**: 489-501 [PMID: 25400134]

32 **Mizuno MS**, Crisma AR, Borelli P, Castelucci P. Expression of the P2X receptor in different classes of ileum myenteric neurons in the female obese ob/ob mouse. *World J Gastroenterol* 2012; **18**: 4693-4703 [PMID: 23002338 DOI: 10.3748/wjg.v18.i34.4693]

33 **Mizuno MS**, Crisma AR, Borelli P, Schäfer BT, Silveira MP, Castelucci P. Distribution of the P2X2 receptor and chemical coding in ileal enteric neurons of obese male mice (ob/ob). *World J Gastroenterol* 2014; **20**: 13911-13919 [PMID: 25320527 DOI: 10.3748/wjg.v20.i38.13911]

34 **Phillips RJ**, Powley TL. Innervation of the gastrointestinal tract: patterns of aging. *Auton Neurosci* 2007; **136**: 1-19 [PMID: 17537681 DOI: 10.1016/j.autneu.2007.04.005]

35 **Santer RM**, Baker DM. Enteric neuron numbers and sizes in Auerbach's plexus in the small and large intestine of adult and aged rats. *J Auton Nerv Syst* 1988; **25**: 59-67 [PMID: 3225382 DOI: 10.1016/0165-1838(88)90008-2]

36 **Saffrey MJ**. Cellular changes in the enteric nervous system during ageing. *Dev Biol* 2013; **382**: 344-355 [PMID: 23537898 DOI: 10.1016/j.ydbio.2013.03.015]

37 **Hoyle CH**, Saffrey MJ. Effects of aging on cholinergic neuromuscular transmission in isolated small intestine of ad libitum fed and calorically-restricted rats. *Neurogastroenterol Motil* 2012; **24**: 586-592 [PMID: 22435850 DOI: 10.1111/j.1365-2982.2012.01913.x]

38 **Zanesco MC**, Souza RR. Morphoquantitative study of the submucous plexus (of Meissner) of the jejunum-ileum of young and old guinea pigs. *Arq Neuropsiquiatr* 2011; **69**: 85-90 [PMID: 21359429 DOI: 10.1590/S0004-282X2011000100017]

39 **Silva WJM,** Ferrari CKB. Mitochondrial metabolism, free radicals and aging. *Revista Brasileira de Geriatria e Gerontologia* 2011; **14**: 441-451 [DOI: 10.1590/S1809-98232011000300005]

40 **Kim SB**, Barone B, Secreto HRC, Silva MRR, Egami MJ, Secreto RA, Juliano Y. Morfologia e morfometria da parede do íleo terminal de camundongos expostos ao raio x. *Acta cirurgica Brasileira* 1994; **9**: 174-182

41 **Cheng JK**, Stoilov I, Mecham RP, Wagenseil JE. A fiber-based constitutive model predicts changes in amount and organization of matrix proteins with development and disease in the mouse aorta. *Biomech Model Mechanobiol* 2013; **12**: 497-510 [PMID: 22790326 DOI: 10.1007/s10237-012-0420-9]

42 **Floridi A**, Ippolito E, Postacchini F. Age-related changes in the metabolism of tendon cells. *Connect Tissue Res* 1981; **9**: 95-97 [PMID: 6458452 DOI: 10.3109/03008208109160246]

43 **Wilson SL**, Guilbert M, Sulé-Suso J, Torbet J, Jeannesson P, Sockalingum GD, Yang Y. A microscopic and macroscopic study of aging collagen on its molecular structure, mechanical properties, and cellular response. *FASEB J* 2014; **28**: 14-25 [PMID: 24025727 DOI: 10.1096/fj.13-227579]

44 **Quintas ML**, Rodrigues CJ, Yoo JH, Rodrigues Junior AJ. Age related changes in the elastic fiber system of the interfoveolar ligament. *Rev Hosp Clin Fac Med Sao Paulo* 2000; **55**: 83-86 [PMID: 10983010 DOI: 10.1590/S0041-87812000000300003]

45 **Rodrigues Junior AJ**, Rodrigues CJ, da Cunha AC, Jin Y. Quantitative analysis of collagen and elastic fibers in the transversalis fascia in direct and indirect inguinal hernia. *Rev Hosp Clin Fac Med Sao Paulo* 2002; **57**: 265-270 [PMID: 12612758]

46 **Pereira KF**, Lima VM, Conegero CI, Chopard RP. Histomorfometria das meninges encefálicas de ratos Wistar em diferentes faixas etárias. *Pesquisa Veterinária Brasileira* 2010; **30**: 996-1002 [DOI: 10.1590/S0100-736X2010001100015]

47 **Paniagua R**, Regadera J, Nistal M, Santamaría L. Elastic fibres of the human ductus deferens. *J Anat* 1983; **137** (Pt 3): 467-476 [PMID: 6654739]

48 **Barros EM**, Rodrigues CJ, Rodrigues NR, Oliveira RP, Barros TE, Rodrigues AJ. Aging of the elastic and collagen fibers in the human cervical interspinous ligaments. *Spine J* 2002; **2**: 57-62 [PMID: 14588289 DOI: 10.1016/S1529-9430(01)00167-X]

P- Reviewer: de Talamoni NGT, Pescatori M **S- Editor**: Qi Y
L- Editor: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i3.428

World J Gastrointest Pharmacol Ther 2016 August 6; 7(3): 428-433
ISSN 2150-5349 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Ethnic variations in ulcerative colitis: Experience of an international hospital in Thailand

Vibhakorn Permpoon, Krit Pongpirul, Sinn Anuras

Vibhakorn Permpoon, Sinn Anuras, Digestive Disease Center, Bumrungrad International Hospital, Bangkok 10600, Thailand

Krit Pongpirul, Department of Preventive and Social Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand

Krit Pongpirul, Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, United States

Krit Pongpirul, Bumrungrad Research Center, Bumrungrad International Hospital, Bangkok 10110, Thailand

Author contributions: Permpoon V and Anuras S initiated and designed the study; Pongpirul K helped to collect, analyze, and interpret the data; Permpoon V and Pongpirul K drafted the manuscript; all authors read and approved the final version of the manuscript.

Institutional review board statement: The study was reviewed and approved by the Bumrungrad International Institutional Review Board No. BI/IRB No.146-09-11.

Informed consent statement: All subjects in this study gave their written informed consent. Participant's identifiable information was removed.

Conflict-of-interest statement: The authors declare no conflict of interest.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at doctorkrit@gmail.com. The presented data are anonymized and risk of identification is low.

Manuscript source: Unsolicited manuscript

Correspondence to: Krit Pongpirul, MD, MPH, PhD, Department of Preventive and Social Medicine, Faculty of Medicine, Chulalongkorn University, 1873 Rama IV Rd., Pathumwan, Bangkok 10330, Thailand. doctorkrit@gmail.com
Telephone: +66-86-6055088

Received: February 9, 2016
Peer-review started: February 11, 2016
First decision: March 14, 2016
Revised: April 12, 2016
Accepted: May 7, 2016
Article in press: May 9, 2016
Published online: August 6, 2016

Abstract

AIM: To investigate the clinical characteristics, treatment, medication use, and treatment response in patients with ulcerative colitis (UC) across ethnic groups.

METHODS: This study retrospectively analyzed medical records of all 268465 patients who visited the Bumrungrad International Digestive Disease Center during 2005-2010. The demographics, clinical characteristics, medication use, results of investigations, and medical and surgical management for patients with UC were evaluated. Evaluation included sigmoidoscopy and colonoscopy performed in compliance with the American Society of Gastrointestinal Endoscopy practice guidelines. Patient ethnicities were categorized into seven groups: Thai, Oriental, South Asian (SA), Middle Eastern (ME), Caucasian, African, and Hispanic. UC pathological severity was classified into inactive, mild, moderate, and severe. Associations between categorical variables were analyzed using the χ^2 or Fischer's exact test. Associations between categorical and interval variables were analyzed using

Student's t-test and/or analysis of covariance.

RESULTS: UC was diagnosed in 371 of the 268465 patients: male 56.33%; ME 42%, Caucasian 23%, and Thai 19%. Annual incidence of UC was 82 cases per 100000 with wide ethnic variation, ranging from 29 to 206 cases per 100000 in Oriental and ME patients, respectively. Of the patients with UC, 16.71% had severe UC with highest incidence among the patients from ME (20.39%) and lowest among the Caucasian population (11.90%). ME had highest proportion of pancolitis (52.90%), followed by Caucasian (45.35%) and Asian (34.40%). Only 20.93% of Caucasian patients received steroid, compared with 26.40% and 27.10% of Asian and Middle Eastern, respectively (P = 0.732). Overall, 13.72% of UC patients did not respond to steroid therapy, with non-significantly higher proportions of non-responders among Asian and Middle Eastern patients (15.22% and 15.04%, respectively) (P = 0.781). On average, 5.93% underwent surgical management with ethnic variation, ranging from 0% in African to 18% in SA. Cancer was found in three (Thai, ME, and African) cases (0.82 institution-specific incidence).

CONCLUSION: Incidence, symptom duration, pathological severity, clinical manifestations, medication use, treatment response, need for surgical consultation, and cancer incidence of patients with UC potentially vary by ethnicity.

Key words: Ulcerative colitis; Ethnic groups; Anatomical pathological conditions; Medical tourism; Retrospective studies

Core tip: Incidence and prevalence of ulcerative colitis have been shown to vary across geographical areas and ethnic groups. Patients from different ethnic origins and/or healthcare systems have been managed using the same guidelines for diagnosis and treatment of ulcerative colitis. In this study, comparative analysis of symptom duration, pathological severity, extra-intestinal manifestations, surgical consultation need, medication use, and cancer incidence across ethnic groups were presented. Understanding how these attributes vary by ethnicity is useful for service delivery design, especially in this facility that is responsible for the care of patients from diverse backgrounds.

Permpoon V, Pongpirul K, Anuras S. Ethnic variations in ulcerative colitis: Experience of an international hospital in Thailand. *World J Gastrointest Pharmacol Ther* 2016; 7(3): 428-433 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i3/428.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i3.428

INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory condition of the colon in genetically susceptible individuals exposed to environmental risk factors[1]; it is also an emerging global disease[2]. Ethnicity has long been hypothesized as one of the determinants for developing UC based on the varying incidence and prevalence across geographical boundaries[2-5]. In general, Asian and Middle Eastern populations have a lower incidence of UC than Caucasian individuals (6.3 *vs* 24.3 per 100000 person-years)[2]. As patients in low-income countries have been diagnosed less frequently than those in richer countries, UC has been believed to be associated with industrialization of nations[2].

Health care systems in each country may play significant roles in the diagnosis and management of UC. Although existing epidemiological data are useful for design of health service delivery within a country, these data might not be adequate for a healthcare institution that provides medical services in one setting to patients from various origins. Although developed countries have a higher incidence of UC, the effective health care systems have better clinical outcomes than that of less developed ones. In contrast, the epidemiologic findings are more likely to be affected by the genetics of the population than by the health care system.

Better understanding of the clinical course of UC can help to guide clinical decisions. Patients are symptomatic for varying lengths of time before the definitive diagnosis is made and their symptoms vary in severity at presentation. To accurately diagnose the disease properly and avoid disease progression due to delay in diagnosis, the patient should be evaluated by endoscopy with biopsy. Distribution of the lesions could guide medication choices and routes. A patient suffering pancolitis for longer period of time (*i.e.,* more than 10 years) has an increased risk of cancer and therefore should be re-assessed with colonoscopy at optimal time intervals. Recommended indications for surgical management have recently been updated but still rely on clinical judgment[6].

As a large private hospital for medical tourism in Asia, Bumrungrad International Hospital (BIH) serves more than one million patients from at least 190 countries annually. This allows comparative analyses across ethnic groups. This study compared clinical characteristics including incidence and severity, medication use, treatment response, surgical consultation need, and cancer incidence across ethnic groups.

MATERIALS AND METHODS

This retrospective study analyzed demographics, clinical characteristics, results of investigations, as well as medical and surgical management information in medical records of all patients who visited the Digestive Disease Center (BIDDC) during 2005-2010. Colonoscopy and sigmoidoscopy were performed in compliance with the American Society of Gastrointestinal Endoscopy (ASGE) practice guidelines. Ulcerative colitis was diagnosed based on clinical grounds and supported by the appropriate findings on total colonoscopy, biopsy, and by negative

Table 1 Ethnicity distribution of ulcerative colitis (2008-2010) and total gastrointestinal patients (2005-2010)

Ethnicity	2005	2006	2007	2008	2009	2010	Total	%Total	UC	UC	Annual Incidence
Thai	18820	20093	20256	23423	24224	23456	130272	49%	69	19%	32
Oriental	5333	6737	7060	8237	8614	9614	45595	17%	23	6%	29
South Asian	2329	2206	2003	2344	2566	2935	14383	5%	33	9%	140
Middle Eastern	4567	5831	6418	7828	7726	9483	41853	16%	155	42%	206
Caucasian	4088	4712	5264	5209	5658	5572	30503	11%	86	23%	174
African	488	598	836	1050	1287	1193	5452	2%	5	1%	47
Hispanic	44	86	60	73	67	77	407	0%	0	0%	0
Total	35669	40263	41897	48164	50142	52330	268465		371		82[1]

[1]Overall annual incidence was calculated from UC cases identified from all patients during 2008-2010 and therefore was not equal to the column average annual incidence. UC: Ulcerative colitis.

Table 2 Duration of ulcerative colitis symptoms and severity at presentation by ethnicity

Ethnicity	Duration (mo)	95%CI	Inactive	Mild	Moderate	Severe	Severe%	Extra-intestinal	Surgery
Thai	6.34	2.67-10.00	2	41	16	10	14.49%	2.90%	5.80%
Oriental	13.44	-10.42-37.31	0	9	10	4	17.39%	0.00%	8.70%
South Asian	14.04	3.45-24.63	2	16	8	6	18.75%	15.15%	12.12%
Middle Eastern	18.46	10.63-26.29	8	55	58	31	20.39%	9.68%	3.87%
Caucasian	6.74	0.08-13.41	6	35	33	10	11.90%	9.30%	6.98%
African	34.67	-29.98-99.32	0	3	2	0	0.00%	40.00%	0.00%
Overall	13.06	9.05-17.07	18	159	127	61	16.71%	8.63%	5.93%

stool examination for infectious causes. All patients received total colonoscopy to confirm the distribution of the colitis. The Montreal classification was used to classify severity of the disease.

Patient ethnicities were arbitrarily categorized into seven groups: Thai, Oriental, South Asian, Middle Eastern, Caucasian, African, and Hispanic. With sample size limitation, some analyses were done using the patients in three major ethnic groups: Asian (Thai, Oriental, South Asian), Middle Eastern, and Caucasian. UC severity was classified based on pathological findings into inactive, mild, moderate, and severe using standard, well-accepted published criteria. The need for surgical consultation was based on the content of relevant operative note in medical record; only colon-related surgeries (*i.e.,* partial colectomy and total procto-colectomy, colostomy, and ileo-anal pouch) were included. A patient with clinical response to high-dose glucocorticoids (prednisone 40 to 60 mg/d or equivalent) within 30 d for oral therapy or 7 to 10 d for intravenous therapy was classified as steroid responsive. Steroid dependence was defined if glucocorticoids cannot be tapered to less than 10 mg/d within three months of starting steroids, without recurrent disease, or if relapse occurs within 3 mo of stopping glucocorticoids. A patient without a meaningful clinical response to glucocorticoids up to doses of prednisone 40 to 60 mg/d (or equivalent) within 30 d for oral therapy or 7 to 10 d for intravenous therapy was classified as steroid refractory.

Descriptive statistics were used where appropriate. Association between categorical variables was analyzed using χ^2 test or Fischer's exact test. Association between categorical and interval variables was analyzed using Student's *t*-test and/or analysis of covariance where appropriate. The statistical analysis of this study was performed by the corresponding author who had formal biostatistics training as part of his doctoral education. This study was approved by Bumrungrad International Institutional Review Board (BI/IRB No.146-09-11).

RESULTS

Of 268465 individuals who visited BIDDC during 2005-2010, half were Thai (49%) (Table 1). The distribution of ethnicity of patients visiting the BIDDC was slightly different from hospital patient ethnic profiles (Thai: Non-Thai = 60:40). UC was diagnosed in 371 patients (Male 56.33%), 42% of which were Middle Eastern, 23% Caucasian and 19% Thai. Based on 2008-2010 data, overall annual facility-specific incidence of UC was estimated to be 82 cases per 100000 with wide ethnic variation, ranging from 29 to 206 cases per 100000 in Oriental and Middle Eastern patients, respectively.

Eighty-one percent of the patients presented with no more than one year of symptoms. Patients experienced UC symptoms for a mean of 13.06 mo (95%CI: 9.05-17.07) before their first visit to BIDDC (Table 2). Thai and Caucasian patients presented with a mean of 6.34 and 6.74 mo of UC symptoms, respectively. Middle Eastern patients had symptoms for more than 18 mo on average at presentation.

Overall, 16.71% of patients had severe UC with highest incidence among Middle Eastern patients (20.39%) and lowest among Caucasian (11.90%). Extra-intestinal manifestations were found in 8.63% of the patients with great ethnic variation (40% African *vs* 0% Oriental Non-Thai) (Tables 2 and 3). On average, 5.93% of the patients

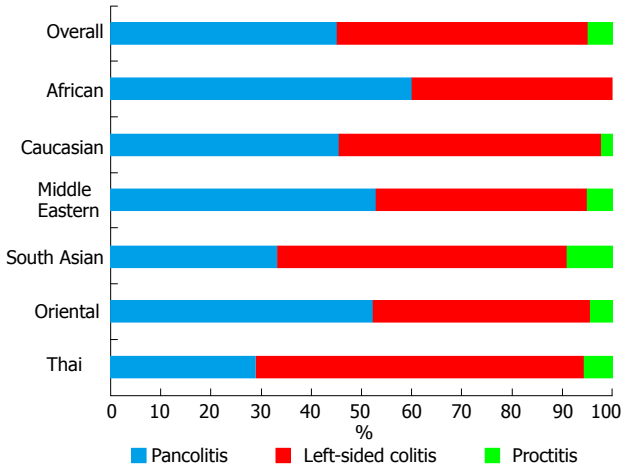

Figure 1 Distribution of ulcerative colitis across ethnic groups.

underwent surgical management with ethnic variation, ranging from 0% in African to 18% in South Asian patients (Table 2).

Pancolitis, left-sided colitis, and proctitis were identified in 45.02%, 50.13%, and 4.85%, respectively (Figure 1). Thai patients had significantly higher proportion of left-sided colitis (65.22%) than the other ethnic origins (P = 0.005). When compared across major ethic groups, Middle Eastern patients had highest prevalence of pancolitis (52.90%), followed by Caucasian (45.35%) and Asian (34.40%) patients (P = 0.021).

Only 20.93% of Caucasian patients received steroid, compared with 26.40% and 27.10% of Asian and Middle Eastern, respectively (P = 0.732). Overall, 13.72% of UC patients did not respond to steroid therapy, with non-significantly higher proportions of non-responders among Asian and Middle Eastern patients (15.22% and 15.04%, respectively) (P = 0.781). Of 277 cases that received thiopurine, 8 patients were non-responders (19.05%), including 5 Middle Eastern patients. Caucasians were relatively the best responders to both steroids and thiopurine.

Cancer was found in three individuals (one Thai, one Middle Eastern, and one African) out of 366 cases, resulting in 0.82 institution-specific incidence. Low and high grade dysplasia was found in 2 and 1 cases, respectively.

DISCUSSION

Although existing incidence and prevalence of UC have been showed to vary across geographical areas[2,4] and ethnic groups[5], evidence from our study provided more data on the Middle Eastern patients and also suggested the potential variation of many other aspects (duration of UC symptoms, severity, distribution, and response to medications) across patients of differing ethnic origins. These attributes are useful for service delivery design, especially in this facility that is responsible for the care of patients from diverse backgrounds.

Based on the clinical findings, we hypothesized that there is potential association between symptom duration and disease severity at presentation. UC has been a disease that progresses over time; hence, earlier and more aggressive management might be needed[7]. As Middle Eastern patients had longer duration of symptoms and more severity at presentation than Thai and Caucasian, we observed that the "progressive" nature might be different across ethnicities and the degree of clinical management should therefore be different. Further study is still required to prove this concept, however.

Table 3 Extra-intestinal manifestations

Organs	Manifestations (No.)
Skin	Psoriasis (4)
	Erythema nodosum (1)
	Pyoderma gangrenosum (1)
	Dermatitis (1)
Musculoskeletal	Arthritis (4)
	Sacroilitis (1)
	Osteoporosis (4)
Liver	Sclerosing cholangitis (4)
Miscellaneous	Oropharyngeal ulcer (2)

Different initial anatomic locations of inflammation present with various clinical patterns that have different prognoses and different rates of complications which could lead to the need for surgery[8]. The variation of anatomic distribution of the UC across ethnic groups suggested an association between anatomic location and patient ethnicity. We therefore propose that the association between anatomic location and clinical outcomes of UC could be confounded by patient ethnicity[9].

Assuming comparably high socio-economic status of our patients, the Thai population had two times lower incidence of pancolitis than Oriental. This finding is useful for both clinicians and patients to choose "optimal" investigation when expense, invasiveness, and yield are of concern. That is, a Thai patient who had mild-to-moderate left-sided colitis from initial colonoscopy and prefers gentle procedure and/or has cost concern might be more likely to get sigmoidoscopy than a Japanese patient with similar conditions for follow-up visits. This is supported by our findings on different anatomic locations of UC across patient ethnic groups presented above. The dynamics of the clinical decision-making process would become more personalized, especially when a unified international standard of care for the procedure is not available.

Our institutional data revealed that Middle Eastern patients had almost twice the incidence of UC as that of Caucasian patients. Based on our informal customer interview, majority of the Middle Eastern patients either could not find a specialized center for inflammatory bowel disease or preferred to travel for care outside their countries. Our clinical practice has taken this into account by tailoring the initial investigations to meet the different needs. For example, although a Thai patient who presents with chronic diarrhea would receive stool examination and culture, a Middle Eastern patient with the exact same condition would also be tested for fecal

calprotectin[10]. Ideally, tailored clinical services to patients from different origins should be based on the standard guidelines of the countries of origin. In reality, however, clinical practice guidelines are not readily available for all countries and the service delivery design, therefore, must be based on our institutional data.

Findings from our study might also be beneficial for modification of current international standard guidelines[11,12]. Standard guidelines have been developed based on evidence from studies in specific populations therefore limited generalizability. We propose that each of the components in a guideline can be modified for optimal care for patients from each of the ethnic groups. For example, based on our data, 20.39% and 3.87% of Middle Eastern patients were severe UC and underwent surgical management, respectively. If a patient from this ethnic origin, were diagnosed as having severe UC and asked about his/her probability of surgical need, we would be able to calculate the conditional probability of 18%.

Some limitations of our study should be noted. First, generalization of our institutional incidence data was limited by potential selection bias. However, the main purpose of our study was to customize our medical services to meet relatively different needs of patients from various origins rather than to conclude about UC incidence of an ethnic origin. Second, anatomical change over time could be present in some patients but was not adjusted for in our analysis presented here. Some study limitations should be noted. First, the uses of institutional data may either under- or over-estimate the incidence. Although annual incidence of 1.2 to 20.3 cases per 100000 persons have been reported[4], our institution-specific annual incidence of 82 per 100000 populations was much higher with great variation across ethnic origins. The main objective of this study was not to present population-based epidemiological data; the incidence data presented here therefore do not represent an ethnic group as a whole. Current literature on this topic has been dominated by Caucasian data whereas other ethnicities are less well represented. Our institution is a rare setting that serves patients from many geographic origins with significant ethnic variation. Although wide variation of the patients' country of origin existed, we do not have differential selection of patients.

ACKNOWLEDGMENTS

The authors would like to thank the Knowledge Angels of Bumrungrad Research Center for their kind help with data collection and analysis and Professor Dr. James S Miser for language edits.

COMMENTS

Background

Ulcerative colitis (UC) is a chronic inflammatory condition of the colon in genetically susceptible individuals exposed to environmental risk factors. Incidence and prevalence of ulcerative colitis vary across geographical areas and ethnic groups. In an era of globalization and medical tourism, a healthcare institution is more likely to provide care to patients of differing ethnic origins.

Research frontiers

In general, Asian and Middle Eastern populations have a lower incidence of UC than Caucasian individuals. Patients from different ethnic origins and/or healthcare systems have been managed using the same guidelines for diagnosis and treatment of UC.

Innovations and breakthroughs

In this study, comparative analysis of symptom duration, pathological severity, extra-intestinal manifestations, surgical consultation need, medication use, and cancer incidence across ethnic groups were presented.

Applications

Understanding how these attributes vary by ethnicity is useful for service delivery design, especially in this facility that is responsible for the care of patients from diverse backgrounds.

Peer-review

In this paper, the authors conducted a retrospective single-center study to investigate the clinical characteristics, treatment, medication use, and treatment response of patients with UC in Thailand. It is interesting that the results showed ethnic differences in severity, distribution, and response to treatments for UC.

REFERENCES

1 **Podolsky DK**. Inflammatory bowel disease. *N Engl J Med* 2002; **347**: 417-429 [PMID: 12167685 DOI: 10.1056/NEJMra020831]
2 **Molodecky NA**, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, Benchimol EI, Panaccione R, Ghosh S, Barkema HW, Kaplan GG. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology* 2012; **142**: 46-54.e42; quiz e30 [PMID: 22001864 DOI: 10.1053/j.gastro.2011.10.001]
3 **Ahuja V**, Tandon RK. Inflammatory bowel disease in the Asia-Pacific area: a comparison with developed countries and regional differences. *J Dig Dis* 2010; **11**: 134-147 [PMID: 20579217 DOI: 10.1111/j.1751-2980.2010.00429.x]
4 **Loftus EV**. Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. *Gastroenterology* 2004; **126**: 1504-1517 [PMID: 15168363 DOI: 10.1053/j.gastro.2004.01.063]
5 **Hou JK**, El-Serag H, Thirumurthi S. Distribution and manifestations of inflammatory bowel disease in Asians, Hispanics, and African Americans: a systematic review. *Am J Gastroenterol* 2009; **104**: 2100-2109 [PMID: 19471256 DOI: 10.1038/ajg.2009.190]
6 **Ross H**, Steele SR, Varma M, Dykes S, Cima R, Buie WD, Rafferty J. Practice parameters for the surgical treatment of ulcerative colitis. *Dis Colon Rectum* 2014; **57**: 5-22 [PMID: 24316941 DOI: 10.1097/DCR.0000000000000030]
7 **Torres J**, Billioud V, Sachar DB, Peyrin-Biroulet L, Colombel JF. Ulcerative colitis as a progressive disease: the forgotten evidence. *Inflamm Bowel Dis* 2012; **18**: 1356-1363 [PMID: 22162423 DOI: 10.1002/ibd.22839]
8 **Farmer RG**, Easley KA, Rankin GB. Clinical patterns, natural history, and progression of ulcerative colitis. A long-term follow-up of 1116 patients. *Dig Dis Sci* 1993; **38**: 1137-1146 [PMID: 8508710 DOI: 10.1007/BF01295733]
9 **VanderWeele TJ,** Shpitser I. On the definition of a confounder. *Ann Stat* 2013; **41**: 196-220 [PMID: 25544784 DOI: 10.1214/12-AOS1058]
10 **Stragier E**, Van Assche G. The use of fecal calprotectin and lactoferrin in patients with IBD. Review. *Acta Gastroenterol Belg* 2013; **76**: 322-328 [PMID: 24261027]
11 **Kornbluth A**, Sachar DB. Ulcerative colitis practice guidelines in adults: American College Of Gastroenterology, Practice Parameters

Committee. *Am J Gastroenterol* 2010; **105**: 501-523; quiz 524 [PMID: 20068560 DOI: 10.1038/ajg.2009.727]
12 **Stenke E**, Hussey S. Ulcerative colitis: management in adults, children and young people (NICE Clinical Guideline CG166). *Arch Dis Child Educ Pract Ed* 2014; **99**: 194-197 [PMID: 24821990 DOI: 10.1136/archdischild-2013-305512]

P- Reviewer: Devendra CD, Inoue T **S- Editor**: Qi Y **L- Editor**: A
E- Editor: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i3.434

World J Gastrointest Pharmacol Ther 2016 August 6; 7(3): 434-439
ISSN 2150-5349 (online)

ORIGINAL ARTICLE

Retrospective Study

Incidence of leukopenia after intraperitoneal *vs* combined intravenous/intraperitoneal chemotherapy in pseudomyxoma peritonei

Philipp Horvath, Stefan Beckert, Florian Struller, Alfred Königsrainer, Ingmar Königsrainer

Philipp Horvath, Stefan Beckert, Florian Struller, Alfred Königsrainer, Ingmar Königsrainer, Department of General, Visceral and Transplant Surgery, University of Tübingen, Comprehensive Cancer Center, 72076 Tübingen, Germany

Author contributions: Horvath P designed and performed the research and wrote the paper; Beckert S performed the statistical analysis and supervised the report; Struller F contributed to the research; Königsrainer A supervised the report; Königsrainer I interpreted the data, supervised the report and made the final revision of the paper.

Institutional review board statement: We confirm that retrospective data collection and dealing with personal data was conducted in accordance to the guidelines of the local ethics committee.

Informed consent statement: We confirm that all patients gave written or oral informed consent prior to their inclusion.

Conflict-of-interest statement: We declare that we have no conflict of interest.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

Correspondence to: Ingmar Königsrainer, MD, Department of General, Visceral and Transplant Surgery, University of Tübingen, Comprehensive Cancer Center, Hoppe-Seyler-Strasse 3, 72076 Tübingen, Germany. koenigsrainer@med.uni-tuebingen.de
Telephone: +49-7071-2986620
Fax: +49-7071-295588

Received: February 12, 2016
Peer-review started: February 12, 2016
First decision: March 18, 2016
Revised: March 18, 2016
Accepted: May 10, 2016
Article in press: May 11, 2016
Published online: August 6, 2016

Abstract

AIM: To investigate the clinical impact of post-hyperthermic intraperitoneal chemotherapy (HIPEC) leukopenia, intraperitoneal and combined intravenous/intraperitoneal drug administrations were compared.

METHODS: Two patient cohorts were retrospectively analyzed regarding the incidence of postoperative leukopenia. The first cohort (n = 32) received Mitomycin C (MMC)-based HIPEC intraperitoneally (35 mg/m² for 90 min) and the second cohort (n = 10) received a bi-directional therapy consisting of oxaliplatin (OX) (300 mg/m² for 30 min) intraperitoneally and 5-fluorouracil (5-FU) 400 mg/m² plus folinic acid 20 mg/m² intravenously. The following data were collected retrospectively: Age, sex, length of operation, length of hospital stay, amount of resection including extent of peritonectomy, peritoneal cancer index, CC (completeness of cytoreduction)-status and leukocyte-count before cytoreductive surgery (CRS) and HIPEC, on days 3, 7 and 14 after CRS and HIPEC. HIPEC leukopenia was defined as < 4000 cells/m³.

RESULTS: Leukopenia occurred statistically more often in the MMC than in the OX/5-FU-group (10/32 *vs* 0/10; P = 0.042). Leukopenia set-on was on day 7 after CRS and MMC-HIPEC and lasted for two to three days. Three patients (33%) required medical treatment. Patients affected by leukopenia were predominantly female (7/10 patients) and older than 50 years (8/10 patients). The

length of hospital stay tended to be higher in the MMC-group without reaching statistical significance (22.5 ± 11 *vs* 16.5 ± 3.5 d). Length of operation (08:54 ± 01:44 *vs* 09:48 ± 02:28 h) were comparable between patients with and without postoperative leukopenia. Prior history of systemic chemotherapy did not trigger post-HIPEC leukopenia. Occurrence of leucopenia did not trigger surgical site infections, intraabdominal abscess formations, hospital-acquired pneumonia or anastomotic insufficiencies.

CONCLUSION: Surgeons must be aware that there is a higher incidence of postoperative leukopenia in MMC-based HIPEC protocols primarily affecting females and older patients.

Key words: Pseudomyxoma peritonei; Mitomycin C; Oxaliplatin; Hyperthermic intraperitoneal chemotherapy; Postoperative leukopenia

Core tip: Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (HIPEC) are considered the therapy of choice for patients with pseudomyxoma peritonei. Nevertheless this treatment is a major undertaking associated with elevated morbidity. The occurrence of postoperative leukopenia can deteriorate the patient's outcome by triggering complications like anastomotic insufficiencies or intraabdominal abscess formations so that surgeons must be aware that special patient subsets (primarily older patients and females) exist that are at a higher risk for developing post-HIPEC leukopenia.

Horvath P, Beckert S, Struller F, Königsrainer A, Königsrainer I. Incidence of leukopenia after intraperitoneal *vs* combined intravenous/intraperitoneal chemotherapy in pseudomyxoma peritonei. *World J Gastrointest Pharmacol Ther* 2016; 7(3): 434-439 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i3/434.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i3.434

INTRODUCTION

Pseudomyxoma peritonei (PMP) is a rare clinical condition generally associated with perforated appendiceal neoplasmas. It is characterized by huge amounts of intra-abdominal gelatinous fluid collections accompanied by mucinous implants on the peritoneum. Preferred areas for these implants are areas of reduced persistaltic movement such as the ileocoecal region, sigmoid colon and ligament of Treitz. Despite controversy regarding the pathological classification, PMP is nowadays classified as low-grade or high-grade disease.

Cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) are the treatment of choice in patients with PMP, creating 5-year-survival rates of 62% to 100% for low-grade, and 0% to 65% for high-grade PMP[1]. Also for many other gastrointestinal and gynecological tumors this dual-approach was able to achieve a survival benefit[1]. A part from its clinical benefits, morbidity rates after CRS and HIPEC remain high (up to 70%). Reasons are long operations, multivisceral resections including stripping of peritoneum thus creating large wound areas and the side-effects of HIPEC itself. Frequently used chemotherapeutic agents for HIPEC in PMP are Mitomycin C (MMC) und oxaliplatin (OX). These two combine beneficial properties for intraperitoneal administration. A high molecular weight resulting in decelerated systemic absorption and prolonged local toxicity makes these agents attractive for intraabdominal administration. Furthermore, enhanced toxicity is achieved by hyperthermia of the dialysate.

Systemic absorption of the chemotherapeutic agents and consecutive leukopenia can account for a variety of postoperative complications and might depend on the agent used and partly on the amount of stripped peritoneum, leading to a larger area of exposed sub-peritoneal veins, thus facilitating systemic absorption.

This study investigates the incidence of postoperative leukopenia in patients either treated with MMC only intraperitoneally or with OX/5-fluorouracil (5-FU) intravenously/intraperitoneally.

MATERIALS AND METHODS

From 2007 to 2015, 42 patients diagnosed with PMP originating from mucinous appendiceal tumors were included. All patients gave informed consent prior to their inclusion. Thirty-two patients were treated with an MMC-based HIPEC protocol (35 mg/m²) for 90 min in an open-closed technique at 42.5 ℃ as described elsewhere[2]. Ten patients were treated according to an OX-based HIPEC protocol (300 mg/m²) for 30 min in the same way and additionally 5-FU (400 mg/m²) and folinic acid (20 mg/m²) were administered intravenously prior to HIPEC.

The following data were collected retrospectively: age, sex, length of operation, length of hospital stay, amount of resection including extent of peritonectomy, peritoneal cancer index (PCI), completeness of cytoreduction (CC)-status and leukocyte-count before CRS and HIPEC, on days 3, 7 and 14 after CRS and HIPEC. HIPEC leukopenia was defined as < 4000 cells/m³.

All complications were graded using the Clavien-Dindo classification of surgical complications[2].

Prior to CRS and HIPEC all patients underwent clinical examinations and blood tests to guarantee adequate performance status and computed tomography was performed to rule out extraabdominal disease.

CRS was conducted according to a standardized procedure consisting of midline laparotomy and screening of the abdomen for peritoneal tumor implants in order to define the PCI-score as described by Königsrainer *et al*[3]. After maximal cytoreduction, achieving a CC-0/1 status, HIPEC was administered. All anastomoses were

Table 1 Clinicopathological characteristics of patients with Mitomycin C and oxaliplatin/5-fluorouracil treatment

	MMC	OX/5-FU
No.	32	10
Gender		
Male (*n* = 20; 47%)	15	5
Female (*n* = 22; 53%)	17	5
Age (yr)	50 ± 14	54 ± 10
Length of operation (h)	09:41 ± 2:27	09:07 ± 1:44
CC-status		
CC-0	21	3
CC-1	11	7
PCI	20 ± 11	25 ± 10
Length of hospital stay (d)	22.5 ± 11	16.5 ± 3.5
Resections		
Total peritonectomy	30	10
Splenectomy	11	3
Omentectomy	25	3
Cholecystectomy	19	7
Colon	18	3
Small bowel	4	1
Anastomotic insufficiencies	0	0
Leucopenia	10	0
G-CSF treatment	3	0
HAP	2	0
SSI	1	1
PE	2	2
IAA	0	1
UTI	1	0

HAP: Hospital-acquired pneumonia; SSI: Surgical site infections; IAA: Intraabdominal abscess formation; PE: Pleural effusion; UTI: Urinary tract infection; G-CSF: Granulozyte-colony stimulating factor; MMC: Mitomycin C; OX/5-FU: Oxaliplatin/5-fluorouracil.

completed before HIPEC started. Total peritonectomy was defined as complete removal of the parietal peritoneum. In total six tubes were transcutaneously inserted into the abdomen to guarantee high-volume influx and efflux of the dialysate. Temperature was monitored to ensure an influx-temperature of 42.5 ℃. After HIPEC and removal of all chemotherapy-containing fluid, the abdomen was lavaged again and then closed.

Statistical analysis

SPSS ver. 12.0 (SPSS Inc. Chicago, IL, United States) was used for statistical analysis and data are written as mean ± SD. A $P < 0.05$ was considered statistically significant when using the chi-square test and the *t*-test. The χ^2 test was used for nominal variables and the *t*-test for continuous variables.

RESULTS

In total 40 patients diagnosed with PMP underwent CRS and HIPEC. Complete data were available on all patients. Table 1 shows patients and treatment characteristics. Of the patients 53 (53%) were female. Mean age and PCI-score were comparable in the MMC- and OX/5-FU-groups without being statistically significant (50 ± 14 years *vs* 54 ± 10 years; PCI 20 ± 11 *vs* 25 ± 10). In 58% a CC-0 status and in 42% a CC-1 status was achieved. Three patients in the MMC- and one patient in the OX/5-FU-group received systemic chemotherapy prior to CRS and HIPEC. These 4 patients underwent a FOLFOX regimen. Only one patient (MMC-group) with prior history of systemic chemotherapy developed postoperative leukopenia. The length of hospital stay tended to be greater in the MMC-group without reaching statistical significance (22.5 ± 11 d *vs* 16.5 ± 3.5 d). Total peritonectomy was conducted in 30 of 32 patients in the MMC-group and in all patients in the OX/5-FU-group. Splenectomy was necessary due to tumor involvement in 14 of 40 patients (12 in the MMC-group and 2 in the OX/5-FU-group). All anastomoses were performed prior to HIPEC and no anastomotic insufficiencies occurred. No statistically significant differences were observed between the MMC- and the OX/5-FU-group regarding occurrence of hospital-acquired pneumonia (HAP) (2 *vs* 0 patients), pleural effusion (PE) (2 *vs* 2 patients) surgical site infections (SSI) (one *vs* one patient), intraabdominal abscess formations (IAA) (0 *vs* 1 patient) and urinary tract infections (UTI) (1 *vs* 0 patient). Patients with HAP required antibiotic treatment (Clavien-Dindo grade Ⅱ). Patients with SSI required bed-side wound treatment but no antibiotic treatment (Clavien-Dindo grade Ⅰ). Two of four patients with PE needed pleural drainage (Clavien-Dindo grade Ⅲa). One patient with IAA required antibiotic treatment (Clavien-Dindo grade Ⅱ) and one patient with a UTI also required antibiotic treatment (Clavien-Dindo grade Ⅱ).

Leukopenia occurred in ten of 32 patients (31%) in the MMC-group. Of these ten patients with MMC-associated leukopenia seven were female. No patient in the OX/5-FU-group developed postoperative leukopenia. Figure 1 shows the postoperative course of the leukocyte count. Leukopenia occurred in all patients between day 6 and day 7 after CRS and HIPEC was administered for 2 to 3 d. Three patients required medical treatment with filgastrim till leukocyte counts were in normal range. Length of operation (08:54 ± 01:44 h *vs* 09:48 ± 02:28 h) and of hospital stay (25 ± 12 d *vs* 20 ± 10 d) were comparable and not statistically significant in patients with or without postoperative leukopenia (Table 2). Splenectomy was necessary in two (20%) of 10 patients with leukopenia and in twelve (40%) of 32 patients without leukopenia. Of 10 patients with leukopenia eight were older than 50 years which was statistically significant (60 ± 16 years *vs* 48.5 ± 11 years; $P = 0.01$).

Occurrence of postoperative leukopenia did not trigger surgical site infections, intraabdominal abscess formations, anastomotic insufficiencies or urinary tract infections.

DISCUSSION

PMP is a rare clinical condition arising in the vast majority of cases from ruptured appendiceal malignancies. In the past debulking surgery accompanied by systemic

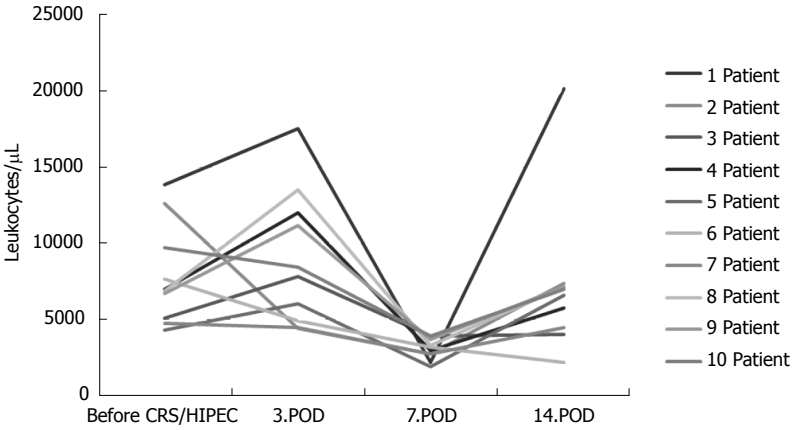

Figure 1 Course of leucocyte count/μL. POD: Postoperative day; CRS: Cytoreductive surgery; HIPEC: Post-hyperthermic intraperitoneal chemotherapy.

Table 2 Clinicopathological characteristics of patients with and without leukopenia

	Leukopenia	No leukopenia
No.	10	32
Gender		
Male	3	16
Female	7	16
Age (yr)	60 ± 16	48.5 ± 11
MMC	10	22
OX/5-FU	0	10
Splenectomy	2	12
Hospital stay (d)	25 ± 12	20 ± 10
Length of operation (h)	08:54 ± 01:44	09:48 ± 02:28
HAP	2	0
SSI	1	1
PE	2	2
IAA	0	1
UTI	1	0

HAP: Hospital-acquired pneumonia; SSI: Surgical site infections; IAA: Intraabdominal abscess formation; PE: Pleural effusion; UTI: Urinary tract infection; MMC: Mitomycin C; OX/5-FU: Oxaliplatin/5-fluorouracil.

chemotherapy was applied. Due to the efforts of Sugarbaker PH this aforementioned strategy has been widely abandoned and superseded by a dual-approach therapy consisting of CRS and HIPEC, resulting in a 15-year survival rate of up to 60%[1,4-8]. Furthermore Chua *et al*[1] were able to demonstrate an impressive median progression-free survival rate of 8.2 years after CRS and HIPEC thus once more emphasizing the efficacy of this dual-approach in disease control. Nevertheless, every surgeon dealing with CRS and HIPEC has to be aware that this therapy is a major undertaking accompanied by long operating times and multivisceral resections and is thus associated with high morbidity rates of up to 70% and mortality rates of up to 11%[9-15]. MMC and OX are the most frequently used intraperitoneally administered drugs in PMP-patients. Both are alkylating chemotherapeutics, interfering with DNA and DNA-synthesis without being cell-cycle dependent[16]. In order to potentiate the activity of the intraperitoneally administered OX, patients are given intravenous 5-FU and folinic acid 30 min prior to HIPEC. Because of a pH incompatibility 5-FU cannot be mixed with OX for intraperitoneal use[17,18]. Despite its advantageous pharmacokinetic properties MMC-induced leukopenia due to bone marrow toxicity after HIPEC is a known and frequently encountered side-effect of this treatment[19]. In our study the incidence of MMP-induced leukopenia was 31% (n = 10/32 patients), which was lower than in other reports in the literature[20,21]. None of the patients in the OX/5-FU-group developed postoperative leukopenia. This might be related to the different route of drug elimination. Oxaliplatin is predominantly excreted *via* urine, by tissue-binding and by renal elimination, whereas MMC undergoes hepatic metabolism which might contribute to systemic accumulation thus promoting occurrence of leukopenia. In accordance with other studies[20,21], prior systemic chemotherapy was not associated with a higher risk of leukopenia. Only four patients (10%) in our study population were not chemo-naïve and one patient, having received MMC-HIPEC, developed postoperative leukopenia. Patients who received systemic chemotherapy prior to CRS and HIPEC and had a history of chemotherapy-associated leukopenia are at greater risk for further episodes of leukopenia. This is the main reason why some authors recommend a dose reduction in MMC-HIPEC in this special patient subgroup[21]. In our analysis female sex and age (> 50 years) were associated with the occurrence of MMC-induced leukopenia. The phenomenon of female gender being associated with a greater risk for chemotherapy-induced leukopenia has been reported previously, but its reasons are still unknown. Bécouarn *et al*[17] provide an explanation for the association between leukopenia and female gender. The authors speculate that women harbor a relatively large surface area of the peritoneum combined with a smaller plasma volume as compared with men of equal weight[22]. As it is a body-surface-area (BSA)-based MMC dose, women with equal weight and a smaller plasma volume have higher MMC-plasma concentrations than do males in the case of equal absorption, thus explaining a higher cytotoxic effect of MMC. Due to these circumstances some HIPEC-centers introduced lower doses of chemotherapeutics in HIPEC for female patients[23].

Our data show that splenectomy was not associated

with a higher incidence of MMC-induced leukopenia. Only two (20%) of ten patients suffered from leukopenia after splenectomy whereas twelve (40%) of 30 patients without postoperative leukopenia were splenectomized. These data might suggest a protective effect of splenectomy on leukopenia due to post-splenectomy leukocytosis. As far as this is concerned the literature presents controversial results. Bécouarn *et al*[17] found a higher, although not significant, incidence of neutropenia in the splenectomized patients after MMC-HIPEC, whereas Bidus *et al*[24] reported also a potentially protective effect of splenectomy on leukopenia in patients receiving adjuvant systemic chemotherapy. The time when chemotherapy was administered could be the decisive variable for these conflicting results.

The role of peritonectomy in the pathophysiology of post-HIPEC leukopenia was negligible in our study because 38 out of 40 patients received total peritonectomy, so that we could not evaluate the definite effect of total peritonectomy on the incidence of post-HIPEC leukopenia.

In our study patients with post-HIPEC leukopenia tended to have a longer hospital stay (25 d *vs* 20 d) without reaching statistical significance. In accordance with the study by Hompes *et al*[16] MMC-induced leukopenia neither elevated the risk of postoperative infections and anastomotic insufficiencies nor prolonged the patient's hospital stay. A larger study population might have found a higher global infection risks in patients with MMC-induced leukopenia. Nonetheless, this clinical condition should not be underestimated and especially in females, older patients and patients with a prior history of chemotherapy-induced leukopenia the possibility that a balance between optimal oncological treatment and systemic cytotoxicity, maybe achieved by a dose reduction in HIPEC, should be taken into account.

COMMENTS

Background

Hyperthermic intraperitoneal chemotherapy (HIPEC) followed by complete cytoreductive surgery is the therapy of choice for patients with pseudomyxoma peritonei (PMP). In the vast majority of cases ruptured appendiceal neoplasms are causal for PMP. HIPEC protocols for the treatment of PMP after complete cytoreduction include only intraperitoneal or concomitant intravenous/intraperitoneal drug administration. Mitomycin C (MMC) and oxaliplatin (OX)/fluorouracil (5-FU) are the most frequently used agents. The aim of the study was to find out the incidence of postoperative leukopenia depending on the chemotherapy regimen used.

Research frontiers

The bi-directional therapy consisting of HIPEC and complete cytoreduction is used in the vast majority of patients with PMP. Nevertheless this treatment is associated with high morbidity rates due to long operative times, multivisceral resections and by the HIPEC itself. HIPEC-associated leukopenia can further contribute to postoperative morbidity. The results show that especially in MMC-based HIPEC protocols and in female and in elderly patients post-HIPEC leukopenia can occur.

Innovations and breakthroughs

In this study the author demonstrated that MMC-HIPEC protocols provoke post-HIPEC leukopenia between day six and seven after operation. Every surgeon dealing with this therapy should be aware of the fact and especially in women and older patients the incidence of post-HIPEC leukopenia is elevated, thus this special patient subset should be even more monitored in the postoperative course. Previous studies reported similar results and some of them also suggested a dose reduction in MMC-HIPEC in women and older patients.

Applications

This study suggests that in women and elderly patients a-priori a dose reduction should be taken into account in order to decreased the incidence of post-HIPEC leukopenia in MMC-protocols.

Terminology

HIPEC: Hyperthermic intraperitoneal chemotherapy: Combined with complete cytoreduction it is the treatment of choice for pseudomyxoma peritonei.

Peer-review

This paper presents an essential and interesting data. In my opinion this is a professional report of an important and currently still discussed in a lack number of papers problematic leukopenia incidence occurring after HIPEC. For me as a person who is working scientifically and clinically on HIPEC method this is a brief but very professional work which is very worthy. The properly presented data and good quality of English are a very strong plus points of this paper.

REFERENCES

1 **Chua TC**, Moran BJ, Sugarbaker PH, Levine EA, Glehen O, Gilly FN, Baratti D, Deraco M, Elias D, Sardi A, Liauw W, Yan TD, Barrios P, Gómez Portilla A, de Hingh IH, Ceelen WP, Pelz JO, Piso P, González-Moreno S, Van Der Speeten K, Morris DL. Early- and long-term outcome data of patients with pseudomyxoma peritonei from appendiceal origin treated by a strategy of cytoreductive surgery and hyperthermic intraperitoneal chemotherapy. *J Clin Oncol* 2012; **30**: 2449-2456 [PMID: 22614976 DOI: 10.1200/JCO.2011.39.7166]

2 **Clavien PA**, Barkun J, de Oliveira ML, Vauthey JN, Dindo D, Schulick RD, de Santibañes E, Pekolj J, Slankamenac K, Bassi C, Graf R, Vonlanthen R, Padbury R, Cameron JL, Makuuchi M. The Clavien-Dindo classification of surgical complications: five-year experience. *Ann Surg* 2009; **250**: 187-196 [PMID: 19638912 DOI: 10.1097/SLA.0b013e3181b13ca2]

3 **Königsrainer I**, Horvath P, Struller F, Forkl V, Königsrainer A, Beckert S. Risk factors for recurrence following complete cytoreductive surgery and HIPEC in colorectal cancer-derived peritoneal surface malignancies. *Langenbecks Arch Surg* 2013; **398**: 745-749 [PMID: 23456355 DOI: 10.1007/s00423-013-1065-6]

4 **Mazzei MA**, Khader L, Cirigliano A, Cioffi Squitieri N, Guerrini S, Forzoni B, Marrelli D, Roviello F, Mazzei FG, Volterrani L. Accuracy of MDCT in the preoperative definition of Peritoneal Cancer Index (PCI) in patients with advanced ovarian cancer who underwent peritonectomy and hyperthermic intraperitoneal chemotherapy (HIPEC). *Abdom Imaging* 2013; **38**: 1422-1430 [PMID: 23744439 DOI: 10.1007/s00261-013-0013-9]

5 **Gough DB**, Donohue JH, Schutt AJ, Gonchoroff N, Goellner JR, Wilson TO, Naessens JM, O'Brien PC, van Heerden JA. Pseudomyxoma peritonei. Long-term patient survival with an aggressive regional approach. *Ann Surg* 1994; **219**: 112-119 [PMID: 8129481 DOI: 10.1097/00000658-199402000-00002]

6 **Miner TJ**, Shia J, Jaques DP, Klimstra DS, Brennan MF, Coit DG. Long-term survival following treatment of pseudomyxoma peritonei: an analysis of surgical therapy. *Ann Surg* 2005; **241**: 300-308 [PMID: 15650641 DOI: 10.1097/01.sla.0000152015.76731.1f]

7 **Youssef H**, Newman C, Chandrakumaran K, Mohamed F, Cecil TD, Moran BJ. Operative findings, early complications, and long-term survival in 456 patients with pseudomyxoma peritonei syndrome of appendiceal origin. *Dis Colon Rectum* 2011; **54**: 293-299 [PMID: 21304299 DOI: 10.1007/DCR.0b013e318202f026]

8 **Yan TD**, Bijelic L, Sugarbaker PH. Critical analysis of treatment failure after complete cytoreductive surgery and perioperative

intraperitoneal chemotherapy for peritoneal dissemination from appendiceal mucinous neoplasms. *Ann Surg Oncol* 2007; **14**: 2289-2299 [PMID: 17541772 DOI: 10.1245/s10434-007-9462-0]

9 **Jacquet P**, Stephens AD, Averbach AM, Chang D, Ettinghausen SE, Dalton RR, Steves MA, Sugarbaker PH. Analysis of morbidity and mortality in 60 patients with peritoneal carcinomatosis treated by cytoreductive surgery and heated intraoperative intraperitoneal chemotherapy. *Cancer* 1996; **77**: 2622-2629 [PMID: 8640714 DOI: 10.1002/(SICI)1097-0142(19960615)77: 12<2622: : AID-CNCR28>3.0.CO; 2-T]

10 **Loggie BW**, Fleming RA. Complications of heated intraperitioneal chemotherapy and strategies for prevention. *Cancer Treat Res* 1996; **82**: 221-233 [PMID: 8849953 DOI: 10.1007/978-1-4613-1247-5_14]

11 **Witkamp AJ**, de Bree E, Kaag MM, van Slooten GW, van Coevorden F, Zoetmulder FA. Extensive surgical cytoreduction and intraoperative hyperthermic intraperitoneal chemotherapy in patients with pseudomyxoma peritonei. *Br J Surg* 2001; **88**: 458-463 [PMID: 11260116 DOI: 10.1046/j.1365-2168.2001.01701.x]

12 **Loungnarath R**, Causeret S, Bossard N, Faheez M, Sayag-Beaujard AC, Brigand C, Gilly F, Glehen O. Cytoreductive surgery with intraperitoneal chemohyperthermia for the treatment of pseudomyxoma peritonei: a prospective study. *Dis Colon Rectum* 2005; **48**: 1372-1379 [PMID: 15909071 DOI: 10.1007/s10350-005-0045-5]

13 **Smeenk RM**, Verwaal VJ, Zoetmulder FA. Toxicity and mortality of cytoreduction and intraoperative hyperthermic intraperitoneal chemotherapy in pseudomyxoma peritonei--a report of 103 procedures. *Eur J Surg Oncol* 2006; **32**: 186-190 [PMID: 16303281 DOI: 10.1016/j.ejso.2005.08.009]

14 **Butterworth SA**, Panton ON, Klaassen DJ, Shah AM, McGregor GI. Morbidity and mortality associated with intraperitoneal chemotherapy for Pseudomyxoma peritonei. *Am J Surg* 2002; **183**: 529-532 [PMID: 12034386 DOI: 10.1016/S0002-9610(02)00843-7]

15 **Deraco M**, Baratti D, Inglese MG, Allaria B, Andreola S, Gavazzi C, Kusamura S. Peritonectomy and intraperitoneal hyperthermic perfusion (IPHP): a strategy that has confirmed its efficacy in patients with pseudomyxoma peritonei. *Ann Surg Oncol* 2004; **11**: 393-398 [PMID: 15070599 DOI: 10.1245/ASO.2004.07.002]

16 **Hompes D**, D'Hoore A, Wolthuis A, Fieuws S, Mirck B, Bruin S, Verwaal V. The use of Oxaliplatin or Mitomycin C in HIPEC treatment for peritoneal carcinomatosis from colorectal cancer: a comparative study. *J Surg Oncol* 2014; **109**: 527-532 [PMID: 24375059 DOI: 10.1002/jso.23546]

17 **Bécouarn Y**, Ychou M, Ducreux M, Borel C, Bertheault-Cvitkovic F, Seitz JF, Nasca S, Nguyen TD, Paillot B, Raoul JL, Duffour J, Fandi A, Dupont-André G, Rougier P. Phase II trial of oxaliplatin as first-line chemotherapy in metastatic colorectal cancer patients. Digestive Group of French Federation of Cancer Centers. *J Clin Oncol* 1998; **16**: 2739-2744 [PMID: 9704726 DOI: 10.1186/1744-8069-7-29]

18 **Elias D**, Benizri E, Di Pietrantonio D, Menegon P, Malka D, Raynard B. Comparison of two kinds of intraperitoneal chemotherapy following complete cytoreductive surgery of colorectal peritoneal carcinomatosis. *Ann Surg Oncol* 2007; **14**: 509-514 [PMID: 17096054 DOI: 10.1245/s10434-006-9167-9]

19 **Schnake KJ**, Sugarbaker PH, Yoo D. Neutropenia following perioperative intraperitoneal chemotherapy. *Tumori* 1999; **85**: 41-46 [PMID: 10228496]

20 **Sugarbaker PH**, Alderman R, Edwards G, Marquardt CE, Gushchin V, Esquivel J, Chang D. Prospective morbidity and mortality assessment of cytoreductive surgery plus perioperative intraperitoneal chemotherapy to treat peritoneal dissemination of appendiceal mucinous malignancy. *Ann Surg Oncol* 2006; **13**: 635-644 [PMID: 16523363 DOI: 10.1245/ASO.2006.03.079]

21 **Lambert LA**, Armstrong TS, Lee JJ, Liu S, Katz MH, Eng C, Wolff RA, Tortorice ML, Tansey P, Gonzalez-Moreno S, Lambert DH, Mansfield PF. Incidence, risk factors, and impact of severe neutropenia after hyperthermic intraperitoneal mitomycin C. *Ann Surg Oncol* 2009; **16**: 2181-2187 [PMID: 19475451 DOI: 10.1245/s10434-009-0523-4]

22 **Sugarbaker PH**. Management of peritoneal surface malignancies using intraperitoneal chemotherapy and cytoreductive surgery. A manual for physicians and nurses. 3rd ed. Grand rapids; MI: Ludann Co., 1998

23 **Sugarbaker PH**, Stuart OA, Carmignani CP. Pharmacokinetic changes induced by the volume of chemotherapy solution in patients treated with hyperthermic intraperitoneal mitomycin C. *Cancer Chemother Pharmacol* 2006; **57**: 703-708 [PMID: 16096789 DOI: 10.1007/s00280-005-0074-8]

24 **Bidus MA**, Krivak TC, Howard R, Rose GS, Cosin J, Dainty L, Elkas JC. Hematologic changes after splenectomy for cytoreduction: implications for predicting infection and effects on chemotherapy. *Int J Gynecol Cancer* 2006; **16**: 1957-1962 [PMID: 17177832 DOI: 10.1111/j.1525-1438.2006.00725.x]

P- Reviewer: Alzahrani NA, Ihemelandu C, Nowacki M
S- Editor: Qiu S **L- Editor**: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i3.440

World J Gastrointest Pharmacol Ther 2016 August 6; 7(3): 440-446
ISSN 2150-5349 (online)

ORIGINAL ARTICLE

Retrospective Study

Increase in colonic diverticular hemorrhage and confounding factors

Ken Kinjo, Toshiyuki Matsui, Takashi Hisabe, Hiroshi Ishihara, Shinichiro Maki, Kenta Chuman, Akihiro Koga, Kensei Ohtsu, Noritaka Takatsu, Fumihito Hirai, Kenshi Yao, Masakazu Washio

Ken Kinjo, Toshiyuki Matsui, Takashi Hisabe, Hiroshi Ishihara, Shinichiro Maki, Kenta Chuman, Akihiro Koga, Kensei Ohtsu, Noritaka Takatsu, Fumihito Hirai, Kenshi Yao, Department of Gastroenterology, Fukuoka University Chikushi Hospital, Fukuoka 818-8502, Japan

Masakazu Washio, Department of Community Health and Clinical Epidemiology, St. Mary's College, Fukuoka 818-8502, Japan

Author contributions: Kinjo K collected and analyzed the data, and drafted the manuscript; Matsui T designed and supervised the study; Washio M performed the statistical analysisl; Hisabe T, Ishihara H, Maki S, Chuman K, Koga A, Ohtsu K, Takatsu N, Hirai F and Yao K performed the colonoscopy and revised the manuscript for important intellectual content; all authors have read and approved the final version to be published.

Institutional review board statement: This study was approved by the institutional review board of Fukuoka University Chikushi Hospital (R15-024) and was conducted in accordance with the Declaration of Helsinki.

Informed consent statement: In this study, we do not necessarily need the individual agreement from a study subject. Because this study is retrospective study and based on the ethical guidelines for medical studies in which human body samples are not used informed consent procedure No.12 was applied. Information about the implementation of this research was presented on Fukuoka University Chikushi Hospital clinical research support center home page.

Conflict-of-interest statement: In connection with this manuscript, there is no Conflict of Interest to be disclosed with any companies.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at matsui@fukuoka-u.ac.jp. Participants gave informed consent was not obtained but the presented data are anonymized and risk of identification is low.

Manuscript source: Unsolicited manuscript

Correspondence to: Toshiyuki Matsui, MD, PhD, Department of Gastroenterology, Fukuoka University Chikushi Hospital, 1-1-1 Zokumyoin, Chikushino, Fukuoka 818-8502, Japan. matsui@fukuoka-u.ac.jp
Telephone: +81-92-9211011
Fax: +81-92-9292630

Received: March 13, 2016
Peer-review started: March 14, 2016
First decision: April 5, 2016
Revised: April 17, 2016
Accepted: May 7, 2016
Article in press: May 9, 2016
Published online: August 6, 2016

Abstract

AIM: To classify changes over time in causes of lower gastrointestinal bleeding (LGIB) and to identify factors associated with changes in the incidence and characteristics of diverticular hemorrhage (DH).

METHODS: A total of 1803 patients underwent colonoscopy for overt LGIB at our hospital from 1995 to 2013. Patients were divided into an early group (EG, 1995-2006, *n* = 828) and a late group (LG, 2007-2013, *n* = 975), and specific diseases were compared between groups. In addition, antithrombotic drug (ATD) use and nonsteroidal anti-inflammatory drug (NSAID) use were compared

between patients with and without DH.

RESULTS: Older patients (⩾ 70 years old) and those with colonic DH were more frequent in LG than in EG ($P < 0.01$). Patients using ATDs as well as NSAIDs, male sex, obesity (body mass index ⩾ 25 kg/m^2), smoking, alcohol drinking, and arteriosclerotic diseases were more frequent in patients with DH than in those without.

CONCLUSION: Incidence of colonic DH seems to increase with aging of the population, and factors involved include use of ATDs and NSAIDs, male sex, obesity, smoking, alcohol drinking, and arteriosclerotic disease. These factors are of value in handling DH patients.

Key words: Lower gastrointestinal bleeding; Colonic diverticular hemorrhage; Increase of incidence; Aging

Core tip: Colonic diverticular hemorrhage (DH) is the most frequent cause of lower gastrointestinal bleeding. A rapid increase in the incidence of colonic DH has been seen with the aging population. One reason is the widespread adoption of antithrombotic drugs (ATDs) since the early 2000s, based on guidelines to prevent ischemic heart disease and ischemic cerebrovascular disease. DH is more likely in patients who are older, are men, obesity, use nonsteroidal anti-inflammatory drugs or ATDs, and have hypertension and diabetes associated with arteriosclerotic disease. These factors are of value in handling DH patients.

Kinjo K, Matsui T, Hisabe T, Ishihara H, Maki S, Chuman K, Koga A, Ohtsu K, Takatsu N, Hirai F, Yao K, Washio M. Increase in colonic diverticular hemorrhage and confounding factors. *World J Gastrointest Pharmacol Ther* 2016; 7(3): 440-446 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i3/440.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i3.440

INTRODUCTION

Lower gastrointestinal bleeding (LGIB) is often diagnosed in patients with overt bleeding, positive results for fecal occult blood, abdominal symptoms, and anemia. Colonoscopy is often the first test performed as an approach to the diagnosis and treatment of LGIB. In the United States, endoscopy is recommended "in the early evaluation of severe acute LGIB"[1].

Use of oral antithrombotic drugs (ATDs) and non-steroidal anti-inflammatory drugs (NSAIDs) is increasing with the aging of the population, and the number of patients with diseases causing LGIB is increasing[2,3]. In addition, the types of diseases being encountered are also changing. In particular, the prevalence of colonic diverticulum is increasing. The aging population in Japan, as in Western countries, is showing an increase in diverticulosis[4,5]. DH is also increasing in Japan. In fact, diverticular hemorrhage (DH) is now one of the most common causes of LGIB in Japan and Western countries[2,3,6].

The present study examined changes over time in diseases causing LGIB that are associated with aging. In particular, this study sought to identify factors associated with changes in the incidence and patient characteristics of colonic DH.

MATERIALS AND METHODS

Among 42540 patients who underwent colonoscopy at our hospital during the 19-year period between January 1995 and December 2013, this retrospective study included those who underwent colonoscopy for overt LGIB. Our hospital is one of the emergency hospitals in Chikushino city, Fukuoka, Japan.

Our hospital also diagnoses and treats a large number of patients with inflammatory bowel disease (IBD). Patients with IBD (ulcerative colitis, Crohn's disease, Behçet's disease, intestinal tuberculosis) were excluded because of differences from other diseases in terms of diagnosis and treatment. We also excluded patients who developed bleeding after endoscopic treatment (*e.g.,* biopsy, polypectomy), or who experienced bleeding from the small bowel. As a result, this study included 1803 patients, all of whom were Japanese. This study was approved by the institutional review board of Fukuoka University Chikushi Hospital (R15-024) and was conducted in accordance with the Declaration of Helsinki. Also, since this was a retrospective study, the need to obtain informed consent to participate in the study was waived by the review board.

Factors evaluated included sex (male/female ratio) and age ⩾ 70 years, Obesity [body mass index (BMI) ⩾ 25 kg/m^2]. Patients were categorized into 2 groups according to the use of oral ATDs [antiplatelet drugs (n = 266)]: Low-dose aspirin, ticlopidine, clopidogrel, cilostazol, limaprost alfadex, ethyl icosapentate, dipyridamole, sarpogrelate hydrochloride, beraprost sodium, dilazep; or anticoagulants (n = 58): Warfarin and dabigatran: No ATDs; ⩾ 1 oral ATDs. Use of NSAIDs (loxoprofen, diclofenac, ibuprofen, etodolac, meloxicam, or celecoxib) was also examined. Two-group comparisons included the use or non-use of NSAIDs and the use or non-use of NSAIDs in combination with an antithrombotic drug.

Two-group comparisons for lifestyle factors included smoking or non-smoking and use or non-use of alcohol. We treated current and past smoking as positive for smoking, while only current drinkers were defined as positive for alcohol. Other factors examined other underlying diseases (cerebrovascular disease, ischemic heart disease, hypertension, hyperlipidemia, hyperuricemia, diabetes mellitus, chronic liver disease, and chronic kidney disease). Comorbidity was quantified using the

Table 1 Baseline characteristics of all patients undergoing colonoscopy for overt lower gastrointestinal bleeding

Age, sex and other factors	Overall *n* = 1803 *n* (%)
Sex	
Male	913 (50.6)
Female	890 (49.4)
Age, yr	
Mean	59.0 ± 18.6
⩾ 70	582 (32.3)
< 70	1221 (67.7)
BMI, kg/m^2	
< 18.5	212 (11.8)
18.5 to < 25	1049 (58.2)
⩾ 25	337 (18.7)
Unknown	205 (11.4)
Oral drugs	
ATDs	308 (17.1)
NSAIDs	115 (6.4)
ATDs + NSAIDs	26 (1.4)
Lifestyle habits	
Smoking (current/past)	551 (30.6)
Alcohol drinking (current)	625 (34.7)
Underlying disease	
Cerebrovascular disease	171 (9.5)
Ischemic heart disease	143 (7.9)
Hypertension	658 (36.5)
Hyperlipidemia	355 (19.7)
Hyperuricemia	91 (5.0)
Diabetes	207 (11.5)
Chronic liver disease	106 (5.9)
Chronic kidney disease	43 (2.4)
Charlson Risk Index	
⩽ 1	1413 (78.4)
⩾ 2	390 (21.6)
Blood transfusion	130 (7.2)

Each set of values represents mean ± SD or *n* (%). BMI: Body mass index; ATDs: Antithrombotic drugs; NSAIDs: Nonsteroidal anti-inflammatory drugs.

Charlson Risk Index (CRI)[7]. Two groups were compared: CRI ⩽ 1; and CRI ⩾ 2. Transfusion requiring ⩾ 2 units of blood was also examined as a factor.

Diagnosis

Colonoscopy was generally performed on the day of or the day after overt bleeding. Depending on the clinical symptoms and physical examination findings in each patient, colonoscopy was performed without pretreatment, or after pretreatment with an enema or bowel-cleansing agent. Olympus colonoscopes were used (PCF-240AZI, PCF-240AI, CF-240AZI, CF-240ZI, PCF-260AZI, PCF-PQ260I, and CF-260AI; Olympus, Tokyo, Japan). Colonic DH was defined as follows[8].

Definite colonic DH (*n* = 207): (1) Active bleeding observed from a diverticulum; and (2) Presence of blood clots, erosions, or an exposed vessel near a diverticulum.

Either (1) or (2), absence of blood in the terminal ileum on total colonoscopy, and no other obvious cause of bleeding.

Suspected colonic DH (*n* = 66): Obvious bloody stools, no blood in the terminal ileum on colonoscopy after bowel preparation, and the only likely cause of bleeding is DH.

Diseases causing LGIB were classified into hemorrhoids, ischemic colitis, DH, advanced colon cancer, early colon cancer/polyps/adenomas, infectious enteritis, angiodysplasia, drug-related enteritis, and no abnormal findings. Rectal ulcers, stercoral ulcers, rectal mucosal prolapse, pneumatosis cystoides intestinalis, enteric endometriosis, submucosal tumors, radiation enteritis, and nonspecific inflammation were categorized as "Others".

Study period

The 1803 patients who underwent colonoscopy for overt LGIB were divided into two groups by time period, with each consisting of about half of the patients. The early group (EG, *n* = 828) was treated from January 1995 to December 2006, and the late group (LG, *n* = 975) was treated from January 2007 to December 2013. Incidence of each disease during these two periods was compared.

Statistical analysis

All statistical analyses were conducted using the Statistical Analysis System package (SAS Institute, Cary, NC). The χ^2 test was used to compare categorical variables between the two groups. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Colonoscopy

A total of 1803 patients underwent colonoscopy for overt LGIB at our hospital between 1995 and 2013. Table 1 summarizes the patient characteristics. Figure 1 shows the number of patients who underwent colonoscopy for overt LGIB each year, required hospitalization, required transfusions, and used oral ATDs and NSAIDs. The number of patients with overt LGIB tended to increase each year.

Comparison of specific diseases in EG and LG

Table 2 summarizes the specific diseases in the 1803 patients who underwent colonoscopy for overt LGIB. In EG, the most common cause of overt LGIB was hemorrhoids in 212 patients (25.6%), followed by ischemic colitis in 143 patients (17.3%), advanced cancer in 80 patients (9.7%), early cancer/adenomas/polyps in 53 patients (6.4%), and colonic DH in 49 patients (5.9%). In LG, the most common cause of overt LGIB was colonic DH in 224 patients (23.0%), followed by hemorrhoids in 220 patients (22.6%), ischemic colitis in 173 patients (17.7%), advanced cancer in 73 patients (7.5%), and early cancer/adenomas/polyps in 58 patients (5.9%).

Compared with LG, EG showed lower frequencies of patients ⩾ 70 years old ($P < 0.01$) and bleeding from colonic diverticulum ($P < 0.01$). The number of patients with DH tended to increase each year, with a marked increase after 2003 (Figure 2).

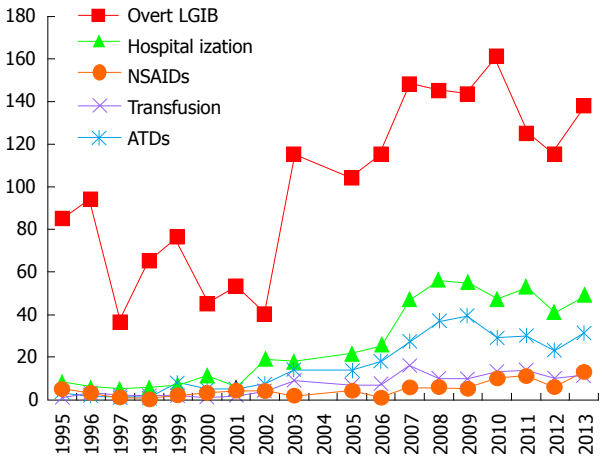

Figure 1 Annual totals and changes over time in number of patients undergoing colonoscopy for overt lower gastrointestinal bleeding, requiring hospitalization, requiring transfusions, and using antithrombotic drugs and nonsteroidal anti-inflammatory drugs (1995 to 2013). The incidence of lower gastrointestinal bleeding (LGIB) started to increase rapidly in 2002-2003, associated with increases in the number of patients hospitalized, receiving blood transfusions, using antithrombotic drugs (ATDs), and using nonsteroidal anti-inflammatory drugs (NSAIDs).

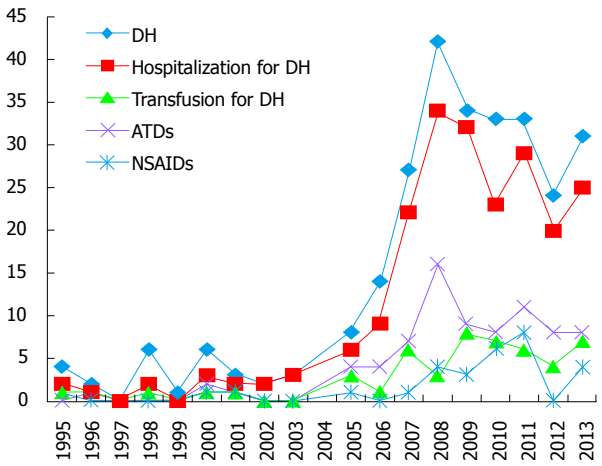

Figure 2 Changes in the number of patients with colonic diverticular hemorrhage: Number of patients requiring hospitalization, requiring transfusion, and using antithrombotic drugs or nonsteroidal anti-inflammatory drugs (1995 to 2013). The number of patients with diverticular hemorrhage (DH) started to increase rapidly in 2003, and peaked in 2008. This was associated with an increase in the number of patients hospitalized, receiving blood transfusions, using antithrombotic drugs (ATDs), and using nonsteroidal anti-inflammatory drugs (NSAIDs).

Comparison of patient characteristics between DH and non-DH patients

As shown in Table 3, compared with non-DH, DH showed higher frequencies of patients ⩾ 70 years old ($P < 0.01$) and male patients ($P < 0.01$). Seventy-nine (28.9%) of the 273 DH patients and 229 (15.0%) of the 1530 non-DH patients received ATDs. ATDs were thus more commonly used in DH patients than in non-DH patients ($P < 0.01$). Thirty (11.0%) of the 273 DH patients and 85 (5.6%) of the 1530 non-DH patients took oral NSAIDs. Oral NSAIDs were thus also more commonly used in DH patients than in non-DH patients ($P < 0.01$).

In addition, obesity ($P < 0.01$), smoking ($P < 0.01$), alcohol drinking ($P < 0.01$), hypertension ($P < 0.01$), hyperlipidemia ($P = 0.02$), diabetes ($P < 0.01$), and requirement for blood transfusion ($P < 0.01$) were significantly more frequent in DH patients than in non-DH patients.

DISCUSSION

The incidence of LGIB increased from 20.5/100000 inhabitants/year in the early 1990s[6] to 87/100000 inhabitants/year in 2010[3]. In particular, the incidence of LGIB is high in elderly patients (690/100000 inhabitants/year)[3], and is expected to continue increasing with the aging of the population in Japan. The aging rate (⩾ 65 years old) of Japan was 12% in 1990, 20% in 2005. However, the aging rate of 2013 is higher than 25%, and aging advances[9]. Our study also showed an annual increase in the number of patients undergoing colonoscopy for overt LGIB. One background factor was the approval of low-dose aspirin 100 mg in Japan as an antiplatelet drug in 2000, with initial marketing in 2001. In addition, ATDs such as clopidogrel and warfarin became more widely used for risk reduction and secondary prevention of cerebral and cardiovascular events in Japan, based on 2002-2009 guidelines from the American College of Cardiology and American Heart Association[10,11]. ATDs and NSAIDs have been reported as risk factors for LGIB[2,3]. These background factors, together with the aging of the population, are thought to be key reasons for the rapid increase in the incidence of LGIB.

In particular, the incidence of colonic DH increased markedly from 5.9% in EG to 23.0% in LG, with a pronounced change in the specific diseases causing LGIB. In LG, colonic DH was the most common disorder causing LGIB. Moreover, colonic DH has also recently been reported as the most common cause of LGIB in Japan[2,12]. Compared to a few decades ago, changes in the aging-associated diseases that cause LGIB have been occurring. In particular, the incidence of colonic DH has increased.

Colonic diverticulosis itself is increasing. During the period from 1960 to the 1980s, Kubo *et al*[13] reported that the prevalence of colonic diverticula was only 8.9% among patients who underwent barium enema examination. Since the 1990s, the prevalence of diverticulosis seen on barium enema examination or colonoscopy has increased to 15%-25%[5,14]. In addition, more than 80% of patients who had diverticulosis were elderly. Diverticulosis is thus thought to increase with aging[5]. As the absolute number of diverticula in colonic diverticulosis increases with the aging of the population, the risk of DH is increased. Moreover, as mentioned previously, the availability of low-dose aspirin has probably also contributed to the rapid rise in DH starting in 2001.

Of course, bleeding does not occur in all patients with diverticulosis. In fact, bleeding only occurs in 2%-5% of

Table 2 Comparison between early group and late group among patients undergoing colonoscopy for overt lower gastrointestinal bleeding from 1995-2013 *n* (%)

Age, sex and cause/site of bleeding	All patients (1995-2013) *n* = 1803	EG (1995-2006) *n* = 828	LG (2007-2013) *n* = 975	*P*-value	Adjusted *P*-value
Old age (≥ 70 yr)	582 (32.3)	196 (23.7)	386 (39.6)	< 0.01	< 0.01
Male	913 (50.6)	419 (56.0)	495 (50.8)	0.94	0.94
External/internal hemorrhoids	432 (24.0)	212 (25.6)	220 (22.6)	0.13	0.46
Ischemic colitis	316 (17.5)	143 (17.3)	173 (17.7)	0.79	0.1
Colonic DH	273 (15.1)	49 (5.9)	224 (23.0)	< 0.01	< 0.01
Advanced colonic cancer	153 (8.5)	80 (9.7)	73 (7.5)	0.1	0.06
Early colon cancer/colon adenomas/polyps	111 (6.2)	53 (6.4)	58 (5.9)	0.69	0.73
Infectious enteritis	68 (3.8)	37 (4.5)	31 (3.2)	0.15	0.4
Angiodysplasia	27 (1.5)	15 (1.8)	12 (1.2)	0.31	0.26
Drug-related enteritis	23 (1.3)	13 (1.6)	10 (1.0)	0.3	0.32
Others	248 (13.8)	137 (16.5)	111 (11.4)	-	-
No abnormal findings	152 (8.4)	89 (10.7)	63 (6.5)	-	-
Total	1803 (100)	828 (100)	975 (100)	-	-

Adjusted *P*-value age- and sex-adjusted P-value; No abnormal findings no site identified as origin of bleeding; Each set of values represents number (%). χ^2 test. EG: Early group: 1995-2006; LG: Late group: 2007-2013; DH: Diverticular hemorrhage.

Table 3 Comparison of old age, sex, and use of antithrombotic drugs or nonsteroidal anti-inflammatory drugs between patients with and without diverticular hemorrhage *n* (%)

	DH (*n* = 273)	Non-DH (*n* = 1530)	Adjusted *P* value
Old age (≥ 70 yr)	136 (49.8)	446 (29.2)	< 0.01
Male sex	172 (63.0)	741 (48.4)	< 0.01
Obesity (BMI ≥ 25 kg/m^2)	82 (30.0)	255 (16.7)	< 0.01
ATDs	79 (28.9)	229 (15.0)	< 0.01
NSAIDs	30 (11.0)	85 (5.6)	< 0.01
Smoking (current/past)	124 (45.4)	427 (27.9)	< 0.01
Alcohol drinking (current)	139 (50.9)	486 (31.8)	< 0.01
Cerebrovascular disease	36 (13.2)	134 (8.8)	0.67
Ischemic heart disease	34 (12.5)	109 (7.1)	0.33
Hypertension	178 (65.2)	480 (31.4)	< 0.01
Hyperlipidemia	74 (27.1)	281 (18.4)	< 0.05
Hyperuricemia	20 (7.3)	71 (4.6)	0.78
Diabetes	44 (16.1)	163 (10.7)	< 0.01
Chronic liver disease	13 (4.8)	93 (6.1)	0.1
Chronic kidney disease	8 (2.9)	35 (2.3)	0.94
Charlson Risk Index ≥ 2	79 (28.9)	311 (20.3)	0.67
Blood transfusion	56 (20.5)	74 (4.8)	< 0.01

P value age- and sex-adjusted *P*-value; χ^2 test: Each set of values represents *n* (%). DH: Diverticular hemorrhage; ATDs: Antithrombotic drugs; NSAIDs: Nonsteroidal anti-inflammatory drugs; BMI: body mass index.

patients during the natural history of diverticulosis, and most cases are relatively mild, resolving spontaneously in 75%-93% of cases[15,16].

In a case-control study in Japan, risk factors for DH included the use of ATDs and NSAIDs, hypertension, diabetes, arteriosclerotic diseases such as ischemic disease mellitus and chronic kidney disease, age ≥ 70 years, obesity[13,15,17,18]. Our study also found higher rates of use for both ATDs and NSAIDs, higher age and male sex, obesity, smoking, alcohol drinking, and arteriosclerotic diseases in patients with colonic DH compared to those with bleeding from other causes. ATDs and NSAIDs thus represent risk factors for bleeding in diverticulosis. Moreover, higher rates of DH in patients who are older, obesity, smoke, or drink alcohol may be related to the association between older age, obesity, smoking, and alcohol drinking with arteriosclerotic disease.

Colonic diverticula develop at sites where the vasa recta penetrate the large intestinal wall when intestinal pressure increases. Blood vessels in the diverticula are separated from the bowel lumen only by the mucosa, and are easily injured. With repeated mechanical stimuli, intimal thickening of the vasa recta occurs, often with thinning of the media. These changes can cause segmental weakening of the vasa recta, which may lead to arterial hemorrhage in the bowel lumen[19]. Thus, because DH represents a form of arterial bleeding, transfusion may be required more often than with bleeding due to other disorders.

Arteriosclerosis also plays a role in the pathogenesis

leading to rupture of blood vessels in diverticula[20]. However, not all patients with diverticulosis experience bleeding; indeed, most patients remain asymptomatic. Therefore, in addition to arteriosclerosis, other factors increase the fragility of blood vessels in diverticula.

Our study was performed with adjustment for factors including sex and age, so analysis was performed independently of arteriosclerosis. The results identified NSAIDs and ATDs as a significant risk factor, suggesting that NSAIDs and ATDs have synergistic effects on injury to blood vessels in diverticula. Most NSAIDs inhibit prostaglandin synthesis, which disrupts the microcirculation and inhibits platelet aggregation. As a result, NSAIDs can lead to bleeding in patients with diverticulosis[17]. DH in Japan is more common in men, whereas in Western countries, DH occurs equally in men and women[21]. These findings suggest ethnic differences, but the exact factors involved are not yet well understood. DH in Japan is more common in men, because it may be one of the reasons the men listed as the risk of arteriosclerosis in the Japan Atherosclerosis Society Guidelines[22].

Our study examined patients who underwent colonoscopy for LGIB over a 19-year period, including detailed information about underlying diseases and medications, and found an increase in overt LGIB during this time. The investigation included a relatively large cohort of 273 patients with colonic DH for comparison and analysis. We compared the baseline data of DH patients and those of non-DH patients among all subjects in this cohort. Therefore, there may have been little recall bias or selection bias in our study. We divided it in 2004 when we divided it for the same period or in 2000 when low-dose aspirin was approved as division of EG and LG. However, number of cases included a difference too much in EG and LG and was inappropriate for analysis. Thus, the 1803 patients were divided into two groups by time period, with each consisting of about half of the patients.

However, some limitations to the study must be considered. One was the retrospective nature of the study design, and the fact that only a single institution was involved. In addition, this was not a strictly controlled study, as no comparison with non-bleeding diverticulosis was conducted. We had not evaluated by carotid artery ultrasonography for arteriosclerosis in this study.

In conclusion, a rapid increase in the incidence of colonic DH has been seen with the aging population. One reason is the widespread adoption of ATDs since the early 2000s, based on guidelines to prevent ischemic heart disease and ischemic cerebrovascular disease. Colonic DH is the most frequent cause of LGIB.

DH is more likely in patients who are older, are men, obesity, use NSAIDs or ATDs, and have hypertension and diabetes associated with arteriosclerotic disease. These patients are also likely to have more severe anemia and require blood transfusions. These factors should be kept in mind when treating patients with LGIB.

ACKNOWLEDGMENTS

We thank Mr. Matt Morgan Forte Inc for assistance with the translation to English.

COMMENTS

Background

With the aging of the population in Japan, dramatic changes in the incidence of lower gastrointestinal bleeding (LGIB) have been seen, particularly as an increase in colonic diverticular hemorrhage.

Research frontiers

Colonic diverticular hemorrhage (DH) is more likely in patients who are older, are men, obesity, use nonsteroidal anti-inflammatory drugs (NSAIDs) or antithrombotic drugs (ATDs), and have hypertension and diabetes associated with arteriosclerotic disease.

Innovations and breakthroughs

Older patients and those with colonic DH were more frequent in late group than in early group ($P < 0.01$). Patients using ATDs as well as NSAIDs, male sex, obesity, smoking, alcohol drinking, and arteriosclerotic diseases were more frequent in patients with DH than in those without.

Applications

Incidence of colonic DH seems to increase with aging of the population, and factors involved include use of ATDs and NSAIDs, male sex, obesity, smoking, alcohol drinking, and arteriosclerotic disease. These factors should be kept in mind when treating patients with LGIB.

Terminology

NSAIDs and ATDs have synergistic effects on injury to blood vessels in diverticula. Most NSAIDs inhibit prostaglandin synthesis, which disrupts the microcirculation and inhibits platelet aggregation. As a result, NSAIDs can lead to bleeding in patients with diverticulosis, including severe DH.

Peer-review

This is an interesting manuscript which analyses the spectrum of LGIB in a single center retrospective analysis at Fukuoka University Hospital in chikushino city in Japan, and provides further data on the incidence and risk factors for colonic DH. Amongst a total of 1803 japanese patients with LGIB with a mean age of 59 years, 273 patients with colonic DH were separated into an early (1995-2006) and late (2007-2013) group.

REFERENCES

1 **Davila RE**, Rajan E, Adler DG, Egan J, Hirota WK, Leighton JA, Qureshi W, Zuckerman MJ, Fanelli R, Wheeler-Harbaugh J, Baron TH, Faigel DO. ASGE Guideline: the role of endoscopy in the patient with lower-GI bleeding. *Gastrointest Endosc* 2005; **62**: 656-660 [PMID: 16246674 DOI: 10.1016/j.gie2005.07.032]

2 **Nagata N**, Niikura R, Aoki T, Shimbo T, Kishida Y, Sekine K, Tanaka S, Okubo H, Watanabe K, Sakurai T, Yokoi C, Akiyama J, Yanase M, Mizokami M, Uemura N. Lower GI bleeding risk of nonsteroidal anti-inflammatory drugs and antiplatelet drug use alone and the effect of combined therapy. *Gastrointest Endosc* 2014; **80**: 1124-1131 [PMID: 25088922 DOI: 10.1016/j.gie.2014.06.039]

3 **Hreinsson JP**, Gumundsson S, Kalaitzakis E, Björnsson ES. Lower gastrointestinal bleeding: incidence, etiology, and outcomes in a population-based setting. *Eur J Gastroenterol Hepatol* 2013; **25**: 37-43 [PMID: 23013623 DOI: 10.1097/MEG.0b013e32835948e3]

4 **Nakaji S**, Sugawara K, Saito D, Yoshioka Y, MacAuley D, Bradley T, Kernohan G, Baxter D. Trends in dietary fiber intake in Japan over the last century. *Eur J Nutr* 2002; **41**: 222-227 [PMID: 12395216 DOI: 10.1007/s00394-002-0379-x]

5 **Nagata N**, Niikura R, Aoki T, Shimbo T, Itoh T, Goda Y, Suda R, Yano H, Akiyama J, Yanase M, Mizokami M, Uemura N. Increase in colonic diverticulosis and diverticular hemorrhage in an aging society: lessons from a 9-year colonoscopic study of 28,192 patients in Japan. *Int J Colorectal Dis* 2014; **29**: 379-385 [PMID: 24317937

DOI: 10.1007/s00384-013-1808-4]
6 **Longstreth GF**. Epidemiology and outcome of patients hospitalized with acute lower gastrointestinal hemorrhage: a population-based study. *Am J Gastroenterol* 1997; **92**: 419-424 [PMID: 9068461]
7 **Charlson ME**, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis* 1987; **40**: 373-383 [PMID: 3558716]
8 **Jensen DM**, Machicado GA, Jutabha R, Kovacs TO. Urgent colonoscopy for the diagnosis and treatment of severe diverticular hemorrhage. *N Engl J Med* 2000; **342**: 78-82 [PMID: 10631275 DOI: 10.1056/NEJM200001133420202]
9 Ministry of Internal Affairs and Communications, Population prediction. [accessed 2015 Jan 14]. Available from: URL: http://www.soumu.go.jp/johotsusintokei/whitepaper/ja/h25/html/nc123110.html.
10 **Antithrombotic Trialists' Collaboration.** Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. *BMJ* 2002; **324**: 71-86 [PMID: 11786451 DOI: 10.1136/bmj.324.7329.71]
11 ACC/AHA/ESC 2006 Guidelines for the Management of Patients With Atrial Fibrillation: Executive Summary. *Circulation* 2006; **114**: 700-752
12 **Yamada A**, Sugimoto T, Kondo S, Ohta M, Watabe H, Maeda S, Togo G, Yamaji Y, Ogura K, Okamoto M, Yoshida H, Kawabe T, Kawase T, Omata M. Assessment of the risk factors for colonic diverticular hemorrhage. *Dis Colon Rectum* 2008; **51**: 116-120 [PMID: 18085336 DOI: 10.1007/s10350-007-9137-8]
13 **Kubo A**, Kagaya T, Nakagawa H. Studies on complications of diverticular disease of the colon. *Jpn J Med* 1985; **24**: 39-43 [PMID: 3873561]
14 **Nakada I**, Ubukata H, Goto Y, Watanabe Y, Sato S, Tabuchi T, Soma T, Umeda K. Diverticular disease of colon at a regional general hospital in Japan. *Dis colon Rectum* 1995; **38**: 755-9 [PMID 7607039]
15 **Niikura R**, Nagata N, Shimbo T, Aoki T, Yamada A, Hirata Y, Sekine K, Okubo H, Watanabe K, Sakurai T, Yokoi C, Mizokami M, Yanase M, Akiyama J, Koike K, Uemura N. Natural history of bleeding risk in colonic diverticulosis patients: a long-term colonoscopy-based cohort study. *Aliment Pharmacol Ther* 2015; **41**: 888-894 [PMID: 25715746 DOI: 10.1111/apt.13148]
16 **McGuire HH**. Bleeding colonic diverticula. A reappraisal of natural history and management. *Ann Surg* 1994; **220**: 653-656 [PMID: 7979613]
17 **Aldoori WH**, Giovannucci EL, Rimm EB, Wing AL, Willett WC. Use of acetaminophen and nonsteroidal anti-inflammatory drugs: a prospective study and the risk of symptomatic diverticular disease in men. *Arch Fam Med* 1998; **7**: 255-260 [PMID: 9596460]
18 **Nagata N**, Sakamoto K, Arai T, Niikura R, Shimbo T, Shinozaki M, Aoki T, Sekine K, Okubo H, Watanabe K, Sakurai T, Yokoi C, Yanase M, Akiyama J, Noda M, Uemura N. Visceral fat accumulation affects risk of colonic diverticular hemorrhage. *Int J Colorectal Dis* 2015; **30**: 1399-1406 [PMID: 26099321 DOI: 10.1007/s00384-015-2295-6]
19 **Meyers MA**, Alonso DR, Baer JW. Pathogenesis of massively bleeding colonic diverticulosis: new observations. *AJR Am J Roentgenol* 1976; **127**: 901-908 [PMID: 1087123]
20 **Chen CY**, Wu CC, Jao SW, Pai L, Hsiao CW. Colonic diverticular bleeding with comorbid diseases may need elective colectomy. *J Gastrointest Surg* 2009; **13**: 516-520 [PMID: 19005733 DOI: 10.1007/s11605-008-0731-4]
21 **Stollman N**, Raskin JB. Diverticular disease of the colon. *Lancet* 2004; **363**: 631-639 [PMID: 14987890 DOI: 10.1016/S0140-6736(04)15597-9]
22 **Japan Atherosclerosis Society**. [Japan Atherosclerosis Society (JAS) guidelines for prevention of atherosclerotic cardiovascular diseases]. *J Atheroscler Thromb* 2007; **4**: 5-57 [PMID: 17566346]

P- Reviewer: Liu DL, Ozuner G, Reichert MC, Wani HU
S- Editor: Qi Y **L- Editor**: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i3.447

World J Gastrointest Pharmacol Ther 2016 August 6; 7(3): 447-452
ISSN 2150-5349 (online)

ORIGINAL ARTICLE

Prospective Study

Faecal incontinence and health related quality of life in inflammatory bowel disease patients: Findings from a tertiary care center in South Asia

Duminda Subasinghe, Navarathna Mudiyanselage Meththananda Navarathna, Dharmabandhu Nandadeva Samarasekera

Duminda Subasinghe, Navarathna Mudiyanselage Meththananda Navarathna, Dharmabandhu Nandadeva Samarasekera, Department of Surgery, Faculty of Medicine, University Surgical Unit, the National Hospital of Sri Lanka, Colombo 08, Sri Lanka

Author contributions: Subasinghe D, Navarathna NMM and Samarasekera DN were involved in study planning, data collection and analysis, and writing of the manuscript; all authors read and approved the final manuscript.

Institutional review board statement: This study was approved by the ethics review committee of the National Hospital of Sri Lanka.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: The authors of this manuscript having no conflicts of Interest to disclose.

Data sharing statement: There is no additional data available.

Correspondence to: Dharmabandhu Nandadeva Samarasekera, MBBS (Colombo), MS, MD (East Anglia), FRCS (Eng), FRCS (Edin), PG Cert MedEd (Dundee), Professor of Surgery and Head, Department of Surgery, Faculty of Medicine, University Surgical Unit, the National Hospital of Sri Lanka, Kynsey Road, Colombo 08, Sri Lanka. samarasekera58@yahoo.co.uk
Telephone: +94-77-5761000
Fax: +94-11-2691581

Received: December 9, 2015
Peer-review started: December 11, 2015
First decision: January 4, 2016
Revised: March 9, 2016
Accepted: April 14, 2016
Article in press: April 18, 2016
Published online: August 6, 2016

Abstract

AIM: To analyze the frequency and severity of faecal incontinence (FI) and its effect on the quality of life (QOL) in inflammatory bowel disease (IBD) patients.

METHODS: All patients who attended surgical and medical gastroenterology outpatient clinics in a tertiary care center with an established diagnosis of either ulcerative colitis (UC) or Crohn's disease (CD) over a period of 10 mo were included in this study. Before enrollment into the study, the patients were explained about the study and informed consent was obtained. The patients with unidentified colitis were excluded. The data on demographics, disease characteristics, FI (Vaizey score), and quality of life (IBD-Q) were collected. Data were analyzed using SPSS version 21.

RESULTS: There were 184 patients (women = 101, 54.9%; UC = 153, 83.2%) with a female preponderance for UC (male/female ratio = 1:1.5) and a male preponderance for CD (male/female = 2:1). Forty-eight (26%) patients reported symptoms of FI. Among the patients with FI, 70.8% were women (n = 34) and 29.2% were men (n = 14) with an average age of 52.7 years (range, 20-78 years). Average age of onset of FI was 48.6 (range, 22-74) years. Ten percent (n = 5) reported regular FI.

Incontinence to flatus was seen in 33.3% (n = 16), to liquid faeces in 56.2% (n = 27), to solid faeces in 6.2% (n = 3) and to all three in 4.1% (n = 2). Twenty-one percent (n = 10) complained of disruption of their physical and social activity. There was no association between FI and type of IBD. Significant associations were found between FI and age (P = 0.005) and gender (P < 0.001). QOL in our cohort of patients was significantly affected by FI.

CONCLUSION: In our study, nearly a quarter of patients reported FI. There was a significant correlation between FI and QOL. Therefore, enquiring about FI in IBD patients can lead to identification of this debilitating condition. This will enable early referral for continence care in this group of patients.

Key words: Inflammatory bowel disease; Quality of life; Faecal incontinence; Crohn's disease

Core tip: This was a prospective study involving 184 patients with inflammatory bowel disease (IBD). It was designed to analyze the frequency and severity of faecal incontinence (FI) and its effect on the quality of life (QOL) in IBD patients in a tertiary care center. In our study, nearly 25% of patients reported the symptoms of FI. There was a significant correlation between FI and QOL. Therefore, enquiring about FI in IBD patients can lead to identification of this debilitating condition.

Subasinghe D, Navarathna NMM, Samarasekera DN. Faecal incontinence and health related quality of life in inflammatory bowel disease patients: Findings from a tertiary care center in South Asia. *World J Gastrointest Pharmacol Ther* 2016; 7(3): 447-452 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i3/447.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i3.447

INTRODUCTION

Faecal incontinence (FI) is defined as the involuntary passage of solid or liquid stools, which is a hygienic and social problem[1]. It is a devastating personal and social problem which causes emotional distress leading to social isolation and loss of self-confidence[2]. The prevalence rates of FI in the community vary between 2.2%-15% in adults[3-7]. It is widely accepted that many patients with anal incontinence do not seek medical advice, thus making the true prevalence uncertain. Therefore under-reporting is common due to social embarrassment[8,9].

FI can lead to social isolation. It also can adversely affect ability to maintain relationships, occupation and self-esteem aspects of the quality of life (QOL)[10,11]. Ulcerative colitis (UC) and Crohn's disease (CD) are chronic inflammatory conditions related to the gastrointestinal tract. There is a paucity of knowledge of FI in patients with inflammatory bowel disease (IBD), except in patients with fistulas and those who underwent restorative proctocolectomy with an ileal pouch[12]. FI is also known to be associated with vaginal delivery in women[13,14]. In addition, in both genders, FI can be associated with a range of pelvic floor disorders and perianal surgeries (*e.g.*, haemorrhoidectomy and sphincterotomy)[15,16].

The only estimation of FI in IBD is from patients attending special clinics and the data from the Crohn's and Colitis Foundation of the United Kingdom, and the incidence ranged from 22%-33.5%[17-19]. No previous study has reported on FI among patients with IBD in Sri Lanka or in South Asia.

Therefore, the main aim of this study was to determine the frequency and severity of FI, and its effect on QOL in IBD patients who presented to a tertiary care center in Sri Lanka, which is a South Asian country.

MATERIALS AND METHODS

Patients and methods

This study was conducted at the National Hospital of Sri Lanka, which is a tertiary care hospital. The patients were interviewed prospectively over a period of 10 mo. Before the interview, the patients were educated about the study and informed consent was obtained. All the patients who attended outpatient clinics with an established histological diagnosis of either UC or CD were included in the study. Diagnosis of IBD was made based on clinical, endoscopic, radiological and histological findings. Age younger than 18 years, lack of cooperation, diagnosed psychiatric illness, being too ill to participate, patients with neurological disorders and those with a previous traumatic anal sphincter injury were excluded from the study. The study was approved by the ethics review committee of the hospital.

All IBD patients were interviewed using an interviewer administered questionnaire, which consisted of two parts. The first part consisted of personal details of the patients including socio-demographic data, disease characteristics, management details and history. The second part of the questionnaire included FI severity (Vaizey score)[20] and quality of life (IBD-Q) score.

FI

FI was assessed based on Vaizey score with a four point scale: Never, rarely, sometimes, and regularly. Vaizey score was selected because it has shown high clinical validity and utility[20]. The Vaizey Incontinence questionnaire consists of seven questions. A score of 0 suggests no problems with bowel continence and a score of 24 suggests very severe problems with incontinence.

QOL

IBD-Q32 evaluates QOL in four main aspects (bowel symptoms, emotional health, systemic symptoms and social symptoms). Cumulative score reflects the overall QOL. For each aspect under specific category, score varies from one to seven. Score of one indicates very poor QOL and that of seven indicates excellent QOL. Total IBDQ

Table 1 Demographic characteristics of the study population *n* (%)

	Total IBD	UC	CD
Age at the diagnosis (yr)			
⩽ 10	2 (1.1)	1 (0.7)	1 (3.2)
11-19	17 (9.2)	13 (8.5)	4 (12.9)
20-29	42 (22.8)	25 (16.3)	17 (54.8)
30-39	53 (28.8)	49 (32.0)	4 (12.9)
40-49	39 (21.2)	36 (23.5)	3 (9.7)
50-59	21 (11.4)	19 (12.4)	2 (6.5)
60-69	8 (4.3)	8 (5.2)	-
70-79	2 (1.1)	2 (1.3)	-
Gender			
Male	83 (45.1)	62 (40.5)	21 (67.7)
Female	101 (54.9)	91 (59.5)	10 (32.3)
Education			
Primary (Grade 1-5)	40 (21.7)	35 (22.9)	5 (16.1)
Secondary (Grade 6-13)	118 (64.1)	101 (66.0)	17 (54.8)
Higher (University or above)	26 (14.1)	17 (11.0)	9 (29.0)
Employment			
None	72 (39.1)	64 (41.8)	8 (25.8)
Student	11 (6.0)	11 (7.2)	-
Labourer	63 (34.2)	50 (32.7)	13 (41.9)
Professional	38 (20.7)	28 (18.3)	10 (32.3)

IBD: Inflammatory bowel disease; UC: Ulcerative colitis; CD: Crohn's disease.

score can range from 32 (very poor QOL) to 224 (perfect HRQOL).

Both IBDQ and Vaizey score were selected because of their simplicity and precision, which make those ideal for clinical practice to identify patients who require specialist help and in the clinical research setting to provide a sensitive measure of FI[20,21].

Statistical analysis

The associations between categorical data were examined using χ^2 test. The association between categorical variables and IBDQ-32 scores was determined using Student's *t*-test. Factors statistically significant in the univariate analysis were included in a multivariate regression model to examine their associations with FI score and QOL. The differences were considered significant when $P \leqslant 0.05$. Data were analyzed using SPSS (Version 21, Chicago, IL, United States).

RESULTS

Demographic and disease characteristics

There were 184 patients (M:F = 83:101) with a mean age of 44.5 (range, 20-78) years. The majority of patients (83.2%, *n* = 153) had UC. The mean duration of disease was 8.17 (range, 1-28) years, while 33.7% (*n* = 62) of patients had IBD for more than 10 years. The participation rate of our population was high (184/188 = 97.87%). The majority of UC patients were female, with a male to female ratio of 1:1.5. A male preponderance was noted in CD (male to female ratio = 2:1). None of our patients had a positive family history. Mean age at diagnosis for UC was 36.3 (range, 7-71) years. The patients with CD were diagnosed at a significantly younger age than UC patients (27.35 ± 10.22 years *vs* 38.14 ± 13.05 years, $P < 0.0001$). Peak age of onset was in the fourth decade for UC and in the third decade for CD (Table 1). Out of females (*n* = 101, UC = 91, CD = 10), the majority were unmarried (*n* = 55, UC = 47, CD = 8). Out of married females (*n* = 46), 25 had undergone lower segment caesarian sections and 10 had undergone vaginal deliveries, while 11 had no childbirth yet. There were no females with ongoing pregnancy in our sample.

Table 2 Descriptive statistics for the four domains and overall score of the IBDQ-32 and categories

	Minimum (Reference)	Maximum (Reference)	Mean
IBDQbowel	10	56	22.33
IBDQSystemic	6	34	12.5
IBDQEmotinal	18	84	34.87
IBDQSocial	5	35	12.3
IBDQTotal	51 (32)	215 (224)	94.28

QOL

The mean of IBDQ-32 scores of enrolled patients was 94.28 (51 to 215). Mean IBDQ scores of bowel symptoms, systemic, emotional, social categories of IBDQ are shown in Table 2. The social symptom and systemic symptom categories had the lowest HRQOL scores (12.3 and 12.5, respectively).

There was no significant difference between CD and UC, with regard to the mean IBDQ-32 (80.26 for CD and 79.52 for UC, $P = 0.778$) or mean Vaizey score (UC 13.79 *vs* CD 14.45, $P = 0.629$). Also, there was no significant difference in mean scores of bowel symptom (21.11 *vs* 22.39, $P = 0.220$), systemic (12.46 *vs* 12, $P = 0.560$) social (11.91 *vs* 11.10, $P = 0.297$) and emotional symptoms (34.04 *vs* 34.77, $P = 0.607$) between the two categories of UC and CD.

Determinants of QOL

Although females had a slightly higher mean IBDQ score (79.9 *vs* 79.34), it was not statistically significant ($P = 0.769$). In subgroup analysis, there was no significant difference in the four aspects of IBDQ categories ($P > 0.05$). Females had significantly higher incontinence scores than males (mean Vaizey score 79.9 *vs* 79.34, $P < 0.05$).

Twenty-six (14.1%) patients of the total study population underwent surgical treatment. In the UC group, 8.5% (*n* = 13) underwent surgical treatment, the commonest surgical procedure was restorative proctocolectomy (*n* = 12) and one patient underwent sigmoid colectomy. IBD patients who underwent surgery had significantly higher IBDQ bowel (23.48 *vs* 21, $P < 0.05$) and IBDQ total scores (81.83 *vs* 79.34, $P < 0.05$) compared to the patients who were on long-term medical management. However, the difference

Table 3 Details of surgical procedures for inflammatory bowel disease

Surgical procedure	Indication	*n* (%)
UC		
Restorative proctocolectomy and ileoanal pouch	Steriod resistance-7 Atypia on histology-4 Sigmoid colon cancer-1	12 (7.8)
Sigmoid colectomy	Stricture of sigmoid colon	1 (0.7)
CD		
Drainage and fistulectomy	Perianal abscess and fistula	1 (3.2)
Fistulectomy and repair	Recurrent enterocutaneous fistula	1 (3.2)
Incision and drainage	R/Ischiorectal fossa abscess	1 (3.2)
Repair of the fistula	Enrerocutaneous fistula	2 (6.4)
R/hemicolectomy and ileo transverse anastomosis	Strictures of the colon	4 (12.9)
Total colectomy and ileostomy	Strictures of colon	2 (6.4)
Repair of the fistula	Recto vaginal fistula	1 (3.2)
Strictureplasty, R/hemicolectomy and ileo transverse	Two long segment narrowings –distal ileum	1 (3.2)
Anastomosis	multiple narrowings > 10 in jejunum and proximal ileum and strictures of ascending colon	-

UC: Ulcerative colitis; CD: Crohn's disease.

Table 4 Correlation between quality of life components and incontinence scores

Association	Pearson correlation coefficient (Rho value)
IBDQbowel *vs* Vaizey score	0.74
IBDQSystemic *vs* Vaizey score	0.13
IBDQEmotinal *vs* Vaizey score	0.09
IBDQSocial *vs* Vaizey score	0.3
IBDQTotal *vs* Vaizey score	0.61

of incontinence scores was not significantly different between the two groups.

Mean IBDQ-emotional and IBDQ-social scores had significant association with the extent of colonic involvement by the disease. The mean total IBDQ scores did not show significant differences in relation to education level ($P = 0.676$), age ($P = 0.343$), duration ($P = 0.884$), extent of IBD ($P = 0.92$) or current symptoms of the disease ($P = 0.3$).

The relationships between psychosocial, clinical, and demographic variables and the overall score of IBDQ-32 are shown in Table 3.

FI vs QOL

The extent of colitis was significantly associated with the Vaizey scores ($P = 0.002$), where patients with distal colitis had higher scores. Association of total IBDQ and Vaizey score was statistically significant ($P < 0.001$). Pearson correlation was performed to determine the correlation between Vaizey score and components of QOL scores and total IBD-Q score. QOL scores for emotional and systemic components showed a weak association (Rho < 0.3), QOL score of social component showed a moderate association (Rho 0.3-0.7) and that of bowel symptoms showed a strong association (Rho > 0.7) (Table 4).

DISCUSSION

It is noted that the incidence of IBD is increasing in the Asian population[22,23]. They are among the group of chronic disorders associated with periods of remission and unpredictable relapses. QOL measurement is especially pertinent in IBD, because it is a chronic disabling disease[24] which commonly occurs in early adulthood and hence affects all aspects of life, mainly physical, social and psychological. The peculiarities of chronic disease over acutely resolving conditions are that they often have a long-term negative effect on the emotional and social life, which are most of the time not visually apparent[25]. Feeling dirty and smelly following loss of bowel control, with resultant offensive body odours, unfulfilled potential in the work place and issues related to sexual relationships were the highly ranked concern in a survey of patients with IBD[26].

In addition, fear of loss of bowel control and its unpredictability can lead to a profound effect on the individual's behaviour. In the majority of patients with IBD, this factor can lead to an avoidance of routine social events or impairment of daily activities[27,28]. Recent work by Daniel *et al*[27] and Hall *et al*[29] showed that these patients only attend places with toilet facilities or avoid public places all together.

Our results showed that IBD patients who underwent surgery for UC and CD had significantly higher IBDQ bowel (23.48 *vs* 21, $P < 0.05$) and IBDQ total scores (81.83 *vs* 79.34, $P < 0.05$) than those who was on long-term medical management. This may be due to the long-term symptom relief and avoidance of chronic medicine intake leading to more convenient life style. According to our results, higher Vaizey scores were associated with lower IBDQ scores ($P < 0.001$). This shows that the fear of anal incontinence and its unpredictability had a profound effect on the individual's day-to-day activities. In our study, we found important variables significantly

related with lower QOL, suggesting that HRQOL analysis has an important role in understanding the true impact of the disease on patients. QOL score of social component showed a moderate association (rho 0.3-0.7) and QOL of bowel symptom component showed a strong association (rho > 0.7) with FI. This shows the significant impact of incontinence on social activities.

In conclusion, FI has adverse effects on social, emotional and other aspects of QOL in patients with IBD. Given the availability of specialist FI interventions and support, we recommend that sensitive questioning regarding FI should be part of routine disease surveillance in the outpatient setting to cater for this unmet need.

COMMENTS

Background

Severity and impact of faecal incontinence (FI) on quality of life (QOL) of inflammatory bowel disease (IBD) are not widely investigated. In general FI has adverse effects on daily activities, hence on QOL. The current study was designed to evaluate the severity and frequency of FI and its effect on QOL in IBD patients presented to a tertiary care center in a South Asia country.

Research frontiers

This study has showed that FI has more adverse effects on social, emotional and other aspects of QOL in IBD. Given the availability of specialist FI interventions and support, the authors recommend that sensitive questioning regarding FI should be part of routine disease surveillance in the outpatient setting.

Innovations and breakthrough

Current literature suggests various strategies to improve the management and outcome of chronic diseases such as IBD. This study provides evidence on improvement QOL by considering the FI as an important aspect of the management.

Applications

This study has showed that FI correlates with HRQOL in IBD patients. Therefore, these aspects should be addressed to improve the management of these patients having this chronic disease.

Terminology

FI is defined as the involuntary passage of passage of solid or liquid stools, which is a social and hygienic problem. Ulcerative colitis/Crohn's disease are chronic IBD affecting gastrointestinal tract.

Peer-review

A well-timed piece with pertinent clinical insight, and the information provided is relevant and could be interesting enough to warrant readers' attention.

REFERENCES

1 **Norton C**, Whitehead WE, Bliss DZ, Harari D, Lang J. Management of fecal incontinence in adults. *Neurourol Urodyn* 2010; **29**: 199-206 [PMID: 20025031 DOI: 10.1002/nau.20803]

2 **Crowell MD**, Schettler VA, Lacy BE, Lunsford TN, Harris LA, DiBaise JK, Jones MP. Impact of anal incontinence on psychosocial function and health-related quality of life. *Dig Dis Sci* 2007; **52**: 1627-1631 [PMID: 17211712 DOI: 10.1007/s10620-006-9249-3]

3 **Johanson JF**, Lafferty J. Epidemiology of fecal incontinence: the silent affliction. *Am J Gastroenterol* 1996; **91**: 33-36 [PMID: 8561140]

4 **Thomas TM**, Egan M, Walgrove A, Meade TW. The prevalence of faecal and double incontinence. *Community Med* 1984; **6**: 216-220 [PMID: 6478780]

5 **Macmillan AK**, Merrie AE, Marshall RJ, Parry BR. The prevalence of fecal incontinence in community-dwelling adults: a systematic review of the literature. *Dis Colon Rectum* 2004; **47**: 1341-1349 [PMID: 15484348 DOI: 10.1007/s10350-004-0593-0]

6 **Perry S**, Shaw C, McGrother C, Matthews RJ, Assassa RP, Dallosso H, Williams K, Brittain KR, Azam U, Clarke M, Jagger C, Mayne C, Castleden CM. Prevalence of faecal incontinence in adults aged 40 years or more living in the community. *Gut* 2002; **50**: 480-484 [PMID: 11889066 DOI: 10.1136/gut.50.4.480]

7 **Whitehead WE**, Borrud L, Goode PS, Meikle S, Mueller ER, Tuteja A, Weidner A, Weinstein M, Ye W. Fecal incontinence in US adults: epidemiology and risk factors. *Gastroenterology* 2009; **137**: 512-517, 517.e1-2 [PMID: 19410574]

8 **Leigh RJ**, Turnberg LA. Faecal incontinence: the unvoiced symptom. *Lancet* 1982; **1**: 1349-1351 [PMID: 6123650 DOI: 10.1016/S0140-6736(82)92413-8]

9 **Bartlett L**, Nowak M, Ho YH. Reasons for non-disclosure of faecal incontinence: a comparison between two survey methods. *Tech Coloproctol* 2007; **11**: 251-257 [PMID: 17676265 DOI: 10.1007/s10151-007-0360-z]

10 **Deutekom M**, Terra MP, Dobben AC, Dijkgraaf MG, Baeten CG, Stoker J, Bossuyt PM. Impact of faecal incontinence severity on health domains. *Colorectal Dis* 2005; **7**: 263-269 [PMID: 15859965 DOI: 10.1111/j.1463-1318.2005.00772.x]

11 **Bartlett L**, Nowak M, Ho YH. Impact of fecal incontinence on quality of life. *World J Gastroenterol* 2009; **15**: 3276-3282 [PMID: 19598304 DOI: 10.3748/wjg.15.3276]

12 **Wuthrich P**, Gervaz P, Ambrosetti P, Soravia C, Morel P. Functional outcome and quality of life after restorative proctocolectomy and ileo-anal pouch anastomosis. *Swiss Med Wkly* 2009; **139**: 193-197 [PMID: 19350425]

13 **Nichols CM**, Ramakrishnan V, Gill EJ, Hurt WG. Anal incontinence in women with and those without pelvic floor disorders. *Obstet Gynecol* 2005; **106**: 1266-1271 [PMID: 16319251 DOI: 10.1097/01.AOG.0000187303.43011.12]

14 **Chin K**. Obstetrics and fecal incontinence. *Clin Colon Rectal Surg* 2014; **27**: 110-112 [PMID: 25320570 DOI: 10.1055/s-0034-1383903]

15 **Norton C**. Faecal incontinence in adults. 1: Prevalence and causes. *Br J Nurs* 1996; **5**: 1366-1368, 1370-1374 [PMID: 9025365]

16 **Lunniss PJ**, Gladman MA, Hetzer FH, Williams NS, Scott SM. Risk factors in acquired faecal incontinence. *J R Soc Med* 2004; **97**: 111-116 [PMID: 14996955 DOI: 10.1258/jrsm.97.3.111]

17 **Enck P**, Bielefeldt K, Rathmann W, Purrmann J, Tschöpe D, Erckenbrecht JF. Epidemiology of faecal incontinence in selected patient groups. *Int J Colorectal Dis* 1991; **6**: 143-146 [PMID: 1744485 DOI: 10.1007/BF00341234]

18 **Dibley L**, Norton C. Experiences of fecal incontinence in people with inflammatory bowel disease: self-reported experiences among a community sample. *Inflamm Bowel Dis* 2013; **19**: 1450-1462 [PMID: 23624884 DOI: 10.1097/MIB.0b013e318281327f]

19 **Norton C**, Dibley LB, Bassett P. Faecal incontinence in inflammatory bowel disease: associations and effect on quality of life. *J Crohns Colitis* 2013; **7**: e302-e311 [PMID: 23228710 DOI: 10.1016/j.crohns.2012.11.004]

20 **Vaizey CJ**, Carapeti E, Cahill JA, Kamm MA. Prospective comparison of faecal incontinence grading systems. *Gut* 1999; **44**: 77-80 [PMID: 9862829 DOI: 10.1136/gut.44.1.77]

21 **Olopade FA**, Norman A, Blake P, Dearnaley DP, Harrington KJ, Khoo V, Tait D, Hackett C, Andreyev HJ. A modified Inflammatory Bowel Disease questionnaire and the Vaizey Incontinence questionnaire are simple ways to identify patients with significant gastrointestinal symptoms after pelvic radiotherapy. *Br J Cancer* 2005; **92**: 1663-1670 [PMID: 15856043 DOI: 10.1038/sj.bjc.6602552]

22 **Yang SK**, Hong WS, Min YI, Kim HY, Yoo JY, Rhee PL, Rhee JC, Chang DK, Song IS, Jung SA, Park EB, Yoo HM, Lee DK, Kim YK. Incidence and prevalence of ulcerative colitis in the Songpa-Kangdong District, Seoul, Korea, 1986-1997. *J Gastroenterol*

Hepatol 2000; **15**: 1037-1042 [PMID: 11059934 DOI: 10.1046/j.1440-1746.2000.02252.x]
23 **Yao T**, Matsui T, Hiwatashi N. Crohn's disease in Japan: diagnostic criteria and epidemiology. *Dis Colon Rectum* 2000; **43**: S85-S93 [PMID: 11052483 DOI: 10.1007/BF02237231]
24 **Peyrin-Biroulet L**, Loftus EV, Colombel JF, Sandborn WJ. The natural history of adult Crohn's disease in population-based cohorts. *Am J Gastroenterol* 2010; **105**: 289-297 [PMID: 19861953 DOI: 10.1038/ajg.2009.579]
25 **Pizzi LT**, Weston CM, Goldfarb NI, Moretti D, Cobb N, Howell JB, Infantolino A, Dimarino AJ, Cohen S. Impact of chronic conditions on quality of life in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2006; **12**: 47-52 [PMID: 16374258 DOI: 10.1097/01.MIB.0000191670.04605.e7]
26 **de Rooy EC**, Toner BB, Maunder RG, Greenberg GR, Baron D, Steinhart AH, McLeod R, Cohen Z. Concerns of patients with inflammatory bowel disease: results from a clinical population. *Am J Gastroenterol* 2001; **96**: 1816-1821 [PMID: 11419835 DOI: 10.1016/S0002-9270(01)02440-6]
27 **Daniel JM**. Young adults' perceptions of living with chronic inflammatory bowel disease. *Gastroenterol Nurs* 2002; **25**: 83-94 [PMID: 12055376 DOI: 10.1097/00001610-200205000-00002]
28 **Dudley-Brown S**. Living with ulcerative colitis. *Gastroenterol Nurs* 1996; **19**: 60-64 [PMID: 8717674 DOI: 10.1097/00001610-199603000-00004]
29 **Hall NJ**, Rubin GP, Dougall A, Hungin AP, Neely J. The fight for 'health-related normality': a qualitative study of the experiences of individuals living with established inflammatory bowel disease (ibd). *J Health Psychol* 2005; **10**: 443-455 [PMID: 15857873 DOI: 10.1177/1359105305051433]

P- Reviewer: Flores C, Pastorelli L, Sandberg KC
S- Editor: Gong ZM **L- Editor**: Wang TQ **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i3.453

World J Gastrointest Pharmacol Ther 2016 August 6; 7(3): 453-462
ISSN 2150-5349 (online)

SYSTEMATIC REVIEWS

Efficacy and safety of botulinum toxin in treatment of anismus: A systematic review

Sameh Hany Emile, Hossam Ayman Elfeki, Hosam Ghazy Elbanna, Mohamed Youssef, Waleed Thabet, Tito M Abd El-Hamed, Basem Said, Ahmed Lotfy

Sameh Hany Emile, Hossam Ayman Elfeki, Hosam Ghazy Elbanna, Mohamed Youssef, Waleed Thabet, Tito M Abd El-Hamed, Basem Said, Ahmed Lotfy, General Surgery Department, Colorectal Surgery Unit, Mansoura Faculty of Medicine, Mansoura University, Mansoura City 35516, Dakahlia Providence, Egypt

Author contributions: Emile SH designed the review; Emile SH and Elfeki HA conducted the search and acquisition and analysis of data; Emile SH, Elfeki HA and Youssef M wrote the manuscript; Elbanna HG, Abd El-Hamed TM and Said B contributed to data interpretation, writing and critical revision of the manuscript; Thabet W, Said B and Lotfy A shared in data analysis, drafting and critical revision of the manuscript; all authors approved the final version of the manuscript.

Conflict-of-interest statement: All the authors declare that they have no competing interests.

Data sharing statement: Technical appendix, statistical code, and dataset are available from the corresponding author at sameh200@hotmail.com. Informed consents of patients were obtained by the original studies included in the review.

Manuscript source: Invited manuscript

Correspondence to: Sameh Hany Emile, MD, Lecturer of General Surgery, General Surgery Department, Colorectal Surgery Unit, Mansoura Faculty of Medicine, Mansoura University, Elgomhuoria Street, Mansoura City 35516, Dakahlia Providence, Egypt. sameh200@hotmail.com
Telephone: +20-100-6267150
Fax: +20-50-239733

Received: February 29, 2016
Peer-review started: February 29, 2016
First decision: June 16, 2016
Revised: June 20, 2016
Accepted: July 11, 2016
Article in press: July 13, 2016
Published online: August 6, 2016

Abstract

AIM: To evaluate the efficacy and safety of botulinum toxin type A (BTX-A) in the management of patients with anismus.

METHODS: An organized search of published literature was conducted using electronic databases including: PubMed/MEDLINE, and Cochrane Central Register of Controlled Trials, also an internet-based search using "Google Scholar" service was conducted. Both comparative and observational studies were included. We excluded irrelevant articles, editorials, case reports, reviews, and meta-analyses. The studies that followed the patients less than 6 mo were excluded. Variables collected were demographic data of the patients, technique of BTX-A injection and number of sessions, short-term and long-term clinical improvement, post-injection changes in electromyography (EMG), defecography, manometry, and balloon expulsion test, and complications recorded after BTX-A injection.

RESULTS: Seven studies comprising 189 patients were included in the review. The median age of the patients was 41.2 years and female-to-male ratio was 1.3:1. The median dose of BTX-A injected per procedure was 100 IU (range, 20-100 IU). Lateral injection was done in five trails and combined lateral and posterior injections in two trials. Three studies used endorectal ultrasonography-guided technique, one study used EMG-guided technique,

whereas the remaining three studies used manual palpation with the index finger. The median percentage of patients who reported initial improvement of symptoms was 77.4% (range 37.5%-86.7%), this percentage declined to a median of 46% (range 25%-100%) at 4 mo after injection of BTX-A. Rates of improvement evaluated by balloon expulsion test, EMG, and defecography ranged between (37.5%-80%), (54%-86.7%), and (25%-86.6%), respectively. Fourteen (7.4%) patients developed complications after injection of BTX-A. Complication rates across the studies ranged from 0% to 22.6%.

CONCLUSION: Initial satisfactory improvement of symptoms after BTX-A injection remarkably deteriorated after 3 mo of the procedure. However, repeated injection may provide better sustained results with no additional morbidities. Further analysis of more patients is necessary to conclude the safety of BTX-A for the treatment of anismus.

Key words: Botulinum toxin; Botulinum toxin type A; Botox; Anismus; Puborectalis syndrome; Efficacy

Core tip: Injection of botulinum toxin type A (BTX-A) is a simple, technically feasible outpatient procedure. The initial satisfactory improvement of symptoms after BTX-A injection remarkably deteriorates after three months of the procedure from a median rate of 77.4% to 46%. However, repeated injections may provide better sustained results with no additional morbidities. The endorectal ultrasonography- and electromyography-guided techniques do not add significant value regarding both initial and long-term improvement. Combined lateral and posterior injections do not offer better results than lateral injection alone, on the contrary they can lead to higher complication rates. Although most of the studies reported very low complication rates after BTX-A injection; further studies on a larger number of patients are necessary to conclude the safety of this treatment.

Emile SH, Elfeki HA, Elbanna HG, Youssef M, Thabet W, Abd El-Hamed TM, Said B, Lotfy A. Efficacy and safety of botulinum toxin in treatment of anismus: A systematic review. *World J Gastrointest Pharmacol Ther* 2016; 7(3): 453-462 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i3/453.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i3.453

INTRODUCTION

Anismus is a functional disorder of the defecation process that entails failure of relaxation or even paradoxical contraction of the puborectalis muscle and external anal sphincter (EAS) during defecation[1]. The term "Anismus" was first described by Preson and Lennard-Jones[2] in 1985. Anismus, also known as pelvic floor dyssynergia and puborectalis syndrome[3], commonly affects young and middle-aged females. The exact incidence of anismus is still unknown; however, it ranges between 20% and 70% of the general population[4].

The pathophysiology of anismus is not clearly defined yet. Certain predisposing factors as physical and emotional stress, previous anorectal surgery or hysterectomy, and psychological disorders are associated with anismus[5]. Sexual assault or abuse in childhood may also contribute to the development of anismus[6].

Patients with anismus typically complain of symptoms of outlet obstruction and defecation difficulties. Frequent attempts of evacuation, prolonged straining, anal pain, and sense of incomplete evacuation are the common presenting features of this condition[7]. On digital rectal examination (DRE), the puborectalis muscle and EAS fail to relax during straining, and sometimes a paradoxical contraction may occur. Although DRE can preliminarily diagnose anismus, additional physiologic tests such as anorectal manometry[8], balloon expulsion test[2], electromyography (EMG) of the puborectalis muscle and EAS[9], and defecography[10] are required to establish the diagnosis.

Anismus is initially managed in a conservative manner, starting with dietary modification focusing on high fiber diet, then using enemas and laxatives in increasing doses. However, conservative measures usually fail to solve the problem. Biofeedback (BFB) retraining was introduced by Bleijenberg and Kuijpers[11] for the treatment of anismus. Results of BFB were conflicting with efficacy rates ranging from 31% to 89%[3]. Surgical treatment in the form of partial miotomy of the puborectalis muscle has been described in a few reports with long-term success reaching up to 67% of patients[12].

Botulinum toxin, the product of Clostridium botulinum anaerobic bacterium, divides into seven subtype (A-G) that share similar structure, yet have different antigenic properties. Botulinum toxin type A (BTX-A) functions through extracellular binding to glycoprotein structures on the presynaptic cholinergic nerve endings which prevents the secretion of acetylcholine causing neuromuscular blockage and muscle paralysis. In addition, BTX-A blocks the efferent autonomic fibers to the smooth muscles and to the exocrine glands. While BTX-A does not induce direct central nervous system effects, some indirect effects as reflex inhibition and intra-cortical inhibition have been observed[13].

Injection of BTX-A neurotoxin directly into the puborectalis muscle is a non-operative method for the treatment of anismus[14]. Similar to BFB, the results of BTX-A injection were also conflicting. While the short-term results were highly satisfactory, the long-term outcome was disappointing with success rates of around 50% necessitating repeated injections in order to maintain the initial clinical improvement[15].

The primary objective of the current review was to analyze all the eligible articles that have evaluated the efficacy of BTX-A with regard to short and long-term

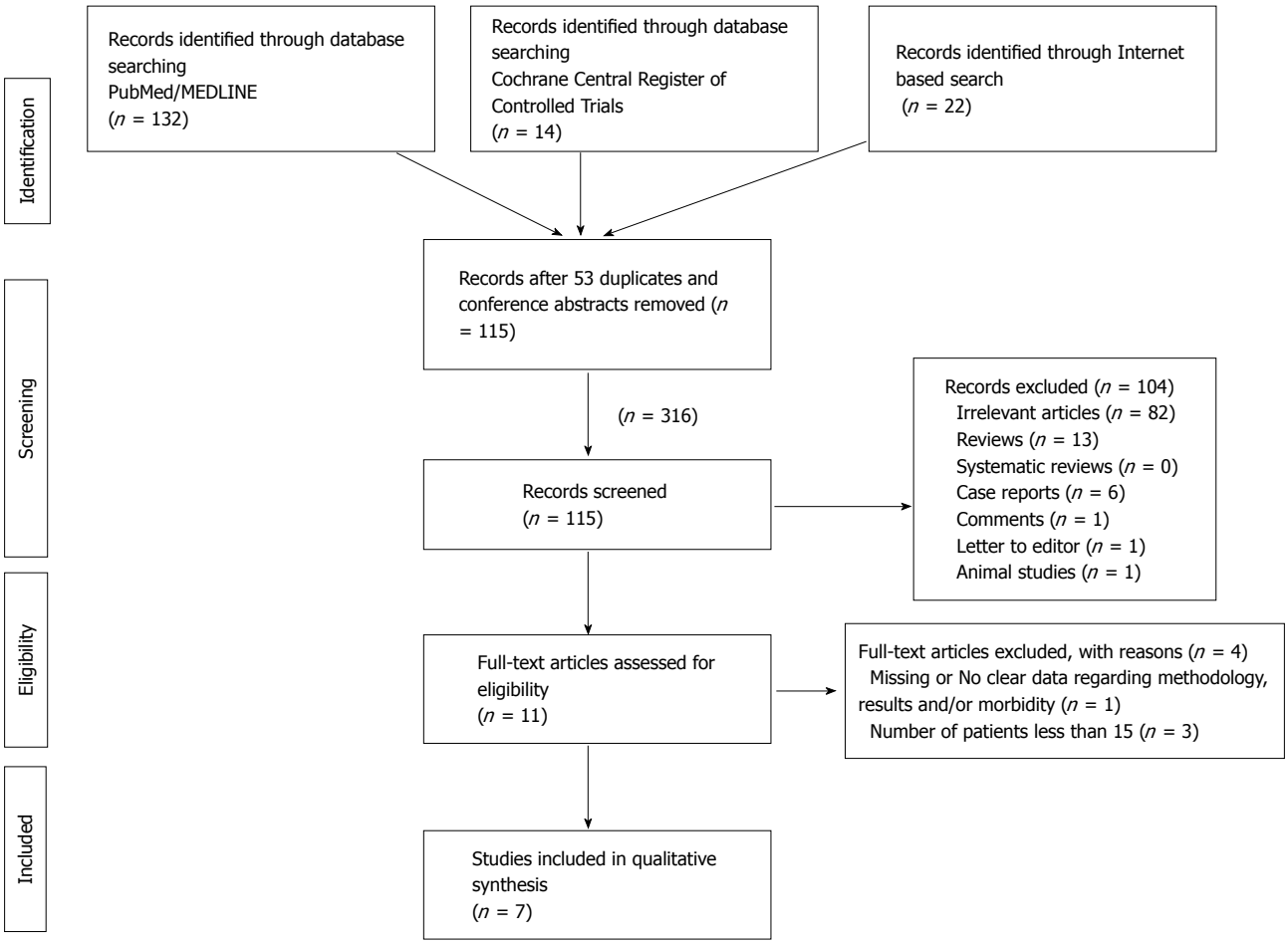

Figure 1 PRISMA flow-diagram.

outcomes. The secondary objective was to assess the side effects and complications encountered after the procedure to establish an evidence-based approach of treatment of anismus with BTX-A injection.

MATERIALS AND METHODS

Registration

This systematic review was registered online in the PROSPERO project under the registration number of CRD42016033892.

Search strategy

A systematic review of the literature for the role of BTX-A in the treatment of anismus was conducted following the screening guidelines established by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Figure 1)[16]. Electronic databases including: PubMed/MEDLINE, and Cochrane Central Register of Controlled Trials were searched for published studies until October 2015. A parallel internet-based search using "Google Scholar" service was also conducted. The PubMed function "related articles" was used to search further articles.

The keywords used in the preliminary search process included: Anismus, puborectalis, botulinum toxin, BTA-A, puborectalis syndrome, efficacy, and safety. The following keywords syntax was utilized in the search process: (Botulinum toxin OR BTX-A) AND (Anismus OR Paradoxical contraction of puborectalis OR puborectalis syndrome).

Relevant articles mentioned in the references section of the initial publications were obtained and the related articles were also screened to add any relevant publications to the results. The full text versions of the selected articles were screened by two independent reviewers (Emile SH and Elfeki HA) to check eligibility.

Inclusion criteria

This systematic review included studies that involved patients with anismus who were treated with BTX-A injection. We included both comparative and non-comparative trails that evaluated BTX-A therapy for treatment of anismus with a sample size of at least 15 patients. No language restrictions were applied.

Exclusion criteria

We have excluded irrelevant articles, editorials, case reports, reviews, and meta-analyses. The studies that followed the patients less than six months were excluded. Duplicate reports were identified and excluded from this review. Articles that did not report the aim,

Table 1 Assessment of the methodological qualities of the comparative studies included in the review

Items	Farid *et al*[12], 2009	Farid *et al*[20], 2009
Inclusion criteria	1	1
Exclusion criteria	1	1
Comparable demographics	1	1
Number of participating centers is stated?	1	1
The number of surgeons is stated	0	0
Reporting where the authors were on the learning curve	0	0
Clearly stated diagnostic criteria for clinical outcomes	1	1
Adequate description of surgical technique	1	1
Standard surgical technique	1	1
Standard perioperative care	0	0
Age and range are given for patients in BTX-A group	1	1
Statement about any missing data	0	0
Age and range are given for patients in the comparative group	1	1
Patients in each group treated along similar timelines	1	1
The patients asking to enter the study, did they actually take part to it?	0	0
Statement about drop-out rates	0	0
Clearly defined outcomes	1	1
Availability of blind assessors	0	0
Assessment tools were standardized	1	1
Was the analysis by intention to treat?	0	0
Score	12	12

Yes = 1, no (not reported) = 0; Total score = 21, < 8 = poor quality; 8-14 = fair quality and > 14 = good quality. BTX-A: Botulinum toxin type A.

methodology, demographic data of patients, final results, and conclusion clearly were excluded after second thorough revision.

Types of included studies

After reviewing the full text of 11 articles, seven of them[12,17-22] met the eligibility criteria of the review. Two studies were randomized comparative trials[12,20], comparing BTX-A injection with BFB or partial division of the puborectalis muscle. The remaining five trials were observational cohort studies assessing the efficacy and complications of BTX-A injection.

Unfortunately, due to the high degree of heterogeneity among the studies included a formal meta-analysis could not be conducted in this review as two studies were comparative and five were case series studies. In addition, the studies reviewed had different methods for assessment of improvement of anismus and variable follow-up durations.

Assessment of the methodological quality and risk of bias within the studies included

Two reviewers (Emile SH and Elfeki HA) have independently assessed the methodological quality and risk of bias in each study. The revised grading system of the Scottish Intercollegiate Guidelines Network (SIGN)[23] was used to assess comparative studies. The checklist for the quality of case series of the National Institute for Health and Clinical Excellence (NICE)[24] was used for assessment of case series studies. Six of the assessed studies were of fair quality and one study[18] was of good quality (Tables 1 and 2, Figure 2).

Variables of interest

We have extracted the following data from each study: The demographic data of the patients, technique of BTX-A injection and number of sessions, short-term and long-term clinical improvement, post-injection changes in EMG, defecography, manometry, and balloon expulsion test, and complications recorded after BTX-A injection.

The clinical diagnostic criteria of anismus in the studies included were based on the established Rome criteria[25]. The short-term and long-term clinical improvements were defined as the subjective feeling of improvement of symptoms within one month, and four months after injection, respectively.

Statistical analysis

The statistical methods of this study were reviewed by Professor Basem Eldeek, PhD, Mansoura University, Faculty of medicine. Data were extracted from the original articles into fields of Microsoft Excel spreadsheet. SPSS (Statistical Package for Social Science) version 22 under Microsoft Windows was used in the analysis of the collected data. Variables were expressed as median, normal range, and percentage of patients reported in each variable. *P* value less than 0.05 was considered significant.

RESULTS

Characteristics of the studies and the patients

Seven studies met the inclusion criteria of this review and were included. Two studies were retrospective and five were prospective. The median duration of follow-up was 14.6 (range, 6-19.2) mo.

The studies comprised 189 patients who were 108 (57%) female and 81 (43%) male with a female-to-male ratio of 1.3:1. The median age of the patients was 41.2 (range, 23.7-56) years. All patients complained

Table 2 Assessment of the methodological qualities of case-series studies included in the review

Items	Shafik *et al*[17]	Ron *et al*[18]	Maria *et al*[19]	Hompes *et al*[21]	Zhang *et al*[22]
Multi-center study	0	0	0	0	0
Clearly defined objective	1	1	1	1	1
Reported inclusion exclusion criteria	0	1	1	0	0
Clearly defined outcomes	0	1	1	1	1
Prospective data collection	1	1	1	1	1
Patients were recruited consecutively	0	1	0	0	0
Clearly described results of the study?	1	1	1	1	1
Stratified outcomes	1	1	1	1	1
Total Score	4	7	6	5	5

Yes = 1, no (not reported) = 0; Total score, 8; ⩽ 3 = poor quality; 4-6 = fair quality; ⩾ 7 = good quality.

Table 3 Characteristics of the studies included

Ref.	Country	Type	*n*	Male	Mean age (yr)	Duration of complaint (mo)	Follow up (mo)	Dose of BTX-A (IU)	Site of injection
Shafik *et al*[17]	Egypt	Prospective	15	2	41.2	105.6	14.6	25	Lateral (3, 9 o'clock)
Ron *et al*[18]	Israel	Prospective	24	9	23.7	Not reported	61.0	10-20	Lateral and posterior
Maria *et al*[19]	Italy	Prospective	24	10	56.0	28.0	39.0	60	Lateral (3, 9 o'clock)
Farid *et al*[12]	Egypt	Prospective RCT	15	15	34.7	71.1	14.7	100	Lateral (5, 7 o'clock)
Farid *et al*[20]	Egypt	Prospective RCT	24	7	34.7	Not reported	12.0	100	Lateral (5, 7 o'clock)
Hompes *et al*[21]	United Kingdom	Retrospective	56	20	47.5	Not reported	19.2	100	Lateral (3, 9 o'clock)
Zhang *et al*[22]	China	Retrospective	31	18	50.1	67.2	8.4	100	Lateral and posterior (3, 6, 9 o'clock)

RCT: Randomized controlled trial; BTX-A: Botulinum toxin type A.

of symptoms of outlet obstruction constipation for a median duration of 69.1 (range, 28-105.6) mo. The characteristics of each study are shown in Table 3. Only in two studies[17,22] patients completed a course of BFB retraining before they were considered unresponsive and were shifted to BTX-A injection.

Technique of injection

The studies used BTX-A under variable commercial names (Dysport®, Botox® and Allergan®). Injection of BTX-A was performed as a day-case procedure, except in one study[22] where patients were hospitalized after BTX-A injection. The injection was conducted under local anesthesia in one study[21], caudal anesthesia in one study[23], sedation in one study[18], and without anesthesia in four studies[12,17,19,20].

The median dose of BTX-A injected per procedure was 100 IU (range, 20-100 IU). The site of injection varied; five trials employed lateral injection either at 5 and 7 o' clock[12,20], or at 3 and 9 o'clock[17,19,21]. The remaining two trials used a combination of lateral and posterior injections[18,22].

Three studies[18,19,22] used endorectal ultrasonography-guided technique for injection, one study[17] used an EMG-guided technique, whereas the remaining three studies used manual palpation with the index finger.

A single session of BTX-A injection was conducted in four studies, two sessions were conducted in two studies[17,18], and more than two injection sessions were required in one study[19]. Auxiliary pelvic floor rehabilitation (BFB) program was employed after BTX-A injection in one study[22].

Efficacy of BTX-A injection

Clinical improvement: The clinical improvement of symptoms was classified into initial and long-term improvement. The median percentage of patients who reported initial improvement of symptoms was 77.4% (range, 37.5%-86.7%). This percentage declined to a median of 46% (range 25%-100%) at 4 mo after injection of BTX-A (Figure 3). One study[19] that employed repeated injections of BTX-A at two and four months reported long-term improvement in all patients.

Symptom assessment scores were not routinely used as only two studies[12,22] submitted patients to Wexner constipation scale[22] before and after BTX-A injection. The mean Wexner scores dropped from 11.2 and 14.3 before injection to 8.2 and 6.4 after injection, respectively. None of the studies used any of the scores designated for obstructed defecation syndrome.

Improvement according to anorectal manometry: Two studies[18,20] reported post-injection manometric relaxation in 28.5% and 70.8% of patients, respectively. No significant changes in anal pressures after BTX-A injection were observed according to two studies[12,21]. Conversely, two studies[19,22] reported significant decrease in the mean resting and squeeze anal pressures 3 mo

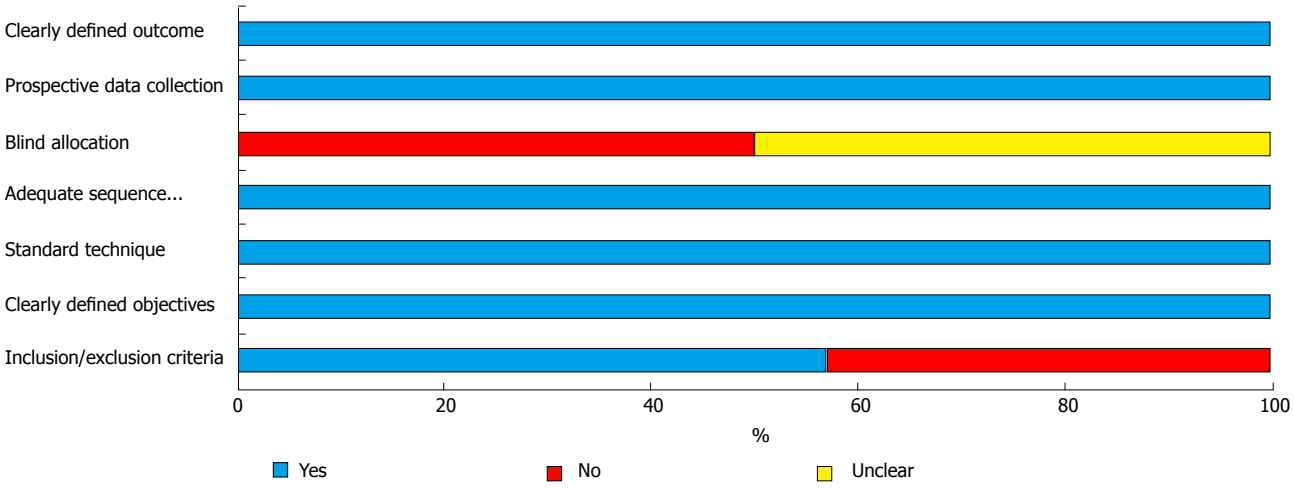

Figure 2 Assessment of the methodological quality of the studies included.

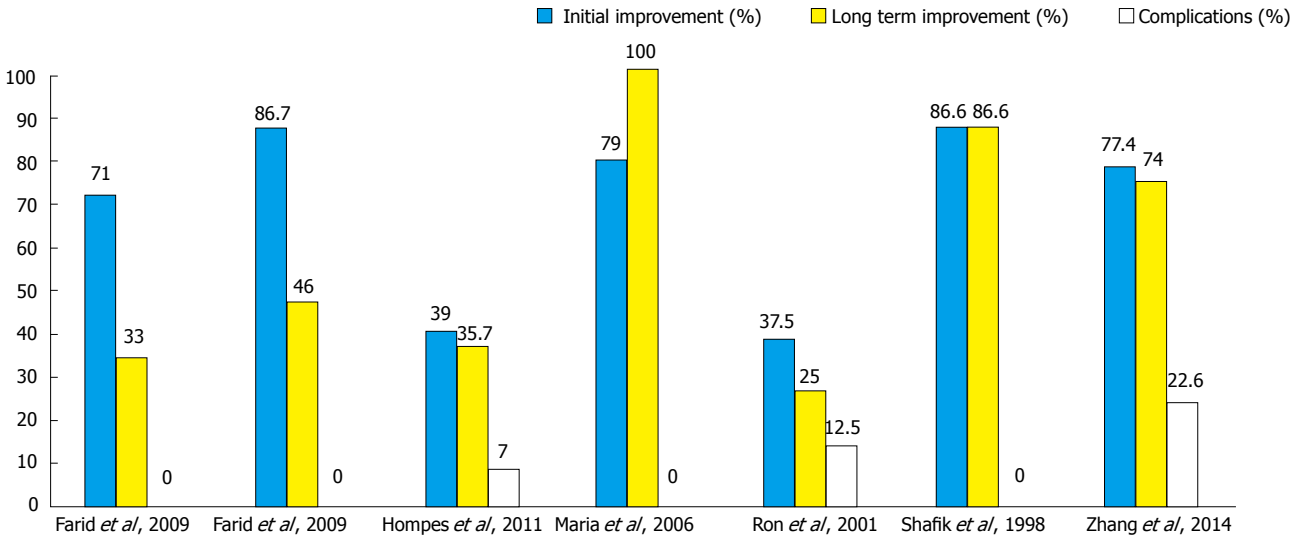

Figure 3 Improvement of symptoms and complications after injection of botulinum toxin type A.

after injection.

Improvement according to balloon expulsion test: Positive balloon expulsion was reported in four studies[12,18,20,22], with a median rate of 74.6% ranging from 37.5%-80%. Ninety-four patients were subjected to balloon expulsion test before and after injection, all of them failed the test before injection and 62 (66%) of them had a positive test within three month after BTX-A injection.

Improvement according to EMG: Based on EMG, three studies[12,17,20] reported post-injection improvement of anismus ranging between 54% and 86.7%. Fifty-four patients were subjected to EMG before and after injection, 39 (72%) of them showed improvement in their post-injection EMG.

Improvement according to defecography: Four studies[12,17,19,20] reported improvement of 25%-86.6% of patients in the post-injection defecogram. Seventy-eight patients underwent defecography before and after injection, 50 (64%) of them showed resolution of signs of anismus in the post-injection defecogram. One study[19] evaluated the anorectal angle (ARA) before and after injection, and reported a significant increase of the ARA during straining from 97° ± 11° to 127° ± 10° at two months after injection.

Complications

Fourteen (7.4%) patients developed complications after the injection of BTX-A. The median rate of complications across the studies was zero ranging from 0%-22.6% (Figure 3). Eleven (5.8%) patients developed minor transient fecal incontinence (FI) as reported by two studies[21,22]. Two patients developed acute posterior anal fissure[18], and one patient developed complete rectal prolapse[18].

DISCUSSION

Anismus is a complex functional disorder with unclear pathophysiology and elusive diagnosis rendering it a difficult condition to treat. Conservative measures for treatment of constipation usually fail to provide any significant improvement to the patients with anismus. BFB retraining, surgical division of puborectalis muscle, and BTX-A injection are the main options for treatment of anismus that were described in literature. Nevertheless, the optimal treatment of anismus is still debatable.

BFB retraining depends on the concept of operant conditioning. During BFB patients learn how to control an unconscious physiologic function with the aid of an instrument that provides visual, auditory or verbal feedback of an action that can be reinforced until a satisfactory response is accomplished[26,27].

Since the first report[11] that concluded the utility of BFB in pelvic floor disorders, several other studies tried to evaluate the efficacy of BFB in the treatment of anismus. Gilliland *et al*[28] reported a success rate of 63% in patients who completed their training programs. Similarly, Rhee *et al*[29] reported that about 69% of anismus patients showed complete response on completion of their BFB training program. However, most of these studies were small non-controlled series with short follow-up durations.

A meta-analysis of randomized controlled trials[30] evaluating BFB in the treatment of pelvic floor disorders concluded that symptomatic relief of anismus after BFB was six fold that obtained with other methods. Moreover, clinical improvement of symptoms after EMG-BFB was seven times higher than that after non-EMG BFB.

Surgical miotomy of the puborectalis muscle was described since 1960s with initial satisfactory results[1,31]. However, subsequent trials reported disappointing outcomes and unacceptably high rates of FI following division of the puborectalis muscle[32,33]. A recent pilot study[34] devised a modified semi-closed technique in dividing puborectalis muscle stating that symptomatic improvement occurred in 75% of patients without encountering any significant postoperative complications. Nonetheless, the authors recommended conducting more studies before considering this technique a validated procedure.

Hallan *et al*[35] described direct injection of BTX-A into the puborectalis muscle. BTX-A is a potent neurotoxin that causes muscle paralysis by inhibition of release of acetylcholine at the presynaptic region[13,14]. Injection of BTX-A emerged as a promising option in the treatment of anismus with the advantages of being less costly and technically easier than BFB retraining[19]. BTX-A injection, unlike BFB, does not depend on patient's cooperation and compliance which are merely subjective.

As the effect of BTX-A is temporary for around three months after administration, BTX-A injection therapy was considered successful in terms of short-term symptomatic improvement of anismus. Longer term improvement necessitates repeated injections in order to maintain the achieved clinical improvement[18].

The objective of the current review was to assess the efficacy and safety of BTX-A injection in the management of anismus. Only seven studies were eligible to be included which reflects the paucity of trials in this regard. Patients were mostly middle-aged females coping with the literature[4]. Most of the studies used BTX-A injection as a primary treatment except two studies[18,23] that resorted to BTX-A after failure of BFB therapy.

Despite the availability of designated scores for obstructed defecation[36-38], none of the studies reviewed employed any of these scores to assess patients with anismus. Instead, two studies used Wexner constipation score which is not specific for obstructed defecation syndrome. The clinical utility of the obstructed defecation scores in anismus remains debatable and needs further studies to be ascertained.

Some studies used endorectal ultrasonography- or EMG-guided techniques for BTX-A injection, yet none obtained superior results compared to the studies that used manual guidance, concluding no clear benefits for the guided techniques. Although Zhang *et al*[22] found ultrasonography-guided injection simplified the localization of the injection site which led to a long-term improvement rate of 74%; the adjuvant BFB course they have applied to the patients after BTX-A injection could have contributed to this good outcome, rather than the guided technique of injection.

Only two studies[18,22] used combined lateral and posterior injections technique which was associated with higher complication rates with almost the same efficacy obtained by lateral injection alone. We can explain this phenomenon that posterior injection potentially affects part of EAS at the anorectal ring, subsequently this will lead to weakening of the sphincter complex and development of FI. While the site of injection played an important role in the development of complications, the dose of BTX-A did not have any special significance since the studies that used the least dose[17,18] reported conflicting results with an efficacy close to that of higher doses.

The median rate of initial improvement of symptoms after injection was 77.4% reaching up to 86%. Unfortunately, these initial good results did not last longer as they dropped to a median of 46% after three months necessitating repeated injections of BTX-A in three studies. The studies that reported satisfactory long- term results had to repeat the injection twice or more. The reason why repeated injections attained better long-term results can be attributed to the cumulative effect of BTX-A on the puborectalis muscle. Interestingly, we found that the repeated injections do not necessarily induce higher complication rates, therefore repeated BTX-A injection can potentially provide sustained improvement in cases where BFB fails and surgical miotomy is contraindicated or refused by the patient.

Improvement of anismus as assessed by the physiologic tests was variable and rather confounding.

Anorectal manometry reported a decrease in anal pressures in two studies[19,21]. Conversely, the remaining studies showed no significant change in the anal pressures, although clinical improvement was evident. The rate of improvement of anismus evaluated by balloon expulsion test, EMG, and defecography ranged between (37.5%-80%), (54%-86.7%), and (25%-86.6%), respectively. Interestingly, the highest rates of improvement according to clinical examination, EMG, and defecography were the same (86%) implying the harmony of these tests with the clinical examination.

Complications after BTX-A injection were detected in 7.4% of patients. The most common complication was FI which was only transient, for two weeks, and of a minor grade. FI was reported in two studies[18,22], both applied combined lateral and posterior injections. Other morbidities as posterior anal fissure and complete rectal prolapse were observed only by one study[18] that also used posterior injection in addition to lateral injection, hence demonstrating the negative impact of posterior injection that induces further weakness to the sphincter muscles.

In summary, BTX-A injection has distinct advantages as technical feasibility, lack of need for general or spinal anesthesia, being an outpatient procedure, and excellent initial results. On the other hand, BTX-A injection proved to be a temporary short-term solution with disappointing outcome on the long term. However, longer term results can be improved further by repeated injections, although satisfactory results are still not guaranteed.

Limitations

The heterogeneity of the studies included was a major limitation during the analysis and interpretation of their results, thus, a meta-analysis could not be conducted. Another limitation was the lack of data of some investigations that were not reported by some studies. In addition, most of the studies were observational with low grade of evidence; only two studies were randomized controlled trials which may influence the final outcome of the review.

Conclusions

The injection of BTX-A is a simple, technically feasible outpatient procedure. The initial satisfactory improvement of symptoms after BTX-A injection remarkably deteriorated after three months of the procedure. However, repeated injections may provide better sustained results with no additional morbidities.

The endorectal ultrasonography- and EMG-guided techniques did not add significant value regarding both initial and long-term improvement. Combined lateral and posterior injections technique did not achieve better results than lateral injection alone, on the contrary the studies that employed the combined injections technique reported higher complication rates. Overall, further analysis of more patients is necessary to conclude the safety of BTX-A in the treatment of anismus.

Recommendations

The present review suggests that injection of BTX-A in the puborectalis muscle is an effective short-term method for treatment of anismus, hence in case of deterioration of the initial satisfactory amelioration of clinical symptoms we recommend further sessions of BTX-A injection in order to maintain the clinical improvement.

From the results we obtained, we don't recommend combined lateral and posterior injections since this technique can result in higher complication rates, yet with no substantial benefits.

COMMENTS

Background

Anismus is considered one of the most important causes of obstructed defecation syndrome. The precise diagnosis and management of anismus have been a challenging problem for surgeons. While biofeedback (BFB) confers excellent results in many patients; some patients fail to respond to BFB, hence alternative methods for treatment are indicated. The injection of botulinum toxin type A (BTX-A) in the puborectalis muscle provided satisfactory short-term results, yet these good results tend to deteriorate with time. The aim of this review was to determine the overall efficacy and safety of BTX-A in treatment of anismus

Research frontiers

BTX-A has various indications in surgery as cervical dystonia, severe axillary hyperhidrosis, strabismus, and upper limb spasticity. Earlier attempts of using BTX-A for treatment of anismus date back to the nineties. BTX-A prevents the release of acetylcholine by binding to glycoprotein structures on the cholinergic nerve terminals, inducing neuromuscular blockage.

Innovations and breakthroughs

A number of trials have used BTX-A for treating anismus and pelvic floor dyssynergia using different approaches and dosage of BTX-A. Some authors used endorectal ultrasonography and EMG as a guide for the injection process. While some authors used lateral injection method; others tried combined lateral and posterior injections. The studies evaluating the efficacy and safety of BTX-A were reviewed by the authors and the data were extracted using a standardized collection tool.

Applications

This review suggests that BTX-A can be an effective method for treatment of anismus; however, the remarkable deterioration of symptom improvement may necessitate injection of further doses of BTA-X within an interval of 3-6 mo after the first injection.

Terminology

BTX-A stands for botulinum toxin type A, EMG stands for electromyography, and BFB stands for biofeedback.

Peer-review

This is a short review about the botulinum toxin treatment for patients with anismus. This review is well written.

REFERENCES

1 **Wasserman IF**. Puborectalis syndrome (rectal stenosis due to anorectal spasm). *Dis Colon Rectum* 1964; **7**: 87-98 [PMID: 14128302 DOI: 10.1007/BF02616902]

2 **Preston DM**, Lennard-Jones JE. Anismus in chronic constipation. *Dig Dis Sci* 1985; **30**: 413-418 [PMID: 3987474 DOI: 10.1007/BF01318172]

3 **Park UC**, Choi SK, Piccirillo MF, Verzaro R, Wexner SD. Patterns of anismus and the relation to biofeedback therapy. *Dis Colon Rectum* 1996; **39**: 768-773 [PMID: 8674369 DOI: 10.1007/BF02054442]

4 **Rao SS**, Tuteja AK, Vellema T, Kempf J, Stessman M. Dyssynergic defecation: demographics, symptoms, stool patterns, and quality of life. *J Clin Gastroenterol* 2004; **38**: 680-685 [PMID: 15319652 DOI: 10.1097/01.mcg.0000135929.78074.8c]

5 **Gosselink MJ**, Schouten WR. Rectal sensory perception in females with obstructed defecation. *Dis Colon Rectum* 2001; **44**: 1337-1344 [PMID: 11584213 DOI: 10.1007/BF02235485]

6 **Shelton AA**, Welton ML. The pelvic floor in health and disease. *West J Med* 1997; **167**: 90-98 [PMID: 9291746]

7 **Kuijpers HC**, Bleijenberg G. The spastic pelvic floor syndrome. A cause of constipation. *Dis Colon Rectum* 1985; **28**: 669-672 [PMID: 4053909 DOI: 10.1007/BF02553449]

8 **Weber J**, Ducrotte P, Touchais JY, Roussignol C, Denis P. Biofeedback training for constipation in adults and children. *Dis Colon Rectum* 1987; **30**: 844-846 [PMID: 3677958]

9 **Johansson C**, Nilsson BY, Mellgren A, Dolk A, Holmström B. Paradoxical sphincter reaction and associated colorectal disorders. *Int J Colorectal Dis* 1992; **7**: 89-94 [PMID: 1613301 DOI: 10.1007/BF00341293]

10 **Kuijpers HC**, Bleijenberg G, de Morree H. The spastic pelvic floor syndrome. Large bowel outlet obstruction caused by pelvic floor dysfunction: a radiological study. *Int J Colorectal Dis* 1986; **1**: 44-48 [PMID: 3598314 DOI: 10.1007/BF01648836]

11 **Bleijenberg G**, Kuijpers HC. Treatment of the spastic pelvic floor syndrome with biofeedback. *Dis Colon Rectum* 1987; **30**: 108-111 [PMID: 3803114]

12 **Farid M**, Youssef T, Mahdy T, Omar W, Moneim HA, El Nakeeb A, Youssef M. Comparative study between botulinum toxin injection and partial division of puborectalis for treating anismus. *Int J Colorectal Dis* 2009; **24**: 327-334 [PMID: 19039596 DOI: 10.1007/s00384-008-0609-7]

13 **Dressler D**, Saberi FA, Barbosa ER. Botulinum toxin: mechanisms of action. *Arq Neuropsiquiatr* 2005; **63**: 180-185 [PMID: 15830090 DOI: 10.1590/S0004-282X2005000100035]

14 **Jankovic J**, Brin MF. Therapeutic uses of botulinum toxin. *N Engl J Med* 1991; **324**: 1186-1194 [PMID: 2011163]

15 **Maria G**, Brisinda G, Bentivoglio AR, Cassetta E, Albanese A. Botulinum toxin in the treatment of outlet obstruction constipation caused by puborectalis syndrome. *Dis Colon Rectum* 2000; **43**: 376-380 [PMID: 10733120 DOI: 10.1007/BF02258305]

16 **Liberati A**, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, Clarke M, Devereaux PJ, Kleijnen J, Moher D. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ* 2009; **339**: b2700 [PMID: 19622552 DOI: 10.1136/bmj.b2700]

17 **Shafik A**, El-Sibai O. Botulin toxin in the treatment of nonrelaxing puborectalis syndrome. *Dig Surg* 1998; **15**: 347-351 [PMID: 9845612 DOI: 10.1159/000018630]

18 **Ron Y**, Avni Y, Lukovetski A, Wardi J, Geva D, Birkenfeld S, Halpern Z. Botulinum toxin type-A in therapy of patients with anismus. *Dis Colon Rectum* 2001; **44**: 1821-1826 [PMID: 11742168 DOI: 10.1007/BF02234461]

19 **Maria G**, Cadeddu F, Brandara F, Marniga G, Brisinda G. Experience with type A botulinum toxin for treatment of outlet-type constipation. *Am J Gastroenterol* 2006; **101**: 2570-2575 [PMID: 17029615 DOI: 10.1111/j.1572-0241.2006.00791.x]

20 **Farid M**, El Monem HA, Omar W, El Nakeeb A, Fikry A, Youssef T, Yousef M, Ghazy H, Fouda E, El Metwally T, Khafagy W, Ahmed S, El Awady S, Morshed M, El Lithy R. Comparative study between biofeedback retraining and botulinum neurotoxin in the treatment of anismus patients. *Int J Colorectal Dis* 2009; **24**: 115-120 [PMID: 18719924 DOI: 10.1007/s00384-008-0567-0]

21 **Hompes R**, Harmston C, Wijffels N, Jones OM, Cunningham C, Lindsey I. Excellent response rate of anismus to botulinum toxin if rectal prolapse misdiagnosed as anismus ('pseudoanismus') is excluded. *Colorectal Dis* 2012; **14**: 224-230 [PMID: 21689279 DOI: 10.1111/j.1463-1318.2011.02561.x]

22 **Zhang Y**, Wang ZN, He L, Gao G, Zhai Q, Yin ZT, Zeng XD. Botulinum toxin type-A injection to treat patients with intractable anismus unresponsive to simple biofeedback training. *World J Gastroenterol* 2014; **20**: 12602-12607 [PMID: 25253964 DOI: 10.3748/wjg.v20.i35.12602]

23 **Baird AG**, Lawrence JR. Guidelines: is bigger better? A review of SIGN guidelines. *BMJ Open* 2014; **4**: e004278 [PMID: 24500613 DOI: 10.1136/bmjopen-2013-004278]

24 **National Institute for Health and Clinical Excellence**. NICE clinical guidelines, Appendix 4 Quality of case series form. Available from: URL: http://www.nice.org.uk/nicemedia/pdf/Appendix_04_qualityofcase_series_form_preop.pdf

25 **Rome Foundation**. Rome III Diagnostic Questionnaires. Constipation Module. [accessed 2016 Jan 30]. Available from: URL: http://www.romecriteria.org/pdfs/ConstMode.pdf

26 **Agachan F**, Chen T, Pfeifer J, Reissman P, Wexner SD. A constipation scoring system to simplify evaluation and management of constipated patients. *Dis Colon Rectum* 1996; **39**: 681-685 [PMID: 8646957 DOI: 10.1007/BF02056950]

27 **Chiarioni G**, Heymen S, Whitehead WE. Biofeedback therapy for dyssynergic defecation. *World J Gastroenterol* 2006; **12**: 7069-7074 [PMID: 17131466 DOI: 10.3748/wjg.v12.i44.7069]

28 **Gilliland R**, Heymen S, Altomare DF, Park UC, Vickers D, Wexner SD. Outcome and predictors of success of biofeedback for constipation. *Br J Surg* 1997; **84**: 1123-1126 [PMID: 9278659 DOI: 10.1002/bjs.1800840825]

29 **Rhee PL**, Choi MS, Kim YH, Son HJ, Kim JJ, Koh KC, Paik SW, Rhee JC, Choi KW. An increased rectal maximum tolerable volume and long anal canal are associated with poor short-term response to biofeedback therapy for patients with anismus with decreased bowel frequency and normal colonic transit time. *Dis Colon Rectum* 2000; **43**: 1405-1411 [PMID: 11052518 DOI: 10.1007/BF02236637]

30 **Koh CE**, Young CJ, Young JM, Solomon MJ. Systematic review of randomized controlled trials of the effectiveness of biofeedback for pelvic floor dysfunction. *Br J Surg* 2008; **95**: 1079-1087 [PMID: 18655219 DOI: 10.1002/bjs.6303]

31 **Wallace WC**, Madden WM. Experience with partial resection of the puborectalis muscle. *Dis Colon Rectum* 1969; **12**: 196-200 [PMID: 5767850 DOI: 10.1007/BF02617812]

32 **Barnes PR**, Hawley PR, Preston DM, Lennard-Jones JE. Experience of posterior division of the puborectalis muscle in the management of chronic constipation. *Br J Surg* 1985; **72**: 475-477 [PMID: 4016517 DOI: 10.1002/bjs.1800720623]

33 **Kamm MA**, Hawley PR, Lennard-Jones JE. Lateral division of the puborectalis muscle in the management of severe constipation. *Br J Surg* 1988; **75**: 661-663 [PMID: 3416122 DOI: 10.1002/bjs.1800750713]

34 **Asciore L**, Pescatori LC, Pescatori M. Semi-closed bilateral partial miotomy of the puborectalis for anismus: a pilot study: Partial miotomy of the puborectalis for anismus. *Int J Colorectal Dis* 2015; **30**: 1729-1734 [PMID: 26255256 DOI: 10.1007/s00384-015-2330-7]

35 **Hallan RI**, Williams NS, Melling J, Waldron DJ, Womack NR, Morrison JF. Treatment of anismus in intractable constipation with botulinum A toxin. *Lancet* 1988; **2**: 714-717 [PMID: 2901570 DOI: 10.1016/S0140-6736(88)90188-2]

36 **Altomare DF**, Spazzafumo L, Rinaldi M, Dodi G, Ghiselli R, Piloni V. Set-up and statistical validation of a new scoring system for obstructed defaecation syndrome. *Colorectal Dis* 2008; **10**: 84-88 [PMID: 17441968]

37 **Renzi A**, Brillantino A, Di Sarno G, d'Aniello F. Five-item score for obstructed defecation syndrome: study of validation. *Surg Innov* 2013; **20**: 119-125 [PMID: 22599920 DOI: 10.1177/1553350612446354]

38 **Emile S**, Youssef M, Thabet W, Omar W, Abd El-Hamed TM,

Elshobaky A, El Nakeeb A, Magdy A, El-Yamani F, Farid M. Mansoura Numeroalphabetic Constipation Score in Obstructed Defecation Syndrome (ODS): Validation of a New Score. *IJAR* 2015; **3**: 264-275

P- Reviewer: Amornyotin S, Onda M, Soriano-Ursua MA
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i3.463

World J Gastrointest Pharmacol Ther 2016 August 6; 7(3): 463-468
ISSN 2150-5349 (online)

CASE REPORT

Response of irritable bowel syndrome with constipation patients administered a combined quebracho/conker tree/ *M. balsamea Willd* extract

Kenneth Brown, Brandi Scott-Hoy, Linda W Jennings

Kenneth Brown, Brandi Scott-Hoy, KBS Research LLC, Dallas, TX 75248, United States

Linda W Jennings, Annette C and Harold C Simmons Transplant Institute, Baylor Scott and White Health, Dallas, TX 75246, United States

Author contributions: Brown K and Scott-Hoy B contributed equally to this work; Brown K and Scott-Hoy B substantially contributed to conception, design and interpretation of the case series data, critical revisions of the manuscript, and final approval of the manuscript; Jennings LW contributed substantially to the analysis and interpretation of the case series data, critical revision and important intellectual concepts in the manuscript and final approval of the manuscript.

Institutional review board statement: The study is exempt from IRB review and oversight pursuant to the terms of the United States Department of Health and Human Service's Policy for Protection of Human Research Subjects at 45 CFR and 46.101(b) since the data already exists in patient medical charts and this data was accumulated retrospectively. There was no experimentation on patients. The botanical extract was recommended to patients who chose to take the formulation.

Informed consent statement: All patients gave their verbal consent for publication of their anonymous medical chart data.

Conflict-of-interest statement: No potential conflicts of interest relevant to this article were reported.

Correspondence to: Brandi Scott-Hoy, MD, KBS Research LLC, 15660 N Dallas Parkway, Suite 700, Dallas, TX 75248, United States. brandi@kbsresearch.com
Telephone: +1-214-9843724

Received: February 23, 2016
Peer-review started: February 23, 2016
First decision: March 30, 2016
Revised: May 8, 2016
Accepted: June 14, 2016
Article in press: June 16, 2016
Published online: August 6, 2016

Abstract

The aim of this case series was to retrospectively examine the symptom response of irritable bowel syndrome with constipation (IBS-C) patients administered an herbal extract in a real-world setting. Twenty-four IBS-C patients in a community office practice were provided a combination over-the-counter dietary supplement composed of quebracho (150 mg), conker tree (470 mg) and *M. balsamea Willd* (0.2 mL) extracts (Atrantil™) and chose to take the formulation for a minimum of 2 wk in an attempt to manage their symptoms. Patient responses to the supplement were assessed by visual analogue scale (VAS) for abdominal pain, constipation and bloating at baseline and at 2 wk as part of standard-of-care. Patient scores from VAS assessments recorded in medical chart data were retrospectively compiled and assessed for the effects of the combined extract on symptoms. Sign tests were used to compare changes from baseline to 2 wk of taking the extract. Significance was defined as $P < 0.05$. Twenty-one of 24 patients (88%) responded to the dietary supplement as measured by individual improvements in VAS scores for abdominal pain, bloating and constipation symptoms comparing scores prior to administration of the extract against those reported after 2 wk. There were also significant improvements in individual as well as mean VAS scores after 2 wk of administration of the combined

extract compared to baseline for abdominal pain [8.0 (6.5, 9.0) *vs* 2.0 (1.0, 3.0), $P < 0.001$], bloating [8.0 (7.0, 9.0) *vs* 1.0 (1.0, 2.0), $P < 0.001$] and constipation [6.0 (3.0, 8.0) *vs* 2.0 (1.0, 3.0), $P < 0.001$], respectively. In addition, 21 of 24 patients expressed improved quality of life while taking the formulation. There were no reported side effects to administration of the dietary supplement in this practice population suggesting excellent tolerance of the formulation. This pilot retrospective analysis of symptom scores from patients before and after consuming a quebracho/conker tree/*M. balsamea Willd* extract may support the formulation's use in IBS-C.

Key words: Irritable bowel syndrome; Constipation; Abdominal pain; Bloating; Dietary supplement; Herbal; Botanical; Extract; Peppermint

Core tip: Irritable bowel syndrome with constipation (IBS-C) is a diagnosis by exclusion which is defined by abdominal pain accompanied by reduced stool frequency and painful, hard bowel movements. Gas and bloating may also be present in many patients with this condition suggesting a role in fermentation of food producing gas by bacteria in the gut. Safe tannin byproducts from wineries used in cows to reduce gas that can impair milk and meat production are combined with saponins, shown to be antibacterial and promote intestinal motility, and peppermint oil for abdominal pain in this combination extract (Atrantil™) to manage key IBS-C symptoms.

Brown K, Scott-Hoy B, Jennings LW. Response of irritable bowel syndrome with constipation patients administered a combined quebracho/conker tree/*M. balsamea Willd* extract. *World J Gastrointest Pharmacol Ther* 2016; 7(3): 463-468 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i3/463.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i3.463

INTRODUCTION

One third of diagnosed irritable bowel syndrome (IBS) in the United States is constipation predominant and includes symptoms of abdominal pain, bloating, and constipation[1]. Women experience IBS symptoms about twice as frequently as men[2]. Irritable bowel syndrome with constipation (IBS-C) has a huge impact on quality of life and productivity especially in women[3] with one investigator suggesting that IBS patients have worse health-related quality of life compared to patients with diabetes and end-stage renal disease[4]. The symptom-driven quality of life altering condition can be due to the production of gas (hydrogen, methane) which causes bloating and contributes to alterations in motility in IBS-C patients. Gas production has been linked to the presence of methanogenic archaebacteria[5,6]. Methane production has been found to be associated with delayed transit time[7,8]. Individuals diagnosed with small intestinal bacterial overgrowth (SIBO) also produce more hydrogen and methane which can lead to abdominal pain and constipation[9]. Fiber supplements[10] and probiotics[11] as well as drugs like rifaximin, neomycin[12], laxatives[13,14], lubiprostone[15], and linaclotide[16] all have variable effects in patients with IBS-C. There is still a need for safe agents to support GI health in patients with IBS-C.

Atrantil™, a dietary supplement composed of Quebracho, Conker Tree and *M. balsamea Willd* extracts, has been shown against placebo to statistically improve constipation and bloating in IBS-C subjects[17]. Quebracho extract contains tannins which are large delocalized flavonoid structures that have been used safely in wine for decades[18]. Tannins potentially have dual function[19]: They act as molecular "sponges" for excess hydrogen and methane[20] as well as disrupt and destroy bacterial lipid bilayers. Conker tree extract contains escins, also known as saponins. Saponins act as an antimicrobial agents, promote intestinal motility[21] and directly reduce methane production/emission[22,23]. *M. balsamea Willd* extract contains peppermint oil which has been shown to reduce abdominal pain and discomfort[11].

Patients from a single, community physician practice, who had failed to respond to conventional therapy, chose to take a recommended over-the-counter dietary supplement composed of quebracho, conker tree and *M. balsamea Willd* extracts in attempt to manage symptoms of abdominal pain, bloating and constipation associated with IBS-C. Their medical chart responses were retrospectively analyzed for improvement in symptomology.

CASE REPORT

Patient charts were retrospectively examined from a single physician's practice in this case report of 24 IBS-C patients who took the dietary supplement, Atrantil™ [quebracho (150 mg), conker tree (470 mg) and *M. balsamea Willd* (0.2 mL) extracts], after experiencing incomplete management of symptoms with other therapies. The quebracho extract has a 80%-82% polyphenol content with 72%-74% soluble tannins, primarily consisting by of profisetinidin subunits as part of trimeric, tetrameric and pentameric condensed tannins (about 75%) determined by MALDI-TOF and ^{1}H- and ^{13}C-NMR fingerprint analysis. The Conker Tree extract is standardized to 20% saponin content by UV-Visible spectrophotometry and high performance thin layer chromatography (HPTLC) densitometry. Finally, pure peppermint oil content from *M. balsamea Willd* was determined by specific gravity, angular rotation and refractive index (USP29).

No IRB review or oversight was required in this analysis according to the terms of the United States Department of Health and Human Service's Policy for Protection of Human Research Subjects at 45 CFR and 46.101(b) since the data already existed in patient medical charts and this data was accumulated retrospectively. There was

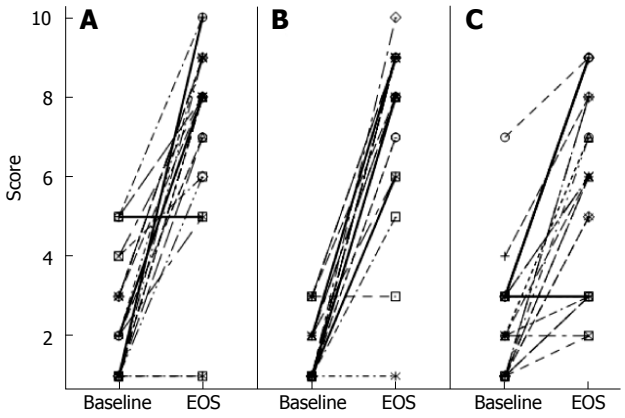

Figure 1 Visual Analogue Scores (0 = worst symptoms, 10 = no symptoms) were taken prior to administration of the dietary supplement (baseline) and at 2 wk end of analysis (end of analysis) for abdominal pain (A), bloating (B), and constipation (C). Each symbol represents a different, individual patient in the analysis.

no experimentation on patients. The dietary supplement was recommended to patients who chose to take the formulation after failing to respond to other treatments. The patients in this analysis were diagnosis with IBS-C for at least 6 mo prior to enrollment into the study (according to Rome Ⅲ criteria) and had a history of uncontrolled symptoms of abdominal pain, bloating and constipation. Patients were previously on the FODMAP diet, probiotics and/or traditional drug treatments. The combined extract was administered for two weeks. Patient response to the combined extract was assessed by visual analogue scale (VAS) at baseline (before administration) and after 2 wk [End of Analysis (EOA)] for abdominal pain, bloating, and constipation as part of standard-of-care. The median and the 25th and 75th percentiles, the interquartile range (IQR), were used to summarize the scores. Sign tests, which make no assumptions about the shape of the distribution, were used to compare changes over time. Significance was defined as $P < 0.05$. Changes in therapy for rescue due to increased symptoms and side effects were also noted. All patients consented to have their data published.

The patients in this retrospective chart analysis (n = 24) ranged in age from 18 to 58. The population consisted of 2 men and 22 women with a racial composition of 21 Caucasian, 2 Hispanic or Latino, as well as 1 African American. There were also various co-morbidities of gastroesophageal reflux disease, rosacea, hypertension and fatigue, which did not contribute to their gastrointestinal condition. Patients were not taking any other therapies for IBS-C or SIBO when they were first administered the combined extract. By EOA, 21 out of 24 patients had responded with improved VAS scores for abdominal pain (Figure 1A), bloating (Figure 1B), and constipation (Figure 1C).

Overall, 88% of this gastroenterology practice population which had incomplete relief with traditional therapies responded to the combined herbal extract in the dietary supplement. A comparison of mean VAS scores for abdominal pain, bloating and constipation from baseline and EOA showed a significant improvement in all three symptoms over time for the entire population while on the combined extract (Table 1).

Table 1 Symptom response of irritable bowel syndrome with constipation patient population to combined herbal extract

Symptom	Baseline median (IQR)	EOA median (IQR)	EOA-baseline median (IQR)	*P* value
Abdominal pain	2.0 (1.0, 3.0)	8.0 (6.5, 9.0)	5.5 (3.5, 7.0)	< 0.001
Bloating	1.0 (1.0, 2.0)	8.0 (7.0, 9.0)	6.5 (5.5, 8.0)	< 0.001
Constipation	2.0 (1.0, 3.0)	6.0 (3.0, 8.0)	4.0 (2.0, 5.5)	< 0.001

EOA: End of Analysis (2 wk); IQR: Interquartile range (25%, 75%).

A response rate of 88% in IBS-C patients with a significant reduction in abdominal pain, bloating, and constipation suggests very good efficacy in this difficult to treat population. No rescue medication was needed during the 2 wk course of the observation and there were no reported adverse events suggesting excellent tolerance of the herbal extract.

DISCUSSION

Over 90% of IBS patients suffer from bloating which is directly linked to abdominal pain and distention[24]. These symptoms may be caused by SIBO or dysbiosis. No matter the cause, current therapeutics may not meet the needs of all patients. In a 10 wk study of rifaximin (550 mg TID) *vs* placebo in IBS patients, for example, the overall response rate was 40.8% *vs* 31.2% for placebo (P = 0.01)[25]. Using a similar retrospective medical chart analysis to the one utilized in this study, Yang *et al*[26] found a 69% response rate to rifaximin and 44% to neomycin in 98 lactulose breath test positive IBS patients. Another study found that patients who had an abnormal lactulose breath test with follow up testing (n = 47) when treated with neomycin had a 75% response rate[9]. Even with the success of antibiotic treatment, relapse remains a significant problem in SIBO patients[27].

Other agents are also used for constipated patients. In an open-label extension study of lubiprostone (n = 522), a locally acting chloride channel activator, demonstrated a response rate of about 40%, but about 32% of participants in the extension part of the study required a rescue medication[28]. Adverse effects for lubiprostone include dose-related nausea and dyspnea with chest tightness. For idiopathic constipation, lanaclotide demonstrated about 50% response rate for pain and increase in stool frequency compared to placebo responses of about 35% and about 25%, respectively[29,30]. About 20% of patients on linaclotide experienced diarrhea compared to about 3% in the placebo groups.

Nutritional approaches to IBS-C and SIBO include dietary fiber, the FODMAP (Fermentable oligosaccharides, disaccharides, monosaccharides and polyols) diet

and probiotics. Fiber can be effective in managing constipation, but bloating, distension, flatulence and cramping may limit the use of insoluble fiber. Water intake with fiber is very important. In patients with IBS, soluble fiber, such as psyllium may be effective, but insoluble fiber can exacerbate symptoms[10,31]. The FODMAP diet has been found to decrease abdominal pain and bloating, but adherence to the diet can be difficult[32]. Probiotics containing *Bifidobacterium lactis* DN-173 010, *Lactobacillus casei* Shirota, and *Escherichia coli* Nissle 1917 demonstrate favorable data on defecation frequency and stool consistency[33]. Other approaches are still needed for patients with IBS-C and SIBO.

In a 2 wk randomized, double-blind placebo-controlled study of patients previously diagnosed with IBS-C (n = 16), there were significant improvements in the average constipation (P = 0.0034), bloating (P < 0.001) and constipation plus bloating scores (P < 0.001) in the Atrantil™ group compared to no improvement for the placebo arm[17]. There were also no reports of AEs over the 2 wk period. In this retrospective chart analysis of 24 patients administered Atrantil™, there was a 3.2-fold average improvement in abdominal pain, a 5.1-fold improvement in bloating, and a 2.7-fold improvement in constipation. Twenty-one of 24 patients responded to therapy for an overall response rate of 88%. There were also no reported side effects to therapy. These consistent data suggest that the combined herbal extracts of quebracho, conker tree and *M. balsamea Willd* present in Atrantil™ decreases symptoms associated with IBS-C.

The quebracho extract consists primarily of tannins, the same used in for over 50 years to change the taste and texture of wine[18]. Tannins are highly delocalized structures which are able to act as antiradical sinks or antioxidants[34]. Tannins also directly limit methanogenesis by inhibiting the growth of methane producing bacteria by reducing the availability of hydrogen[35]. Conker Tree extract contains the antimicrobial saponins[21] which can also reduce the production as well as emission of methane presumably by limiting hydrogen availability[22,23]. Saponins have also been found to improve intestinal motility in mice[36] and improve passage of gas, GI sounds and bowel movements in postoperative colorectal surgery patients[37]. The *M. balsamea Willd* extract contains peppermint oil which has been found to help reduce abdominal pain[11]. Peppermint oil has also been shown to act as an antispasmodic attenuating contractile responses to acetylcholine, histamine, 5-hydroxytryptamine, and substance P[38,39]. The combination of these extracts in Atrantil™ may have limited the availability of hydrogen by preventing growth of microorganisms which produce methane that contributes to abdominal pain, bloating and constipationin this IBS-C patient population. In addition, the combination extracts may also improve motility and intestinal transit time.

Though this pilot medical chart analysis was performed in a relatively small number of patients (n = 24) with IBS-C, the response rate was very high (88%). The small number of patients, the fact that they were drawn from a single site and the uncontrolled nature of the analysis with only therapy adherent individuals being evaluated are limitations for this study. Still, the statistical improvement in symptoms of abdominal pain, bloating and constipation found in this retrospective study are consistent with a previous placebo-controlled clinical trial[17]. Therefore, the results of this small open-label study of Atrantil™ may be a useful intervention for patients with IBS-C and SIBO. Further, larger double-blind, placebo-controlled studies are needed to confirm these results.

COMMENTS

Case characteristics

The primary symptoms experienced by this clinical practice cohort of patients were abdominal pain, bloating and constipation.

Clinical diagnosis

Significant improvements in abdominal pain, bloating and constipation were found after a 2 wk administration of the mixed quebracho/conker Tree/*M. balsamea Willd* extracts in Atrantil™ in irritable bowel syndrome with constipation (IBS-C) patients.

Differential diagnosis

Organic causes of constipation were excluded first for all patients in this practice cohort which were then diagnosed with IBS-C according to Rome III criteria for functional constipation including at least two of the following: (1) two or fewer defecations in the toilet per week; (2) At least one episode of fecal incontinence per week; (3) History of retentive posturing or excessive volitional stool retention; (4) History of painful or hard bowel movements; (5) Presence of a large fecal mass in the rectum; and (6) History of large diameter stools which may obstruct the toilet.

Laboratory diagnosis

Since there is no tissue or blood marker for IBS-C, no laboratory testing was performed in this case series.

Treatment

Twenty-four IBS-C patients in a single clinical practice were provided a combination over-the-counter dietary supplement composed of quebracho (150 mg), conker tree (470 mg) and *M. balsamea Willd* (0.2 mL) extracts (Atrantil™) and chose to take the formulation for a minimum of 2 wk in an attempt to manage abdominal pain, bloating and constipation.

Related reports

This case series is a follow up to a well-controlled pilot clinical study in IBS-C patients (Brown *et al*, 2015) testing the same dietary supplement in IBS-C patients composed of quebracho (150 mg), conker tree (470 mg) and *M. balsamea Willd* (0.2 mL) extracts (Atrantil™).

Term explanation

All terms in this case series are standard and used in the field of gastroenterology.

Experiences and lessons

This case series shows the utility of a dietary supplement in drug refractory IBS-C patients formulated to act as a molecular sink for gas ions in the intestine, a bacteriostatic agent to inhibit the impact of bacteria in the small bowel and a component to aid in abdominal discomfort.

Peer-review

The limitations of this case series were that it was in a relatively small cohort of patients biased for compliance in consuming the therapeutic agent in an uncontrolled setting. This case series in combination with the previously

published pilot clinical trial suggests promise for Atrantil™ in IBS-C patients with the caveat that a larger, well-controlled study is needed.

REFERENCES

1 **Su AM**, Shih W, Presson AP, Chang L. Characterization of symptoms in irritable bowel syndrome with mixed bowel habit pattern. *Neurogastroenterol Motil* 2014; **26**: 36-45 [PMID: 23991913 DOI: 10.1111/nmo.12220]

2 **Lovell RM**, Ford AC. Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. *Clin Gastroenterol Hepatol* 2012; **10**: 712-721.e4 [PMID: 22426087 DOI: 10.1016/j.cgh.2012.02.029]

3 **Everhart JE**, Ruhl CE. Burden of digestive diseases in the United States part II: lower gastrointestinal diseases. *Gastroenterology* 2009; **136**: 741-754 [PMID: 19166855 DOI: 10.1053/j.gastro.2009.01.015]

4 **Mönnikes H**. Quality of life in patients with irritable bowel syndrome. *J Clin Gastroenterol* 2011; **45** Suppl: S98-101 [PMID: 21666428 DOI: 10.1097/MCG.0b013e31821fbf44]

5 **Pimentel M**, Mayer AG, Park S, Chow EJ, Hasan A, Kong Y. Methane production during lactulose breath test is associated with gastrointestinal disease presentation. *Dig Dis Sci* 2003; **48**: 86-92 [PMID: 12645795]

6 **Attaluri A**, Jackson M, Valestin J, Rao SS. Methanogenic flora is associated with altered colonic transit but not stool characteristics in constipation without IBS. *Am J Gastroenterol* 2010; **105**: 1407-1411 [PMID: 19953090 DOI: 10.1038/ajg.2009.655]

7 **Pimentel M**, Lin HC, Enayati P, van den Burg B, Lee HR, Chen JH, Park S, Kong Y, Conklin J. Methane, a gas produced by enteric bacteria, slows intestinal transit and augments small intestinal contractile activity. *Am J Physiol Gastrointest Liver Physiol* 2006; **290**: G1089-G1095 [PMID: 16293652]

8 **Triantafyllou K**, Chang C, Pimentel M. Methanogens, methane and gastrointestinal motility. *J Neurogastroenterol Motil* 2014; **20**: 31-40 [PMID: 24466443 DOI: 10.5056/jnm.2014.20.1.31]

9 **Pimentel M**, Chow EJ, Lin HC. Eradication of small intestinal bacterial overgrowth reduces symptoms of irritable bowel syndrome. *Am J Gastroenterol* 2000; **95**: 3503-3506 [PMID: 11151884]

10 **Moayyedi P**, Quigley EM, Lacy BE, Lembo AJ, Saito YA, Schiller LR, Soffer EE, Spiegel BM, Ford AC. The effect of fiber supplementation on irritable bowel syndrome: a systematic review and meta-analysis. *Am J Gastroenterol* 2014; **109**: 1367-1374 [PMID: 25070054 DOI: 10.1038/ajg.2014.195]

11 **Ford AC**, Moayyedi P, Lacy BE, Lembo AJ, Saito YA, Schiller LR, Soffer EE, Spiegel BM, Quigley EM. American College of Gastroenterology monograph on the management of irritable bowel syndrome and chronic idiopathic constipation. *Am J Gastroenterol* 2014; **109** Suppl 1: S2-26; quiz S27 [PMID: 25091148 DOI: 10.1038/ajg.2014.187]

12 **Pimentel M**, Chang C, Chua KS, Mirocha J, DiBaise J, Rao S, Amichai M. Antibiotic treatment of constipation-predominant irritable bowel syndrome. *Dig Dis Sci* 2014; **59**: 1278-1285 [PMID: 24788320 DOI: 10.1007/s10620-014-3157-8]

13 **Chapman RW**, Stanghellini V, Geraint M, Halphen M. Randomized clinical trial: macrogol/PEG 3350 plus electrolytes for treatment of patients with constipation associated with irritable bowel syndrome. *Am J Gastroenterol* 2013; **108**: 1508-1515 [PMID: 23835436 DOI: 10.1038/ajg.2013.197]

14 **Kamm MA**, Mueller-Lissner S, Wald A, Richter E, Swallow R, Gessner U. Oral bisacodyl is effective and well-tolerated in patients with chronic constipation. *Clin Gastroenterol Hepatol* 2011; **9**: 577-583 [PMID: 21440672 DOI: 10.1016/j.cgh.2011.03.026]

15 **Drossman DA**, Chey WD, Johanson JF, Fass R, Scott C, Panas R, Ueno R. Clinical trial: lubiprostone in patients with constipation-associated irritable bowel syndrome--results of two randomized, placebo-controlled studies. *Aliment Pharmacol Ther* 2009; **29**: 329-341 [PMID: 19006537 DOI: 10.1111/j.1365-2036.2008.03881.x]

16 **Videlock EJ**, Cheng V, Cremonini F. Effects of linaclotide in patients with irritable bowel syndrome with constipation or chronic constipation: a meta-analysis. *Clin Gastroenterol Hepatol* 2013; **11**: 1084-1092.e3; quiz e68 [PMID: 23644388 DOI: 10.1016/j.cgh.2013.04.032]

17 **Brown K,** Scott-Hoy B, Jennings. Efficacy of a Quebracho, Conker Tree, and M. balsamea Willd blended extract in patients with irritable bowel syndrome with constipation. *J Gasterenterol Hepatol Res* 2015; **4**: 1762-1767 [DOI: 10.17554/j.issn.2224-3992.2015.04.560]

18 **Bertoldi D**, Santato A, Paolini M, Barbero A, Camin F, Nicolini G, Larcher R. Botanical traceability of commercial tannins using the mineral profile and stable isotopes. *J Mass Spectrom* 2014; **49**: 792-801 [PMID: 25230175 DOI: 10.1002/jms.3457]

19 **Nakayama T**, Hashimoto T, Kajiya K, Kumazawa S. Affinity of polyphenols for lipid bilayers. *Biofactors* 2000; **13**: 147-151 [PMID: 11237174]

20 **Hook SE**, Wright AD, McBride BW. Methanogens: methane producers of the rumen and mitigation strategies. *Archaea* 2010; **2010**: 945785 [PMID: 21253540 DOI: 10.1155/2010/945785]

21 **Fu F**, Hou Y, Jiang W, Wang R, Liu K. Escin: inhibiting inflammation and promoting gastrointestinal transit to attenuate formation of postoperative adhesions. *World J Surg* 2005; **29**: 1614-1120 [PMID: 16311848]

22 **Guo YQ**, Liu JX, Lu Y, Zhu WY, Denman SE, McSweeney CS. Effect of tea saponin on methanogenesis, microbial community structure and expression of mcrA gene, in cultures of rumen micro-organisms. *Lett Appl Microbiol* 2008; **47**: 421-426 [PMID: 19146532 DOI: 10.1111/j.1472-765X.2008.02459.x]

23 **Li W.** Using Saponins to Reduce Gaseous Emissions from Steers: Doctor of Philosophy Dissertation, Michigan State University, Department of Animal Science, 2012

24 **Ringel Y,** Williams RE, Kalilani L, Cook SF. Prevalence, characteristics, and impact of bloating symptoms in patients with irritable bowel syndrome. *Clin Gastroenterol Hepatol* 2009; **7**: 68-72 [PMID: 19124113 DOI: 10.1016/j.cgh.2008.07.008]

25 **Pimentel M**, Lembo A, Chey WD, Zakko S, Ringel Y, Yu J, Mareya SM, Shaw AL, Bortey E, Forbes WP. Rifaximin therapy for patients with irritable bowel syndrome without constipation. *N Engl J Med* 2011; **364**: 22-32 [PMID: 21208106 DOI: 10.1056/NEJMoa1004409]

26 **Yang J**, Lee HR, Low K, Chatterjee S, Pimentel M. Rifaximin versus other antibiotics in the primary treatment and retreatment of bacterial overgrowth in IBS. *Dig Dis Sci* 2008; **53**: 169-174 [PMID: 17520365]

27 **Pimentel M**, Morales W, Chua K, Barlow G, Weitsman S, Kim G, Amichai MM, Pokkunuri V, Rook E, Mathur R, Marsh Z. Effects of rifaximin treatment and retreatment in nonconstipated IBS subjects. *Dig Dis Sci* 2011; **56**: 2067-2072 [PMID: 21559740 DOI: 10.1007/s10620-011-1728-5]

28 **Chey WD**, Drossman DA, Johanson JF, Scott C, Panas RM, Ueno R. Safety and patient outcomes with lubiprostone for up to 52 weeks in patients with irritable bowel syndrome with constipation. *Aliment Pharmacol Ther* 2012; **35**: 587-599 [PMID: 22251419 DOI: 10.1111/j.1365-2036.2011.04983.x]

29 **Chey WD**, Lembo AJ, Lavins BJ, Shiff SJ, Kurtz CB, Currie MG, MacDougall JE, Jia XD, Shao JZ, Fitch DA, Baird MJ, Schneier HA, Johnston JM. Linaclotide for irritable bowel syndrome with constipation: a 26-week, randomized, double-blind, placebo-controlled trial to evaluate efficacy and safety. *Am J Gastroenterol* 2012; **107**: 1702-1712 [PMID: 22986437]

30 **Rao S**, Lembo AJ, Shiff SJ, Lavins BJ, Currie MG, Jia XD, Shi K, MacDougall JE, Shao JZ, Eng P, Fox SM, Schneier HA, Kurtz CB, Johnston JM. A 12-week, randomized, controlled trial with a 4-week randomized withdrawal period to evaluate the efficacy and safety of linaclotide in irritable bowel syndrome with constipation. *Am J Gastroenterol* 2012; **107**: 1714-124; quiz p.1725 [PMID: 22986440]

31 **Eswaran S**, Muir J, Chey WD. Fiber and functional gastrointestinal disorders. *Am J Gastroenterol* 2013; **108**: 718-727 [PMID: 23545709 DOI: 10.1038/ajg.2013.63]

32 **Halmos EP**, Power VA, Shepherd SJ, Gibson PR, Muir JG. A diet low in FODMAPs reduces symptoms of irritable bowel syndrome. *Gastroenterology* 2014; **146**: 67-75.e5 [PMID: 24076059 DOI: 10.1053/j.gastro.2013.09.046]

33 **Chmielewska A**, Szajewska H. Systematic review of randomised controlled trials: probiotics for functional constipation. *World J Gastroenterol* 2010; **16**: 69-75 [PMID: 20039451]

34 **Hagerman AE**, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW, Riechel TL. High molecular weight plant polyphenolics (tannins) as biological antioxidant. *J Agric Food Chem* 1998; **46**: 1887-1892 [DOI: 10.1021/jf970975b]

35 **Tavendale MH,** Meagher LP, Pacheco D, Walker N, Attwood GT, Sivakumaran S. Methane production from in vitro rumen incubations with Lotus pedunculatus and Medicago sativa, and effects of extractable condensed tannin fractions on methanogenesis. *Animal Feed Sci Technol* 2005; **123**: 403-419

36 **Matsuda H**, Li Y, Yoshikawa M. Effects of escins Ia, Ib, IIa, and IIb from horse chestnuts on gastrointestinal transit and ileus in mice. *Bioorg Med Chem* 1999; **7**: 1737-1741 [PMID: 10482465]

37 **Xie Q**, Zong X, Ge B, Wang S, Ji J, Ye Y, Pan L. Pilot postoperative ileus study of escin in cancer patients after colorectal surgery. *World J Surg* 2009; **33**: 348-354 [PMID: 19052813 DOI: 10.1007/s00268-008-9816-1]

38 **de Sousa AA**, Soares PM, de Almeida AN, Maia AR, de Souza EP, Assreuy AM. Antispasmodic effect of Mentha piperita essential oil on tracheal smooth muscle of rats. *J Ethnopharmacol* 2010; **130**: 433-436 [PMID: 20488237 DOI: 10.1016/j.jep.2010.05.012]

39 **Khanna R**, MacDonald JK, Levesque BG. Peppermint oil for the treatment of irritable bowel syndrome: a systematic review and meta-analysis. *J Clin Gastroenterol* 2014; **48**: 505-512 [PMID: 24100754 DOI: 10.1097/MCG.0b013e3182a88357]

P- Reviewer: Gomes A **S- Editor**: Qi Y **L- Editor**: A
E- Editor: Lu YJ

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

ISSN 2150-5349 (online)

World Journal of *Gastrointestinal Pharmacology and Therapeutics*

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 469-586

Published by Baishideng Publishing Group Inc

WJGPT

World Journal of Gastrointestinal Pharmacology and Therapeutics

A peer-reviewed, online, open-access journal of gastrointestinal pharmacology and therapeutics

Editorial Board

2016-2019

The *World Journal of Gastrointestinal Pharmacology and Therapeutics* Editorial Board consists of 394 members, representing a team of worldwide experts in surgery research. They are from 45 countries, including Argentina (3), Australia (12), Austria (3), Belarus (1), Belgium (4), Brazil (10), Canada (10), China (39), Czech Repoublic (1), Egypt (3), Estonia (1), Finland (1), France (6), Germany (19), Greece (7), Hungary (4), India (17), Iran (5), Ireland (1), Israel (5), Italy (32), Japan (34), Lebanon (1), Lithuania (3), Mexico (2), Netherlands (10), New Zealand (2), Norway (2), Pakistan (1), Philippines (1), Poland (3), Portugal (2), Romania (1), Russia (1), Saudi Arabia (3), Singapore (3), Slovenia (1), South Africa (1), South Korea (14), Spain (13), Sweden (3), Thailand (4), Turkey (6), United Kingdom (19) and United States (80).

Duo Li, *Hangzhou*
Yu-Yuan Li, *Guangzhou*
Xiong Ma, *Shanghai*
Qin Pan, *Shanghai*
Guo-Ping Sun, *Hefei*
Xue-Ying Sun, *Harbin*
Ming-Fu Wang, *Hong Kong*
Che-Yuen Justin Wu, *Hong Kong*
De Xiang Xu, *Hefei*
Ruian Xu, *Xiamen*
Ming-Xian Yan, *Jinan*
Yong-Feng Yang, *Nanjing*
Thomas Yau, *Hong Kong*
Win-Nei Yeo, *Hong Kong*
Long Yu, *Guangzhou*
Jian-Ping Yuan, *Guangzhou*
Man-Fung Yuen, *Hong Kong*
Liqun Zhang, *Beijing*
Min-Sheng Zhu, *Nanjing*

Czech Republic

Rene Kizek, *Brno*

Denmark

Rene Kizek, *Brno*

Egypt

Omar ME Abdel-Salam, *Cairo*
Ahmed Osman Abdel-Zaher, *Assiut*
Osama Ahmed Badary, *Cairo*

Estonia

Riin Tamm, *Tartu*

Finland

Riitta Korpela, *Helsinki*

France

Tarik Asselah, *Clichy*
Ferrand Audrey, *Toulouse*
Frederic Batteux, *Paris*
Thierry Capiod, *Paris*
Frederic Lagarce, *Angers*
Hang Thi Thu Nguyen, *Clermont-Ferrand*

Germany

Susanne Beckebaum, *Essen*
Jürgen Borlak, *Hannover*
Güralp Onur Ceyhan, *Munich*
Walee Chamulitrat, *Heidelberg*
Anton Gillessen, *Munster*
Dirk Heitzmann, *Muenster*
Jens Michael Heyn, *München*
Joachim Labenz, *Siegen*
Thomas Müller, *Berlin*
Belal Naser, *Salzgitter*
Beate Niesler, *Heidelberg*
Matthias Ocker, *Marburg*
Andreas Marc Palmer, *Konstanz*
Dirk Rades, *Luebeck*
Fuat Hakan Saner, *Essen*
Manfred V Singer, *Mannheim*
Konrad Ludwig Streetz, *Aachen*
Frank Tacke, *Aachen*
Gerhard Treiber, *Balingen*

Greece

Moses S Elisaf, *Ioannina*
Anastasios Koulaouzidis, *Greek*
Ioannis E Koutroubakis, *Heraklion*
Spilios Manolakopoulos, *Athens*
Konstantinos Christou Mountzouris, *Athens*
George V Papatheodoridis, *Athens*
Konstantinos Tziomalos, *Thessaloniki*

Hungary

Zsolt Barta, *Debrecen*
László Czakó, *Szeged*
Bela Molnar, *Budapest*
Gyula Mózsik, *Pecs*

India

Anil Kumar Agarwal, *New Delhi*
Sandip Basu, *Bombay*
Chiranjib Chakraborty, *Vellore*
Rukhsana Chowdhury, *Kolkata*
C M Habibullah, *Hyderabad*
Mohandas K Mallath, *Mumbai*
Balraj Mittal, *Lucknow*
Rama Devi Mittal, *Lucknow*
Asish K Mukhopadhyay, *Kolkata*
Lekha Saha, *Chandigarh*
Darisetty Santosh, *Hyderabad*
Shiv Kumar Sarin, *New Delhi*
Jayshri Ankur Shah, *Mumbai*
Sonu Sundd Singh, *Gurgaon*
Sikta Swarnakar, *Calcutta*
Rakesh Kumar Tandon, *New Delhi*
Asna Urooj, *Mysore*

Iran

Seyed Mohsen Dehghani, *Shiraz*
Ahmad Reza Dehpour, *Tehran*
Sara Farhang, *Tabriz*
Parisa Hasanein, *Hamadan*
Amir Mohammad Mortazavian, *Tehran*

Ireland

Zaid S Heetun, *Dublin*

Israel

Nimer Assy, *Safed*
Simon Bar-Meir, *Tel-Hashomer*
Rami Eliakim, *Haifa*
Tiberiu Hershcovici, *Jerusalem*
Haim Shmuel Odes, *Beer Sheva*

Italy

Giovanni C Actis, *Turin*
Bruno Annibale, *Roma*
Leonardo Baiocchi, *Rome*
Gabrio Bassotti, *San Sisto*
Francesca Borrelli, *Napoli*
Giuseppe Brisinda, *Rome*
Renzo Caprilli, *Rome*
Mauro Antonio Maria Carai, *Cagliari*
Renato Caviglia, *Rome*
Carolina Ciacci, *Naples*
Mario Cottone, *Trabucco*
Roberto De Giorgio, *Bologna*
Luca Elli, *Milano*
Alessandro Granito, *Bologna*
Francesco William Guglielmi, *Bari*
Mario Guslandi, *Milano*
Pietro Invernizzi, *Monza*
Mariano Malaguarnera, *Catania*
Massimo C Mauri, *Milan*
Massimo Montalto, *Rome*
Giovanni Monteleone, *Rome*
Gerardo Nardone, *Naples*
Fabio Pace, *Seriate*
Raffaele Pezzilli, *Bologna*
Rita Rezzani, *Brescia*
Carmelo Scarpignato, *Parma*
Generoso Uomo, *Napoli*
Paolo Usai-Satta, *Cagliari*
Maurizio Vecchi, *Milan*
Massimiliano Veroux, *Catania*

Japan

Akira Andoh, *Otsu*
Yuichiro Eguchi, *Saga*
Munechika Enjoji, *Fukuoka*
Norihiro Furusyo, *Fukuoka*
Naoki Hotta, *Nagoya*
Shigeo Ikegawa, *Higashi-Osaka*
Susumu Ito, *Okinawa*
Satoru Kakizaki, *Maebashi*
Terumi Kamisawa, *Tokyo*
Motoyori Kanazawa, *Miyagi*
Takuma Kato, *Mie*
Takashi Kawai, *Tokyo*
Shirao Kuniaki, *Oita*
Nobuyuki Matsuhashi, *Tokyo*
Tatsuya Matsura, *Yonago*
Teruo Murakami, *Hiroshima*
Yuji Naito, *Kyoto*
Katsuyuki Nakajima, *Maebashi Gunma*
Hiroshi Nakase, *Kyoto*
Nobuhiro Ohkohchi, *Tsukuba*
Shogo Ohkoshi, *Niigata*
Tomohiko Shimatani, *Kure*
Yasuhiko Sugawara, *Tokyo*
Yoshitaka Takuma, *Okayama*
Tatsuhiro Tsujimoto, *Nara*
Takato Ueno, *Fukuoka*
Kenji Watanabe, *Osaka*
Toshiaki Watanabe, *Tokyo*

Jiro Watari, *Nishinomiya*
Satoshi Yamagiwa, *Niigata*
Takayuki Yamamoto, *Mie*
Norimasa Yoshida, *Kyoto*
Hitoshi Yoshiji, *Kashihara*
Katsutoshi Yoshizato, *Higashihiroshima*

Lebanon

AIa Sharara, *Beirut*

Lithuania

Dalia Adukauskiene, *Kaunas*
Giedrius Barauskas, *Kaunas*
Laimas Jonaitis, *Kaunas*

Mexico

Pablo Muriel, *Mexico City*
Guillermo Benito Robles-Díaz, *Mexico City*

Netherlands

Judith Elisabeth Baars, *Rotterdam*
Albert J Bredenoord, *Nieuwegein*
Nanne KH De Boer, *Amsterdam*
Pieter Jan Floris De Jonge, *Rotterdam*
Wouter J de Jonge, *Amsterdam*
Mireille A Edens, *Groningen*
Chris JJ Mulder, *Amsterdam*
Paul E Sijens, *Groningen*
Vera Esther Valkhoff, *Rotterdam*

New Zealand

Momir M Mikov, *Dunedin*
Maxim Petrov, *Auckland*

Norway

Guanglin Cui, *Tromso*
Reidar Fossmark, *Trondheim*

Pakistan

Anwar Hassan Gilani, *Karachi*

Philippines

Mark Anthony Ayuyao De Lusong, *Quezon City*

Poland

Halina Cichoz-Lach, *Lublin*
Jaroslaw Czyz, *Cracow*
Julian Teodor Swierczynski, *Gdansk*

Portugal

Cristina Freire, *Hull*
Ana Isabel Lopes, *Lisbon*

Romania

Dan Lucian Dumitrascu, *Cluj*

Russia

Tatyana A Korolenko, *Novosibirsk*

Saudi Arabia

Mohammad S Khuroo, *Riyadh*
Moamen Salah Refat, *Taif*
Shahab Uddin, *Riyadh*

Singapore

Kok-Ann Gwee, *Singapore*
Khek-Yu Ho, *Singapore*
Kok-Yuen Ho, *Singapore*

Slovenia

Rok Orel, *Ljubljana*

South Africa

Christoffel Van Rensburg, *Cape Town*

South Korea

Ki Baik Hahm, *Inchon*
Seok Joo Han, *Seoul*
Jeong Won Jang, *Seoul*
Dong Joon Kim, *Gangwon-do*
Jae J Kim, *Seoul*
Kyoung Mee Kim, *Seoul*
Nayoung Kim, *Songnam*
Sung-Bae Kim, *Seoul*
Byung-Hoon Lee, *Seoul*
Kwan S Lee, *Seoul*
Sang-Han Lee, *Daegu*
Yun Jeong Lim, *Goyang*
Ji-Young Park, *Seoul*
Uy Dong Sohn, *Seoul*

Spain

Matias Antonio Avila, *Pamplona*
Luis Bujanda, *San Sebastián*
Maria Carmen Collado, *Valencia*
Conrado M Fernandez-Rodriguez, *Madrid*
ángel Lanas, *Zaragoza*
Juan-R Malagelada, *Barcelona*
Jose JG Marin, *Salamanca*
Antonio Ruiz Medina, *Jaén*
Maria J Monte, *Salamanca*
Miguel Munoz, *Seville*
Jesus Prieto, *Pamplona*
Victor Manuel Victor, *Valencia*
Maria D Yago, *Granada*

Sweden

Bodil Ohlsson, *Malmo*
Henrik Thorlacius, *Malmo*
Curt Tysk, *Orebro*

Thailand

Weekitt Kittisupamongkol, *Bangkok*
Abhasnee Sobhonslidsuk, *Bangkok*
Suporn Treepongkaruna, *Bangkok*
Sombat Treeprasertsuk, *Bangkok*

Turkey

Fusun Acarturk, *Etiler-Ankara*
Engin Altintas, *Mersin*
Güldeniz K Cakmak, *Kozlu Zonguldak*
Hayrullah Derici, *Balkesir*
Mukaddes Esrefoglu, *Istanbul*
Ilker Tasci, *Ankara*

United Kingdom

Nadeem Ahmad Afzal, *Hampshire*
Qasim Aziz, *London*
Hugh Barr, *Gloucester*
Ian Leonard Phillip Beales, *Norwich*
Barbara Braden, *Oxford*
Susan J Duthie, *Aberdeen*
Eyad Elkord, *Manchester*
Anton Vignaraj Emmanuel, *London*
Konstantinos C Fragkos, *London*
Nusrat Husain, *Manchester*
Jin-Yong Kang, *London*
Mariusz Madalinski, *Ipswich*
Srinivasan Madhusudan, *Nottingham*
Subramanian Mahadevan, *Birmingham*
John Francis Mayberry, *Leicester*
Chuka Uche Nwokolo, *Coventry*
Ajith Kumar Siriwardena, *Manchester*
Her-Hsin Tsai, *Hull*
Craig LC Williams, *Glasgow*

United States

Zubair H Aghai, *Camden*
Shrikant Anant, *Oklahoma*
Kondala R Atkuri, *Stanford*
Cheryl Hunt Baker, *Orlando*
James M Becker, *Boston*
Qiang Cai, *Atlanta*
Jian-De Chen, *Galveston*
Liang Cheng, *Indianapolis*

WJGPT

World Journal of Gastrointestinal Pharmacology and Therapeutics

Contents

Quarterly Volume 7 Number 4 November 6, 2016

World Journal of Gastrointestinal Pharmacology and Therapeutics
Volume 7 Number 4 November 6, 2016

Contents

NAME OF JOURNAL
World Journal of Gastrointestinal Pharmacology and Therapeutics

ISSN
ISSN 2150-5349 (online)

LAUNCH DATE
May 6, 2010

FREQUENCY
Quarterly

EDITORIAL OFFICE
Xiu-Xia Song, Director
Fang-Fang Ji, Vice Director
World Journal of Gastrointestinal Pharmacology and Therapeutics
Baishideng Publishing Group Inc
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Inc
8226 Regency Drive,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLICATION DATE
November 6, 2016

INSTRUCTIONS TO AUTHORS
http://www.wjgnet.com/bpg/gerinfo/204

ONLINE SUBMISSION
http://www.wjgnet.com/esps/

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i4.469

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 469-476
ISSN 2150-5349 (online)

REVIEW

Bilirubin in coronary artery disease: Cytotoxic or protective?

Nancy Gupta, Tavankit Singh, Rahul Chaudhary, Sushil K Garg, Gurprataap Singh Sandhu, Varun Mittal, Rahul Gupta, Roxana Bodin, Sachin Sule

Nancy Gupta, Sachin Sule, Department of Medicine, Westchester Medical Center, New York Medical College, Valhalla, NY 10595, United States

Tavankit Singh, Department of Medicine, Cleveland Clinic, Cleveland, OH 44195, United States

Rahul Chaudhary, Department of Medicine, Sinai Hospital of Baltimore, Johns Hopkins University, Baltimore, MD 21218, United States

Sushil K Garg, Department of Medicine, University of Minnesota, Minneapolis, MN 55455, United States

Gurprataap Singh Sandhu, Department of Medicine, University of Pittsburgh, Pittsburgh, PA 15260, United States

Varun Mittal, Einstein Healthcare Network, Philadelphia, PA 19141, United States

Rahul Gupta, Government Medical College, Amritsar, Punjab 143001, India

Roxana Bodin, Division of Gastroenterology and Hepatobiliary Disease, New York Medical College, Valhalla, NY 10595, United States

Author contributions: All authors contributed to the manuscript; Gupta N, Singh T and Chaudhary R have contributed equally for the paper.

Conflict-of-interest statement: No potential conflicts of interest relevant to this article were reported.

Manuscript source: Invited manuscript

Correspondence to: Nancy Gupta, MD, Department of Medicine, Westchester Medical Center, New York Medical College, 40 Sunshine Cottage Rd, Valhalla, NY 10595, United States. drnancygupta87@gmail.com
Telephone: +1-914-5361563
Fax: +1-914-4935827

Received: April 2, 2016
Peer-review started: April 7, 2016
First decision: June 6, 2016
Revised: June 7, 2016
Accepted: August 27, 2016
Article in press: August 29, 2016
Published online: November 6, 2016

Abstract

Bilirubin has traditionally been considered a cytotoxic waste product. However, recent studies have shown bilirubin to have anti-oxidant, anti-inflammatory, vaso-dilatory, anti-apoptotic and anti-proliferative functions. These properties potentially confer bilirubin a new role of protection especially in coronary artery disease (CAD), which is a low grade inflammatory process exacerbated by oxidative stress. In fact, recent literature reports an inverse relationship between serum concentration of bilirubin and the presence of CAD. In this article, we review the current literature exploring the association between levels of bilirubin and risk of CAD. We conclude that current evidence is inconclusive regarding the protective effect of bilirubin on CAD. A causal relationship between low serum bilirubin level and increased risk of CAD is not currently established.

Key words: Bilirubin; Cytotoxic; Protective; Anti-oxidant; Anti-inflammatory; Anti coronary artery disease; Lipid peroxidation; Gilbert

Core tip: Bilirubin has traditionally been considered a cytotoxic waste product. However, recent studies have shown bilirubin to have anti-oxidant, anti-inflammatory, vasodilatory, anti-apoptotic and anti-proliferative functions. These properties potentially confer bilirubin a new role of protection especially in coronary artery disease (CAD), which is a low grade inflammatory process exacerbated by oxidative stress. In fact, recent literature reports an inverse relationship between serum concentration of bilirubin and the presence of CAD. In this article, we review the current literature exploring the association between levels of bilirubin and risk of CAD.

Gupta N, Singh T, Chaudhary R, Garg SK, Sandhu GS, Mittal V, Gupta R, Bodin R, Sule S. Bilirubin in coronary artery disease: Cytotoxic or protective? *World J Gastrointest Pharmacol Ther* 2016; 7(4): 469-476 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i4/469.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i4.469

INTRODUCTION

Traditionally, bilirubin, a product of heme metabolism was thought to be a cytotoxic waste product, toxic to neurons[1]. However, it was later observed to possess other properties: Vasodilatory, anti-oxidant, anti-inflammatory, anti-mutagenic, immune-modulatory, antiproliferative and anti-apoptotic on vascular cells[2,3]. It has also been suggested to have a lipid lowering effect by reducing plasma and low-density lipid peroxidation[3]. By virtue of these properties, bilirubin was hypothesized to have a protective effect in coronary artery disease (CAD)[4]. Vitek *et al*[5] studied this relation in patients with Gilbert syndrome (a hereditary disorder leading to unconjugated hyperbilirubinemia with normal liver chemistries) and reported a 2% prevalence of ischemic CAD with Gilbert syndrome (n = 50) compared to 12.1% in the general population (n = 2296, P < 0.05)[5].

BILIRUBIN METABOLISM

Hemoglobin is released from the senescent red blood cells and non-erythroid hemoproteins. Hemoglobin is broken down into heme pigment and globin chains. Heme pigment is oxidatively metabolized by heme oxygenase into biliverdin, carbon monoxide and free iron. Biliverdin is further degraded by biliverdin reductase into unconjugated bilirubin. Following its uptake by the hepatocytes, unconjugated bilirubin is converted to conjugated bilirubin by the action of uridine-diphosphoglucuronate glucuronosyltransferase (UDP-GT). The gene that codes for UDP-GT is *UGT1A1* gene and a genetic variation in the promoter region of *UGT1A1* gene is associated with Gilbert syndrome. This genetic variation involves insertion of an additional thymine-adenine base pair in the TATA box of the *UGT1A1* gene instead of the normal 6 pairs[4,6] (Figure 1). This leads to deficiency of the enzyme that leads to accumulation of unconjugated bilirubin within the blood[4]. Patients with Gilbert have otherwise normal serum liver chemistries[6]. However, another condition called Crigler-Najjar syndrome with unconjugated hyperbilirubinemia stems from complete or near complete loss of UGT1A1 activity. Type 1 Crigler Najjar is a rare and lethal recessive disorder compared to type 2 Crigler Najjar where the UGT1A1 activity is still maintained, albeit at a minimal level[7]. Patients with Criggler Najjar Type 1 develop severe neurological impairment and carry a high early mortality unless they receive liver transplantation.

Conjugated bilirubin is excreted into the bile canaliculus by the canalicular membrane transporter multidrug resistance related protein 2 (MRP2). Mutations in the gene that affects this transport protein leads to conjugated hyperbilirubinemia. This condition is called Dubin-Johnson syndrome[8]. Another autosomal recessive disorder, in which patients have multiple defects in hepatocyte uptake and excretion of bilirubin, leads to increase in conjugated bilirubin and is called Rotor syndrome.

PROTECTIVE PROPERTIES OF BILIRUBIN

Several mechanisms have been proposed highlighting the protective effects of bilirubin: (1) Bilirubin has anti-oxidant properties independent of whether it is free or albumin bound, conjugated or unconjugated. Bilirubin increases in response to the oxidative stress and acts as a scavenger of the reactive oxygen species[9,10]. Bilirubin sub-fractions (Bu and Bc) have demonstrated inhibition of low-density lipoproteins oxidation, which in turn retards the peroxidation of lipids, hence could potentially restrict the progression of atherosclerosis[10]. Of note, unconjugated bilirubin in concentrations as low as 10 nmol/L has been reported to protect neuronal cultures from the oxidative stress generated by 10000 times higher concentrations of hydrogen peroxide[11]. This anti-oxidative effect of bilirubin is amplified by the recycling of bilirubin to biliverdin and so forth *via* redox reactions (Figure 1)[3]. This recycling of bilirubin amplifies its anti-oxidant potential up to 10000 times; (2) Bilirubin has been shown to be inversely associated with increased arterial stiffness[12,13]. Pre-clinical studies have observed this effect to be mediated by preservation of vascular nitric oxide, which mediates endothelial relaxation[2,13]. Decreased levels of nitric oxide impair the ability of the coronary vessels to dilate during exercise or stress, thus, provoking myocardial ischemia in patients with CAD[14]. Besides vaso-relaxation, nitric oxide also inhibits leukocyte adhesion to endothelium, vascular smooth muscle cell migration and proliferation, platelet aggregation and neointimal formation[2,15]. Thus, preservation of nitric oxide offers a significant protection against atherosclerosis[12]; (3) Bilirubin has

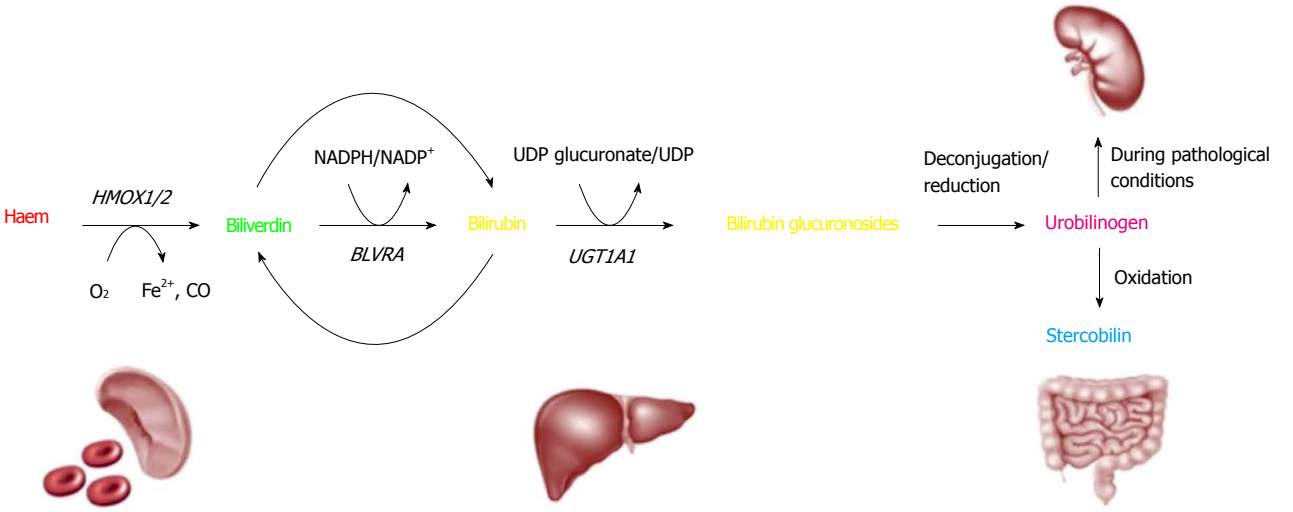

Figure 1 Bilirubin metabolism (from heme to bilirubin). Hemoglobin is cleaved to yield globin and heme (red). Heme is enzymatically converted to biliverdin (green) by liberating iron, *via* oxidation with loss of a carbon atom (CO). This, in turn, yields bilirubin (orange) after enzymatic reduction of biliverdin. In the liver, bilirubin is conjugated to enable excretion, requiring the enzyme UGT1A1[4].

also been seen to reduce platelet aggregation. Kundur *et al*[3] showed that when unconjugated bilirubin is added to platelet rich plasma at concentrations seen in Gilbert syndrome (0.99-5.85 mg/dL), it inhibits both - collagen induced and adenosine diphosphate induced platelet aggregation, in a dose dependent fashion for the latter. P and E-selectin are markers of platelet activation and released from activated platelets and endothelium. They predominantly mediate adhesion of platelets and inflammatory cells to endothelium and facilitate the formation of large stable platelet-leukocyte aggregates that can lead to thrombus formation. Bilirubin, biliverdin and inducible heme oxygenase have an inhibitory effect on P- and E-selectin[16]. This is supported by studies showing reduced levels of circulating inflammatory biomarkers, P selectin and CD 40 ligand in patients with Gilbert syndrome[3]; (4) Bilirubin and heme oxygenase exhibit anti-inflammatory properties and prevent oxidant induced microvascular leukocyte adhesion. Heme oxygenase (the rate-limiting enzyme in bilirubin production) also functions as a vasodilatory and anti- proliferative agent during vascular injury[15]. An inverse association has been demonstrated between total bilirubin levels and the markers of inflammation, namely, C-reactive protein (direct marker); neutrophil to lymphocyte ratio and red cell distribution width (indirect markers)[17]. In animal models, bilirubin has also been seen to exhibit anti-complement effect *in vitro*, thus, conferring protection against increased thrombogenicity and clot formation[3,17-19]. This anti-inflammatory effect has been hypothesized to antagonize the process of atherosclerosis, which is a low-grade chronic inflammatory state; (5) preclinical studies on mice have demonstrated a protective effect of bilirubin on angiotensin-Ⅱ induced hypertension. Angiotensin-Ⅱ is known to cause superoxide production, which is attenuated by induction of heme oxygenase *via* redox reactions. This reaction leads to production of bilirubin and carbon monoxide; hence an increased bilirubin level is associated with increased attenuation of superoxide production[20]. This protective effect, in conjunction with carbon monoxide, by inhibition of angiotensin-Ⅱ has also been suggested and extrapolated in the cardiomyocytes preventing left ventricular hypertrophy[21-23]; and (6) Bilirubin has also been described to solubilize cholesterol and promotes its clearance through the bile[24,25]. This finding is also supported by the evidence of reduced levels of total cholesterol, low-density lipoprotein (LDL) and apolipoprotein B/apolipoprotein-A1 ratio and elevated high density lipoprotein (HDL) to LDL ratio in patients with Gilbert syndrome[3].

EVIDENCE SUPPORTING THE PROTECTIVE EFFECT OF BILIRUBIN ON CAD

Several studies have suggested a cardioprotective role of bilirubin. Schwertner *et al*[26] was the first to report this protective effect of high level of bilirubin in CAD. They compared 619 subjects in training set (complete data on all risk factors considered was available) *vs* 258 subjects in test set (some risk factor data was not available). They observed a statistically significant inverse association between bilirubin and CAD. Fifty percent reduction in total bilirubin was associated with 47% increased odds of CAD, both univariate and multivariate after adjustment for other risk factors[26]. In 1996, Hopkins *et al*[27] evaluated 161 patients with early familial CAD and compared them to 155 control subjects. Patients with familial CAD had significantly lower bilirubin concentration as compared to controls (8.9 ± 6.1 micromol/L *vs* 12.4 ± 8.1 micromol/L, P = 0.0001). After adjustment for other risk factors, bilirubin

was found to be an independent protective factor with an odds ratio of 0.25 (P = 0.001) for an increase of 1 mg/dL. The benefits of elevated bilirubin were seen to be comparable to those of HDL. In a meta-analysis published in 2003 by Novotny *et al*[28] a significant inverse relationship was shown between serum bilirubin levels and the severity of atherosclerosis. Eleven relevant studies were used for analysis and the subjects involved were males. The relation between serum bilirubin levels and severity of atherosclerosis had a spearman rank coefficient of r = -0.31 (P < 0.0001).

Subsequently, in another study by Erdogan *et al*[29] in 2012, 179 patients undergoing angiography were analyzed to evaluate for CAD. Out of them, 110 patients had good collateral formation and 69 had poor collateral development. Higher serum bilirubin levels were associated with good collateral development as compared to poor collateral development (0.80 ± 0.27 mg/dL *vs* 0.53 ± 0.19 mg/dL, P < 0.001). These findings suggest a possible protective effect of elevated serum bilirubin levels against myocardial ischemia in patients with chronic total coronary occlusion with collaterals limiting infarct size and providing additional blood flow to the ischemic area[29]. Wei *et al*[30] also showed similar results with a significant inverse correlation between CAD and total bilirubin (n = 1260) in patients who underwent coronary angiography**.**

In 2013, Stojanov *et al*[31] reported cardioprotective effects of increased levels of bilirubin in 628 healthy subjects. The subjects were 442 men and 186 women aged 18 to 22 years. They divided the subjects into 2 groups based on levels of bilirubin. Subjects with level below the upper limit of reference were classified as "low bilirubin" (⩽ 0.95 mg/dL in women and ⩽ 1.4 mg/dL in men) and those with value above the upper limit of reference were classified as "high bilirubin". Men with high bilirubin concentration (> 1.4 mg/dL) had higher concentration of albumin and uric acid (P < 0.001) and lower level of oxidized LDL (P < 0.05). In females, high bilirubin (> 0.95 mg/dL) was associated with significantly higher albumin (P < 0.05) and lower thiobarbituric acid-reacting substances (TBARS) (P < 0.05). These findings support the evidence of an anti-oxidant effect of bilirubin secondary to inverse association with ox-LDL and anti-inflammatory effect secondary to direct correlation with albumin, which is a negative acute phase reactant in inflammatory response[31]. Shortly after that study, Canpolat *et al*[18] used computed tomographic angiography (CTA) to evaluate the relationship between bilirubin levels and nature of coronary plaques. The study included 1115 subjects who underwent CTA for evaluation of CAD. Patients were divided into 4 quartiles depending on the total bilirubin level. Patients with any coronary plaque were observed to have statistically significant lower levels of serum bilirubin (P = 0.002). Patients with critical stenosis (> 50% obstruction) had lower bilirubin levels compared to non-critical stenosis (0.57 ± 0.18 mg/dL *vs* 0.70 ± 0.24 mg/dL, P < 0.001). The authors concluded that lower serum levels of bilirubin were significantly associated with the presence, severity and the noncalcified morphology of atherosclerotic plaques.

Later, Song *et al*[32] designed a prospective cohort study with 8593 subjects followed over a period of 4 years. Low bilirubin levels (< 0.32 mg/dL) were observed to be an independent risk factor associated with an increased risk of CAD development (n = 80, 0.9% of total subjects) with adjusted hazard ratio (HR) of 1.890 (95%CI: 1.088-3.284, P = 0.024). Low bilirubin levels were shown to further increase the risk of CAD development six fold in patients with metabolic syndrome with HR of 2.016 (95%CI: 1.069-3.800, P = 0.030). The authors concluded by suggesting addition of bilirubin level to the risk assessment tool for assessing CAD in patients. Similar results showing an inverse association of bilirubin levels with coronary artery calcification were reported by Mahabadi *et al*[33]. However, they attributed the cardioprotective effects from bilirubin to a more favorable cardiovascular risk profile observed in their patients with CAD and elevated bilirubin levels in their study[33].

Akboga *et al*[17] conducted another study evaluating anti-inflammatory properties of bilirubin. In a retrospective cross-sectional study, they included 1501 patients who underwent coronary angiography. They divided them into 3 groups based on Gensini scores: No CAD (control group, n = 380), mild CAD (n = 497) and severe CAD (n = 624), with the objective of establishing anti-inflammatory effects of bilirubin in addition to its anti-oxidant effects. A significant inverse correlation between total bilirubin and C-reactive protein (r = -0.112, P < 0.001), neutrophil to lymphocyte ratio (r = -0.070, P = 0.026) and red cell distribution width (r = -0.074, P = 0.027) was observed. These findings helped establish anti-inflammatory properties of bilirubin in addition to their anti-oxidant effects. They also re-confirmed the inverse association of bilirubin with CAD severity [spearman's rank correlation coefficient (r) = -0.173, P < 0.001].

GENETIC POLYMORPHISMS OF *UGT1A1*28* AND THEIR RELATION WITH CAD

Polymorphisms in the *UGT1A1* gene (also known as *UGT1A1*28*) leads to unconjugated hyperbilirubinemia due to deficiency or decreased activity of *UGT1A1*[34]. Thus, patients with *UGT1A1*28* allele have shown to have higher levels of bilirubin[35]. Whereas, patients with wild type allele, *i.e.,* normal genotype have normal levels of bilirubin. To understand the true role of bilirubin it is prudent to look into the association between *UGT1A1*28* and CAD. Establishing an inverse association between the two would strengthen the hypothesis of bilirubin being protective in CAD.

In 2003, the Rotterdam study (case control study of 114 patients) hypothesized that since individuals

homozygous for *UGT1A1*28* have higher serum bilirubin, they would have a lower risk of CAD. They found that the relative risk of myocardial infarction (MI) for heterozygous genotype was 0.9 (95%CI: 0.7-1.3) and with homozygous *UGT1A1*28* was 1.3 (95%CI: 0.8-2.2). After adjusting for factors like age, gender, smoking, body mass index, diabetes mellitus, systolic blood pressure, total cholesterol, and HDL-cholesterol, the risk estimate was 1.0 (0.7-1.4) for heterozygotes and 1.2 (0.7-2.1) for homozygotes. Authors argued that the protective effect could have been missed because of the lack of power to detect such an effect. However, no association was seen between low serum bilirubin and the risk for CAD[36].

The Framingham offspring study (a prospective cohort study) in 2006 observed 1780 participants over 24 years and found that subjects homozygous for the gene *UGT1A1*28* had approximately one-third the risk for CAD compared to individuals homozygous for the wild type allele. However, the association between the incidence of myocardial infarction (MI) and gene polymorphism was not significant although the trend was similar. It was concluded that the carriers homozygous for *UGT1A1*28* allele with higher bilirubin concentrations exhibited a strong association with lower risk of cardiovascular disease[34].

Lingenhel *et al*[37] studied two polymorphisms in the promoter region of *UGT1A1* gene to analyze whether *UGTA1A* gene polymorphisms or bilirubin levels are independently associated with risk of CAD development. This case-control study enlisted 477 patients with premature, familial CAD and 619 controls that were matched for age and gender. Bilirubin levels were found to be significantly lower in the familial CAD group as compared to the controls ($P = 1.2 \times 10^{-10}$ in men and 1.9×10^{-9} in women). The low bilirubin levels were found to be significantly associated to CAD whereas *UGTA1A* polymorphisms were not, with odds ratio of 0.9 (CI: 0.86-0.94, $P = 2.6 \times 10^{-6}$) for men and 0.77 (CI: 0.68-0.87, $P = 3.2 \times 10^{-5}$) for women respectively for each 0.1mg/dl increase in bilirubin levels[37]. Hence, indicating that increased bilirubin levels and not genetic polymorphisms are associated with reduced risk of CAD.

Hsieh *et al*[38] sought to explore the association of UGTA1A polymorphisms with risk of CAD development. A case-control design was set up ($n = 135$; cases = 61, controls = 74) and although bilirubin levels in the control group were found to be significantly higher than CAD group, no significant differences were observed in the polymorphism of UGTA1A between the two groups.

Rantner *et al*[39] in their prospective case control study, investigated plasma bilirubin concentration and UGT1A1 promoter TA repeat polymorphism in a cohort of patients with peripheral arterial disease and age and diabetes matched control group. They observed significantly lower bilirubin concentrations in patients than in controls. UGT1A1 polymorphism was strongly associated with bilirubin concentration in both patients and controls. However, UGT1A1 polymorphism was not associated with peripheral arterial disease.

EVIDENCE NOT SUPPORTING THE PROTECTIVE EFFECT OF BILIRUBIN ON CAD

Contrary to the evidence presented above, several studies have negated the protective effect of bilirubin on CAD. British Regional Health Study (BRHS) was a prospective study designed to examine the relationship between the level of bilirubin and risk of ischemic CAD. Subjects ($n = 7685$) were followed up for a mean of 11.5 years, out of which 737 individuals were seen to develop major ischemic CAD. A U-shaped relationship was observed between serum bilirubin and risk of ischemic CAD with increased risk at bilirubin concentrations < 0.4 mg/dL and at > 0.7 mg/dL (RR = 0.99, CI: 0.73-1.34)[40]. A similar U-shaped relationship between serum bilirubin level and risk of developing CAD was observed in the Prospective Epidemiological Study of Myocardial Infarction (PRIME) study. In the PRIME study, 216 individuals who had developed CAD at 5-year follow up were designated as cases, and 434 individuals as matched controls. Individuals with bilirubin levels < 0.33 mg/dL and > 0.8 mg/dL were seen to have higher incidence of development of CAD, even after adjustment of other risk factors. However, the association between bilirubin levels > 0.8 mg/dL and the incidence of CAD was not statistically significant (OR = 0.68, CI: 0.34-1.39, $P = 0.29$). This U-shaped association was suggested as being due to reduced endogenous antioxidants with insufficient dietary intake[41]. The clinical significance of this U-shaped relation remains unclear.

Acet *et al*[42] investigated patients ($n = 360$) undergoing percutaneous coronary intervention (PCI) within 12 h of symptom onset with the aim to establish a relation between bilirubin levels and infarct-related artery patency in the setting of ST-segment elevation myocardial infarction (STEMI). The group with elevated total bilirubin was seen to have higher impaired flow (defined as pre-PCI TIMI $\leqslant$ 2 flow) than normal flow (Pre-PCI TIMI > 3) ($P < 0.001$). Furthermore, the in-hospital mortality and major adverse cardiac events were significantly higher in the high total bilirubin group ($P = 0.002$, $P < 0.001$ respectively). However, an important point to note is that the study did not exclude patients with elevated markers of hepatocellular injury thus making the true relationship between isolated elevated bilirubin levels and CAD difficult to interpret.

Ayaz *et al*[43] reported an independent positive association between bilirubin and left ventricular mass/hypertrophy in a population with untreated hypertension ($n = 114$). After performing a linear and logistical regression, total bilirubin ($P = 0.011$) was shown to be an independent risk factor for CAD. The authors attribute this to the suppression of reactive oxygen species[44]. A similar effect has been seen in pre-clinical studies

in rats, which showed a protective effect of elevated bilirubin on left ventricular hypertrophy in spontaneously hypertensive rats. They hypothesized the role of liver growth factor inhibition by bilirubin. However, this relationship still needs to be better elucidated.

In another study, 221 patients who were evaluated for CAD by coronary angiography showed a moderate but significant positive correlation between direct bilirubin levels and the Gensini score (r = 0.158, P = 0.019). However, no such significant correlation was demonstrated between total bilirubin and the Gensini score. This study was limited by its small sample size (n = 221)[5]. Authors interpreted that the relationship between bilirubin and CAD was unlikely causal. However, the cardiovascular risk factors in CAD have shown to be additive and in presence of several risk factors the beneficial effects of bilirubin might get masked. Thus, despite the results of this study, elevated serum bilirubin levels might confer protective effects in patients with favorable risk profile for CAD[5].

EXPERT COMMENTARY

While the available evidence regarding the effects of bilirubin are not definitive and different studies provide contradictory findings, some useful conclusions can be drawn.

First, it is possible that the protective effects seen with higher bilirubin levels are possibly mediated through heme oxygenase or by other substrates involved in the pathway of bilirubin production, namely, biliverdin and carbon monoxide. Although few studies have reported an inverse association between bilirubin and the risk of CAD, no such association was seen with *UGT1A1* gene polymorphism and the risk of CAD. Thus, a conclusion can be safely inferred that if at all bilirubin is protective in CAD, it is likely that bilirubin production (by induction of heme oxygenase and accompanied by production of carbon monoxide) and not just its excretion indirectly confers the protective effect observed with CAD. This would reflect as bilirubin having a protective effect on CAD whereas, in reality it is only a mediator or a marker.

Second, low bilirubin levels can be indicative of decreased heme oxygenase activity (a powerful anti-oxidant) or could be indicative of high oxidative stress in patients leading to consumption of the natural anti-oxidants including bilirubin. Hence, lower levels of bilirubin are perhaps not the causal factor for CAD but may indicate patients at an increased risk of developing CAD[45].

Third, bilirubin requires vitamin E as the co-oxidant, hence patients with a high bilirubin and deficiency of vitamin E, have less atheroprotective effect that weakens the inverse association between elevated bilirubin levels and the risk of CAD[46].

Also, Grosser *et al*[47-49] have reported induction of heme oxygenase with statin and aspirin therapy. Induction of heme oxygenase increases the bilirubin production. Individuals with *UGT1A1* gene variants have a lower capability of exclusion of bilirubin; hence, bilirubin accumulation is to be expected in individuals on statins and aspirin. Hence as per this hypothesis, patients with CAD or at an increased risk of CAD with *UGT1A1* gene polymorphism, on aspirin and statin should have increased levels of bilirubin. In that case, bilirubin might be looked upon as a marker of the drug activity.

Mendelian randomization is done to establish a causal relationship[50]. As mentioned above, a lack of significant association between the gene polymorphisms of UGT1A1 and risk for CAD goes in favor of bilirubin being a marker than a primary mediator for the cardioprotective effects observed with CAD. Moreover, it also points out towards incomplete penetrance of the *UGT1A1* gene. Inconsistent results further support the need for further exploration of the underlying mechanisms and a prospective study with a high power to establish a definite causal relationship between bilirubin levels and CAD.

REFERENCES

1 **Tenhunen R**, Marver HS, Schmid R. The enzymatic conversion of heme to bilirubin by microsomal heme oxygenase. *Proc Natl Acad Sci USA* 1968; **61**: 748-755 [PMID: 4386763 DOI: 10.1073/pnas.61.2.748]

2 **Pae HO**, Son Y, Kim NH, Jeong HJ, Chang KC, Chung HT. Role of heme oxygenase in preserving vascular bioactive NO. *Nitric Oxide* 2010; **23**: 251-257 [PMID: 20713168 DOI: 10.1016/j.niox.2010.08.002]

3 **Kundur AR**, Singh I, Bulmer AC. Bilirubin, platelet activation and heart disease: a missing link to cardiovascular protection in Gilbert's syndrome? *Atherosclerosis* 2015; **239**: 73-84 [PMID: 25576848 DOI: 10.1016/j.atherosclerosis.2014.12.042]

4 **Wagner KH**, Wallner M, Mölzer C, Gazzin S, Bulmer AC, Tiribelli C, Vitek L. Looking to the horizon: the role of bilirubin in the development and prevention of age-related chronic diseases. *Clin Sci* (Lond) 2015; **129**: 1-25 [PMID: 25881719 DOI: 10.1042/CS20140566]

5 **Vítek L**, Jirsa M, Brodanová M, Kalab M, Marecek Z, Danzig V, Novotný L, Kotal P. Gilbert syndrome and ischemic heart disease: a protective effect of elevated bilirubin levels. *Atherosclerosis* 2002; **160**: 449-456 [PMID: 11849670 DOI: 10.1016/S0021-9150(01)00601-3]

6 **VanWagner LB**, Green RM. Evaluating elevated bilirubin levels in asymptomatic adults. *JAMA* 2015; **313**: 516-517 [PMID: 25647209 DOI: 10.1001/jama.2014.12835]

7 **Erlinger S**, Arias IM, Dhumeaux D. Inherited disorders of bilirubin transport and conjugation: new insights into molecular mechanisms and consequences. *Gastroenterology* 2014; **146**: 1625-1638 [PMID: 24704527 DOI: 10.1053/j.gastro.2014.03.047]

8 **Strassburg CP**. Hyperbilirubinemia syndromes (Gilbert-Meulengracht, Crigler-Najjar, Dubin-Johnson, and Rotor syndrome). *Best Pract Res Clin Gastroenterol* 2010; **24**: 555-571 [PMID: 20955959 DOI: 10.1016/j.bpg.2010.07.007]

9 **Liao SL**. The role of bilirubin and phototherapy in the oxidative/antioxidant balance. *Pediatr Neonatol* 2015; **56**: 77-78 [PMID: 25735960 DOI: 10.1016/j.pedneo.2015.01.001]

10 **Wu TW**, Fung KP, Wu J, Yang CC, Weisel RD. Antioxidation of human low density lipoprotein by unconjugated and conjugated bilirubins. *Biochem Pharmacol* 1996; **51**: 859-862 [PMID: 8602883 DOI: 10.1016/0006-2952(95)02395-X]

11 **Baranano DE**, Rao M, Ferris CD, Snyder SH. Biliverdin reductase: a major physiologic cytoprotectant. *Proc Natl Acad Sci USA* 2002; **99**: 16093-16098 [PMID: 12456881 DOI: 10.1073/pnas.252626999]

12 **Tanındı A**, Erkan AF, Alhan A, Töre HF. Arterial stiffness and

central arterial wave reflection are associated with serum uric acid, total bilirubin, and neutrophil-to-lymphocyte ratio in patients with coronary artery disease. *Anatol J Cardiol* 2015; **15**: 396-403 [PMID: 25430407 DOI: 10.5152/akd.2014.5447]

13 **Liu J**, Wang L, Tian XY, Liu L, Wong WT, Zhang Y, Han QB, Ho HM, Wang N, Wong SL, Chen ZY, Yu J, Ng CF, Yao X, Huang Y. Unconjugated bilirubin mediates heme oxygenase-1-induced vascular benefits in diabetic mice. *Diabetes* 2015; **64**: 1564-1575 [PMID: 25475440 DOI: 10.2337/db14-1391]

14 **Cannon RO**. Role of nitric oxide in cardiovascular disease: focus on the endothelium. *Clin Chem* 1998; **44**: 1809-1819 [PMID: 9702990]

15 **Kang SJ**, Lee C, Kruzliak P. Effects of serum bilirubin on atherosclerotic processes. *Ann Med* 2014; **46**: 138-147 [PMID: 24720602 DOI: 10.3109/07853890.2014.895588]

16 **Vachharajani TJ**, Work J, Issekutz AC, Granger DN. Heme oxygenase modulates selectin expression in different regional vascular beds. *Am J Physiol Heart Circ Physiol* 2000; **278**: H1613-H1617 [PMID: 10775141]

17 **Akboga MK**, Canpolat U, Sahinarslan A, Alsancak Y, Nurkoc S, Aras D, Aydogdu S, Abaci A. Association of serum total bilirubin level with severity of coronary atherosclerosis is linked to systemic inflammation. *Atherosclerosis* 2015; **240**: 110-114 [PMID: 25770689 DOI: 10.1016/j.atherosclerosis.2015.02.051]

18 **Canpolat U**, Aytemir K, Yorgun H, Hazırolan T, Kaya EB, Şahiner L, Sunman H, Tokgözoğlu L, Kabakcı G, Oto A. Association of serum total bilirubin levels with the severity, extent and subtypes of coronary atherosclerotic plaques detected by coronary CT angiography. *Int J Cardiovasc Imaging* 2013; **29**: 1371-1379 [PMID: 23546648 DOI: 10.1007/s10554-013-0209-7]

19 **Bisht K**, Wegiel B, Tampe J, Neubauer O, Wagner KH, Otterbein LE, Bulmer AC. Biliverdin modulates the expression of C5aR in response to endotoxin in part via mTOR signaling. *Biochem Biophys Res Commun* 2014; **449**: 94-99 [PMID: 24814708 DOI: 10.1016/j.bbrc.2014.04.150]

20 **Kelsen S**, Patel BJ, Parker LB, Vera T, Rimoldi JM, Gadepalli RS, Drummond HA, Stec DE. Heme oxygenase attenuates angiotensin II-mediated superoxide production in cultured mouse thick ascending loop of Henle cells. *Am J Physiol Renal Physiol* 2008; **295**: F1158-F1165 [PMID: 18701634 DOI: 10.1152/ajprenal.00057.2008]

21 **Hu CM**, Chen YH, Chiang MT, Chau LY. Heme oxygenase-1 inhibits angiotensin II-induced cardiac hypertrophy in vitro and in vivo. *Circulation* 2004; **110**: 309-316 [PMID: 15226216 DOI: 10.1161/01.CIR.0000135475.35758.23]

22 **Li Volti G**. Letter regarding article by Hu et al, "heme oxygenase-1 inhibits angiotensin II-induced cardiac hypertrophy in vitro and in vivo". *Circulation* 2005; **111**: 100 [PMID: 15710770 DOI: 10.1161/01.CIR.0000155250.27345.02]

23 **Otterbein LE**, Soares MP, Yamashita K, Bach FH. Heme oxygenase-1: unleashing the protective properties of heme. *Trends Immunol* 2003; **24**: 449-455 [PMID: 12909459 DOI: 10.1016/S1471-4906(03)00181-9]

24 **Ockner RK**. Laboratory tests in liver disease. In: Wyngaarden JB, Smith LI (eds.). Cecil textbook of medicine. 16th ed. Philadelphia: WB Saunders, 1982: 775-778

25 **Routh JI.** Liver function. 1n: Tietz NW (eds.). Fundamentals of clinical chemistry. Philadelphia: WB Saunders, 1976: 1026-1062

26 **Schwertner HA**, Jackson WG, Tolan G. Association of low serum concentration of bilirubin with increased risk of coronary artery disease. *Clin Chem* 1994; **40**: 18-23 [PMID: 8287538]

27 **Hopkins PN**, Wu LL, Hunt SC, James BC, Vincent GM, Williams RR. Higher serum bilirubin is associated with decreased risk for early familial coronary artery disease. *Arterioscler Thromb Vasc Biol* 1996; **16**: 250-255 [PMID: 8620339 DOI: 10.1161/01.ATV.16.2.250]

28 **Novotný L**, Vítek L. Inverse relationship between serum bilirubin and atherosclerosis in men: a meta-analysis of published studies. *Exp Biol Med* (Maywood) 2003; **228**: 568-571 [PMID: 12709588]

29 **Erdogan T**, Ciçek Y, Kocaman SA, Canga A, Cetin M, Durakoglugil E, Satiroglu O, Temiz A, Ergül E, Sahin I, Bostan M. Increased serum bilirubin level is related to good collateral development in patients with chronic total coronary occlusion. *Intern Med* 2012; **51**: 249-255 [PMID: 22293798 DOI: 10.2169/internalmedicine.51.6417]

30 **Wei S**, Gao C, Wei G, Chen Y, Zhong L, Li X. The level of serum bilirubin associated with coronary lesion types in patients with coronary artery disease. *J Cardiovasc Med* (Hagerstown) 2012; **13**: 432-438 [PMID: 21799439 DOI: 10.2459/JCM.0b013e32834a3967]

31 **Stojanov M**, Stefanovic A, Dzingalasevic G, Ivanisevic J, Miljkovic M, Mandic-Radic S, Prostran M. Total bilirubin in young men and women: association with risk markers for cardiovascular diseases. *Clin Biochem* 2013; **46**: 1516-1519 [PMID: 23827733 DOI: 10.1016/j.clinbiochem.2013.06.020]

32 **Song YS**, Koo BK, Cho NH, Moon MK. Effect of low serum total bilirubin levels (≤0.32 mg/dl) on risk of coronary artery disease in patients with metabolic syndrome. *Am J Cardiol* 2014; **114**: 1695-1700 [PMID: 25288499 DOI: 10.1016/j.amjcard.2014.08.043]

33 **Mahabadi AA**, Lehmann N, Möhlenkamp S, Kälsch H, Bauer M, Schulz R, Moebus S, Jöckel KH, Erbel R, Heusch G. Association of bilirubin with coronary artery calcification and cardiovascular events in the general population without known liver disease: the Heinz Nixdorf Recall study. *Clin Res Cardiol* 2014; **103**: 647-653 [PMID: 24633678 DOI: 10.1007/s00392-014-0697-z]

34 **Lin JP**, O'Donnell CJ, Schwaiger JP, Cupples LA, Lingenhel A, Hunt SC, Yang S, Kronenberg F. Association between the UGT1A1*28 allele, bilirubin levels, and coronary heart disease in the Framingham Heart Study. *Circulation* 2006; **114**: 1476-1481 [PMID: 17000907 DOI: 10.1161/CIRCULATIONAHA.106.633206]

35 **Hunt SC,** Kronenberg F, Eckfeldt JH, Hopkins PN, Myers RH, Heiss G. Association of plasma bilirubin with coronary heart disease and segregation of bilirubin as a major gene trait: the NHLBI family heart study. *Atherosclerosis* 2001; **154**: 747-754 [PMID: 11257278 DOI: 10.1016/S0021-9150(00)00420-2]

36 **Bosma PJ**, van der Meer IM, Bakker CT, Hofman A, Paul-Abrahamse M, Witteman JC. UGT1A1*28 allele and coronary heart disease: the Rotterdam Study. *Clin Chem* 2003; **49**: 1180-1181 [PMID: 12816916 DOI: 10.1373/49.7.1180]

37 **Lingenhel A**, Kollerits B, Schwaiger JP, Hunt SC, Gress R, Hopkins PN, Schoenborn V, Heid IM, Kronenberg F. Serum bilirubin levels, UGT1A1 polymorphisms and risk for coronary artery disease. *Exp Gerontol* 2008; **43**: 1102-1107 [PMID: 18790042 DOI: 10.1016/j.exger.2008.08.047]

38 **Hsieh CJ**, Chen MJ, Liao YL, Liao TN. Polymorphisms of the uridine-diphosphoglucuronosyltransferase 1A1 gene and coronary artery disease. *Cell Mol Biol Lett* 2008; **13**: 1-10 [PMID: 17952380 DOI: 10.2478/s11658-007-0030-1]

39 **Rantner B**, Kollerits B, Anderwald-Stadler M, Klein-Weigel P, Gruber I, Gehringer A, Haak M, Schnapka-Köpf M, Fraedrich G, Kronenberg F. Association between the UGT1A1 TA-repeat polymorphism and bilirubin concentration in patients with intermittent claudication: results from the CAVASIC study. *Clin Chem* 2008; **54**: 851-857 [PMID: 18375480 DOI: 10.1373/clinchem.2007.102046]

40 **Breimer LH**, Wannamethee G, Ebrahim S, Shaper AG. Serum bilirubin and risk of ischemic heart disease in middle-aged British men. *Clin Chem* 1995; **41**: 1504-1508 [PMID: 7586525]

41 **Troughton JA**, Woodside JV, Young IS, Arveiler D, Amouyel P, Ferrières J, Ducimetière P, Patterson CC, Kee F, Yarnell JW, Evans A. Bilirubin and coronary heart disease risk in the Prospective Epidemiological Study of Myocardial Infarction (PRIME). *Eur J Cardiovasc Prev Rehabil* 2007; **14**: 79-84 [PMID: 17301631 DOI: 10.1097/01.hjr.0000230097.81202.9f]

42 **Acet H**, Ertş F, Akıl MA, Polat N, Aydın M, Akyüz A, Ayçiçek H, Alan S. A novel predictor of infarct-related artery patency before percutaneous intervention and in-hospital outcomes for ST-segment elevation myocardial infarction patients: serum bilirubin level. *Postepy Kardiol Interwencyjnej* 2014; **10**: 91-97 [PMID: 25061454 DOI: 10.5114/pwki.2014.43513]

43 **Ayaz T**, Durakoğlugil ME, Kocaman SA, Durakoğlugil T, Erdoğan T, Sahin OZ, Sahin SB, Çiçek Y, Satiroğlu O. Bilirubin Level is Associated with Left Ventricular Hypertrophy Independent of Blood Pressure in Previously Untreated Hypertensive Patients. *Korean Circ J* 2014; **44**: 336-343 [PMID: 25278987 DOI: 10.4070/

kcj.2014.44.5.336]
44 **Ndisang JF**, Jadhav A. Upregulating the heme oxygenase system suppresses left ventricular hypertrophy in adult spontaneously hypertensive rats for 3 months. *J Card Fail* 2009; **15**: 616-628 [PMID: 19700139 DOI: 10.1016/j.cardfail.2009.02.003]
45 **Lin R**, Wang Y, Wang Y, Fu W, Zhang D, Zheng H, Yu T, Wang Y, Shen M, Lei R, Wu H, Sun A, Zhang R, Wang X, Xiong M, Huang W, Jin L. Common variants of four bilirubin metabolism genes and their association with serum bilirubin and coronary artery disease in Chinese Han population. *Pharmacogenet Genomics* 2009; **19**: 310-318 [PMID: 19238116 DOI: 10.1097/FPC.0b013e328328f818]
46 **Neuzil J**, Stocker R. Free and albumin-bound bilirubin are efficient co-antioxidants for alpha-tocopherol, inhibiting plasma and low density lipoprotein lipid peroxidation. *J Biol Chem* 1994; **269**: 16712-16719 [PMID: 8206992]
47 **Grosser N**, Abate A, Oberle S, Vreman HJ, Dennery PA, Becker JC, Pohle T, Seidman DS, Schröder H. Heme oxygenase-1 induction may explain the antioxidant profile of aspirin. *Biochem Biophys Res Commun* 2003; **308**: 956-960 [PMID: 12927812 DOI: 10.1016/S0006-291X(03)01504-3]
48 **Grosser N**, Hemmerle A, Berndt G, Erdmann K, Hinkelmann U, Schürger S, Wijayanti N, Immenschuh S, Schröder H. The antioxidant defense protein heme oxygenase 1 is a novel target for statins in endothelial cells. *Free Radic Biol Med* 2004; **37**: 2064-2071 [PMID: 15544924 DOI: 10.1016/j.freeradbiomed.2004.09.009]
49 **Grosser N**, Erdmann K, Hemmerle A, Berndt G, Hinkelmann U, Smith G, Schröder H. Rosuvastatin upregulates the antioxidant defense protein heme oxygenase-1. *Biochem Biophys Res Commun* 2004; **325**: 871-876 [PMID: 15541371 DOI: 10.1016/j.bbrc.2004.10.123]
50 **Relton CL**, Davey Smith G. Two-step epigenetic Mendelian randomization: a strategy for establishing the causal role of epigenetic processes in pathways to disease. *Int J Epidemiol* 2012; **41**: 161-176 [PMID: 22422451 DOI: 10.1093/ije/dyr233]

P- Reviewer: Canpolat U, Jorgensen ME, Zhou T **S- Editor**: Qi Y
L- Editor: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i4.477

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 477-489
ISSN 2150-5349 (online)

REVIEW

Local ablative treatments for hepatocellular carcinoma: An updated review

Antonio Facciorusso, Gaetano Serviddio, Nicola Muscatiello

Antonio Facciorusso, Nicola Muscatiello, Gastroenterology Unit, Department of Medical and Surgical Sciences, University of Foggia, 71100 Foggia, Italy

Gaetano Serviddio, Internal Medicine Unit, University of Foggia, 71100 Foggia, Italy

Author contributions: Muscatiello N performed the bibliographic research; Facciorusso A wrote the paper; Serviddio G revised the manuscript; all the authors contributed to the article.

Conflict-of-interest statement: None of the authors have received fees for serving as a speaker or are consultant/advisory board member for any organizations. None of the authors have received research funding from any organizations. None of the authors are employees of any organizations. None of the authors own stocks and/or share in any organizations. None of the authors own patents. None of the authors has conflicts of interest to declare.

Manuscript source: Invited manuscript

Correspondence to: Antonio Facciorusso, MD, Gastroenterology Unit, Department of Medical and Surgical Sciences, University of Foggia, Viale L. Pinto, 1, 71100 Foggia, Italy. antonio.facciorusso@virgilio.it
Telephone: +39-0881-732154
Fax: +39-0881-732135

Received: May 22, 2016
Peer-review started: May 23, 2016
First decision: July 4, 2016
Revised: July 16, 2016
Accepted: August 17, 2016
Article in press: August 19, 2016

Published online: November 6, 2016

Abstract

Ablative treatments currently represent the first-line option for the treatment of early stage unresectable hepatocellular carcinoma (HCC). Furthermore, they are effective as bridging/downstaging therapies before orthotopic liver transplantation. Contraindications based on size, number, and location of nodules are quite variable in literature and strictly dependent on local expertise. Among ablative therapies, radiofrequency ablation (RFA) has gained a pivotal role due to its efficacy, with a reported 5-year survival rate of 40%-70%, and safety. Although survival outcomes are similar to percutaneous ethanol injection, the lower local recurrence rate stands for a wider application of RFA in hepato-oncology. Moreover, RFA seems to be even more cost-effective than liver resection for very early HCC (single nodule $\leqslant$ 2 cm) and in the presence of two or three nodules $\leqslant$ 3 cm. There is increasing evidence that combining RFA to transarterial chemoembolization may increase the therapeutic benefit in larger HCCs without increasing the major complication rate, but more robust prospective data is still needed to validate these pivotal findings. Among other thermal treatments, microwave ablation (MWA) uses high frequency electromagnetic energy to induce tissue death *via* coagulation necrosis. In comparison to RFA, MWA has several theoretical advantages such as a broader zone of active heating, higher temperatures within the targeted area in a shorter treatment time and the lack of heat-sink effect. The safety concerns raised on the risks of this procedure, due to the broader and less predictable necrosis areas, have been recently overcome. However, whether MWA ability to generate a larger ablation zone will translate into a survival gain remains unknown. Other treatments, such as high-intensity focused ultrasound ablation, laser ablation, and cryoablation, are less investigated but showed promising results in early HCC patients and could be a valuable therapeutic option in the

next future.

Key words: Liver cancer; Hepatocellular carcinoma; Radiofrequency ablation; Microwave ablation; Radiofrequency ablation

Core tip: Ablative treatments currently represent the first-line option for the treatment of early stage unresectable hepatocellular carcinoma. Among ablative therapies, radiofrequency ablation has gained a pivotal role due to its efficacy, with a reported 5-year survival rate of 40%-70%, and safety. Among other thermal treatments, microwave ablation, high-intensity focused ultrasound ablation, laser ablation, and cryoablation, are less investigated but showed promising results.

Facciorusso A, Serviddio G, Muscatiello N. Local ablative treatments for hepatocellular carcinoma: An updated review. *World J Gastrointest Pharmacol Ther* 2016; 7(4): 477-489 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i4/477.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i4.477

INTRODUCTION

Hepatocellular carcinoma (HCC) represents a life-threatening condition and constitutes the main cause of death among cirrhotic patients[1,2]. In the last years, the accurate screening programs and more refined diagnostic imaging have made early HCC diagnosis feasible in 30%-60% of cases[3].

Local ablation represents the standard of care for patients at early stage, who are not suitable to surgery or orthotopic liver transplantation (OLT). Among ablative treatments, thermal ablative therapies have gained an increasing role in the last decade due to their efficacy in preventing local recurrence as well as in prolonging overall survival (OS). Thermal ablative treatments are classified as hyperthermic, such as radiofrequency ablation (RFA), microwave ablation (MWA), high-intensity focused ultrasound (HIFU) or laser therapy, or hypothermic such as cryoablation.

These procedures are usually performed by means of a percutaneous approach but in particular conditions (for instance in cases of nodules in "at-risk" location) laparoscopic ablation may be recommended.

In this review we aim to provide a comprehensive overview on the main thermal therapies for HCC with the up-to-date data on their efficacy and safety.

INDICATION TO TREATMENT

Thermal ablative treatments represent the standard of care for unresectable HCC in very early/early stage according to Barcelona Clinic Liver Cancer (BCLC) system[2,4]. The term "unresectable" covers a broad spectrum of pathological conditions, from single nodule in a deep location (therefore not easy to treat by surgery) to multinodular disease in patients with deteriorated liver function. Therefore, percutaneous therapies are a valuable option in non-optimal candidates to surgery due to tumor size, number, location, liver function, or comorbidities.

Another indication to thermal treatment is the pre-transplant setting, where RFA has been proved to be effective both as downstaging and as bridging therapy[5-7].

Main absolute and relative contraindications to thermal treatments are described in Table 1. Absolute contraindications, shared with other locoregional treatments, are the presence of extrahepatic liver disease, altered mental status, active infection, tumor abutting a major hepatic duct, impaired liver function (particularly in presence of ascites); relative contraindications are more than 4 nodules or at least one lesion > 5 cm, severe cardiopulmonary disease and refractory coagulopathy[8].

MECHANISM OF ACTION AND EQUIPMENT OF RFA

The mechanism of action of RFA relies on the destruction of tumoral tissue by the radiofrequency-generated heat. In particular, the injury is due to frictional heat produced by the ionic agitation of particles within tissue as a consequence of the application of alternating current[9-13].

The electrical current in the radiofrequency range (200-1200 MHz) is transmitted by a needle electrode under imaging guidance (usually ultrasonography) and the electrical circuit is completed through grounding pads attached to the thighs or back of the patient. The needle is partially insulated and presents an activated tip that is not insulated. This tip varies in length with the most common size being 3 cm long. Tips may be singular and straight or consisting of an array of expandable tines that form an umbrella fully encompassing the nodule when deployed.

An important aim of the treatment should be to ensure thermal destruction not only of the tumoral nodule but also of a surrounding margin about 1 cm long in order to ablate eventual microsatellites thus preventing local recurrence.

In order to reach this target, multiple electrodes can be applied thus achieving a broader ablation zone and allowing ablation of nodules up to 4-5 cm.

Another aspect to be considered is the "heat-sink effect", namely the dissipation of the thermal output by blood flowing through adjacent vessels thereby decreasing the efficacy of the procedure[14]. This is the reason why nodules close to major vessel are considered a suboptimal target and constitute a relative contraindication for RFA.

Table 1 Contraindications to thermal ablative treatments

Absolute contraindications
Extrahepatic disease
Altered mental status
Active infection
Tumor abutting a major hepatic duct
Liver decompensation (particularly in presence of ascites)
Relative contraindications
Lesions > 5 cm
More than four lesions
Severe pulmonary or cardiac disease
Refractory coagulopathy

The procedure is usually performed under sedation when the percutaneous approach is preferred. In cases of laparoscopic RFA, to be considered in cases of nodules close to the liver capsule or other organs, general anesthesia is needed[15].

SURVIVAL OUTCOMES AFTER RFA FOR HCC

A large number of studies have confirmed the efficacy of RFA in early HCC patients suggesting this procedure as viable therapeutic option in unresectable early stage. Considering the state-of-art of the literature, RFA provided 5-year survival rates of 40%-70% and beyond in HCC series[9,10].

A recent Chinese study reported OS rates of 96.6%, 60.2%, and 27.3% at 1-, 5-, and 10-year[16], similar to those reported by Kim *et al*[17] which were 95.5%, 59.7% and 32.3%, respectively. These results are concordant with other recent Western studies conducted in Milan-in patients (87.0%-99.0% at 1 year, 60.0%-87.4% at 3 years, and 42.3%-74.8% at 5 years)[18,19].

Several studies pointed out different predictors of survival, such as Child-Pugh (CP) score, initial response, serum ferritin, number or size of nodules and AFP levels[19-21].

Our group has recently analyzed predictors of post-recurrence survival (PRS) after RFA, namely the survival time elapsed after tumor recurrence[18]. We found, in line with other studies, baseline CP score, AFP levels and performance status (PS) as predictors of OS in multivariate analysis. However, analysis of PRS showed that in addition to CP score and PS, also tumor burden at the time of recurrence and recurrence pattern significantly influenced PRS[18]. Interestingly, AFP level, one of the main predictors of survival at baseline, became non-significant when evaluated at tumor relapse, confirming the difference between predictors of OS assessed at baseline and at tumor relapse[18].

Of note, local recurrence (LR) did not impact significantly on OS in our study[18] as well as in other reports[17,21,22], probably due to the frequent multi-focality of distant recurrences that makes more difficult the therapeutic approach, while local recurrences, even when multifocal, are confined in one liver segment (namely the same as that previously treated) and may be more easily treated with RFA or a single selective transarterial chemoembolization (TACE) session.

Unlike OS, reported rates of LR after RFA are not univocal ranging from 3.2% to 27% at 5 years[16-21], maybe because of different etiologies of HCC in the published series, different approaches to the problem of insufficient ablative margins, use of combined treatment with TACE and, above all, different definition of radiologic tumor recurrence at imaging. As expected, tumor features such as nodules number, size, histopathological grading, and AFP have been found to be predictors of recurrence[16-21]. Moreover, an insufficient ablation margin after the treatment appear to be an important prognostic factor for LR[23,24].

Intrahepatic distant recurrences are common, ranging from 68% to 74% at 5 years[16-19,21], and are usually associated to poorer prognosis. This type of recurrence is mostly induced by underlying hepatic disease and is often observed after 2 years, which is the time point considered able to differentiate between real recurrences from de novo tumors occurred in the pro-tumorigenic milieu of liver cirrhosis[25].

Therefore, because of their high frequency and aggressive behavior, distal recurrences are a major determinant of patient survival.

PREVENTION OF RECURRENCE AFTER RFA

The issue of the high rates of post-RFA tumor relapse has recently pushed great efforts in studying adjuvant drugs aimed at decreasing the heavy burden of HCC recurrence after ablation.

Although earlier reports showed interesting results[26,27] and in spite of the theoretical beneficial role of sorafenib (Nexavar®, Bayer, Leverkusen, Germany) as adjuvant therapy, an important multicenter randomised controlled trial (RCT) [Sorafenib as Adjuvant Treatment in the Prevention Of Recurrence of Hepatocellular Carcinoma (STORM)], recruiting 1114 HCC patients after surgery or radiofrequency ablation, failed to meet its primary endpoint, namely recurrence-free survival [hazard ratio (HR): 0.940, 95%CI: 0.78-1.13, P = 0.26] and OS (HR: 0.99, 95%CI: 0.76-1.30, P = 0.48)[28]. This daunting finding was at least in part due to the high treatment discontinuation rate (24% *vs* 7% of placebo) and consent withdrawal (17% *vs* 6%) in the sorafenib arm, mainly because of severe adverse events[28].

Similarly, interferon was proven unhelpful as adjuvant treatment because of the high cost and the narrow therapeutic window[29,30].

Therefore, most of the recent research in this field has focused on other drugs. On the basis of the well-described pro-tumorigenic and pro-fibrogenic properties of angiotensin Ⅱ, due to the induction of vascular endothelial growth factor and transforming growth

factor-beta 1 release[31,32], a number of studies have reported significantly reduced HCC relapse rates after RFA when angiotensin converting enzyme inhibitor (ACE Ⅰ) were used in combination with other agents such as branched-chain amino acids or vitamin K[33-35]. However, ACE I did not prove effective in monotherapy and, above all, no significant difference in OS was registered as compared to the control arm[33-35].

Our group has recently published a retrospective report conducted in 153 HCC patients treated with RFA finding a significant benefit both in terms of recurrence and OS in hypertensive subjects in treatment with angiotensin Ⅱ type 1 receptor blockers (sartans) as compared to those under ACE Ⅰ therapy and to non-hypertensive subjects[36]. The apparent superiority of sartans over ACE Ⅰ may be due to the selective inhibition of angiotensin Ⅱ receptor 1, responsible of the pro-fibrogenic and pro-angiogenic activity of angiotensin, while pro-apoptotic and anti-tumorigenic activity of receptor 2 is preserved and even enhanced in patients administered sartans unlike ACE Ⅰ which prevent the binding of angiotensin Ⅱ to both receptors[37]. However, these preliminary results still need further confirmation.

In conclusion, in spite of the great amount of published reports and in absence of broad RCTs, clear evidence in favor of an adjuvant treatment after RFA is still lacking.

ADVERSE EVENTS OF RFA

In a recent systematic review of 9531 patients treated with RFA, treatment-related severe adverse events were registered in 4.1% of cases with a mortality rate of 0.15%[38].

Adverse events include gastrointestinal tract injury with/without perforation (0.06%-0.3%), diaphragm injury (0.03%), pleural effusion (0.2%-2.3%), bile duct stricture (0.06%-0.5%), biloma (0.06%-0.96%), gallbladder injury (0.06%-0.1%), and hepatic infarction (0.03%-0.06%). Other complications, related to direct mechanical injury, are tumor seeding (0.27%), tumor rupture (0.3%), hemoperitoneum (0.3%-1.6%), and hemo/pneumothorax (0.15%-0.8%). Events not related to mechanical or thermal injury to the liver are hepatic abscess (0.1%), grounding pad burn (0.6%), and vasovagal reflex (0.1%)[39]. However, all these complications are not common and RFA can be considered a safe procedure in high-volume centers when proper indications to treatment are followed.

RFA IN PRE-TRANSPLANT SETTING

RFA has gained increasing interest either as bridging and as downstaging therapy prior to transplantation in hepatocarcinoma patients. A number of papers have reported complete pathological response rates (*i.e.*, complete nodule assessed by the pathologist on the explanted liver) up to 47%-75%[5-7,40,41].

In particular, this response was observed in 50%-78% of nodules within 3 cm and between 13% and 43% in larger nodules[5-7,40,41] *vs* 27%-57% of TACE in Milan-in patients[42,43].

Safety concerns previously raised by some authors due to the theoretical risk of tumoral seeding, reported to occur in about 3% of cases[44], have been recently overcome[45]. Therefore, although TACE remains the most used treatment before OLT, RFA has to be preferred in cases of single nodules under 3 cm as provides higher complete necrosis rates and lower risk of recurrence after transplantation[46].

RFA *VS* LIVER RESECTION FOR HCC

Surgery is the first-line option in very early/early patients not fulfilling transplant criteria[2-4]. By the way, no more than 10%-35% of patients are actually suitable to surgery due to tumoral burden, inadequate liver reserve, or poor performance status[2-4]. These patients may be offered RFA as viable option because of its proven efficacy.

The aforementioned striking results of RFA have recently opened debates on whether RFA can be offered particularly in very early patients (namely, those with a single nodule less than 2 cm) as first-line therapy instead of surgery. To address this point, many research groups have conducted retrospective or randomized controlled studies directly comparing the two treatments.

Table 2 reports the main characteristics of the four RCTs[47-50] comparing the two treatments published so far. As one can read in Table 2, the available RCTs report discordant results with the sole study by Huang *et al*[48] demonstrating a superiority of hepatic resection over RFA. However, the different proportions of nodules larger than 2 cm are likely to be responsible of these discordant results, as RFA is recognized as less effective beyond very early stage.

None of the aforementioned RCTs restricted their analysis to single nodules ⩽ 2 cm, while there are five observational studies focused on this specific setting[51-55]. Unfortunately, most of these retrospective studies suffer from selection bias as RFA patients tended to be older and to present more deteriorated liver function than surgical ones, while larger nodules were more likely to be treated with resection. Therefore, OS and relapse outcomes can be biased by covariate distribution. Two of these studies, which tried to obviate to such a bias through propensity score one-to-one match, reported better DFS in surgical patients ($P = 0.031$ and $P < 0.001$) but discordant results with regard to overall survival ($P = 0.296$ and $P = 0.034$, respectively)[52,55]. However, several concerns have been raised on the rigorousness of the statistical procedure adopted, hence such findings require further confirmation[56]. The low level of evidence impairs the findings of several meta-analyses published in this field, which mostly support the superiority of hepatic resection over RFA in early stage without significant differences in single nodules less than 2 cm[57,58].

An interesting study conducted by the Bologna

Table 2 Randomized controlled trials comparing radiofrequency ablation and surgery in hepatocellular carcinoma patients

Ref.	Liver function	Tumor features	Treatment	3-yr SR	5-yr SR	3-yr DFS	5-yr DFS
Chen *et al*[47]	CP A	Single < 5 cm	HR 90	73.40%	NA	69.00%	NA
	ICG-R15 < 30%		RFA 71	71.40%	NA	64.10%	NA
	PLT > 40000/mm^3						
Huang *et al*[48]	CP A/B	Within MC	HR 115	92.20%	75.70%	60.90%	51.30%
	ICG-R15 < 20%		RFA 115	69.60%	54.80%	46.10%	28.70%
	PLT > 50000/mm^3						
		Single ⩽ 3 cm	HR 45	95.60%	82.20%	NA	NA
			RFA 57	77.20%	61.40%	NA	NA
		Single 3-5 cm	HR 44	95.50%	72.30%	NA	NA
			RFA 27	66.70%	51.50%	NA	NA
		Multifocal < 3 cm	HR 26	80.80%	69.20%	NA	NA
			RFA 31	58.10%	45.20%	NA	NA
Feng *et al*[49]	CP A/B	Up to 2 nodules < 4 cm	HR 84	74.80%	NA	61.10%	NA
	ICG-R15 < 30%		RFA 84	67.20%	NA	49.60%	NA
	PLT > 50000 mm^3						
Fang *et al*[50]	CP A/B	Up to 3 nodules ⩽ 3 cm	HR 60	77.50%	NA	41.30%	NA
	PLT > 50000 mm^3		RFA 60	82.50%	NA	55.40%	NA

SR: Survival rate; DFS: Disease-free survival; CP: Child-Pugh; ICG-R15: Indocyanin green retention at 15 min; PLT: Platelets; HR: Hepatic resection; RFA: Radiofrequency ablation; NA: Not available; MC: Milan criteria.

group, based on a Markov model and a Monte Carlo probabilistic sensitivity analysis, demonstrated that in a 10-year perspective RFA provided similar life-expectancy and quality-adjusted life-expectancy (QALY) at a lower cost than surgery in very early HCC patients, hence it was the most cost-effective therapeutic strategy for this stage[59]. In the case of 2 or 3 tumors ⩽ 3 cm, life-expectancy and QALY were very similar between surgery and RFA, but cost-effectiveness was again in favor of RFA[59]. Therefore, the authors concluded that RFA is more cost-effective than surgery in cases of single nodule under 2 cm or 2/3 nodules ⩽ 3 cm, while liver surgery still represents the most valuable option for single larger early stage HCCs[59].

In conclusion, as supported by a decision-making analysis performed by the same group, the superiority or equivalence of a treatment over the other is strictly dependent on the non-linear relationship among tumor number, size and liver function, with RFA to be preferred in cases of smaller tumors and impaired liver function[60].

RFA *VS* PERCUTANEOUS ETHANOL INJECTION IN EARLY HCC PATIENTS

Percutaneous ethanol injection (PEI) is a well-established technique for the treatment of small HCCs and induces coagulative necrosis as a result of cellular dehydration and protein denaturation. However, ethanol diffusion is likely to be impaired by intratumoral fibrotic septa in cases of nodules > 2 cm.

In fact, the efficacy of such a technique in early stage (namely, multiple nodules or single nodule larger than 2 cm) is considerably inferior as compared to RFA with a complete necrosis rate of 70% in nodules of 2-3 cm and 50% in those between 3 and 5 cm[61,62]. On the other hand, RFA showed a significantly higher necrosis rate, up to 71% in non-infiltrating medium-size (*i.e.*, between 3 and 5 cm) nodules[63]. In our recently published experience, overall complete necrosis rate after RFA was 84.4% in a series whose median tumor size was 3 cm[18,20].

However, if it is widely recognized the superiority of RFA over PEI in medium-size and large nodules, a clear advantage in term of survival in small HCCs (less than 3 cm) is still unclear.

In fact, a recent meta-analysis of 8 RCTs found better survival outcomes (HR: 0.67, 95%CI: 0.51-0.87, P < 0.001) and a lower 3-year LR rate [risk ratio (RR): 0.41, 95%CI: 0.30-0.57, P < 0.01] after RFA as compared to PEI[64], but sensitivity analysis confirmed the superiority of RFA only in Asian studies[65-69] while the three included Italian studies[70-72] found only a non-significant trend in favor of RFA as for survival (HR: 0.82, 95%CI: 0.56-1.20, P = 0.30)[64]. Table 3 summarizes the main findings of the aforementioned trials. Quite interestingly, RFA provided similar if not better results as compared to PEI requiring a significant lower number of sessions (Table 3). This aspect has to be taken into account since, although a single PEI treatment has significantly lower costs than RFA, the higher number of PEI sessions makes this benefit vanish and increases the risk of tumoral seeding.

The above described results are in keeping with another systematic review of four RCTs comparing the two techniques in small HCCs under 3 cm which, however, found RFA associated to higher major complication rates and to be more costly than PEI[73].

In conclusion, although whether RFA leads to better survival rates than PEI in small HCCs is still matter of debate, the lower local recurrence rate stands for a wider

Table 3 Randomized controlled trials comparing radiofrequency ablation and percutaneous ethanol injection in hepatocellular carcinoma patients

Ref.	Region	Patients (*n*)	Nodules *n* (1/>1)	Tumor size, cm	No. of sessions	Complete response (%)	3-yr survival (%)	3-yr recurrence (%)
Lin *et al*[65]	Taiwan	RFA (52)	38/14	2.9 ± 0.8	1.6 ± 0.4	96.0	74	18.0
		PEI (52)	40/12	2.8 ± 0.8	6.5 ± 1.6	88.0	50	45.0
Lin *et al*[66]	Taiwan	RFA (62)	49/13	2.5 ± 1.0	1.3 ± 0.3	96.1	74	14.0
		PEI (62)	49/13	2.3 ± 0.8	4.9 ± 1.3	88.1	51	34.0
Shiina *et al*[67]	Japan	RFA (118)	72/46	NA	2.1 ± 1.3	100.0	81	1.7
		PEI (114)	60/54	NA	6.4 ± 2.6	100.0	66	11.0
Wang *et al*[68]	China	RFA (49)	NA	2.4 ± 1.2	NA	93.8	NA	NA
		PEI (49)	NA	2.3 ± 1.4	NA	77.5	NA	NA
Azab *et al*[69]	Egypt	RFA (30)	NA	NA	1.45	85.0	NA	NA
		PEI (30)	NA	NA	7.68	75.0	NA	NA
Giorgio *et al*[70]	Italy	RFA (128)	128/0	2.3 ± 0.4	5.00	100.0	83	7.8
		PEI (143)	143/0	2.2 ± 0.5	8.00	100.0	78	9.4
Lencioni *et al*[71]	Italy	RFA (52)	40/12	2.8 ± 0.6	1.1 ± 0.5	91.0	NA	21.0
		PEI (50)	31/19	2.8 ± 0.8	5.4 ± 1.6	82.0	NA	59.0
Brunello *et al*[72]	Italy	RFA (70)	54/16	2.4 ± 0.5	NA	95.7	59	NA
		PEI (69)	54/15	2.2 ± 0.5	NA	65.6	56	NA

RFA: Radiofrequency ablation; PEI: Percutaneous ethanol injection; NA: Not available.

application of RFA in hepato-oncology.

COMBINED TREATMENT

There is increasing evidence that combining RFA to TACE may increase the therapeutic benefit in larger HCCs. In fact, the two techniques may exert a synergistic effect on inducing nodule necrosis: Occlusion of the tumor arterial supply by TACE would increase the area of coagulation necrosis obtained by RFA minimizing heat loss whereas the heating-related reactive hyperemia induced by RFA would concentrate the chemotherapeutic agent released during TACE in the peripheral residual viable neoplastic tissue and would reduce cell resistance to the drug[74].

A recent meta-analysis of eight RCTs[75-82] including 598 patients indicated that RFA plus TACE determines a significantly higher 3-year OS rate [odds ratio (OR): 2.65, 95%CI: 1.81-3.86, $P < 0.001$] and 3-year RFS rate (OR: 3.00, 95%CI: 1.75-5.13, $P < 0.001$) than RFA alone, with no difference in major complications (OR: 1.20, 95%CI: 0.31-4.62, $P = 0.79$)[83]. Subgroups analysis revealed that most of this benefit was obtained in patients with intermediate- and large-size HCCs, which are likely to be the optimal setting for the combined treatment[83]. These results should be considered with caution as all the included studies had been conducted in Asia with conventional TACE (Table 4), hence the applicability of such findings in the West is still unclear, although a recent small Italian retrospective report confirmed the superiority of RFA combined to drug-eluting beads TACE over RFA alone in single HCCs beyond 3 cm[84].

OTHER THERMAL ABLATION TECHNIQUES

Microwave ablation

MWA aims to induce tumor necrosis by using high frequency (> 900 MHz, usually 2450 MHz) electromagnetic energy which determines continuous rotation of dipole molecules in the microwave's oscillating electric field. This vigorous movement of dipoles (mainly water molecules) generates friction and heat, thus inducing tissue death *via* coagulation necrosis[85].

In comparison to RFA, MWA has several theoretical advantages: It induces a wider area of active heating and warmer temperatures into the target zone in a shorter treatment time as it is not impaired by tissue desiccation and charring[86]; its efficacy is less impaired by heat-sink effect, due to the more pronounced cooling effect of blood flow and the conductive rather than active nature of heating[87]; multiple antennae can be simultaneously activated without the electrical interference phenomena observed in RFA, thus allowing more rapid treatment of large or multifocal tumours[87]. On these premises, MWA mostly shares the applications of RFA, with the above cited advantages in larger nodules and/or close to blood vessel.

Complete ablation rates of 89%-94% and 5-year survival rates of 51%-57% are reported in 3 retrospective studies enrolling mainly CP B patients[88-90].

The safety concerns raised on the risks of the procedure, due to the broader and less predictable necrosis areas induced by MWA, have been recently overcome by a large multicenter Italian study conducted in a series of 736 patients, of which 522 with HCC, where MWA determined a major complication rate of 2.9% with a peri-procedural mortality rate of < 0.01%[91].

There are actually 7 studies (of which one RCT) directly comparing MWA and RFA in HCC patients[92-98] (Table 5). Unfortunately, the sole RCT published did not report long-term survival data but only complete necrosis rates, which were similar in the two treatment groups (89% for MWA *vs* 96% for RFA)[92]. Retrospective studies reported heterogeneous results, particularly with regard

Table 4 Randomized controlled trials comparing transarterial chemoembolization combined to radiofrequency ablation *vs* radiofrequency ablation alone in hepatocellular carcinoma patients

Ref.	Region	Patients (*n*)	Tumor size, cm	CP A/B/C	3-yr survival (%)	3-yr recurrence (%)
Peng *et al*[75]	China	TACE + RFA (69)	≤ 5.01	60/9/0	69.0	45.0
		RFA (70)	-	59/11/0	47.0	18.0
Cheng *et al*[76]	China	TACE + RFA (96)	≤ 7.5	NA	55.0	NA
		RFA (100)	-	NA	32.0	NA
Yang *et al*[77]	China	TACE + RFA (24)	6.6 ± 0.6	NA	NA	NA
		RFA (12)	5.2 ± 0.4	NA	NA	NA
Shibata *et al*[78]	Japan	TACE + RFA (46)	1.7 ± 0.6	32/14/0	84.8	48.8
		RFA (43)	1.6 ± 0.5	33/10/0	84.5	29.7
Morimoto *et al*[79]	Japan	TACE + RFA (19)	3.6 ± 0.7	12/7/0	93.0	NA
		RFA (18)	3.7 ± 0.6	16/2/0	80.0	28.0
Kang *et al*[80]	China	TACE + RFA (19)	6.7 ± 1.1	12/7/0	36.8	NA
		RFA (18)	6.2 ± 1.2	12/6/0	16.7	NA
Shen *et al*[81]	China	TACE + RFA (18)	5.6 (2.2-15.8)	4/14/0	73.3	50.0
		RFA (16)	5.0 (2.3-12.3)	6/10/0	20.4	18.7
Zhang *et al*[82]	China	TACE + RFA (15)	4.6 (2.3-7.1)	NA	NA	NA
		RFA (15)	4.1 (2.4-6.0)	NA	NA	NA

CP: Child-Pugh; TACE: Transarterial chemoembolization; RFA: Radiofrequency ablation; NA: Not available.

Table 5 Studies comparing radiofrequency ablation and microwave ablation in hepatocellular carcinoma patients

Ref.	Arm (*n*)	Study design	Region	CP (A/B/C)	Tumor size (cm)	Number nodules	3-yr survival (%)	Local tumor recurrence (%)
Shibata *et al*[92]	RFA (36)	RCT	Japan	21/15/0	1.6 (0.7-2)	1.08	NA	8.3
	MWA (36)			19/17/0	1.7 (0.8-2)	1.14	NA	17.4
Lu *et al*[93]	RFA (53)	R	China	49/4/0	2.6 (1-6.1)	1.35	37.6	20.9
	MWA (49)			39/10/0	2.5 (0.9-7.2)	2.00	50.5	11.8
Ohmoto *et al*[94]	RFA (34)	R	Japan	20/11/3	1.6 (0.7-2)	1.08	49.0	9.0
	MWA (49)			31/14/4	1.7 (0.8-2)	1.14	70.0	19.0
Ding *et al*[95]	RFA (85)	R	China	49/36/0	2.38 (1-4.8)	1.15	77.6	5.2
	MWA (113)			75/38/0	2.55 (0.8-5)	1.15	82.7	10.9
Zhang *et al*[96]	RFA (78)	R	China	78/0/0	NA	1.24	64.1	11.8
	MWA (77)			77/0/0	NA	1.36	51.7	10.5
Abdelaziz *et al*[97]	RFA (45)	R	Egypt	24/21/0	2.95 ± 1.03+	1.00	NA	13.5
	MWA (66)			25/41/0	2.9 ± 0.97	1.00	NA	3.9
Vogl *et al*[98]	RFA (25)	R	Germany	NA	NA	1.28	72.0	9.4
	MWA (28)			NA	NA	1.28	79.0	8.3

CP: Child-Pugh; RFA: Radiofrequency ablation; MWA: Microwave ablation; RCT: Randomized controlled trial; R: Retrospective.

to local recurrence probably because of different follow-up time length or radiologic criteria adopted (Table 5).

The two meta-analysis published so far in this field reported no difference 3-year OS with MWA outperforming RFA in terms of LR for treatment of larger tumours[99,100]. However, further RCTs are needed to verify whether MWA efficacy in determining broader ablation areas will translate into a real survival benefit.

HIFU ablation

HIFU ablation aims to elevate tissue temperature by focusing high energy ultrasound (US) waves into one small spot[39]. The main advantage of HIFU ablation is the safety and the less invasiveness with, on the other hand, the limitation of a longer procedure time and acoustic shadowing by the rib cage, which may also cause thermal injury of the overlying soft tissue as a result of high US absorption by the bony cortex[39]. This drawback has been partially overcome by novel equipment using a larger transducer to spread the US beams out, thus decreasing the superficial energy wasting, or a multi-element phased-array transducer able to selectively activate only elements corresponding to the intercostal spaces[101]. There are actually few studies on HIFU, mainly conducted in advanced or recurrent cases for palliative purposes. A retrospective study by Chan *et al*[102] did not find any difference in terms of 3-year survival between HIFU and RFA for recurrent HCCs (69.8% *vs* 64.2%, $P = 0.19$). The same group compared the outcomes of HIFU ablation to those of TACE as bridging therapy before OLT and found similar results as for tumor necrosis in explanted livers ($P = 0.35$)[103]. The authors concluded that HIFU ablation was safe even for CP C patients and increased the number of subject receiving bridging therapy from 39.2% to 80.4%[103].

In our opinion, because of the scarce data currently

available and in attendance of further reliable results in the clinical setting, HIFU represents a promising option to be performed in highly-experienced centers and in selected cases.

LA

LA is one of the least investigated ablative treatments.

In this case, ablation is induced by the interaction of light energy (derived by electrical energy) and tissue[104]. Because laser light is coherent and monochromatic, it can be selectively collimated and focused and large amounts of energy can be transmitted over long distances without significant losses. Light is delivered *via* multiple flexible quartz fibers which have flat or cylindrical diffusing tips. The use of water-cooled laser application sheaths enables a higher laser power output (up to 50 W compared with 5 W of previous devices) while preventing carbonization, thus allowing ablative zones of up to 80 mm diameter[105].

Several retrospective cohort studies have shown that LA is a safe and feasible procedure for the treatment of HCC with a complete response rate ranging from 82% to 97%[105-108].

In an Italian multicenter retrospective study, 5-year cumulative survival was 41%, median survival times were 65 and 68 mo in patients with tumor size ⩽ 3 cm and ⩽ 2 cm, respectively, while median time to recurrence was 24 mo[109].

In a recent RCT with 140 Milan-in patients, complete response was observed in 97.4% of patients treated with RFA and 95.7% with LA and mean time-to local progression and overall survival were comparable between the two study groups (P = 0.129 and 0.693, respectively)[110]. The authors concluded that LA resulted non-inferior to RFA and therefore it should be considered as a valuable alternative for thermal ablation of small HCC in cirrhotic patients[110].

However, in spite of the apparently excellent results in terms of safety and of the described efficacy of LA, the low experience available worldwide currently restricts its application to a limited number of high-volume centers.

CRYOABLATION

Cryoablation induces cytotoxicity based on cyclic applications of extremely low temperatures (-20 ℃ to -40 ℃) within the tumour[39]. Multiple cryoprobes of 2-3 mm in diameter are inserted into the target lesion *via* a dilation catheter to ensure the rapid freezing of the nodule. Cryotherapy is delivered by means of multiple cycles and between two consecutive cycles the cryoprobes are rewarmed by an heating system.

Despite being widely used in various other cancers, the application of percutaneous cryoablation in HCC was sparsely reported. Compared to RFA, cryoablation endows several unique advantages including larger ablative zones, more clearly discernible treatment margin, less pain and good visualization by imaging[111,112]. Main drawbacks are: (1) smaller ablation areas generated by each single probe, hence multiple cryoprobes applications are needed; (2) unpredictable area of ablation (4-10 mm or more); and (3) concerns on the risk of complications such as massive haemorrhage due to ice ball fracture, cold injury to adjacent organs, and cryoshock syndrome[113,114].

Nevertheless, with the recent improvements in technology and the increasing experience acquired worldwide, cryoablation represents a promising therapeutic tool in the field of HCC ablation.

An Asian series of 866 patients within Milan criteria who underwent percutaneous cryoablation was recently analyzed: Complete response was achieved in 96.1% of patients with a major complication rate of 2.8% and no treatment-related mortality[115]. Five-year local tumor recurrence rate was 24.2% and 5-year survival rate was 59.5%[115].

A recent meta-analysis including 4 retrospective studies comparing the effect of cryoablation and RFA on hepatic neoplastic lesions concluded that RFA was significantly superior in terms of safety and local recurrence[116]. However, these studies referred not only to HCC but also to other liver malignancies, used several different equipments as laparoscopic or even surgical cryoablation[116] and were mostly conducted several years ago when experience with cryoablation was still low. In a multicenter Asian RCT enrolling 360 patients with one or two HCC lesions ⩽ 4 cm, cryoablation proved superior to RFA according to 3-year local tumor progression (7% *vs* 11%, P = 0.043) while 5-year overall survival was similar between the two groups (40% *vs* 38%, P = 0.747)[117]. Major complications occurred in seven patients (3.9%) following cryoablation and in six patients (3.3%) following RFA (P = 0.776)[117]. These results have been confirmed in an interesting retrospective study comparing cryoablation and RFA combined to microwave coagulation therapy, where hypothermal therapy proved superior to combined regimen as for 2-year local recurrence-free survival (HR: 0.3, 95%CI: 0.1-0.9; P = 0.02) with no difference in safety outcomes[118].

Although further RCTs are needed in order to confirm these promising results, appropriate use of cryoablation could represent a valuable therapeutic option in early stage HCC patients.

CONCLUSION

Ablative treatments, particularly RFA, currently represent the first-line option for early stage unresectable HCC patients. Main indications to ablative treatments are BCLC 0/A patients not suitable to surgical therapies, namely liver resection and OLT, and bridging/down-staging setting before transplantation. Considering the state-of-art of the literature, RFA provided 5-year survival rates of 40%-70% and beyond in HCC series and, although survival rates are similar to PEI, the lower local recurrence rate stands for a wider application of RFA in

hepato-oncology.

In comparison to RFA, MWA has several theoretical advantages such as a wider ablation area, warmer temperatures into the target area in a shorter treatment time and it is not impaired by heat-sink effect. The safety concerns raised on the risks of this procedure, due to the broader and less predictable necrosis areas, have been recently overcome. However, whether MWA ability to induce a broader ablation zone will lead to a real survival benefit is still unclear.

Other treatments, such as HIFU, LA and cryoablation, are less investigated but showed promising results in early HCC patients and could be a valuable therapeutic option in the next future.

REFERENCES

1 **El-Serag HB**. Hepatocellular carcinoma. *N Engl J Med* 2011; **365**: 1118-1127 [PMID: 21992124 DOI: 10.1056/NEJMra1001683]

2 **European Association For The Study Of The Liver**; European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; **56**: 908-943 [PMID: 22424438 DOI: 10.1016/j.jhep.2011.12.001]

3 **Llovet JM**, Zucman-Rossi J, Pikarsky E, Sangro B, Schwartz M, Sherman M, Gores G. Hepatocellular carcinoma. *Nat Rev Dis Primers* 2016; **2**: 16018 [PMID: 27158749 DOI: 10.1038/nrdp.2016.18]

4 **Bruix J**, Sherman M; American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]

5 **Mazzaferro V**, Battiston C, Perrone S, Pulvirenti A, Regalia E, Romito R, Sarli D, Schiavo M, Garbagnati F, Marchianò A, Spreafico C, Camerini T, Mariani L, Miceli R, Andreola S. Radiofrequency ablation of small hepatocellular carcinoma in cirrhotic patients awaiting liver transplantation: a prospective study. *Ann Surg* 2004; **240**: 900-909 [PMID: 15492574 DOI: 10.1097/01.sla.0000143301.56154.95]

6 **Lu DS**, Yu NC, Raman SS, Lassman C, Tong MJ, Britten C, Durazo F, Saab S, Han S, Finn R, Hiatt JR, Busuttil RW. Percutaneous radiofrequency ablation of hepatocellular carcinoma as a bridge to liver transplantation. *Hepatology* 2005; **41**: 1130-1137 [PMID: 15841454 DOI: 10.1002/hep.20688]

7 **Pompili M**, Mirante VG, Rondinara G, Fassati LR, Piscaglia F, Agnes S, Covino M, Ravaioli M, Fagiuoli S, Gasbarrini G, Rapaccini GL. Percutaneous ablation procedures in cirrhotic patients with hepatocellular carcinoma submitted to liver transplantation: Assessment of efficacy at explant analysis and of safety for tumor recurrence. *Liver Transpl* 2005; **11**: 1117-1126 [PMID: 16123960 DOI: 10.1002/lt.20469]

8 **Lencioni R**, Crocetti L. Local-regional treatment of hepatocellular carcinoma. *Radiology* 2012; **262**: 43-58 [PMID: 22190656 DOI: 10.1148/radiol.11110144]

9 **Lencioni R**, Cioni D, Crocetti L, Franchini C, Pina CD, Lera J, Bartolozzi C. Early-stage hepatocellular carcinoma in patients with cirrhosis: long-term results of percutaneous image-guided radiofrequency ablation. *Radiology* 2005; **234**: 961-967 [PMID: 15665226 DOI: 10.1148/radiol.2343040350]

10 **Omata M**, Tateishi R, Yoshida H, Shiina S. Treatment of hepatocellular carcinoma by percutaneous tumor ablation methods: Ethanol injection therapy and radiofrequency ablation. *Gastroenterology* 2004; **127**: S159-S166 [PMID: 15508080 DOI: 10.1053/j.gastro.2004.09.030]

11 **Livraghi T**, Meloni F, Di Stasi M, Rolle E, Solbiati L, Tinelli C, Rossi S. Sustained complete response and complications rates after radiofrequency ablation of very early hepatocellular carcinoma in cirrhosis: Is resection still the treatment of choice? *Hepatology* 2008; **47**: 82-89 [PMID: 18008357 DOI: 10.1002/hep.21933]

12 **Pompili M**, Saviano A, de Matthaeis N, Cucchetti A, Ardito F, Federico B, Brunello F, Pinna AD, Giorgio A, Giulini SM, De Sio I, Torzilli G, Fornari F, Capussotti L, Guglielmi A, Piscaglia F, Aldrighetti L, Caturelli E, Calise F, Nuzzo G, Rapaccini GL, Giuliante F. Long-term effectiveness of resection and radiofrequency ablation for single hepatocellular carcinoma ≤3 cm. Results of a multicenter Italian survey. *J Hepatol* 2013; **59**: 89-97 [PMID: 23523578 DOI: 10.1016/j.jhep.2013.03.009]

13 **Chamberlain RS**, Fong Y. Radiofrequency thermal ablation of liver tumors. In: Blumgart LH, Fong Y. Surgery of the liver and biliary tract. 3rd ed. Toronto: W.B. Saunders, 2000: 1589-1595

14 **Jacobs A**. Radiofrequency Ablation for Liver Cancer. *Radiol Technol* 2015; **86**: 645-664; quiz 665-668 [PMID: 26199436]

15 **Facciorusso A**, Del Prete V, Antonino M, Neve V, Amoruso A, Crucinio N, Di Leo A, Barone M. Conditional survival analysis of hepatocellular carcinoma patients treated with radiofrequency ablation. *Hepatol Res* 2015; **45**: E62-E72 [PMID: 25472869 DOI: 10.1111/hepr.12458]

16 **Shiina S**, Tateishi R, Arano T, Uchino K, Enooku K, Nakagawa H, Asaoka Y, Sato T, Masuzaki R, Kondo Y, Goto T, Yoshida H, Omata M, Koike K. Radiofrequency ablation for hepatocellular carcinoma: 10-year outcome and prognostic factors. *Am J Gastroenterol* 2012; **107**: 569-577; quiz 578 [PMID: 22158026 DOI: 10.1038/ajg.2011.425]

17 **Kim YS**, Lim HK, Rhim H, Lee MW, Choi D, Lee WJ, Paik SW, Koh KC, Lee JH, Choi MS, Gwak GY, Yoo BC. Ten-year outcomes of percutaneous radiofrequency ablation as first-line therapy of early hepatocellular carcinoma: analysis of prognostic factors. *J Hepatol* 2013; **58**: 89-97 [PMID: 23023009 DOI: 10.1016/j.jhep.2012.09.020]

18 **Facciorusso A**, Del Prete V, Antonino M, Crucinio N, Neve V, Di Leo A, Carr BI, Barone M. Post-recurrence survival in hepatocellular carcinoma after percutaneous radiofrequency ablation. *Dig Liver Dis* 2014; **46**: 1014-1019 [PMID: 25085684 DOI: 10.1016/j.dld.2014.07.012]

19 **N'Kontchou G**, Mahamoudi A, Aout M, Ganne-Carrié N, Grando V, Coderc E, Vicaut E, Trinchet JC, Sellier N, Beaugrand M, Seror O. Radiofrequency ablation of hepatocellular carcinoma: long-term results and prognostic factors in 235 Western patients with cirrhosis. *Hepatology* 2009; **50**: 1475-1483 [PMID: 19731239 DOI: 10.1002/hep.23181]

20 **Facciorusso A**, Del Prete V, Antonino M, Neve V, Crucinio N, Di Leo A, Carr BI, Barone M. Serum ferritin as a new prognostic factor in hepatocellular carcinoma patients treated with radiofrequency ablation. *J Gastroenterol Hepatol* 2014; **29**: 1905-1910 [PMID: 24731153 DOI: 10.1111/jgh.12618]

21 **Lee DH**, Lee JM, Lee JY, Kim SH, Yoon JH, Kim YJ, Han JK, Choi BI. Radiofrequency ablation of hepatocellular carcinoma as first-line treatment: long-term results and prognostic factors in 162 patients with cirrhosis. *Radiology* 2014; **270**: 900-909 [PMID: 24475823 DOI: 10.1148/radiol.13130940]

22 **Ng KK**, Poon RT, Lo CM, Yuen J, Tso WK, Fan ST. Analysis of recurrence pattern and its influence on survival outcome after radiofrequency ablation of hepatocellular carcinoma. *J Gastrointest Surg* 2008; **12**: 183-191 [PMID: 17874276]

23 **Koda M**, Tokunaga S, Okamoto T, Hodozuka M, Miyoshi K, Kishina M, Fujise Y, Kato J, Matono T, Sugihara T, Oyama K, Hosho K, Okano J, Murawaki Y, Kakite S, Yamashita E. Clinical usefulness of the ablative margin assessed by magnetic resonance imaging with Gd-EOB-DTPA for radiofrequency ablation of hepatocellular carcinoma. *J Hepatol* 2015; **63**: 1360-1367 [PMID: 26232269 DOI: 10.1016/j.jhep.2015.07.023]

24 **Kim YS**, Lee WJ, Rhim H, Lim HK, Choi D, Lee JY. The minimal ablative margin of radiofrequency ablation of hepatocellular carcinoma (& gt; 2 and & lt; 5 cm) needed to prevent local tumor progression: 3D quantitative assessment using CT image fusion. *AJR Am J Roentgenol* 2010; **195**: 758-765 [PMID: 20729457 DOI: 10.2214/AJR.09.2954]

25 **Cucchetti A**, Piscaglia F, Caturelli E, Benvegnù L, Vivarelli M, Ercolani G, Cescon M, Ravaioli M, Grazi GL, Bolondi L, Pinna AD.

Comparison of recurrence of hepatocellular carcinoma after resection in patients with cirrhosis to its occurrence in a surveilled cirrhotic population. *Ann Surg Oncol* 2009; **16**: 413-422 [PMID: 19034578 DOI: 10.1245/s10434-008-0232-4]

26 **Feng X**, Xu R, Du X, Dou K, Qin X, Xu J, Jia W, Wang Z, Zhao H, Yang S, Guo C, Liu T, Ma K. Combination therapy with sorafenib and radiofrequency ablation for BCLC Stage 0-B1 hepatocellular carcinoma: a multicenter retrospective cohort study. *Am J Gastroenterol* 2014; **109**: 1891-1899 [PMID: 25403366 DOI: 10.1038/ajg.2014.343]

27 **Kan X**, Jing Y, Wan QY, Pan JC, Han M, Yang Y, Zhu M, Wang Q, Liu KH. Sorafenib combined with percutaneous radiofrequency ablation for the treatment of medium-sized hepatocellular carcinoma. *Eur Rev Med Pharmacol Sci* 2015; **19**: 247-255 [PMID: 25683938]

28 **Bruix J**, Takayama T, Mazzaferro V, Chau GY, Yang J, Kudo M, Cai J, Poon RT, Han KH, Tak WY, Lee HC, Song T, Roayaie S, Bolondi L, Lee KS, Makuuchi M, Souza F, Berre MA, Meinhardt G, Llovet JM. Adjuvant sorafenib for hepatocellular carcinoma after resection or ablation (STORM): a phase 3, randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2015; **16**: 1344-1354 [PMID: 26361969 DOI: 10.1016/S1470-2045(15)00198-9]

29 **Mazzaferro V**, Romito R, Schiavo M, Mariani L, Camerini T, Bhoori S, Capussotti L, Calise F, Pellicci R, Belli G, Tagger A, Colombo M, Bonino F, Majno P, Llovet JM. Prevention of hepatocellular carcinoma recurrence with alpha-interferon after liver resection in HCV cirrhosis. *Hepatology* 2006; **44**: 1543-1554 [PMID: 17133492]

30 **Hsu YC**, Ho HJ, Wu MS, Lin JT, Wu CY. Postoperative peg-interferon plus ribavirin is associated with reduced recurrence of hepatitis C virus-related hepatocellular carcinoma. *Hepatology* 2013; **58**: 150-157 [PMID: 23389758 DOI: 10.1002/hep.26300]

31 **Tamaki Y**, Nakade Y, Yamauchi T, Makino Y, Yokohama S, Okada M, Aso K, Kanamori H, Ohashi T, Sato K, Nakao H, Haneda M, Yoneda M. Angiotensin II type 1 receptor antagonist prevents hepatic carcinoma in rats with nonalcoholic steatohepatitis. *J Gastroenterol* 2013; **48**: 491-503 [PMID: 22886508 DOI: 10.1007/s00535-012-0651-7]

32 **Hirose A**, Ono M, Saibara T, Nozaki Y, Masuda K, Yoshioka A, Takahashi M, Akisawa N, Iwasaki S, Oben JA, Onishi S. Angiotensin II type 1 receptor blocker inhibits fibrosis in rat nonalcoholic steatohepatitis. *Hepatology* 2007; **45**: 1375-1381 [PMID: 17518368]

33 **Yoshiji H**, Noguchi R, Toyohara M, Ikenaka Y, Kitade M, Kaji K, Yamazaki M, Yamao J, Mitoro A, Sawai M, Yoshida M, Fujimoto M, Tsujimoto T, Kawaratani H, Uemura M, Fukui H. Combination of vitamin K2 and angiotensin-converting enzyme inhibitor ameliorates cumulative recurrence of hepatocellular carcinoma. *J Hepatol* 2009; **51**: 315-321 [PMID: 19501932 DOI: 10.1016/j.jhep.2009.04.011]

34 **Yoshiji H**, Noguchi R, Ikenaka Y, Kaji K, Aihara Y, Yamazaki M, Yamao J, Toyohara M, Mitoro A, Sawai M, Yoshida M, Morioka C, Fujimoto M, Uemura M, Fukui H. Combination of branched-chain amino acids and angiotensin-converting enzyme inhibitor suppresses the cumulative recurrence of hepatocellular carcinoma: a randomized control trial. *Oncol Rep* 2011; **26**: 1547-1553 [PMID: 21874260 DOI: 10.3892/or.2011.1433]

35 **Kaibori M**, Ishizaki M, Matsui K, Kitade H, Matsui Y, Kwon AH. Evaluation of metabolic factors on the prognosis of patients undergoing resection of hepatocellular carcinoma. *J Gastroenterol Hepatol* 2011; **26**: 536-543 [PMID: 21332549 DOI: 10.1111/j.1440-1746.2010.06439.x]

36 **Facciorusso A**, Del Prete V, Crucinio N, Muscatiello N, Carr BI, Di Leo A, Barone M. Angiotensin receptor blockers improve survival outcomes after radiofrequency ablation in hepatocarcinoma patients. *J Gastroenterol Hepatol* 2015; **30**: 1643-1650 [PMID: 25974743 DOI: 10.1111/jgh.12988]

37 **Du H**, Liang Z, Zhang Y, Jie F, Li J, Fei Y, Huang Z, Pei N, Wang S, Li A, Chen B, Zhang Y, Sumners C, Li M, Li H. Effects of angiotensin II type 2 receptor overexpression on the growth of hepatocellular carcinoma cells in vitro and in vivo. *PLoS One* 2013; **8**: e83754 [PMID: 24391821 DOI: 10.1371/journal.pone.0083754]

38 **Bertot LC**, Sato M, Tateishi R, Yoshida H, Koike K. Mortality and complication rates of percutaneous ablative techniques for the treatment of liver tumors: a systematic review. *Eur Radiol* 2011; **21**: 2584-2596 [PMID: 21858539 DOI: 10.1007/s00330-011-2222-3]

39 **Kim YS**, Lim HK, Rhim H, Lee MW. Ablation of hepatocellular carcinoma. *Best Pract Res Clin Gastroenterol* 2014; **28**: 897-908 [PMID: 25260316 DOI: 10.1016/j.bpg.2014.08.011]

40 **Brillet PY**, Paradis V, Brancatelli G, Rangheard AS, Consigny Y, Plessier A, Durand F, Belghiti J, Sommacale D, Vilgrain V. Percutaneous radiofrequency ablation for hepatocellular carcinoma before liver transplantation: a prospective study with histopathologic comparison. *AJR Am J Roentgenol* 2006; **186**: S296-S305 [PMID: 16632691]

41 **Rodríguez-Sanjuán JC**, González F, Juanco C, Herrera LA, López-Bautista M, González-Noriega M, García-Somacarrera E, Figols J, Gómez-Fleitas M, Silván M. Radiological and pathological assessment of hepatocellular carcinoma response to radiofrequency. A study on removed liver after transplantation. *World J Surg* 2008; **32**: 1489-1494 [PMID: 18373117 DOI: 10.1007/s00268-008-9559-z]

42 **Majno PE**, Adam R, Bismuth H, Castaing D, Ariche A, Krissat J, Perrin H, Azoulay D. Influence of preoperative transarterial lipiodol chemoembolization on resection and transplantation for hepatocellular carcinoma in patients with cirrhosis. *Ann Surg* 1997; **226**: 688-701; discussion 701-703 [PMID: 9409568]

43 **Golfieri R**, Cappelli A, Cucchetti A, Piscaglia F, Carpenzano M, Peri E, Ravaioli M, D'Errico-Grigioni A, Pinna AD, Bolondi L. Efficacy of selective transarterial chemoembolization in inducing tumor necrosis in small (& lt; 5 cm) hepatocellular carcinomas. *Hepatology* 2011; **53**: 1580-1589 [PMID: 21351114 DOI: 10.1002/hep.24246]

44 **Imamura J**, Tateishi R, Shiina S, Goto E, Sato T, Ohki T, Masuzaki R, Goto T, Yoshida H, Kanai F, Hamamura K, Obi S, Yoshida H, Omata M. Neoplastic seeding after radiofrequency ablation for hepatocellular carcinoma. *Am J Gastroenterol* 2008; **103**: 3057-3062 [PMID: 19086957 DOI: 10.1111/j.1572-0241.2008.02153.x]

45 **Lopez KT**, Kuwada SK, Wong LL. Consequences of needle tract seeding of hepatocellular cancer after liver transplant. *Clin Transplant* 2013; **27**: E400-E406 [PMID: 23837571 DOI: 10.1111/ctr.12160]

46 **Clavien PA**, Lesurtel M, Bossuyt PM, Gores GJ, Langer B, Perrier A; OLT for HCC Consensus Group. Recommendations for liver transplantation for hepatocellular carcinoma: an international consensus conference report. *Lancet Oncol* 2012; **13**: e11-e22 [PMID: 22047762 DOI: 10.1016/S1470-2045(11)70175-9]

47 **Chen MS**, Li JQ, Zheng Y, Guo RP, Liang HH, Zhang YQ, Lin XJ, Lau WY. A prospective randomized trial comparing percutaneous local ablative therapy and partial hepatectomy for small hepatocellular carcinoma. *Ann Surg* 2006; **243**: 321-328 [PMID: 16495695]

48 **Huang J**, Yan L, Cheng Z, Wu H, Du L, Wang J, Xu Y, Zeng Y. A randomized trial comparing radiofrequency ablation and surgical resection for HCC conforming to the Milan criteria. *Ann Surg* 2010; **252**: 903-912 [PMID: 21107100 DOI: 10.1097/SLA.0b013e3181efc656]

49 **Feng K**, Yan J, Li X, Xia F, Ma K, Wang S, Bie P, Dong J. A randomized controlled trial of radiofrequency ablation and surgical resection in the treatment of small hepatocellular carcinoma. *J Hepatol* 2012; **57**: 794-802 [PMID: 22634125 DOI: 10.1016/j.jhep.2012.05.007]

50 **Fang Y**, Chen W, Liang X, Li D, Lou H, Chen R, Wang K, Pan H. Comparison of long-term effectiveness and complications of radiofrequency ablation with hepatectomy for small hepatocellular carcinoma. *J Gastroenterol Hepatol* 2014; **29**: 193-200 [PMID: 24224779 DOI: 10.1111/jgh.12441]

51 **Peng ZW**, Lin XJ, Zhang YJ, Liang HH, Guo RP, Shi M, Chen MS. Radiofrequency ablation versus hepatic resection for the treatment of hepatocellular carcinomas 2 cm or smaller: a retrospective comparative study. *Radiology* 2012; **262**: 1022-1033 [PMID: 22357902 DOI: 10.1148/radiol.11110817]

52 **Wang JH**, Wang CC, Hung CH, Chen CL, Lu SN. Survival comparison between surgical resection and radiofrequency ablation for patients in BCLC very early/early stage hepatocellular carcinoma. *J Hepatol* 2012; **56**: 412-418 [PMID: 21756858 DOI: 10.1016/j.jhep.2011.05.020]

53 **Hung HH**, Chiou YY, Hsia CY, Su CW, Chou YH, Chiang JH, Kao

WY, Huo TI, Huang YH, Su YH, Lin HC, Lee SD, Wu JC. Survival rates are comparable after radiofrequency ablation or surgery in patients with small hepatocellular carcinomas. *Clin Gastroenterol Hepatol* 2011; **9**: 79-86 [PMID: 20831902 DOI: 10.1016/j.cgh.2010.08.018]

54 **Takayama T**, Makuuchi M, Hasegawa K. Single HCC smaller than 2 cm: surgery or ablation?: surgeon's perspective. *J Hepatobiliary Pancreat Sci* 2010; **17**: 422-424 [PMID: 19936598 DOI: 10.1007/s00534-009-0239-7]

55 **Liu PH**, Hsu CY, Hsia CY, Lee YH, Huang YH, Chiou YY, Lin HC, Huo TI. Surgical Resection Versus Radiofrequency Ablation for Single Hepatocellular Carcinoma ≤ 2 cm in a Propensity Score Model. *Ann Surg* 2016; **263**: 538-545 [PMID: 25775062 DOI: 10.1097/SLA.0000000000001178]

56 **Cucchetti A**, Piscaglia F, Cescon M, Ercolani G, Pinna AD. Systematic review of surgical resection vs radiofrequency ablation for hepatocellular carcinoma. *World J Gastroenterol* 2013; **19**: 4106-4118 [PMID: 23864773 DOI: 10.3748/wjg.v19.i26.4106]

57 **Cho YK**, Rhim H, Noh S. Radiofrequency ablation versus surgical resection as primary treatment of hepatocellular carcinoma meeting the Milan criteria: a systematic review. *J Gastroenterol Hepatol* 2011; **26**: 1354-1360 [PMID: 21679247 DOI: 10.1111/j.1440-1746.2011.06812.x]

58 **Wang Y**, Luo Q, Li Y, Deng S, Wei S, Li X. Radiofrequency ablation versus hepatic resection for small hepatocellular carcinomas: a meta-analysis of randomized and nonrandomized controlled trials. *PLoS One* 2014; **9**: e84484 [PMID: 24404166 DOI: 10.1371/journal.pone.0084484]

59 **Cucchetti A**, Piscaglia F, Cescon M, Colecchia A, Ercolani G, Bolondi L, Pinna AD. Cost-effectiveness of hepatic resection versus percutaneous radiofrequency ablation for early hepatocellular carcinoma. *J Hepatol* 2013; **59**: 300-307 [PMID: 23603669 DOI: 10.1016/j.jhep.2013.04.009]

60 **Cucchetti A**, Piscaglia F, Cescon M, Serra C, Colecchia A, Maroni L, Venerandi L, Ercolani G, Pinna AD. An explorative data-analysis to support the choice between hepatic resection and radiofrequency ablation in the treatment of hepatocellular carcinoma. *Dig Liver Dis* 2014; **46**: 257-263 [PMID: 24284006 DOI: 10.1016/j.dld.2013.10.015]

61 **Lencioni R**. Loco-regional treatment of hepatocellular carcinoma. *Hepatology* 2010; **52**: 762-773 [PMID: 20564355 DOI: 10.1002/hep.23725]

62 **Livraghi T**, Bolondi L, Lazzaroni S, Marin G, Morabito A, Rapaccini GL, Salmi A, Torzilli G. Percutaneous ethanol injection in the treatment of hepatocellular carcinoma in cirrhosis. A study on 207 patients. *Cancer* 1992; **69**: 925-929 [PMID: 1310435 DOI: 10.1002/1097-0142(19920215)69:4<925::AID-CNCR2820690415>3.0.CO;2-G]

63 **Livraghi T**, Goldberg SN, Lazzaroni S, Meloni F, Ierace T, Solbiati L, Gazelle GS. Hepatocellular carcinoma: radio-frequency ablation of medium and large lesions. *Radiology* 2000; **214**: 761-768 [PMID: 10715043 DOI: 10.1148/radiology.214.3.r00mr02761]

64 **Yang B**, Zan RY, Wang SY, Li XL, Wei ML, Guo WH, You X, Li J, Liao ZY. Radiofrequency ablation versus percutaneous ethanol injection for hepatocellular carcinoma: a meta-analysis of randomized controlled trials. *World J Surg Oncol* 2015; **13**: 96 [PMID: 25889181 DOI: 10.1186/s12957-015-0516-7]

65 **Lin SM**, Lin CJ, Lin CC, Hsu CW, Chen YC. Radiofrequency ablation improves prognosis compared with ethanol injection for hepatocellular carcinoma & lt; or =4 cm. *Gastroenterology* 2004; **127**: 1714-1723 [PMID: 15578509 DOI: 10.1053/j.gastro.2004.09.003]

66 **Lin SM**, Lin CJ, Lin CC, Hsu CW, Chen YC. Randomised controlled trial comparing percutaneous radiofrequency thermal ablation, percutaneous ethanol injection, and percutaneous acetic acid injection to treat hepatocellular carcinoma of 3 cm or less. *Gut* 2005; **54**: 1151-1156 [PMID: 16009687 DOI: 10.1136/gut.2004.045203]

67 **Shiina S**, Teratani T, Obi S, Sato S, Tateishi R, Fujishima T, Ishikawa T, Koike Y, Yoshida H, Kawabe T, Omata M. A randomized controlled trial of radiofrequency ablation with ethanol injection for small hepatocellular carcinoma. *Gastroenterology* 2005; **129**: 122-130 [PMID: 16012942 DOI: 10.1053/j.gastro.2005.04.009]

68 **Wang XW**, Yang S, Lai Z. Clinical effect of radiofrequency ablation therapy and percutaneous ethanol injection therapy on small hepatocellular carcinoma. *Med J West China* 2011; **23**: 1671-1673

69 **Azab M**, Zaki S, El-Shetey AG, Abdel-Moty MF, Alnoomani NM, Gomaa AA, Abdel-Fatah S, Mohiy S, Atia F. Radiofrequency ablation combined with percutaneous ethanol injection in patients with hepatocellular carcinoma. *Arab J Gastroenterol* 2011; **12**: 113-118 [PMID: 22055587 DOI: 10.1016/j.ajg.2011.07.005]

70 **Giorgio A**, Di Sarno A, De Stefano G, Scognamiglio U, Farella N, Mariniello A, Esposito V, Coppola C, Giorgio V. Percutaneous radiofrequency ablation of hepatocellular carcinoma compared to percutaneous ethanol injection in treatment of cirrhotic patients: an Italian randomized controlled trial. *Anticancer Res* 2011; **31**: 2291-2295 [PMID: 21737654]

71 **Lencioni RA**, Allgaier HP, Cioni D, Olschewski M, Deibert P, Crocetti L, Frings H, Laubenberger J, Zuber I, Blum HE, Bartolozzi C. Small hepatocellular carcinoma in cirrhosis: randomized comparison of radio-frequency thermal ablation versus percutaneous ethanol injection. *Radiology* 2003; **228**: 235-240 [PMID: 12759473]

72 **Brunello F**, Veltri A, Carucci P, Pagano E, Ciccone G, Moretto P, Sacchetto P, Gandini G, Rizzetto M. Radiofrequency ablation versus ethanol injection for early hepatocellular carcinoma: A randomized controlled trial. *Scand J Gastroenterol* 2008; **43**: 727-735 [PMID: 18569991 DOI: 10.1080/00365520701885481]

73 **Shen A**, Zhang H, Tang C, Chen Y, Wang Y, Zhang C, Wu Z. Systematic review of radiofrequency ablation versus percutaneous ethanol injection for small hepatocellular carcinoma up to 3 cm. *J Gastroenterol Hepatol* 2013; **28**: 793-800 [PMID: 23432154 DOI: 10.1111/jgh.12162]

74 **Ahrar K**, Newman RA, Pang J, Vijjeswarapu MK, Wallace MJ, Wright KC. 2004 Dr. Gary J. Becker Young Investigator Award: Relative thermosensitivity of cytotoxic drugs used in transcatheter arterial chemoembolization. *J Vasc Interv Radiol* 2004; **15**: 901-905 [PMID: 15361556]

75 **Peng ZW**, Zhang YJ, Liang HH, Lin XJ, Guo RP, Chen MS. Recurrent hepatocellular carcinoma treated with sequential transcatheter arterial chemoembolization and RF ablation versus RF ablation alone: a prospective randomized trial. *Radiology* 2012; **262**: 689-700 [PMID: 22157201 DOI: 10.1148/radiol.11110637]

76 **Cheng BQ**, Jia CQ, Liu CT, Fan W, Wang QL, Zhang ZL, Yi CH. Chemoembolization combined with radiofrequency ablation for patients with hepatocellular carcinoma larger than 3 cm: a randomized controlled trial. *JAMA* 2008; **299**: 1669-1677 [PMID: 18398079 DOI: 10.1001/jama.299.14.1669]

77 **Yang W**, Chen MH, Wang MQ, Cui M, Gao W, Wu W, Wu JY, Dai Y, Yan K. Combination therapy of radiofrequency ablation and transarterial chemoembolization in recurrent hepatocellular carcinoma after hepatectomy compared with single treatment. *Hepatol Res* 2009; **39**: 231-240 [PMID: 19054154 DOI: 10.1111/j.1872-034X.2008.00451.x]

78 **Shibata T**, Isoda H, Hirokawa Y, Arizono S, Shimada K, Togashi K. Small hepatocellular carcinoma: is radiofrequency ablation combined with transcatheter arterial chemoembolization more effective than radiofrequency ablation alone for treatment? *Radiology* 2009; **252**: 905-913 [PMID: 19567647 DOI: 10.1148/radiol.2523081676]

79 **Morimoto M**, Numata K, Kondou M, Nozaki A, Morita S, Tanaka K. Midterm outcomes in patients with intermediate-sized hepatocellular carcinoma: a randomized controlled trial for determining the efficacy of radiofrequency ablation combined with transcatheter arterial chemoembolization. *Cancer* 2010; **116**: 5452-5460 [PMID: 20672352 DOI: 10.1002/cncr.25314]

80 **Kang CB**, Xu HB, Wang SL, Rui B. Treatment of large hepatoma by TACE in combination with RFA. *Zhonghua Gandan Waike Zazhi* 2007; **13**: 828-830

81 **Shen SQ**, Xiang JJ, Xiong CL, Wu SM, Zhu SS. Intraoperative radiofrequency thermal ablation combined with portal vein infusion chemotherapy and transarterial chemoembolization for unresectable HCC. *Hepatogastroenterology* 2005; **52**: 1403-1407 [PMID: 16201083]

82 **Zhang Z**, Wu M, Chen H, Chen D, He J. [Percutaneous

radiofrequency ablation combined with transcatheter arterial chemoembolization for hepatocellular carcinoma]. *Zhonghua Waike Zazhi* 2002; **40**: 826-829 [PMID: 12487855]

83 **Ni JY**, Liu SS, Xu LF, Sun HL, Chen YT. Meta-analysis of radiofrequency ablation in combination with transarterial chemoembolization for hepatocellular carcinoma. *World J Gastroenterol* 2013; **19**: 3872-3882 [PMID: 23840128 DOI: 10.3748/wjg.v19.i24.3872]

84 **Iezzi R**, Pompili M, La Torre MF, Campanale MC, Montagna M, Saviano A, Cesario V, Siciliano M, Annicchiarico E, Agnes S, Giuliante F, Grieco A, Rapaccini GL, De Gaetano AM, Gasbarrini A, Bonomo L. Radiofrequency ablation plus drug-eluting beads transcatheter arterial chemoembolization for the treatment of single large hepatocellular carcinoma. *Dig Liver Dis* 2015; **47**: 242-248 [PMID: 25577299 DOI: 10.1016/j.dld.2014.12.007]

85 **Liang P**, Wang Y. Microwave ablation of hepatocellular carcinoma. *Oncology* 2007; **72** Suppl 1: 124-131 [PMID: 18087193 DOI: 10.1159/000111718]

86 **Skinner MG**, Iizuka MN, Kolios MC, Sherar MD. A theoretical comparison of energy sources--microwave, ultrasound and laser--for interstitial thermal therapy. *Phys Med Biol* 1998; **43**: 3535-3547 [PMID: 9869030 DOI: 10.1088/0031-9155/43/12/011]

87 **McWilliams JP**, Yamamoto S, Raman SS, Loh CT, Lee EW, Liu DM, Kee ST. Percutaneous ablation of hepatocellular carcinoma: current status. *J Vasc Interv Radiol* 2010; **21**: S204-S213 [PMID: 20656230 DOI: 10.1016/j.jvir.2009.11.025]

88 **Dong B**, Liang P, Yu X, Su L, Yu D, Cheng Z, Zhang J. Percutaneous sonographically guided microwave coagulation therapy for hepatocellular carcinoma: results in 234 patients. *AJR Am J Roentgenol* 2003; **180**: 1547-1555 [PMID: 12760916 DOI: 10.2214/ajr.180.6.1801547]

89 **Liang P**, Dong B, Yu X, Yu D, Wang Y, Feng L, Xiao Q. Prognostic factors for survival in patients with hepatocellular carcinoma after percutaneous microwave ablation. *Radiology* 2005; **235**: 299-307 [PMID: 15731369 DOI: 10.1148/radiol.2351031944]

90 **Lu MD**, Chen JW, Xie XY, Liu L, Huang XQ, Liang LJ, Huang JF. Hepatocellular carcinoma: US-guided percutaneous microwave coagulation therapy. *Radiology* 2001; **221**: 167-172 [PMID: 11568335 DOI: 10.1148/radiol.2211001783]

91 **Livraghi T**, Meloni F, Solbiati L, Zanus G; Collaborative Italian Group using AMICA system. Complications of microwave ablation for liver tumors: results of a multicenter study. *Cardiovasc Intervent Radiol* 2012; **35**: 868-874 [PMID: 21833809 DOI: 10.1007/s00270-011-0241-8]

92 **Shibata T**, Iimuro Y, Yamamoto Y, Maetani Y, Ametani F, Itoh K, Konishi J. Small hepatocellular carcinoma: comparison of radiofrequency ablation and percutaneous microwave coagulation therapy. *Radiology* 2002; **223**: 331-337 [PMID: 11997534]

93 **Lu MD**, Xu HX, Xie XY, Yin XY, Chen JW, Kuang M, Xu ZF, Liu GJ, Zheng YL. Percutaneous microwave and radiofrequency ablation for hepatocellular carcinoma: a retrospective comparative study. *J Gastroenterol* 2005; **40**: 1054-1060 [PMID: 16322950]

94 **Ohmoto K**, Yoshioka N, Tomiyama Y, Shibata N, Kawase T, Yoshida K, Kuboki M, Yamamoto S. Comparison of therapeutic effects between radiofrequency ablation and percutaneous microwave coagulation therapy for small hepatocellular carcinomas. *J Gastroenterol Hepatol* 2009; **24**: 223-227 [PMID: 18823439 DOI: 10.1111/j.1440-1746.2008.05596.x]

95 **Ding J**, Jing X, Liu J, Wang Y, Wang F, Wang Y, Du Z. Comparison of two different thermal techniques for the treatment of hepatocellular carcinoma. *Eur J Radiol* 2013; **82**: 1379-1384 [PMID: 23726122 DOI: 10.1016/j.ejrad.2013.04.025]

96 **Zhang L**, Wang N, Shen Q, Cheng W, Qian GJ. Therapeutic efficacy of percutaneous radiofrequency ablation versus microwave ablation for hepatocellular carcinoma. *PLoS One* 2013; **8**: e76119 [PMID: 24146824 DOI: 10.1371/journal.pone.0076119]

97 **Abdelaziz A**, Elbaz T, Shousha HI, Mahmoud S, Ibrahim M, Abdelmaksoud A, Nabeel M. Efficacy and survival analysis of percutaneous radiofrequency versus microwave ablation for hepatocellular carcinoma: an Egyptian multidisciplinary clinic experience. *Surg Endosc* 2014; **28**: 3429-3434 [PMID: 24935203 DOI: 10.1007/s00464-014-3617-4]

98 **Vogl TJ**, Farshid P, Naguib NN, Zangos S, Bodelle B, Paul J, Mbalisike EC, Beeres M, Nour-Eldin NE. Ablation therapy of hepatocellular carcinoma: a comparative study between radiofrequency and microwave ablation. *Abdom Imaging* 2015; **40**: 1829-1837 [PMID: 25601438 DOI: 10.1007/s00261-015-0355-6]

99 **Chinnaratha MA**, Chuang MY, Fraser RJ, Woodman RJ, Wigg AJ. Percutaneous thermal ablation for primary hepatocellular carcinoma: A systematic review and meta-analysis. *J Gastroenterol Hepatol* 2016; **31**: 294-301 [PMID: 26114968 DOI: 10.1111/jgh.13028]

100 **Facciorusso A**, Di Maso M, Muscatiello N. Microwave ablation versus radiofrequency ablation for the treatment of hepatocellular carcinoma: A systematic review and meta-analysis. *Int J Hyperthermia* 2016; In press

101 **Quesson B**, Merle M, Köhler MO, Mougenot C, Roujol S, de Senneville BD, Moonen CT. A method for MRI guidance of intercostal high intensity focused ultrasound ablation in the liver. *Med Phys* 2010; **37**: 2533-2540 [PMID: 20632565]

102 **Chan AC**, Cheung TT, Fan ST, Chok KS, Chan SC, Poon RT, Lo CM. Survival analysis of high-intensity focused ultrasound therapy versus radiofrequency ablation in the treatment of recurrent hepatocellular carcinoma. *Ann Surg* 2013; **257**: 686-692 [PMID: 23426335 DOI: 10.1097/SLA.0b013e3182822c02]

103 **Chok KS**, Cheung TT, Lo RC, Chu FS, Tsang SH, Chan AC, Sharr WW, Fung JY, Dai WC, Chan SC, Fan ST, Lo CM. Pilot study of high-intensity focused ultrasound ablation as a bridging therapy for hepatocellular carcinoma patients wait-listed for liver transplantation. *Liver Transpl* 2014; **20**: 912-921 [PMID: 24753206 DOI: 10.1002/lt.23892]

104 **Jacques SL**. Laser-tissue interactions. Photochemical, photothermal, and photomechanical. *Surg Clin North Am* 1992; **72**: 531-558 [PMID: 1589829]

105 **Giorgio A**, Tarantino L, de Stefano G N, Catalano O, Cusati B, Del Viscovo L A, Caturelli E. Interstitial laser photocoagulation under ultrasound guidance of liver tumors: results in 104 treated patients. *Eur J Ultrasound* 2000; **11**: 181-188 [PMID: 10874193 DOI: 10.1016/S0929-8266(00)00086-0]

106 **Pacella CM**, Bizzarri G, Francica G, Bianchini A, De Nuntis S, Pacella S, Crescenzi A, Taccogna S, Forlini G, Rossi Z, Osborn J, Stasi R. Percutaneous laser ablation in the treatment of hepatocellular carcinoma with small tumors: analysis of factors affecting the achievement of tumor necrosis. *J Vasc Interv Radiol* 2005; **16**: 1447-1457 [PMID: 16319150 DOI: 10.1097/01.RVI.90000172121.82299.38]

107 **Francica G**, Iodice G, Delle Cave M, Sarrantonio R, Lapiccirella G, Molese V, Smeraldo D, Scarano F, De Marino F. Factors predicting complete necrosis rate after ultrasound-guided percutaneous laser thermoablation of small hepatocellular carcinoma tumors in cirrhotic patients: a multivariate analysis. *Acta Radiol* 2007; **48**: 514-519 [PMID: 17520427 DOI: 10.1080/02841850701199942]

108 **Pacella CM**, Bizzarri G, Magnolfi F, Cecconi P, Caspani B, Anelli V, Bianchini A, Valle D, Pacella S, Manenti G, Rossi Z. Laser thermal ablation in the treatment of small hepatocellular carcinoma: results in 74 patients. *Radiology* 2001; **221**: 712-720 [PMID: 11719667 DOI: 10.1148/radiol.2213001501]

109 **Pacella CM**, Francica G, Di Lascio FM, Arienti V, Antico E, Caspani B, Magnolfi F, Megna AS, Pretolani S, Regine R, Sponza M, Stasi R. Long-term outcome of cirrhotic patients with early hepatocellular carcinoma treated with ultrasound-guided percutaneous laser ablation: a retrospective analysis. *J Clin Oncol* 2009; **27**: 2615-2621 [PMID: 19332729 DOI: 10.1200/JCO.2008.19.0082]

110 **Di Costanzo GG**, Tortora R, D'Adamo G, De Luca M, Lampasi F, Addario L, Galeota Lanza A, Picciotto FP, Tartaglione MT, Cordone G, Imparato M, Mattera S, Pacella CM. Radiofrequency ablation versus laser ablation for the treatment of small hepatocellular carcinoma in cirrhosis: a randomized trial. *J Gastroenterol Hepatol* 2015; **30**: 559-565 [PMID: 25251043 DOI: 10.1111/jgh.12791]

111 **Hutchinson M**, Shyn P, Silverman S. Cryoablation of Liver Tumors. In: Dupuy DE, Fong Y, McMullen WN, editors. Image-Guided

Cancer Therapy. New York: Springer Inc., 2013: 491-503
112 **Hu KQ**. Advances in clinical application of cryoablation therapy for hepatocellular carcinoma and metastatic liver tumor. *J Clin Gastroenterol* 2014; **48**: 830-836 [PMID: 25148553 DOI: 10.1097/MCG.0000000000000201]
113 **Mala T**, Samset E, Aurdal L, Gladhaug I, Edwin B, Søreide O. Magnetic resonance imaging-estimated three-dimensional temperature distribution in liver cryolesions: a study of cryolesion characteristics assumed necessary for tumor ablation. *Cryobiology* 2001; **43**: 268-275 [PMID: 11888220]
114 **Sheen AJ**, Siriwardena AK. The end of cryotherapy for the treatment of nonresectable hepatic tumors? *Ann Surg Oncol* 2005; **12**: 202-204 [PMID: 15827810]
115 **Rong G**, Bai W, Dong Z, Wang C, Lu Y, Zeng Z, Qu J, Lou M, Wang H, Gao X, Chang X, An L, Li H, Chen Y, Hu KQ, Yang Y. Long-term outcomes of percutaneous cryoablation for patients with hepatocellular carcinoma within Milan criteria. *PLoS One* 2015; **10**: e0123065 [PMID: 25849963 DOI: 10.1371/journal.pone.0123065]
116 **Wu S**, Hou J, Ding Y, Wu F, Hu Y, Jiang Q, Mao P, Yang Y. Cryoablation Versus Radiofrequency Ablation for Hepatic Malignancies: A Systematic Review and Literature-Based Analysis. *Medicine* (Baltimore) 2015; **94**: e2252 [PMID: 26656371 DOI: 10.1097/MD.0000000000002252]
117 **Wang C**, Wang H, Yang W, Hu K, Xie H, Hu KQ, Bai W, Dong Z, Lu Y, Zeng Z, Lou M, Wang H, Gao X, Chang X, An L, Qu J, Li J, Yang Y. Multicenter randomized controlled trial of percutaneous cryoablation versus radiofrequency ablation in hepatocellular carcinoma. *Hepatology* 2015; **61**: 1579-1590 [PMID: 25284802 DOI: 10.1002/hep.27548]
118 **Ei S**, Hibi T, Tanabe M, Itano O, Shinoda M, Kitago M, Abe Y, Yagi H, Okabayashi K, Sugiyama D, Wakabayashi G, Kitagawa Y. Cryoablation provides superior local control of primary hepatocellular carcinomas of & gt; 2 cm compared with radiofrequency ablation and microwave coagulation therapy: an underestimated tool in the toolbox. *Ann Surg Oncol* 2015; **22**: 1294-1300 [PMID: 25287439 DOI: 10.1245/s10434-014-4114-7]

P- Reviewer: Li Z, Senousy MA, Sheth RA **S- Editor**: Gong XM
L- Editor: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i4.490

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 490-502
ISSN 2150-5349 (online)

REVIEW

Treatment of pregnant women with a diagnosis of inflammatory bowel disease

Sule Poturoglu, Asli Ciftcibasi Ormeci, Ali Erkan Duman

Sule Poturoglu, Asli Ciftcibasi Ormeci, Ali Erkan Duman, Department of Gastroenterology, Haseki Training and Research Hospital, 34096 Istanbul, Turkey

Author contributions: Poturoglu S, Ormeci AC and Duman AE contributed equally to this work; Poturoglu S and Ormeci AC performed the literature search, designed and wrote the manuscript; Poturoglu S and Duman AE was responsible for critical revision of the manuscript for important intellectual content.

Conflict-of-interest statement: No conflicts of interest.

Manuscript source: Invited manuscript

Correspondence to: Sule Poturoglu, MD, Department of Gastroenterology, Haseki Training and Research Hospital, 34096 Istanbul, Turkey. sulepot@mynet.com
Telephone: +90-542-5257271
Fax: +90-212-5896229

Received: March 29, 2016
Peer-review started: April 4, 2016
First decision: May 23, 2016
Revised: August 20, 2016
Accepted: September 21, 2016
Article in press: September 22, 2016
Published online: November 6, 2016

Abstract

The frequency of diagnosis of inflammatory bowel disease (IBD) has increased in younger populations. For this reason, pregnancy in patients with IBD is a topic of interest, warranting additional focus on disease management during this period. The main objective of this article is to summarize the latest findings and guidelines on the management of potential problems from pregnancy to the breastfeeding stage. Fertility is decreased in patients with active IBD. Disease remission prior to conception will likely decrease the rate of pregnancy-related complications. Most of the drugs used for IBD treatment are safe during both pregnancy and breastfeeding. Two exceptions are methotrexate and thalidomide, which are contraindicated in pregnancy. Anti-tumor necrosis factor agents are not advised during the third trimester as they exhibit increased transplacental transmission and potentially cause immunosuppression in the fetus. Radiological and endoscopic examinations and surgical interventions should be performed only when absolutely necessary. Surgery increases the fetal mortality rate. The delivery method should be determined with consideration of the disease site and presence of progression or flare up. Treatment planning should be a collaborative effort among the gastroenterologist, obstetrician, colorectal surgeon and patient.

Key words: Pregnancy; Inflammatory bowel disease; Immunomodulators; Biologics; Breastfeeding; Treatment

Core tip: Active disease prior to conception and during pregnancy increases the rate of pregnancy-related complications; thus, special attention should be given to pregnancy during the disease remission period. The safest drugs for use during pregnancy and breastfeeding are 5-aminosalicylic acid complexes, thiopurines and corticosteroids. Methotrexate and thalidomide are contraindicated. Anti-tumor necrosis factor treatment should be avoided during the third trimester. The risk of venous thromboembolism is increased in patients with moderate-to-severe disease. The delivery method should be selected according to the region of the body involved and disease activity. In this article, the problems

encountered by patients with inflammatory bowel disease from pregnancy to breastfeeding are discussed, and appropriate management strategies are suggested.

Poturoglu S, Ormeci AC, Duman AE. Treatment of pregnant women with a diagnosis of inflammatory bowel disease. *World J Gastrointest Pharmacol Ther* 2016; 7(4): 490-502 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i4/490.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i4.490

INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC) are chronic, idiopathic diseases characterized by relapse and remission periods, and they constitute a major portion of the inflammatory bowel disease (IBD) spectrum. A multicentric epidemiological study performed in Europe found the incidence of UC to be 10.4/10000 and that of CD to be 5.6/10000[1]. The risk of IBD in children is increased 2-13-fold if one parent has IBD[2] and by 33%-36% if both parents have IBD[3,4]. The disease is most frequently diagnosed during the second and third decades of life. CD is diagnosed slightly more frequently in women than men (1.3:1), whereas the ratio is 1:1 for UC. Approximately 25% of female IBD patients are expected to become mothers during their disease period[5]. In other words, the disease affects prospective parents. For this reason, the effect of the disease on possible complications encountered during pregnancy and the effects of the treatment on the fetus, birth method selected and breastfeeding safety are sources of anxiety for patients. To alleviate such worries, physicians must increase their knowledge of and experience with such subjects and share this information with patients and their relatives.

Pregnancy does not cause IBD flare-ups; however, the disease can be exacerbated in IBD patients who become pregnant during the active phase of the disease[6]. Of those patients who become pregnant during the active phase, approximately two-thirds have active disease throughout their pregnancy term[7,8]. Approximately one-third of patients who become pregnant during the remission period experience a disease flare-up[9]. However, these proportions are identical to those in the general population. IBD flare-ups are often due to medication discontinuation during pregnancy, lactation and smoking resumption following birth[10]. Approximately one-third of patients have active disease during conception[11]. Nielsen *et al*[12] reported that the yearly exacerbation rate is 34% during pregnancy and 32% in non-pregnancy. Increased prevalences of premature birth, low birth weight, stillbirth, cesarean section and congenital anomalies have been reported in pregnant women with IBD[13,14]. Such complications are more frequent in patients with CD than those with UC. However, these meta-analyses did not take disease activity or medical treatments into consideration. Congenital malformation rates were increased in IBD patients in case-controlled studies conducted in Hungary[15] and Italy[16], as opposed to other studies that reported similar rates in IBD patients to those of the general population[17,18]. Some authors[19-21] suggest this difference is explained by disease activity, while others suggest it is due to the high numbers of low-activity patients included in the studies[10,22]. The prospective, case-controlled ECCO-EpiCom study[23] found no significant difference in pregnancy outcomes in pregnant IBD patients compared with the general pregnant population. In that study, logistic regression analyses showed that age > 35 years and tobacco smoking were risk factors for premature birth and congenital anomalies in CD patients and for premature birth in UC patients.

The chance of a normal birth is 85% in patients with UC and 83.5% in those with CD if the disease is in remission during conception[9]. Ideally, the patient should be in remission when trying to conceive.

The physician should inform the patient and her partner of the IBD-pregnancy interaction and its effects on pregnancy outcomes, the treatment risk to benefit ratio and the importance of remission maintenance in reducing fetal risk. It is essential to be aware of disease management protocols during pregnancy to alleviate patient fears.

In this article, our main objective is to evaluate the management of IBD patients during pregnancy by reviewing the effects of IBD treatment on the fetus and mother during pregnancy and lactation.

MEDICAL TREATMENT

Discontinuing medical treatment during pregnancy can further harm the fetus by causing a flare-up in the patient. The pregnancy risk profiles for conventional drugs used in IBD treatment, such as 5-aminosalicylic acid (5-ASA), steroids and immunomodulatory agents, are available. Experience with biological agents is also increasing. The United States Food and Drug Administration (FDA) has deemed almost all drugs safe for use during pregnancy and lactation, with the exception of methotrexate and thalidomide, which are pregnancy category X drugs (Tables 1 and 2).

5-ASA

All 5-ASAs (mesalazine, balsalazide, ipsalazide and sulfasalazine) are used to induce and maintain remission in patients with light-to-moderately active UC, and these drugs exert their effects by acting on the intestinal mucosa.

Sulfasalazine

Effect on pregnancy: Sulfasalazine and its metabolite sulfapyridine inhibit folate synthesis. Sulfasalazine and sulfapyridine cross the placental barrier and can be detected in umbilical cord blood at rates similar to those in maternal blood. For this reason, sulfasalazine

Table 1 Food and Drug Administration pregnancy categories

FDA category	Definition
A	Controlled studies in animals and women demonstrate no risks during the first trimester, and the possibility of fetal harm appears remote
B	Studies in animals have not demonstrated a fetal risk, but no controlled studies have been conducted in pregnant women, or animal studies have shown an adverse event that was not confirmed in controlled studies in women during the first trimester. Chance of fetal harm is remote but remains a possibility
C	No controlled studies have been conducted in women, and animal studies have shown adverse effects on the fetus, or studies in humans and animals are not available. Chance of fetal harm. Give only if potential benefit outweighs the risk
D	There are no controlled studies in women or animals, but positive evidence of fetal risk is available. It can still be used for life-threatening or serious diseases when there are no effective alternative drugs
X	Studies in animals or women have demonstrated fetal abnormalities. The drug is contraindicated in women who are pregnant or may become pregnant

FDA: Food and Drug Administration.

Table 2 Safety of inflammatory bowel disease medications during pregnancy and breastfeeding

Medication	FDA category	Comments during pregnancy	Comments during breastfeeding
Adalimumab	B	Low risk: Transported through the placenta late in the second and third trimester; avoid treatment in the last trimester	Compatible
5-Aminosalicylic acid preparations[1]	B	Low risk: Limited data for olsalazine; if using sulfasalazine, folic acid supplementation is mandatory	Enters breast milk; probably compatible
Amoxicillin/clavulanate	B	Low risk	Enters breast milk; probably compatible
Azathioprine/6-mercaptopurine	D	Low risk	Low transfer to infant; appears in the milk 4 h after ingestion
Budesonide/prednisone	C	Probably low risk, avoid during first trimester (potential risk of oral clefts)	Probably compatible; enters breast milk
Certolizumab	B	Low risk	Limited data; probably compatible
Ciprofloxacin	C	Limited data; not recommended	Compatible
Cyclosporine	C	Low risk	Contraindicated
Methotrexate	X	Contraindicated: Teratogenic	Contraindicated
Metronidazole	B	Low risk, avoid during the first trimester	Enters breast milk, not recommended
Natalizumab	C	Limited data; low risk	Limited data; probably compatible
Tacrolimus	C	Limited data; no increase in congenital anomalies	Contraindicated
Thalidomide	X	Contraindicated: Teratogenic	No data available; potential toxicity

[1]Asacol HD (due to the dibutyl phthalate content) and olsalazine are category C drugs. FDA: Food and Drug Administration.

treatment can be continued during pregnancy. However, since it results in folate deficiency, it must be supplemented with 2 mg folic acid daily[24].

A meta-analysis published in 2008 compared 642 pregnant women who used mesalazine, sulfasalazine or olsalazine with 1158 pregnant controls and found no increased risks of congenital anomalies, low birth weight or similar complications[25]. The rates of low birth weight, prematurity, spontaneous abortion, live births and birth defects were similar between sulfasalazine-using mothers and the general population[8].

Effect on breastfeeding: Sulfasalazine and its metabolite sulfapyridine pass into breast milk. However, although sulfasalazine replaces bilirubin, which is a serious issue, no clinically significant cases have been reported[26]. Diarrhea was reported in infants of mothers taking sulfasalazine[27]. In such cases, adjustment of the sulfasalazine dosage or discontinuing treatment is indicated.

Mesalazine

Effect on pregnancy: Although most 5-ASA drugs are pregnancy category B, olsalazine is category C. Mesalazine metabolites, especially N-acetyl mesalazine, cross the placental barrier[28,29]. The rate of congenital anomalies in babies of mesalazine-exposed mothers was no higher than that in babies of the general population[30,31]. Several studies have reported the use of 5-ASA during pregnancy to be generally safe[32-34].

Enteric-coated mesalamine with dibutyl phthalate (DBP) was reported to cause skeletal anomalies and negative effects on the male reproductive system in animal models[35]. Drugs that contain DBP and 5-ASA (Asacol or Asacol HD; Procter and Gamble Pharmaceuticals, Cincinnati, OH, United States) should be switched to another 5-ASA preparation during pregnancy. However, a study that compared 117 pregnant patients who used Asacol with 156 pregnant patients who used non-Asacol aminosalycilate drugs reported no significant differences in terms of congenital anomaly rates[36].

Effect on breastfeeding: 5-ASA is excreted at a low concentration *via* breast milk[37]. Infants of mothers using 5-ASA can develop diarrhea due to allergic reactions. In such cases, the treatment should be stopped immediately.

Steroids

Effect on pregnancy: Corticosteroids, particularly prednisolone, are classified as pregnancy category C drugs. Carmichael *et al*[38] reported increased incidences of cleft palate and cleft lip anomalies with the use of corticosteroids 1 mo before pregnancy or during the first trimester. However, other studies involving larger patient groups reported no such risk[31].

Several studies also suggest that high-dose corticosteroid usage might cause adrenal suppression by affecting the hypothalamus-pituitary-adrenal axis in newborns. However, one study also concluded that the long-term effects are unclear and the absolute effects on the fetus negligible[39].

The use of budesonide is also regarded as safe during pregnancy. Beaulieu *et al*[40] reported no side effects in eight pregnant patients with CD who used budesonide. Moreover, studies involving larger groups of patients who used budesonide for asthma treatment reported no increase in the rate of birth defects or stillbirths[41,42].

Effect on breastfeeding: As the concentrations of steroids that enter breast milk are low, steroid usage during breastfeeding is deemed safe[43]. However, no specific guidelines exist for prednisolone usage during lactation. If the mother is worried about breastfeeding during steroid treatment, she can stop breastfeeding during her steroid treatment and resume once the treatment is discontinued[44].

Thiopurines

Effect on pregnancy: Azathioprine is a pro-drug that is metabolized into 6-mercaptopurine (6-MP). Following its metabolism into 6-MP, it is again metabolized into its active 6-thioguanine (6-TG) and inactive 6-methylmercaptopurine (6-MMP) metabolites. These drugs damage chromosomes by disrupting nucleic acid synthesis. The FDA classifies these drugs as pregnancy category D, since animal models showed teratogenic effects at therapeutic or elevated dosages[45]. Yet, the significantly higher bioavailability of intraperitoneal or parenteral, compared with oral, thiopurines used for IBD treatment in animal models should not be overlooked. Intact azathioprine or 6-MP cannot cross the placental barrier, whereas 6-TG can[46]. In a prospective study that included 30 pregnant women, 6-TG levels decreased but 6-MMP levels increased during pregnancy; however, these changes did not cause myelotoxicity or hepatotoxicity[47]. After pregnancy, both metabolites returned to their pregestational levels. With the exception of a newborn whose mother had severe pre-eclampsia and pancytopenia during delivery and high alkaline phosphatase levels, 6-MMP was not detected in any of the newborns. No major congenital malformations were seen in those newborns. All newborns had normal Apgar scores, but 60% were diagnosed with anemia. Therefore, a complete blood count is advised for newborns whose mothers used thiopurines during pregnancy.

In daily clinical practice, gestational planning for IBD patients who take thiopurines and continuation of thiopurine usage during pregnancy pose a challenge for the physician. This is due to the numerous contradictory studies in the literature. Two more recent publications reported an increase in the risk of congenital anomalies with thiopurine usage[48,49]. However, these studies have been criticized for their small number of patients and other limitations, such as inclusion of both major and minor anomalies[50]. Other than the risk of congenital anomalies, other studies have reported a relationship between thiopurine usage and the incidences of preterm births and low birth weight[48,49,51].

However, a large number of recent studies showed no relationship between thiopurine usage and the risk of congenital anomalies. Goldstein[52] evaluated women who took azathioprine for various indications; after a review of birth defect records, no significant increases in malformation rates were found. The 20-year study by Ban *et al*[53] reported that neither MP nor any other drug is related to an increased risk of congenital anomalies. Beaugerie *et al*[54] and Coelho *et al*[55], *via* the CESAME study in France, compared 89 women exposed to thiopurine during pregnancy and 129 IBD patients without thiopurine exposure and found no increase in the risk of congenital anomalies in a sub-analysis. The meta-analysis published by Akbari *et al*[56] reported no increase in the risk of congenital anomalies or low birth weight but an increased risk of premature birth with thiopurine usage during pregnancy. Casanova *et al*[57] reported that thiopurine usage was not associated with pregnancy complications and actually predicted lower rates of obstetric complications and better pregnancy outcomes. The first results of the ongoing PIANO study[58], published in 2012, showed no increase in the rates of congenital anomalies or pregnancy complications in 317 pregnant women exposed to thiopurine during pregnancy. The infants of those exposed mothers were followed up and exhibited similar or better developmental parameters compared with infants of mothers who were not exposed to thiopurines[59]. This finding supports the results of another study in 2013 in which 30 babies of mothers taking thiopurines during pregnancy for both medical and psychosocial health reasons showed no differences compared with the control groups[60].

To summarize, thiopurine treatment should be continued during pregnancy to prevent flare-ups, as the risk of active disease outweighs the risk of thiopurine usage. A female patient using anti-tumor necrosis factor (TNF) therapy combined with thiopurines who

is planning to become pregnant can discontinue the thiopurines prior to pregnancy, considering the risk of infection[50]. Women who were not taking thiopurines prior to pregnancy are not advised to start thiopurine treatment during pregnancy, as thiopurines take a long time to act and pose a small risk of bone marrow suppression and pancreatitis[61].

Effect on breastfeeding: Thiopurines were detectable in breast milk 4 h after their ingestion, albeit at very low levels compared with serum plasma levels[62]. Thiopurine metabolites are almost undetectable in infants breastfed by mothers taking thiopurines[63]. Furthermore, no increase in the risk of infection was evident in babies of mothers treated with thiopurines[64]. Therefore thiopurine-using mothers have no issues during breastfeeding. However, mothers of infants with weak immune systems should exercise caution while breastfeeding during thiopurine treatment[10].

Methotrexate

Effect on pregnancy: When taken during the organogenesis period, methotrexate can cause methotrexate embryopathy or fetal/methotrexate syndrome, which is characterized by congenital extremity and craniofacial anomalies[65]. When taken during the third trimester, methotrexate can cause fetal toxicity, retardation of development and loss of the fetus[66,67]. Since it remains in the body for a prolonged period, the drug should be discontinued 3-6 mo before conception[68]. It is recommended that men also stop taking methotrexate 3 mo before conception. In addition, folic acid supplementation should commence 3 mo prior to and be continued during pregnancy.

While on methotrexate, the patient should be warned about its toxic effects on the fetus and advised to use at least two contraception methods to prevent pregnancy[69].

Effect on breastfeeding: Methotrexate is present in breast milk and can cause immunosuppression and neutropenia by accumulating in neonatal tissues. Therefore, it should not be used during pregnancy[70].

Cyclosporine

Effect on pregnancy: Cyclosporine is a pregnancy category C drug. Because cyclosporine can cross the placental barrier, it may exert adverse effects on the fetus[71]. In renal transplant patients, cyclosporine caused premature birth, low birth weight, gestational diabetes, maternal hypertension, pre-eclampsia and fluctuations in the levels of other drugs[72]. Another meta-analysis of the effects of cyclosporine usage on pregnancy (15 studies, 410 patients) reported the incidence of premature birth to be 56% and that of congenital anomalies to be 4.1%. However, the rate of congenital malformation is not significantly different from that in the normal population[73]. In a smaller study that included eight pregnant patients, seven were treated successfully for steroid-refractory ulcerative pancolitis, while one required infliximab (IFX) treatment and was treated successfully. Seven patients delivered healthy infants, and one fetus died in utero. Two newborns were premature, and no congenital anomalies were detected in any of the infants[74].

Effect on breastfeeding: Since very small amounts of cyclosporine pass the placental barrier, it is safe to use in nursing mothers. However, the possibility of immunosuppression in the infant must not be overlooked. If treatment with this drug is planned, the potential risks should be discussed with the mother.

Tacrolimus

Effect on pregnancy: Tacrolimus, like cyclosporine, is classified as a pregnancy category C drug by the FDA. A study that followed 37 female liver transplant patients for 13 years with 49 recorded births reported an increase in the rate of premature births but not in the rate of congenital anomalies[75]. In another report, a female patient treated with tacrolimus during pregnancy delivered a healthy baby[76]. As with cyclosporine, there are few data regarding tacrolimus, so the risk to benefit ratio should be evaluated before using this drug.

Effect on breastfeeding: Tacrolimus is reported to enter the breast milk at a rate of 0.05%. Therefore, there is no clear evidence that it must be stopped during breastfeeding[69].

Biological agents

The use of synthetic TNF inhibitors such as IFX, adalimumab, certolizumab pegol and golimumab for the treatment of IBD is increasing. These drugs are classified as pregnancy category B. Natalizumab, which is rarely used, is a pregnancy category C drug. Clinical experience with the use of anti-TNF agents during pregnancy is limited[77,78].

Effect on pregnancy: Transplacental transmission has been reported mostly for monoclonal antibodies (IFX, adalimumab and golimumab) and rarely for fusion proteins (etanercept). Transplacental transmission of monoclonal antibodies increases during pregnancy, and their concentration in umbilical blood becomes equal to or higher than that in maternal blood during the last trimester.

Infliximab

IFX is an IGG1-type monoclonal antibody that inhibits TNF-α. It cannot pass the placental barrier during the first trimester but is transmitted effectively during the third trimester[79]. Therefore, the fetus is not exposed to the drug during the organogenesis period, but following transplacental transmission during the third trimester, IFX remains in infant blood for a few months following

birth. Neither teratogenicity nor toxicity was detected in a study involving 35 pregnant women who used IFX[80]. No difference was seen in terms of pregnancy outcomes between patients using IFX and healthy controls[81]. However, neonatal death caused by intracerebral and pulmonary hemorrhage, premature birth and Fallot's tetralogy have been reported[82].

The TREAT Registry and IFX Safety Database are the two largest studies on this subject[83,84]. In the TREAT Registry, a prospective study involving CD patients, patients on IFX were compared with those not taking IFX. Thirty-six of 66 pregnant women were treated with IFX during pregnancy. Fetal malformations were not detected. In addition, there was no significant difference between the two groups in terms of neonatal complications and miscarriage.

The IFX Safety Database is a retrospective review of 96 patients using IFX. The treatment was generally stopped during the first trimester after the patients realized they were pregnant. Pregnancy outcomes were not different between patients who did and did not take IFX.

The IFX levels of six neonates of mothers taking IFX were higher than those of the mother, but IFX was cleared from the bloodstream of the infants after 2-7 mo[85]. This shows that IFX crosses the placental barrier easily during the third trimester. Moreover, the reticuloendothelial system of the infant is not sufficiently developed to clear the antibodies effectively.

The latest prospective PIANO study[58] included 1232 pregnant women, of whom 264 were treated with IFX, 151 with adalimumab, 67 with certolizumabpegol and 29 with combined biological agent-immunomodulatory therapy. No differences in parameters such as birth defects and infection rates were detected during the first year, but differences in weight and height were detected between infants who were exposed to anti-TNF therapy and those who were not[58]. The odds ratios for developing complications and for preterm birth with combined IFX-immunomodulatory therapy were 1.7 (1.0-2.2) and 2.4 (1.3-4.3), respectively. No difference was seen in the pregnancy outcomes of CD patients according to drug exposure, but increased risks of preterm birth and low birth weight and a prolonged stay in the intensive care unit were seen in UC patients taking combination therapy.

To summarize, IFX can be used during the first two trimesters of gestation. Patients are advised to stop IFX prior to the last trimester (30 wk). In patients with a flare-up caused by IFX treatment discontinuation, short-term corticosteroid treatment can be administered. Discontinuing IFX during the third trimester or at the end of the second trimester decreases IFX transportation to the placenta, reducing the level to which the neonate is exposed. Exposure to IFX during the third trimester can result in infections or a suboptimal response to vaccinations during the neonatal period. Live vaccines should not be administered during the first 6 mo in infants exposed to anti-TNF therapy[79]. Other vaccinations may proceed according to schedule.

Effect on breastfeeding: IFX is found in minute amounts in breast milk, and its oral absorption is minimal; thus, systemic adverse effects are rarely diagnosed[24,86]. IFX levels in an infant whose mother was taking IFX until 4 wk prior to birth decreased after 6 mo even though the mother continued to take IFX while breastfeeding[87].

However, data regarding the safety of IFX during breastfeeding and its local immunosuppression in the gastrointestinal system are insufficient.

Adalimumab

Adalimumab is an antibody targeting IGG1 that is actively transported through the placenta during pregnancy[79,88]. Similar to IFX, adalimumab crosses the placental barrier during the third trimester. Small observational studies on adalimumab during pregnancy have been conducted. It can be used from the time of conception through the first two trimesters of pregnancy. No increased risk of congenital malformation, spontaneous abortion or preterm birth was detected in pregnant women exposed to adalimumab. Although clinical experience with this drug in pregnant women is limited, the Organization for Teratology Information Specialists, which conducted a prospective study on adalimumab involving 27 pregnant women as well as a review of the birth outcomes of 47 pregnant women who used adalimumab during pregnancy, reported that the rates of spontaneous abortion, stillbirth, preterm birth and congenital anomalies were similar to those in the general population[89]. As this medication is used in weekly doses, it is difficult to discontinue treatment at the beginning of the third trimester as this might result in disease flare-ups. It is suggested to discontinue adalimumab 6-8 wk prior to birth.

Effect on breastfeeding: There are insufficient data on the safety of adalimumab use while breastfeeding. The drug passes into breast milk in small amounts, but no adverse effects have been reported[86]. However, discontinuing treatment while breastfeeding should be decided after reviewing the health of the mother and her IBD status.

Certolizumab

Certolizumab is a PEGylated Fab' fragment of an anti-TNFα monoclonal antibody. As opposed to IgG1 antibodies, Fab' fragments pass through the placenta by passive diffusion; therefore, placental transfer is minimal during the third trimester, unlike the cases with IFX and adalimumab[79]. Certolizumab levels in umbilical blood were very low (less than 2 ng/mL) in 10 pregnant women exposed to certolizumab[90]. In theory, it can be used from conception until birth, but the data are insufficient. Certolizumab excretion *via* breast milk is

minimal[78].

Natalizumab

Information regarding the safety of natalizumab use during pregnancy and lactation is insufficient. No increased risk of congenital malformation was detected in a study involving 164 pregnant women treated with natalizumab for CD or multiple sclerosis[91].

Golimumab

Golimumab is a new anti-TNF inhibitor, approved in 2013. Its effects on pregnancy are unclear. Lau *et al*[92] reviewed 42 pregnant women exposed to golimumab (10 pregnant UC patients) for congenital anomalies. These pregnancies resulted in 19 live births, 13 spontaneous abortions (miscarriages) and 6 elective abortions. Of the 13 mothers who experienced miscarriages, 30.8% received simultaneous methotrexate treatment. As golimumab is a new drug, information on the safety of its use during pregnancy is insufficient, and no evidence of its presence in breast milk is available.

Anti-diarrheal agents

Anti-diarrheal agents should be avoided during pregnancy, especially during the early period. Teratogenicity was detected in neonates exposed to diphenoxylate/atropine and loperamide, but whether this was due to chance or to the drugs was unclear[93,94].

Cathartics

A colon cleanse is necessary for sigmoidoscopy during pregnancy. No study has specifically addressed the teratogenic effects of cathartics; however, no congenital anomalies were seen among 22843 pregnant women treated with laxatives[95]. The FDA reclassified cathartics and laxative agents from category B to category X. Cathartics are associated with a risk of dehydration and electrolyte imbalance.

Magnesium citrate

Magnesium citrate has an FDA category B rating and thus is safe to use for constipation or prior to sigmoidoscopy. It might cause electrolyte imbalance and dehydration when used long term.

Polyethylene glycol solution

Polyethylene glycol (PEG) has a FDA category C rating. No data on the safety of PEG use during pregnancy are available.

COMPLICATIONS AND RISKY SITUATIONS IN PREGNANT IBD PATIENTS

Patients who underwent IBD-related surgery prior to pregnancy may experience a temporary increase in stool frequency. Incontinence can be seen especially during the third trimester, but this disappears after birth. Ileostomy can prolapse during the third trimester. Patients with a history of abdominal surgery and ileostomy reported subileus and ileus attacks[96]. The study by Nguyen *et al*[19], which used data from the Nationwide Inpatient Sample (NIS), compared pregnant women with CD or UC with healthy pregnant women without IBD. The frequencies of venous thromboembolism (VTE) and blood transfusions were increased in pregnant women with IBD. The risk of VTE is fourfold greater in pregnant than in non-pregnant IBD patients[97]. The risk of VTE is especially high during the first 6 wk postpartum[98]. Subcutaneous low-molecular-weight heparin is indicated during the peripartum period in high-risk (relapsed and hospitalized patients with moderate-to-severe disease activity) pregnant IBD patients. Heparin prophylaxis is also advised in pregnant IBD patients who are hospitalized for other reasons. VTE is common among UC patients, while the risk of antepartum hemorrhage is at least twofold higher in CD patients[99]. In another study, placental abruption was seen in 2% of IBD patients[19].

Gestational diabetes is another issue. The risk of gestational diabetes did not differ between IBD patients not on steroids and a control group[100]. However, the risk of gestational diabetes was increased in IBD patients who used steroids during pregnancy in the PIANO registry study, which included more than 1000 pregnant IBD patients[58]. Therefore, a pregnancy in a patient with IBD must be considered risky.

NUTRITION AND SUPPLEMENT TREATMENTS

Although nutrition is crucial for all pregnant women, it is especially so in pregnant women with IBD. The prevalence of malnutrition was sixfold higher in pregnant IBD patients compared with healthy controls[19]. A retrospective study in Canada reported less weight gain during pregnancy in IBD patients compared with the general pregnant population[17]. Since malnutrition during pregnancy poses a risk to the fetus, although enteral nutrition is preferred, parenteral nutrition should be used as soon as the need arises. There is no solid evidence of the benefits of specialized diets in terms of IBD remission[101]. A randomized controlled study reported that consumption of fish oil supplements increased the rate of pregnancy without affecting fetal growth[102]. In patients with anti-phospholipid antibody syndrome, fish oil supplements can be used to prevent miscarriage[103]. They also reduce the risk of preterm birth and miscarriage in pregnant IBD patients. Since fish oil is not classified as a drug, it has not been categorized by the FDA for use during pregnancy. Folic acid supplementation is essential during pregnancy for prevention of neural tube defects. If the patient is on sulfasalazine, daily folic acid intake should be increased accordingly (2-5 g/d)[96,102]. Calcium and vitamin D supplementation is advised in patients using steroids to prevent bone

loss. The patient should also be advised not to smoke. Smoking has a negative effect on pregnancy outcomes, particularly for patients with CD.

ENDOSCOPY

Endoscopic retrograde cholangiopancreatography can be performed if indicated[104].

SURGICAL TREATMENT

Surgical treatment indications in both UC (acute, severe or refractory colitis) and CD (perforation, abscess, severe hemorrhage or bowel obstruction) are identical in pregnant and non-pregnant patients. Surgical interventions should be conducted during the second trimester. However, surgeries performed due to acute indications in pregnant patients with IBD carry a high risk of losing the fetus[105]. Few studies have addressed the effect of surgery on maternal morbidity. Ileostomy should be performed in place of primary anastomosis during surgery[106]. Live and healthy births have been reported despite poor prognoses, intraperitoneal sepsis and surgical interventions[107].

There are case reports of colectomy surgery performed during the third trimester in combination with vaginal birth or cesarean section[108-110]. However, medical treatments are preferred to surgical treatments in non-emergency situations.

DELIVERY METHOD

Delivery by cesarean section is more frequent in IBD patients compared with the general population. Indeed, compared with the general population, the frequency of cesarean delivery is 1.5-fold higher in pregnant patients with CD but similar in those with UC[13,19]. Another study reported that the risk of elective cesarean section was twofold higher in pregnant patients with UC and even higher in those with CD[99]. This is likely due to the increased frequency of perianal diseases in CD patients. A retrospective questionnaire study reported that the risk of developing perianal disease was 18% in patients without prior perianal disease who gave birth vaginally (especially in episiotomy cases)[111]. This may also explain the increased preference for cesarean section. However, other studies involving larger patient groups did not support these findings[112,113]. There are insufficient data to recommend this delivery method for IBD patients[85]. Cesarean section is not thought to prevent disease flare-up or development of perianal diseases. It should be performed only when vaginal birth is contradicted in an individual patient. Vaginal birth exacerbates the disease in pregnant CD patients with active perianal disease and in UC patients with active rectal disease with a history of ileal pouch anal anastomosis (IPAA) with colectomy[85,114]. It is vital to prevent pouch dysfunction in IPAA patients and maintain sphincter functionality. Sphincter integrity can be disturbed by mechanical pressure during vaginal birth. Episiotomy has a risk of causing rectovaginal fistulas and non-healing wounds during periods of active perianal disease[115]. Forceps use and uncontrollable tears can negatively affect pelvic floor function. In general, vaginal birth is advised for pregnant IBD patients with light-to-moderate disease activity, whereas cesarean section is preferred for patients with IPAA or with fulminant or active perianal disease[102]. Performance of a cesarean section for reasons other than the above should be decided by the obstetrician for obstetric reasons.

Although there is a risk of complications caused by adhesions in patients who underwent previous pelvic or abdominal surgery or ileostomy or colostomy, vaginal birth is considered safe. Episiotomy can also be performed in such patients[96,116]. A questionnaire administered to 232 pregnant women with IPAA reported no difference in the rates of pouch-related complications or functional problems between vaginal birth and cesarean section[117]. The European Crohn's and Colitis Organization (ECCO) recommends cesarean section for patients with active perianal disease or active rectal involvement[114]. Furthermore, the presence of an ileo-anal pouch or ileo-rectal anastomosis is reported to be relative indications for cesarean section[114].

The delivery method should be decided through collaboration among the patient, obstetrician, gastroenterologist and colorectal surgeon.

CONCLUSION

Since active IBD can have negative effects on both the pregnant patient and the fetus, treatment should be performed in a conscious and energetic manner[118]. In the case of a planned pregnancy, disease remission should be maintained prior to conception. Most studies report increased rates of preterm, stillbirths, low birth weight and spontaneous abortions in pregnant patients with active disease during pregnancy[13,19]. For this reason, maintenance of disease remission is essential.

The drugs used to treat IBD are generally recognized as safe during both pregnancy and lactation, with the exception of methotrexate and thalidomide. Methotrexate and thalidomide should be discontinued in both men and women at least 3 mo prior to conception. Some clinicians tend to stop treatment during the first trimester. Moskovitz *et al*[30] evaluated 207 pregnancies and reported no significant difference in the effects of medication used during the first trimester *vs* any time during pregnancy on pregnancy outcomes. Although they are low risk, methotrexate and 6-MP should not be used in first pregnancies due to a possible risk of bone marrow suppression and pancreatitis. Cyclosporine can be used successfully during pregnancy but can cause preterm birth and low birth weight. As anti-TNF agents cross the placental barrier during the third trimester and might cause immunosuppression in newborns, the ECCO guidelines suggest discontinuing IFX and

adalimumab at 24-26 wk of gestation[114]. In cases in which discontinuing anti-TNF treatment can cause a disease flare-up, certolizumab can be used, as it has a low rate of transplacental transmission during the third trimester.

In the last decade, pregnancy outcomes in IBD patients who became pregnant during a remission period and maintained remission throughout pregnancy were reported to be normal. Patients should be informed of the importance of remission maintenance, and the risk to benefit ratio of continuing treatment during pregnancy should be discussed. The benefits of remission outweigh possible harm to the fetus caused by any potential drugs used.

Since malnutrition occurs more frequently in pregnant patients with IBD, nutritional supplements should be taken. Preventative measures for VTE should be implemented in hospitalized pregnant patients. Endoscopic retrograde cholangiopancreatography should be performed only if absolutely necessary. Surgical treatment indications are identical to those for non-pregnant patients with IBD but carry a high risk of fetal mortality. As previous surgical intervention can negatively affect fertility, laparoscopic methods should be used whenever possible.

The delivery method should be decided in collaboration with the patient. Vaginal birth is deemed safe in patients without perianal disease or severe active rectal involvement who have no complications.

The ECCO guidelines state that 5-ASA preparations, thiopurines, anti-TNF and corticosteroids carry a low risk for the infant[114]. When used during the third trimester, IFX can be transferred to the newborn through the placenta. Therefore, live vaccines are not advised during the first 6 mo after birth. IFX has not been detected in breast milk. However, IBD treatment planning in pregnant patients requires special attention, and decisions should be made on a case-by-case basis. Pregnant patients should be treated more aggressively than non-pregnant patients, as maintaining remission is crucial for pregnancy outcomes. The treatment method should be decided by consensus among the obstetrician, gastroenterologist and colorectal surgeon to reassure the patient.

ACKNOWLEDGMENTS

We would like to thank Yenal Dundar (Department of Health Services Research, University of Liverpool and Mersey Care NHS Trust) for revising the language of the manuscript.

REFERENCES

1 **Binder V**. Epidemiology of IBD during the twentieth century: an integrated view. *Best Pract Res Clin Gastroenterol* 2004; **18**: 463-479 [PMID: 15157821 DOI: 10.1016/j.bpg.2003.12.002]

2 **Orholm M**, Fonager K, Sørensen HT. Risk of ulcerative colitis and Crohn's disease among offspring of patients with chronic inflammatory bowel disease. *Am J Gastroenterol* 1999; **94**: 3236-3238 [PMID: 10566721 DOI: 10.1111/j.1572-0241.1999.01526.x]

3 **Bennett RA**, Rubin PH, Present DH. Frequency of inflammatory bowel disease in offspring of couples both presenting with inflammatory bowel disease. *Gastroenterology* 1991; **100**: 1638-1643 [PMID: 2019369]

4 **Laharie D**, Debeugny S, Peeters M, Van Gossum A, Gower-Rousseau C, Bélaïche J, Fiasse R, Dupas JL, Lerebours E, Piotte S, Cortot A, Vermeire S, Grandbastien B, Colombel JF. Inflammatory bowel disease in spouses and their offspring. *Gastroenterology* 2001; **120**: 816-819 [PMID: 11231934 DOI: 10.1053/gast.2001.22574]

5 **Malgarinos G**, Gikas A, Delicha E, Stamataki A, Georgopoulos F, Papadimitriou A, Stanciu C, Triantafillidis JK. Pregnancy and inflammatory bowel disease: a prospective case-control study. *Rev Med Chir Soc Med Nat Iasi* 2007; **111**: 613-619 [PMID: 18293689]

6 **Ferguson CB**, Mahsud-Dornan S, Patterson RN. Inflammatory bowel disease in pregnancy. *BMJ* 2008; **337**: a427 [PMID: 18599468 DOI: 10.1136/bmj.39566.681458.BE]

7 **Khosla R**, Willoughby CP, Jewell DP. Crohn's disease and pregnancy. *Gut* 1984; **25**: 52-56 [PMID: 6140209 DOI: 10.1136/gut.25.1.52]

8 **Mogadam M**, Dobbins WO, Korelitz BI, Ahmed SW. Pregnancy in inflammatory bowel disease: effect of sulfasalazine and corticosteroids on fetal outcome. *Gastroenterology* 1981; **80**: 72-76 [PMID: 6108894]

9 **Miller JP**. Inflammatory bowel disease in pregnancy: a review. *J R Soc Med* 1986; **79**: 221-225 [PMID: 2871190]

10 **Mahadevan U**, Sandborn WJ, Li DK, Hakimian S, Kane S, Corley DA. Pregnancy outcomes in women with inflammatory bowel disease: a large community-based study from Northern California. *Gastroenterology* 2007; **133**: 1106-1112 [PMID: 17764676 DOI: 10.1053/j.gastro.2007.07.019]

11 **Hosseini-Carroll P**, Mutyala M, Seth A, Nageeb S, Soliman D, Boktor M, Sheth A, Chapman J, Morris J, Jordan P, Manas K, Becker F, Alexander JS. Pregnancy and inflammatory bowel diseases: Current perspectives, risks and patient management. *World J Gastrointest Pharmacol Ther* 2015; **6**: 156-171 [PMID: 26558150 DOI: 10.4292/wjgpt.v6.i4.156]

12 **Nielsen OH**, Andreasson B, Bondesen S, Jarnum S. Pregnancy in ulcerative colitis. *Scand J Gastroenterol* 1983; **18**: 735-742 [PMID: 6669937 DOI: 10.3109/00365528309182088]

13 **Cornish J**, Tan E, Teare J, Teoh TG, Rai R, Clark SK, Tekkis PP. A meta-analysis on the influence of inflammatory bowel disease on pregnancy. *Gut* 2007; **56**: 830-837 [PMID: 17185356 DOI: 10.1136/gut.2006.108324]

14 **Dominitz JA**, Young JC, Boyko EJ. Outcomes of infants born to mothers with inflammatory bowel disease: a population-based cohort study. *Am J Gastroenterol* 2002; **97**: 641-648 [PMID: 11926208 DOI: 10.1111/j.1572-0241.2002.05543.x]

15 **Nørgård B**, Puho E, Pedersen L, Czeizel AE, Sørensen HT. Risk of congenital abnormalities in children born to women with ulcerative colitis: a population-based, case-control study. *Am J Gastroenterol* 2003; **98**: 2006-2010 [PMID: 14499779 DOI: 10.1111/j.1572-0241.2003.07578.x]

16 **Bortoli A**, Saibeni S, Tatarella M, Prada A, Beretta L, Rivolta R, Politi P, Ravelli P, Imperiali G, Colombo E, Pera A, Daperno M, Carnovali M, de Franchis R, Vecchi M. Pregnancy before and after the diagnosis of inflammatory bowel diseases: retrospective case-control study. *J Gastroenterol Hepatol* 2007; **22**: 542-549 [PMID: 17376049 DOI: 10.1111/j.1440-1746.2006.04754.x]

17 **Moser MA**, Okun NB, Mayes DC, Bailey RJ. Crohn's disease, pregnancy, and birth weight. *Am J Gastroenterol* 2000; **95**: 1021-1026 [PMID: 10763954 DOI: 10.1111/j.1572-0241.2000.01852.x]

18 **Naganuma M**, Kunisaki R, Yoshimura N, Nagahori M, Yamamoto H, Kimura H, Sako M, Kawaguchi T, Takazoe M, Yamamoto S, Matsui T, Hibi T, Watanabe M. Conception and pregnancy outcome in women with inflammatory bowel disease: A multicentre study from Japan. *J Crohns Colitis* 2011; **5**: 317-323 [PMID: 21683301 DOI: 10.1016/j.crohns.2011.02.003]

19 **Nguyen GC**, Boudreau H, Harris ML, Maxwell CV. Outcomes of obstetric hospitalizations among women with inflammatory bowel disease in the United States. *Clin Gastroenterol Hepatol* 2009; **7**:

329-334 [PMID: 19027089 DOI: 10.1016/j.cgh.2008.10.022]
20 **Morales M**, Berney T, Jenny A, Morel P, Extermann P. Crohn's disease as a risk factor for the outcome of pregnancy. *Hepatogastroenterology* 2000; **47**: 1595-1598 [PMID: 11149010]
21 **Bush MC**, Patel S, Lapinski RH, Stone JL. Perinatal outcomes in inflammatory bowel disease. *J Matern Fetal Neonatal Med* 2004; **15**: 237-241 [PMID: 15280131 DOI: 10.1080/14767050410001668662]
22 **Nørgård B**, Hundborg HH, Jacobsen BA, Nielsen GL, Fonager K. Disease activity in pregnant women with Crohn's disease and birth outcomes: a regional Danish cohort study. *Am J Gastroenterol* 2007; **102**: 1947-1954 [PMID: 17573787 DOI: 10.1111/j.1572-0241.2007.01355.x]
23 **Bortoli A**, Pedersen N, Duricova D, D'Inca R, Gionchetti P, Panelli MR, Ardizzone S, Sanroman AL, Gisbert JP, Arena I, Riegler G, Marrollo M, Valpiani D, Corbellini A, Segato S, Castiglione F, Munkholm P. Pregnancy outcome in inflammatory bowel disease: prospective European case-control ECCO-EpiCom study, 2003-2006. *Aliment Pharmacol Ther* 2011; **34**: 724-734 [PMID: 21815900 DOI: 10.1111/j.1365-2036.2011.04794.x]
24 **Dignass A**, Van Assche G, Lindsay JO, Lémann M, Söderholm J, Colombel JF, Danese S, D'Hoore A, Gassull M, Gomollón F, Hommes DW, Michetti P, O'Morain C, Oresland T, Windsor A, Stange EF, Travis SP. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Current management. *J Crohns Colitis* 2010; **4**: 28-62 [PMID: 21122489 DOI: 10.1016/j.crohns.2009.12.002]
25 **Rahimi R**, Nikfar S, Rezaie A, Abdollahi M. Pregnancy outcome in women with inflammatory bowel disease following exposure to 5-aminosalicylic acid drugs: a meta-analysis. *Reprod Toxicol* 2008; **25**: 271-275 [PMID: 18242053 DOI: 10.1016/j.reprotox.2007.11.010]
26 **Khan AK**, Truelove SC. Placental and mammary transfer of sulphasalazine. *Br Med J* 1979; **2**: 1553 [PMID: 43760 DOI: 10.1136/b mj.2.6204.1553]
27 **Branski D**, Kerem E, Gross-Kieselstein E, Hurvitz H, Litt R, Abrahamov A. Bloody diarrhea--a possible complication of sulfasalazine transferred through human breast milk. *J Pediatr Gastroenterol Nutr* 1986; **5**: 316-317 [PMID: 2870147 DOI: 10.1097/00005176-198605020-00028]
28 **Järnerot G**, Into-Malmberg MB, Esbjörner E. Placental transfer of sulphasalazine and sulphapyridine and some of its metabolites. *Scand J Gastroenterol* 1981; **16**: 693-697 [PMID: 6119765 DOI: 10.3109/00365528109182032]
29 **Nørgård B**, Fonager K, Pedersen L, Jacobsen BA, Sørensen HT. Birth outcome in women exposed to 5-aminosalicylic acid during pregnancy: a Danish cohort study. *Gut* 2003; **52**: 243-247 [PMID: 12524407 DOI: 10.1136/gut.52.2.243]
30 **Moskovitz DN**, Bodian C, Chapman ML, Marion JF, Rubin PH, Scherl E, Present DH. The effect on the fetus of medications used to treat pregnant inflammatory bowel-disease patients. *Am J Gastroenterol* 2004; **99**: 656-661 [PMID: 15089898 DOI: 10.1111/j.1572-0241.2004.04140.x]
31 **Nørgård B**, Pedersen L, Christensen LA, Sørensen HT. Therapeutic drug use in women with Crohn's disease and birth outcomes: a Danish nationwide cohort study. *Am J Gastroenterol* 2007; **102**: 1406-1413 [PMID: 17437503 DOI: 10.1111/j.1572-0241.2007.01216.x]
32 **Habal FM**, Hui G, Greenberg GR. Oral 5-aminosalicylic acid for inflammatory bowel disease in pregnancy: safety and clinical course. *Gastroenterology* 1993; **105**: 1057-1060 [PMID: 8405849]
33 **Marteau P**, Tennenbaum R, Elefant E, Lémann M, Cosnes J. Foetal outcome in women with inflammatory bowel disease treated during pregnancy with oral mesalazine microgranules. *Aliment Pharmacol Ther* 1998; **12**: 1101-1108 [PMID: 9845399 DOI: 10.1046/j.1365-2036.1998.00417.x]
34 **Trallori G**, d'Albasio G, Bardazzi G, Bonanomi AG, Amorosi A, Del Carlo P, Palli D, Galli M, Pacini F. 5-Aminosalicylic acid in pregnancy: clinical report. *Ital J Gastroenterol* 1994; **26**: 75-78 [PMID: 8032081]
35 **US Food and Drug Administration.** Asacol (mesalamine) delayed release tablets. May 2010
36 **Aparajita S**, Martin CF, Kane SV, Dubinsky M, Nguyen DD, McCabe RP, Rubin DT, Scherl EJ, Mahadevan U. Is Asacol use associated with congenital anomalies? Results from a nationwide prospective pregnancy registry. *Gastroenterology* 2013; **5**: S-379 [DOI: 10.1016/S0016-5085(13)61398-0]
37 **Habal FM**, Huang VW. Review article: a decision-making algorithm for the management of pregnancy in the inflammatory bowel disease patient. *Aliment Pharmacol Ther* 2012; **35**: 501-515 [PMID: 22221203 DOI: 10.1111/j.1365-2036.2011.04967.x]
38 **Carmichael SL**, Shaw GM. Maternal corticosteroid use and risk of selected congenital anomalies. *Am J Med Genet* 1999; **86**: 242-244 [PMID: 10482873 DOI: 10.1002/(SICI)1096-8628(19990917)86:3<242: : AID-AJMG9>3.0.CO; 2-U]
39 **Tegethoff M**, Pryce C, Meinlschmidt G. Effects of intrauterine exposure to synthetic glucocorticoids on fetal, newborn, and infant hypothalamic-pituitary-adrenal axis function in humans: a systematic review. *Endocr Rev* 2009; **30**: 753-789 [PMID: 19837868 DOI: 10.1210/er.2008-0014]
40 **Beaulieu DB**, Ananthakrishnan AN, Issa M, Rosenbaum L, Skaros S, Newcomer JR, Kuhlmann RS, Otterson MF, Emmons J, Knox J, Binion DG. Budesonide induction and maintenance therapy for Crohn's disease during pregnancy. *Inflamm Bowel Dis* 2009; **15**: 25-28 [PMID: 18680195 DOI: 10.1002/ibd.20640]
41 **Källén B**, Rydhstroem H, Aberg A. Congenital malformations after the use of inhaled budesonide in early pregnancy. *Obstet Gynecol* 1999; **93**: 392-395 [PMID: 10074986 DOI: 10.1097/00006250-199903000-00016]
42 **Norjavaara E**, de Verdier MG. Normal pregnancy outcomes in a population-based study including 2,968 pregnant women exposed to budesonide. *J Allergy Clin Immunol* 2003; **111**: 736-742 [PMID: 12704351 DOI: 10.1067/mai.2003.1340]
43 **McKenzie SA**, Selley JA, Agnew JE. Secretion of prednisolone into breast milk. *Arch Dis Child* 1975; **50**: 894-896 [PMID: 1211963 DOI: 10.1136/adc.50.11.894]
44 **Ng SW**, Mahadevan U. Management of inflammatory bowel disease in pregnancy. *Expert Rev Clin Immunol* 2013; **9**: 161-173; quiz 174 [PMID: 23390947 DOI: 10.1586/eci.12.103]
45 **Polifka JE**, Friedman JM. Teratogen update: azathioprine and 6-mercaptopurine. *Teratology* 2002; **65**: 240-261 [PMID: 11967923 DOI: 10.1097/00007890-200104270-00006]
46 **Nielsen OH**, Bjerrum JT, Herfarth H, Rogler G. Recent advances using immunomodulators for inflammatory bowel disease. *J Clin Pharmacol* 2013; **53**: 575-588 [PMID: 23408468 DOI: 10.1002/jcph.2]
47 **Jharap B**, de Boer NK, Stokkers P, Hommes DW, Oldenburg B, Dijkstra G, van der Woude CJ, de Jong DJ, Mulder CJ, van Elburg RM, van Bodegraven AA. Intrauterine exposure and pharmacology of conventional thiopurine therapy in pregnant patients with inflammatory bowel disease. *Gut* 2014; **63**: 451-457 [PMID: 23424097 DOI: 10.1136/gutjnl-2012-303615]
48 **Nørgård B**, Pedersen L, Fonager K, Rasmussen SN, Sørensen HT. Azathioprine, mercaptopurine and birth outcome: a population-based cohort study. *Aliment Pharmacol Ther* 2003; **17**: 827-834 [PMID: 12641505 DOI: 10.1046/j.1365-2036.2003.01537.x]
49 **Cleary BJ**, Källén B. Early pregnancy azathioprine use and pregnancy outcomes. *Birth Defects Res A Clin Mol Teratol* 2009; **85**: 647-654 [PMID: 19343728 DOI: 10.1002/bdra.20583]
50 **McConnell RA**, Mahadevan U. Use of Immunomodulators and Biologics Before, During, and After Pregnancy. *Inflamm Bowel Dis* 2016; **22**: 213-223 [PMID: 26444103 DOI: 10.1097/MIB.0000000000000596]
51 **Bröms G**, Granath F, Linder M, Stephansson O, Elmberg M, Kieler H. Birth outcomes in women with inflammatory bowel disease: effects of disease activity and drug exposure. *Inflamm Bowel Dis* 2014; **20**: 1091-1098 [PMID: 24810137 DOI: 10.1097/MIB.0000000000000060]
52 **Goldstein LH**, Dolinsky G, Greenberg R, Schaefer C, Cohen-Kerem R, Diav-Citrin O, Malm H, Reuvers-Lodewijks ME, Rost

van Tonningen-van Driel MM, Arnon J, Ornoy A, Clementi M, Di Gianantonio E, Koren G, Braunstein R, Berkovitch M. Pregnancy outcome of women exposed to azathioprine during pregnancy. *Birth Defects Res A Clin Mol Teratol* 2007; **79**: 696-701 [PMID: 17847119 DOI: 10.1002/bdra.20399]

53 **Ban L**, Tata LJ, Fiaschi L, Card T. Limited risks of major congenital anomalies in children of mothers with IBD and effects of medications. *Gastroenterology* 2014; **146**: 76-84 [PMID: 24126096 DOI: 10.1053/j.gastro.2013.09.061]

54 **Beaugerie L**, Brousse N, Bouvier AM, Colombel JF, Lémann M, Cosnes J, Hébuterne X, Cortot A, Bouhnik Y, Gendre JP, Simon T, Maynadié M, Hermine O, Faivre J, Carrat F. Lymphoproliferative disorders in patients receiving thiopurines for inflammatory bowel disease: a prospective observational cohort study. *Lancet* 2009; **374**: 1617-1625 [PMID: 19837455 DOI: 10.1016/S0140-6736(09)61302-7]

55 **Coelho J**, Beaugerie L, Colombel JF, Hébuterne X, Lerebours E, Lémann M, Baumer P, Cosnes J, Bourreille A, Gendre JP, Seksik P, Blain A, Metman EH, Nisard A, Cadiot G, Veyrac M, Coffin B, Dray X, Carrat F, Marteau P. Pregnancy outcome in patients with inflammatory bowel disease treated with thiopurines: cohort from the CESAME Study. *Gut* 2011; **60**: 198-203 [PMID: 21115547 DOI: 10.1136/gut.2010]

56 **Akbari M**, Shah S, Velayos FS, Mahadevan U, Cheifetz AS. Systematic review and meta-analysis on the effects of thiopurines on birth outcomes from female and male patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2013; **19**: 15-22 [PMID: 22434610 DOI: 10.1002/ibd.22948]

57 **Casanova MJ**, Chaparro M, Domènech E, Barreiro-de Acosta M, Bermejo F, Iglesias E, Gomollón F, Rodrigo L, Calvet X, Esteve M, García-Planella E, García-López S, Taxonera C, Calvo M, López M, Ginard D, Gómez-García M, Garrido E, Pérez-Calle JL, Beltrán B, Piqueras M, Saro C, Botella B, Dueñas C, Ponferrada A, Mañosa M, García-Sánchez V, Maté J, Gisbert JP. Safety of thiopurines and anti-TNF-α drugs during pregnancy in patients with inflammatory bowel disease. *Am J Gastroenterol* 2013; **108**: 433-440 [PMID: 23318480 DOI: 10.1038/ajg.2012.430]

58 **Mahadevan U**, Martin CF, Sandler RS, Kane SV, Dubinsky M, Lewis JD, Sands B E. 865 PIANO: a 1000 patient prospective registry of pregnancy outcomes in women with IBD exposed to immunomodulators and biologic therapy. *Gastroenterology* 2012; **142**: 149

59 **Lin K**, Martin CF, Chambers C, Kane SV, Dubinsky M, Sandborn W, Sands BE. Achievement of developmental milestones among offspring of women with inflammatory bowel disease: the PIANO registry. *Gastroenterology* 2014; **5**: S-1 [DOI: 10.1016/S0016-5085(14)60002-0]

60 **de Meij TG**, Jharap B, Kneepkens CM, van Bodegraven AA, de Boer NK. Long-term follow-up of children exposed intrauterine to maternal thiopurine therapy during pregnancy in females with inflammatory bowel disease. *Aliment Pharmacol Ther* 2013; **38**: 38-43 [PMID: 23675854 DOI: 10.1111/apt.12334]

61 **Ng SW**, Mahadevan U. My treatment approach to management of the pregnant patient with inflammatory bowel disease. *Mayo Clin Proc* 2014; **89**: 355-360 [PMID: 24582194 DOI: 10.1016/j.mayocp.2013.11.012]

62 **Christensen LA**, Dahlerup JF, Nielsen MJ, Fallingborg JF, Schmiegelow K. Azathioprine treatment during lactation. *Aliment Pharmacol Ther* 2008; **28**: 1209-1213 [PMID: 18761704 DOI: 10.1111/j.1365-2036.2008.03843.x]

63 **Gardiner SJ**, Gearry RB, Roberts RL, Zhang M, Barclay ML, Begg EJ. Exposure to thiopurine drugs through breast milk is low based on metabolite concentrations in mother-infant pairs. *Br J Clin Pharmacol* 2006; **62**: 453-456 [PMID: 16995866 DOI: 10.1111/j.1365-2125.2006.02639.x]

64 **Angelberger S**, Reinisch W, Messerschmidt A, Miehsler W, Novacek G, Vogelsang H, Dejaco C. Long-term follow-up of babies exposed to azathioprine in utero and via breastfeeding. *J Crohns Colitis* 2011; **5**: 95-100 [PMID: 21453877 DOI: 10.1016/j.crohns.2010.10.005]

65 **Milunsky A**, Graef JW, Gaynor MF. Methotrexate-induced congenital malformations. *J Pediatr* 1968; **72**: 790-795 [PMID: 5652604]

66 **Briggs GG**, Wan SR. Drug therapy during labor and delivery, part 1. *Am J Health Syst Pharm* 2006; **63**: 1038-1047 [PMID: 16709890]

67 **Del Campo M**, Kosaki K, Bennett FC, Jones KL. Developmental delay in fetal aminopterin/methotrexate syndrome. *Teratology* 1999; **60**: 10-12 [PMID: 10413333]

68 **Gisbert JP**. Safety of immunomodulators and biologics for the treatment of inflammatory bowel disease during pregnancy and breast-feeding. *Inflamm Bowel Dis* 2010; **16**: 881-895 [PMID: 19885906 DOI: 10.1002/ibd.21154]

69 **Nielsen OH**, Maxwell C, Hendel J. IBD medications during pregnancy and lactation. *Nat Rev Gastroenterol Hepatol* 2014; **11**: 116-127 [PMID: 23897285 DOI: 10.1038/nrgastro.2013.135]

70 **Johns DG**, Rutherford LD, Leighton PC, Vogel CL. Secretion of methotrexate into human milk. *Am J Obstet Gynecol* 1972; **112**: 978-980 [PMID: 5042796]

71 **van der Aa EM**, Peereboom-Stegeman JH, Noordhoek J, Gribnau FW, Russel FG. Mechanisms of drug transfer across the human placenta. *Pharm World Sci* 1998; **20**: 139-148 [PMID: 9762726]

72 **Paziana K**, Del Monaco M, Cardonick E, Moritz M, Keller M, Smith B, Coscia L, Armenti V. Ciclosporin use during pregnancy. *Drug Saf* 2013; **36**: 279-294 [PMID: 23516008 DOI: 10.1007/s40264-013-0034-x]

73 **Bar Oz B**, Hackman R, Einarson T, Koren G. Pregnancy outcome after cyclosporine therapy during pregnancy: a meta-analysis. *Transplantation* 2001; **71**: 1051-1055 [PMID: 11374400]

74 **Branche J**, Cortot A, Bourreille A, Coffin B, de Vos M, de Saussure P, Seksik P, Marteau P, Lemann M, Colombel JF. Cyclosporine treatment of steroid-refractory ulcerative colitis during pregnancy. *Inflamm Bowel Dis* 2009; **15**: 1044-1048 [PMID: 19137604 DOI: 10.1002/ibd.20858]

75 **Ecevit Ç**, Ünal F, Baran M, Aydoğdu S. Parenthood in pediatric liver transplant patients. *Pediatr Transplant* 2012; **16**: 346-349 [PMID: 22404458 DOI: 10.1111/j.1399-3046.2012.01671.x]

76 **Baumgart DC**, Sturm A, Wiedenmann B, Dignass AU. Uneventful pregnancy and neonatal outcome with tacrolimus in refractory ulcerative colitis. *Gut* 2005; **54**: 1822-1823 [PMID: 16120758 DOI: 10.1136/gut.2005.078972]

77 **O'Donnell S**, O'Morain C. Review article: use of antitumour necrosis factor therapy in inflammatory bowel disease during pregnancy and conception. *Aliment Pharmacol Ther* 2008; **27**: 885-894 [PMID: 18284649 DOI: 10.1111/j.1365-2036.2008.03648.x]

78 **Mahadevan U**, Cucchiara S, Hyams JS, Steinwurz F, Nuti F, Travis SP, Sandborn WJ, Colombel JF. The London Position Statement of the World Congress of Gastroenterology on Biological Therapy for IBD with the European Crohn's and Colitis Organisation: pregnancy and pediatrics. *Am J Gastroenterol* 2011; **106**: 214-223; quiz 224 [PMID: 21157441 DOI: 10.1038/ajg.2010.464]

79 **Mahadevan U**, Wolf DC, Dubinsky M, Cortot A, Lee SD, Siegel CA, Ullman T, Glover S, Valentine JF, Rubin DT, Miller J, Abreu MT. Placental transfer of anti-tumor necrosis factor agents in pregnant patients with inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2013; **11**: 286-292; quiz e24 [PMID: 23200982 DOI: 10.1016/j.cgh.2012.11.011]

80 **Katz JA**, Lichtenstein GR, Keenan GF, Healy D, Jacobs SJ. Outcome of pregnancy in women receiving REMICADE® (Infliximab) for the treatment of Crohn's disease or rheumatoid arthritis. *Gastroenterology* 2001; **120**: A69 [DOI: 10.1016/S0016-5085(08)80343-5]

81 **Friedman S**, Regueiro MD. Pregnancy and nursing in inflammatory bowel disease. *Gastroenterol Clin North Am* 2002; **31**: 265-273, xii [PMID: 12122737 DOI: 10.1016/S0889-8553(01)00015-2]

82 **Srinivasan R**. Infliximab treatment and pregnancy outcome in active Crohn's disease. *Am J Gastroenterol* 2001; **96**: 2274-2275 [PMID: 11467677 DOI: 10.1111/j.1572-0241.2001.03987.x]

83 **Lichtenstein GR**, Feagan BG, Cohen RD, Salzberg BA, Diamond RH, Chen DM, Pritchard ML, Sandborn WJ. Serious infections and

mortality in association with therapies for Crohn's disease: TREAT registry. *Clin Gastroenterol Hepatol* 2006; **4**: 621-630 [PMID: 16678077 DOI: 10.1016/j.cgh.2006.03.002]

84 **Katz JA**, Antoni C, Keenan GF, Smith DE, Jacobs SJ, Lichtenstein GR. Outcome of pregnancy in women receiving infliximab for the treatment of Crohn's disease and rheumatoid arthritis. *Am J Gastroenterol* 2004; **99**: 2385-2392 [PMID: 15571587 DOI: 10.1111/j.1572-0241.2004.30186.x]

85 **Dubinsky M**, Abraham B, Mahadevan U. Management of the pregnant IBD patient. *Inflamm Bowel Dis* 2008; **14**: 1736-1750 [PMID: 18626967 DOI: 10.1002/ibd.20532]

86 **Ben-Horin S**, Yavzori M, Kopylov U, Picard O, Fudim E, Eliakim R, Chowers Y, Lang A. Detection of infliximab in breast milk of nursing mothers with inflammatory bowel disease. *J Crohns Colitis* 2011; **5**: 555-558 [PMID: 22115374 DOI: 10.1016/j.crohns.2011.05.006]

87 **Vasiliauskas EA**, Church JA, Silverman N, Barry M, Targan SR, Dubinsky MC. Case report: evidence for transplacental transfer of maternally administered infliximab to the newborn. *Clin Gastroenterol Hepatol* 2006; **4**: 1255-1258 [PMID: 17045211 DOI: 10.1016/j.cgh.2006.07.018]

88 **Zelinkova Z**, van der Ent C, Bruin KF, van Baalen O, Vermeulen HG, Smalbraak HJ, Ouwendijk RJ, Hoek AC, van der Werf SD, Kuipers EJ, van der Woude CJ. Effects of discontinuing anti-tumor necrosis factor therapy during pregnancy on the course of inflammatory bowel disease and neonatal exposure. *Clin Gastroenterol Hepatol* 2013; **11**: 318-321 [PMID: 23103819 DOI: 10.1016/j.cgh.2012.10.024]

89 **Osting VC**, Carter JD. A safety assessment of tumor necrosis factor antagonists during pregnancy. *Expert Opin Drug Saf* 2010; **9**: 421-429 [PMID: 20367495 DOI: 10.1517/14740330903578639]

90 **Wolf D**, Mahadevan U. Certolizumab pegol use in pregnancy: low levels detected in cord blood. *Arthritis Rheu* 2010; **62**: 718 [DOI: 10.1002/art.28486]

91 **Mahadevan U**, Nazareth M, Cristiano L, Kooijmans M, Hogge G. Natalizumab use during pregnancy. *Am J Gastroenterol* 2008; **103**: S449-S450 [DOI: 10.1111/j.1572-0241.2008.02139_9.x]

92 **Lau AG**, Clark M, Harrison DD, Geldho A, Nissinen R, Sanders M. Pregnancy outcomes in women exposed to golimumab. *Arthritis Rheum* 2013; **65**: S870-S871 [DOI: 10.1002/art.38216]

93 **Lewis JH**, Weingold AB. The use of gastrointestinal drugs during pregnancy and lactation. *Am J Gastroenterol* 1985; **80**: 912-923 [PMID: 2864852]

94 **Briggs GG**, Freeman R K, Yaffe S J. Drugs in pregnancy and lactation: a reference guide to fetal and neonatal risk. USA: Lippincott Williams & Wilkins, 2012

95 **Acs N**, Bánhidy F, Puhó EH, Czeizel AE. No association between severe constipation with related drug treatment in pregnant women and congenital abnormalities in their offspring: A population-based case-control study. *Congenit Anom* (Kyoto) 2010; **50**: 15-20 [PMID: 20201964 DOI: 10.1111/j.1741-4520.2009.00252.x]

96 **Tilson RS**, Friedman S. Inflammatory Bowel Disease During Pregnancy. *Curr Treat Options Gastroenterol* 2003; **6**: 227-236 [PMID: 12744822 DOI: 10.1007/s11938-003-0004-y]

97 **Nguyen GC**, Sam J. Rising prevalence of venous thromboembolism and its impact on mortality among hospitalized inflammatory bowel disease patients. *Am J Gastroenterol* 2008; **103**: 2272-2280 [PMID: 18684186 DOI: 10.1111/j.1572-0241.2008.02052.x]

98 **Pomp ER**, Lenselink AM, Rosendaal FR, Doggen CJ. Pregnancy, the postpartum period and prothrombotic defects: risk of venous thrombosis in the MEGA study. *J Thromb Haemost* 2008; **6**: 632-637 [PMID: 18248600 DOI: 10.1111/j.1538-7836.2008.02921.x]

99 **Bröms G**, Granath F, Linder M, Stephansson O, Elmberg M, Kieler H. Complications from inflammatory bowel disease during pregnancy and delivery. *Clin Gastroenterol Hepatol* 2012; **10**: 1246-1252 [PMID: 22922307 DOI: 10.1016/j.cgh.2012.08.018]

100 **Leung YP**, Kaplan GG, Coward S, Tanyingoh D, Kaplan BJ, Johnston DW, Barkema HW, Ghosh S, Panaccione R, Seow CH. Intrapartum corticosteroid use significantly increases the risk of gestational diabetes in women with inflammatory bowel disease. *J Crohns Colitis* 2015; **9**: 223-230 [PMID: 25576754 DOI: 10.1093/ecco-jcc/jjv006]

101 **Zachos M**, Tondeur M, Griffiths AM. Enteral nutritional therapy for inducing remission of Crohn's disease. *Cochrane Database Syst Rev* 2001; **(3)**: CD000542 [PMID: 11686966 DOI: 10.1002/14651858.cd000542.pub2]

102 **Habal FM**, Ravindran NC. Management of inflammatory bowel disease in the pregnant patient. *World J Gastroenterol* 2008; **14**: 1326-1332 [PMID: 18322943 DOI: 10.3748/wjg.14.1326]

103 **Rossi E,** Costa M. Fish oil derivatives as a prophylaxis of recurrent miscarriage associated with antiphospholipid antibodies (APL): a pilot study. *Lupus* 1993; **2**: 319-323 [PMID: 8305926 DOI: 10.1177/096120339300200508]

104 **Cappell MS**, Colon VJ, Sidhom OA. A study at 10 medical centers of the safety and efficacy of 48 flexible sigmoidoscopies and 8 colonoscopies during pregnancy with follow-up of fetal outcome and with comparison to control groups. *Dig Dis Sci* 1996; **41**: 2353-2361 [PMID: 9011442 DOI: 10.1007/BF02100127]

105 **Ferrero S**, Ragni N. Inflammatory bowel disease: management issues during pregnancy. *Arch Gynecol Obstet* 2004; **270**: 79-85 [PMID: 12721680 DOI: 10.1007/s00404-003-0489-6]

106 **Visser BC**, Glasgow RE, Mulvihill KK, Mulvihill SJ. Safety and timing of nonobstetric abdominal surgery in pregnancy. *Dig Surg* 2001; **18**: 409-417 [PMID: 11721118 DOI: 10.1159/000050183]

107 **Hill J**, Clark A, Scott NA. Surgical treatment of acute manifestations of Crohn's disease during pregnancy. *J R Soc Med* 1997; **90**: 64-66 [PMID: 9068432 DOI: 10.1177/014107689709000203]

108 **Watson WJ**, Gaines TE. Third-trimester colectomy for severe ulcerative colitis. A case report. *J Reprod Med* 1987; **32**: 869-872 [PMID: 3430496]

109 **Bohe MG**, Ekelund GR, Genell SN, Gennser GM, Jiborn HA, Leandoer LJ, Klaus GL, Svanberg LK. Surgery for fulminating colitis during pregnancy. *Dis Colon Rectum* 1983; **26**: 119-122 [DOI: 10.1007/BF02562590]

110 **Boulton R**, Hamilton M, Lewis A, Walker P, Pounder R. Fulminant ulcerative colitis in pregnancy. *Am J Gastroenterol* 1994; **89**: 931-933 [PMID: 8198108]

111 **Kornfeld D**, Cnattingius S, Ekbom A. Pregnancy outcomes in women with inflammatory bowel disease--a population-based cohort study. *Am J Obstet Gynecol* 1997; **177**: 942-946 [PMID: 9369849 DOI: 10.1016/S0002-9378(97)70298-9]

112 **Ilnyckyji A**, Blanchard JF, Rawsthorne P, Bernstein CN. Perianal Crohn's disease and pregnancy: role of the mode of delivery. *Am J Gastroenterol* 1999; **94**: 3274-3278 [PMID: 10566729 DOI: 10.1111/j.1572-0241.1999.01537.x]

113 **Beniada A**, Benoist G, Maurel J, Dreyfus M. [Inflammatory bowel disease and pregnancy: report of 76 cases and review of the literature]. *J Gynecol Obstet Biol Reprod* (Paris) 2005; **34**: 581-588 [PMID: 16208201]

114 **van der Woude CJ**, Ardizzone S, Bengtson MB, Fiorino G, Fraser G, Katsanos K, Kolacek S, Juillerat P, Mulders AG, Pedersen N, Selinger C, Sebastian S, Sturm A, Zelinkova Z, Magro F. The second European evidenced-based consensus on reproduction and pregnancy in inflammatory bowel disease. *J Crohns Colitis* 2015; **9**: 107-124 [PMID: 25602023 DOI: 10.1093/ecco-jcc/jju006]

115 **Brandt LJ**, Estabrook SG, Reinus JF. Results of a survey to evaluate whether vaginal delivery and episiotomy lead to perineal involvement in women with Crohn's disease. *Am J Gastroenterol* 1995; **90**: 1918-1922 [PMID: 7484992 DOI: 10.1097/00006254-199606000-00006]

116 **Biedermann L**, Rogler G, Vavricka SR, Seibold F, Seirafi M. Pregnancy and breastfeeding in inflammatory bowel disease. *Digestion* 2012; **86** Suppl 1: 45-54 [PMID: 23051726 DOI: 10.1159/000341941]

117 **Hahnloser D**, Pemberton JH, Wolff BG, Larson D, Harrington J, Farouk R, Dozois RR. Pregnancy and delivery before and after ileal pouch-anal anastomosis for inflammatory bowel disease: immediate and long-term consequences and outcomes. *Dis Colon Rectum* 2004; **47**: 1127-1135 [PMID: 15164253 DOI: 10.1007/s10350-004-0569-0]

118 **Mountifield RE**, Prosser R, Bampton P, Muller K, Andrews JM. Pregnancy and IBD treatment: this challenging interplay from a patients' perspective. *J Crohns Colitis* 2010; **4**: 176-182 [PMID: 21122502 DOI: 10.1016/j.crohns.2009.10.001]

P- Reviewer: Actis GC, Desai DC, Gheita TA **S- Editor**: Gong ZM
L- Editor: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i4.503

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 503-512
ISSN 2150-5349 (online)

REVIEW

Logical hypothesis: Low FODMAP diet to prevent diverticulitis

Yoshiharu Uno, Jennifer C van Velkinburgh

Yoshiharu Uno, Office Uno Column (Medical Science Publisher), Onoe-Cho, Hyogo 675-0025, Japan

Jennifer C van Velkinburgh, van Velkinburgh Initiative for Collaboratory Biomedical Research, Santa Fe, NM 87501, United States

Author contributions: Uno Y contributed to creation of theory, reference collection, and creation of the table and figures; Uno Y and van Velkinburgh JC contributed equally to the writing of the manuscript.

Conflict-of-interest statement: Uno Y was granted a trademark of low FODMAP diet from the Japan Patent Office. van Velkinburgh JC declares no conflicts of interest in relation to the publication of this review.

Manuscript source: Unsolicited manuscript

Correspondence to: Yoshiharu Uno, MD, PhD, Office Uno Column (Medical Science Publisher), Onoe-Cho, Yota 419-2, Hyogo 675-0025, Japan. yoshiharu333@hotmail.com
Telephone: +81-79-4394697
Fax: +81-79-4394697

Received: April 27, 2016
Peer-review started: April 28, 2016
First decision: July 4, 2016
Revised: August 4, 2016
Accepted: August 15, 2016
Article in press: August 17, 2016
Published online: November 6, 2016

Abstract

Despite little evidence for the therapeutic benefits of a high-fiber diet for diverticulitis, it is commonly recommended as part of the clinical management. The ongoing uncertainty of the cause(s) of diverticulitis confounds attempts to determine the validity of this therapy. However, the features of a high-fiber diet represent a logical contradiction for colon diverticulitis. Considering that Bernoulli's principle, by which enlarged diameter of the lumen leads to increased pressure and decreased fluid velocity, might contribute to development of the diverticulum. Thus, theoretically, prevention of high pressure in the colon would be important and adoption of a low FODMAP diet (consisting of fermentable oligosaccharides, disaccharides, monosaccharides, and polyols) may help prevent recurrence of diverticulitis.

Key words: Diverticular disease; High-fiber diet; Low FODMAP diet; Bernoulli's principle; Irritable bowel syndrome

Core tip: The ongoing uncertainty of the cause(s) of diverticulitis confounds attempts to determine the validity of this therapy; however, the features of a high-fiber diet represent a logical contradiction for colon diverticulitis. Prevention of high pressure in the colon may help to avoid or correct diverticulitis, and this may be achieved by adoption of a low FODMAP diet (restriction of fermentable oligosaccharides, disaccharides, monosaccharides, and polyols).

Uno Y, van Velkinburgh JC. Logical hypothesis: Low FODMAP diet to prevent diverticulitis. *World J Gastrointest Pharmacol Ther* 2016; 7(4): 503-512 Available from: URL: http://www.

wjgnet.com/2150-5349/full/v7/i4/503.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i4.503

INTRODUCTION

The theory of colon diverticulitis and diet association was expanded upon by Painter[1], first in 1969 when he reported that diverticular disease (DD) occurred in people who ate a low residue diet with refined flour and sugar, then again in 1970 when he stated there was no DD in Africa[2]. Painter went on to explain that Denis Parsons Burkitt, the famed United Kingdom surgeon and dietary fiber proponent, provided a personal communication of his observation of short oro-anal transit time in Africans. This information helped to inspire Painter to theorize that a low residue intake related to, what he described as, the "civilized diet" would lead to a viscous stool that passes through the colon more slowly, and that this difference in fecal consistency would explain the incidence of DD in the civilized nations. Moreover, Painter recommended a diet with high residue intake, such as that consisting of wholemeal bread, unprocessed bran, porridge and fruit, replace the traditional low-residue diet of the civilized nations to reduce risk of DD. In 1971, Painter and Burkitt[3] jointly published their "fiber hypothesis" for DD, suggesting that a diet based on unrefined, natural foods with adequate fiber may prevent DD.

Subsequent studies found that intake of a higher fiber diet led to increased volume and less viscous feces accompanied by a shorter transit time[4-6], thereby preventing the rise of internal pressure in the large intestine. Advocates of the fiber diet suggested that it would help to spread the lumen of the large intestine, thereby suppressing the excessive contraction that would otherwise be caused by large amounts of compacted feces. These findings have led to the widely accepted theory that DD is strongly related to constipation[7].

The most important data published so far in support of the fiber hypothesis is that showing a correlation between amount of feces and transit time. In particular, the relationship between stool volume and transit time is not inverse, but is exponential [*i.e.,* log(time) = 2.81633 - 0.56057log(weight)][4]. It is not feasible to shorten transit time for stools over 300 g; therefore, theoretically, the effectiveness of high-fiber diet is limited. Methanogenesis has been linked to the presence of diverticulosis[8], and cellulose, which is contained in dietary fibers, is fermented by methane-producing bacteria[9]. A very recent study used a gas-sensing capsule to measure gas produced by diets of various fiber content found that the high-fiber diet produced more gas in the large intestine than the low-fiber diet[10]. Therefore, ingestion of excess dietary fiber may exacerbate the conditions that support accumulation of feces and gas in the intestine.

Although the mechanism underlying diverticula generation remains unknown, the fiber hypothesis has been widely adopted as an appropriate intervention. Indeed, over the past 45 years, the fiber hypothesis itself has become the basis for dietary advice of DD. The most current patient guide published by the American Gastroenterology Association (AGA) formally recommends a daily fiber intake of at least 25 g[11].

Yet, findings from several recent studies have cast doubt on the validity of the high-fiber hypothesis[12-16]. A subsequent study by Peery *et al*[17] found no association between a low-fiber diet and DD. More concerning, however, were their results from the cross-sectional study of 2104 participants between the ages of 30 and 80 years old, and including 878 cases of DD and 1226 controls without DD, which indicated that high total fiber intake was associated with an increased prevalence of multiple diverticula. The particular fiber subtypes that showed significant association with the occurrence of multiple diverticula were grains, insoluble fiber, and soluble fiber. Another study by the same group a year later found no increased risk of diverticulosis in the descending or sigmoid colon associated with either less frequent bowel movements or symptoms of constipation, and no association between dietary fiber intake and diverticulosis[18]. A subsequent study by Braunschmid *et al*[19] found that colonic diverticular disease did not correlate with constipation symptoms.

PATHOPHYSIOLOGY OF DIVERTICULA

Muscle layer of the colon and diverticula

Theoretically, formation of a mucosal hernia requires intraluminal high pressure and a pre-existing defect in the involved muscle layer (*i.e.,* weakened integrity). Normally, the muscle layer of the human colon is composed of circular muscle and longitudinal muscle, the latter of which is bound as three separate formations (*i.e.,* the taenia) that run from the cecum to the distal portion of the sigmoid colon[20]. When surgical specimens of diverticulitis in sigmoid colon were examined using electron microscopy, the taenia showed up-regulated elastin, with concentration levels greater than 200% compared to those in controls. Increased elastin in the region of the colonic tissue afflicted by diverticulitis may cause unequal elasticity and strength compared to the adjacent areas of unafflicted tissues[21]. Moreover, several conditions that cause the colon tissue to thicken, such as inflammatory or infectious conditions, affect elasticity, as increased thickness leads to reduced tensile strength. Finally, prevalence of colonic DD has been correlated with advancing age[22], presumably due to the large intestine wall becoming brittle. Intriguingly, studies of both European, Asians and African populations have shown similar findings of sigmoid colon strength decreasing with age[23]. Japanese have shown descending colon (lateral direction) strength decreasing with age (Figure 1)[24].

Investigation of colonic tearing during colonoscopy showed that when the sigmoid colon wall is affected by pressure forces from the inside the muscularis propria ruptures first, followed by the serosa and the mucosa

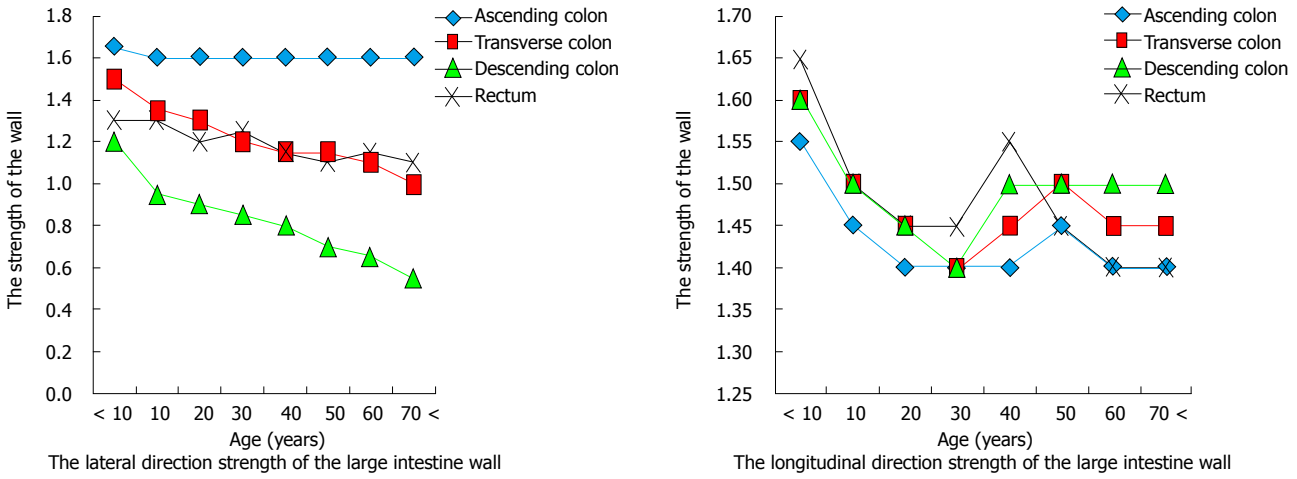

Figure 1 Change of strength with aging of the wall of the large intestine. The descending colon (lateral direction) shows reduction in strength related to aging. In contrast, the ascending colon shows no reduction in strength related to aging.

sequentially[25]. As such, the muscle layer appears to be the weakest point in the wall of the colon, even under physiologic conditions. Thus, considering the collective data of factors that affect the structural integrity of the distal colon, it can be hypothesized that development of diverticula in this area may be related to vulnerability of the intestinal wall that increases with age.

In contrast to the descending colon, the ascending colon shows no reduction in strength associated with aging (Figure 1). Hard stool is usually not present in the proximal colon, and this region does not form a closed space. Theoretically, herniation in the proximal colon requires the presence of a natural vulnerability.

Not all animals have taenia of the colon, and those that do are humans and monkeys among the primates, horses, and guinea pigs and rabbits among the rodents[26]. While natural occurrence of DD has been reported in monkeys and horses, the rodents are used for study of diverticula since the condition can be created experimentally and their small size facilitates convenient research investigation[27-30]. These features of diverticula in the animals with taenia have led to suspicion of a causal relationship between the two.

Colonic DD are most frequently located along the side of taenia[31]. It has thus been speculated that these sites represent the weakest points in the colon muscles, possibly explained by the fact that they are where penetrating vessels cross through[7,32-35]. It is theorized that until diverticulum formation is complete, the outpouching process is advanced by ongoing forces of pressure; although, this hypothesis has not yet been proven experimentally. In line with this theory, however, it is believed that DD does not occur between the tenia libera and tenia omentalis of the transverse colon because of the low vasculature at this site.

A report from 1925 by Lineback[36] demonstrates defects on both sides of the taenia. The circular muscle forms a convergence at the site that is in contact with the longitudinal muscle, and the most frequent site of diverticula is the cleft between the bundles of circular muscle (Figure 2).

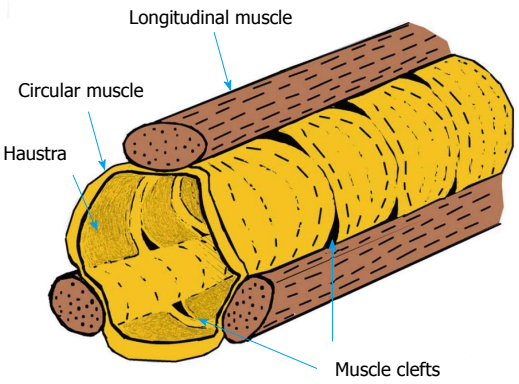

Figure 2 Muscle clefts.

Furthermore, since circular muscles and longitudinal muscles are connected, these clefts may be widened upon contraction of the muscle. This theory does not contradict the fact that multiple diverticula of the same size are present simultaneously. Moreover, these clefts may contain blood vessels[36], and may be present from birth. In line with this theory is the explanation as to DD not occurring between the tenia libera and tenia omentalis of the transverse colon because of the low influence of gas at this site.

Intraluminal pressure after diverticulitis

It is widely believed that feces produced by a high-fiber diet increases colon diameter and in turn decreases intraluminal pressure, in accordance with Laplace's law (wall tension = pressure × radius)[35,37]. This idea is based upon the theory of "segmentation hypothesis" that was first put forth by Painter, in which he described the colon obstructed at both ends as an enclosed space that acts as a series of "little bladders"[1,7]. However, the large intestine is functionally and structurally different from a bladder; indeed, it is a continuous space without

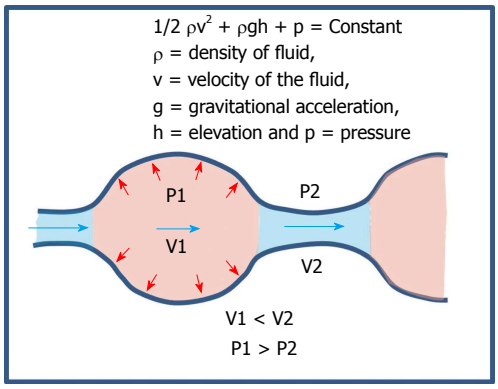

Figure 3 According to Bernoulli's principle, the pressure at the extended site is increased.

complete obstruction, with its outlet at the anus. Besides, in order for this theory to be feasible and valid the colon wall must be flexible enough to adapt the Laplace's law. In patients who have optimal contractility of the intestines, the high-fiber diet may be an effective prevention or intervention measure. However, in patients who have an intestinal wall that is stiff or has excessive contraction, the intestinal tract will not extend adequately in response to the high-fiber diet, leading to higher intraluminal pressure and consequent increased forces acting on the intestinal wall.

Colon affected by diverticulitis shows thickened wall and signs of post-inflammatory fibrosis[38]. *In vivo* studies using colon manometry in patients with diverticulitis showed motility of the intestinal tract as being increased in the descending colon and sigmoid colon but no concomitant increase in the rectum[39,40]. Another study using CT colonography (colonoscopy) to assess symptoms of patients with diverticula in the sigmoid colon showed that pain was associated with air pressure[41]. Considering these data, high bulk feces produced by a high-fiber diet would not be expected to prevent the recurrence of diverticulitis. Not only that but, theoretically, this diet might even promote the symptoms of diverticulitis and pose a risk of recurrence.

Dynamics of fluid and gas

High pressure in the lumen of the colon cannot be produced by small hard stool alone. Although multiple hard stools can accumulate in the intestinal lumen, they cannot exclusively explain the influences of pressure.

In theory, in order to obtain a pressure increase sufficient to affect the intestinal wall, a substance that can rapidly move in the colon is required; such a substance would be gaseous or liquid in form. In 1964, Painter[7,42] used a fluid (barium) to produce internal pressure in the intestinal tract and showed that pressures > 50 mmHg were reached in the closed sigmoid colon; additionally, the author theorized that the pressure levels may have reached > 90 mmHg, but limitations of the equipment precluded accurate measurement. The higher forces (specifically 56-80 mmHg) were confirmed by other studies using barium enema administration from a height of 3 feet (91 cm) above the examination table, and these pressures were considered safe[43]. However, when the barium enema was administered from a height of 6 feet (1.8 m) above the table the intraluminal colonic pressures reached 140-168 mmHg, which surpass the threshold of safety and can cause perforation[43].

Intestinal perforation caused by air pressure has been the subject of many studies[44-49]. The upper limit of the safety air pressure of the endoscope is 80 mmHg[50]. Brayko *et al*[51] studied the characteristics of high-pressure perforation in serosa and mucosa and found that the pressures required for rupture (202 mmHg and 226 mmHg respectively) translated to low risk of perforation of the diverticula in normal endoscopy[51]. Another study, however, showed that the lower pressure forces of CT colonography (38-40 mmHg) can induce abdominal pain[41]. Thus, the human colon can feel pain caused by air pressure at ⩾ 40 mmHg, but the risk of perforation occurs at ⩾ 80 mmHg. While the diverticulum may be induced in the cleft of the muscle layer by a pressure of < 80 mmHg, it is not easily ruptured due to the strength of the mucosa and serosa (which require pressures > 200 mmHg). Considering that most cases of diverticula present as asymptomatic (without abdominal pain), it is likely then that the pressure required for completion of a new diverticulum might be 40 mmHg or less.

Intestinal pressure is affected by the dynamics of liquid as well as air. Compressed gas, according to Boyle's law, has a higher energy than the uncompressed liquid. Therefore, the presence of liquid will increase air pressure in a confined space such as the intestine. A study using barium contrast showed that the transit time in the proximal sigmoid of patients with DD was twice as fast as that in the non-DD control group, but the total time of gastrointestinal emptying was similar in both groups[52]. These results can be explained by Bernoulli's principle, which states that if the diameter of the lumen is large, the pressure will be increased and the fluid velocity will be decreased (Figure 3). Considering this law, narrowing of the rectum will be expected to increase the internal pressure of the sigmoid colon, and when the descending colon is contracted, the pressure of the proximal colon will be expected to be increased. There are seven sphincters located along the length of the colon[53]. The hydrodynamics of each sphincter and influence of its contraction (including the Haustral type) may be explained by Bernoulli's principle (Figure 4). Thus, the difference in frequency of right DD and left DD may be related to differences in pressure at each site.

Japan has a high incidence of DD in the proximal colon[54]. Moreover, study of Japanese cases of DD, but specifically with the condition affecting the right side, showed a high intraluminal pressure (> 20 mmHg) and abnormal motility in the ascending colon[55]. When another group examined the dynamics of gas pressures by scintigraphy they found that gas generated in the right and left colon does not move, as evidenced by

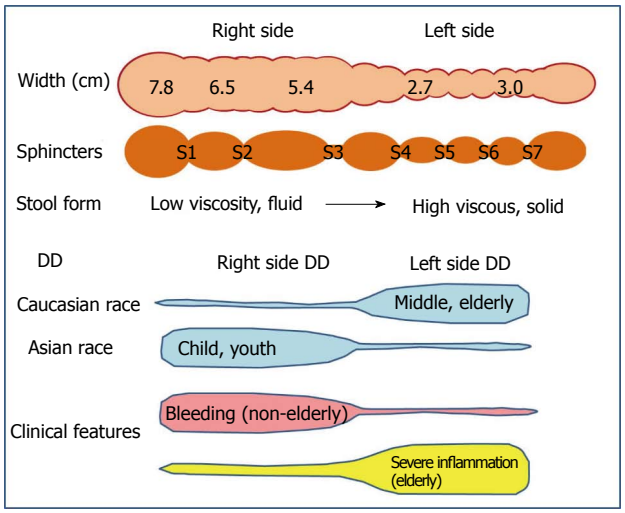

Figure 4 Distinctions in the background and symptoms of right and left diverticular disease. The width of the right colon is about twice that of the left colon[53]. The large intestine contains seven sphincters[53], including S1: Busi ring; S2: Hirsh ring; S3: Cannon ring; S4: Payr-Straus ring; S5: Balli ring; S6: Moultier ring; S7: Rossi ring. Blue-colored areas represent the frequency at the site. In Caucasians, diverticular disease (DD) most frequently occurs on the left side and after middle age. In Asians, DD most frequently occurs on the right side and during childhood. Right-side diverticula is more likely to bleed but less likely to develop the severe diverticula-associated complications of perforation, abscess formation, fistulation, or structuring[7,54].

observations at 60 min post-injection when gas injected into the jejunum remained in the cecum and the right colon and gas injected into the rectum remained in the recto-sigmoid colon[56]. Thus, the segmental location at which gas is fermented will be impacted by the corresponding pressure.

It has already been established that excessive pressure in the colon is related to the intraluminal concentrations of both gas and water. The next question of interest is then, what is the cause of increased levels of gas and the water in the colon?

Dietary fiber increases gas within the colon[10,57]. The primary dietary fiber contained in vegetable and fruit is inulin, and its intake leads to flatulence[58]. The 2015 AGA guidelines cited at the beginning of this article recommend that dietary fiber be obtained through intake of legumes (as lentils), yogurt, and fresh fruit[11]. However, yogurt and beans contain oligosaccharides, both of which are known to generate gas in the gut by fermentation[59,60]. In the case of individuals with fructose intolerance, eating of fresh fruit can result in abdominal pain, belching, bloating, an uncomfortable feeling of fullness, indigestion, and diarrhea[61]. Therefore, the diet components recommended by the AGA are expected to produce a substantial amount of gas in the intestines.

ALTERNATIVE DIET FOR DIVERTICULITIS

Recently, there has been increasing interest in developing diet-based therapies for IBS, and the diet consisting of low fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAP) appears a promising candidate. FODMAPs are not digested or absorbed in the small intestine[62,63]. Therefore, their intake causes increased fluid in the ileum due to the corresponding high osmotic pressure; in addition, they lead to a large amount of gas produced by fermentation in the colon. The daily adoption of the low FODMAP diet by patients with IBS has led to significant improvements in symptoms[64,65].

The detrimental impact of a high FODMAP diet on the intestinal tract has been shown by a study in which the participants drank lactulose[66], which has been demonstrated by MRI to increase fluid content in the ileum[67]. Since this study measured the lactulose-induced increase of gas indirectly, with the hydrogen breath test, a more recent study of the FODMAP diet obtained a direct confirmation of the increasing intestinal gas by abdominal X-ray[68]; these findings, thus, support the theory that the FODMAP diet increases the intraluminal volume of gas and fluid.

The breath test, however, remains a valid method of analysis. Jang *et al*[69] performed a breath test in patients with right DD at 180 min following ingestion of 10 g of lactulose and determined that methane gas was increased but hydrogen gas was decreased in these individuals as compared to controls. It is important to note here, though, that gas volume is known to significantly increase in response to lactulose ingestion at time points greater than the 180 min used in that study, specifically at 240 to 300 min after the ingestion[68]. Therefore, to more accurately investigate the influence of colon gas on clinical symptoms, it is necessary that the study design allow for adequate time for fermentation to occur in the large intestine.

The low FODMAP diet purports avoidance of foods that contain lactose. Lactose not only causes an increase in intestinal water content (*via* increased osmotic load in the ileum) but is also readily fermented by the colonic microbiota, which leads to production of short-chain fatty acids and gas: Mainly hydrogen (H_2), carbon dioxide (CO_2), and methane (CH_4)[70]. Thus, individuals with lactose intolerance can experience diarrhea and abdominal distension as a result of dietary intake.

Prevalence of lactose intolerance is high in Asia and Africa, and lower in Caucasian populations. From this point forward, the article will discuss the potential correlation between DD of right colon (RDD) and lactose intolerance. Among the nine countries with reports of RDD and lactose intolerance in the publicly available literature (Table 1)[34,71-84], all show a strong correlation (r^2 = 0.9524, Figure 5). Incidence of RDD is lowest in European countries, while the incidence of lactose intolerance is lowest in the United States; however, the Asian countries show high incidences for both RDD and lactose intolerance. These findings support a hypothesis of lactose intolerance and RDD.

In Japan, DD in young individuals almost exclusively involves the right side of the colon (Figure 6)[77]. LDD risk

Table 1 The proportion of diverticular disease of right colon and lactose intolerance

Country	Lactose intolerance (%)	RDD (%)[1]
Singapore	100[71]	42[72]
Thailand	98[73]	55[74]
China	95[73]	55[75]
Japan	90[76]	45[77]
South Korea	88[78]	52[79]
Poland	27[80]	15[81]
India	20[73]	12[82]
United Kingdom (White)	6[83]	3[84]
Australia (White)	4[74]	5[34]

[1]Not including pan-DD and bilateral DD. DD: Diverticular disease; RDD: DD of right colon.

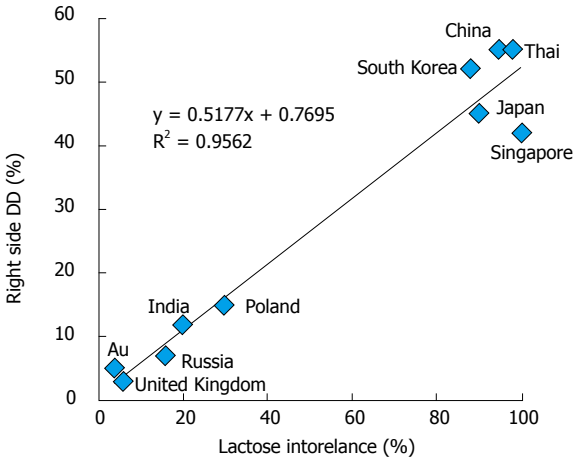

Figure 5 Relationship between lactose intolerance and the right-side diverticular disease. Au: Australia; DD: Diverticular disease.

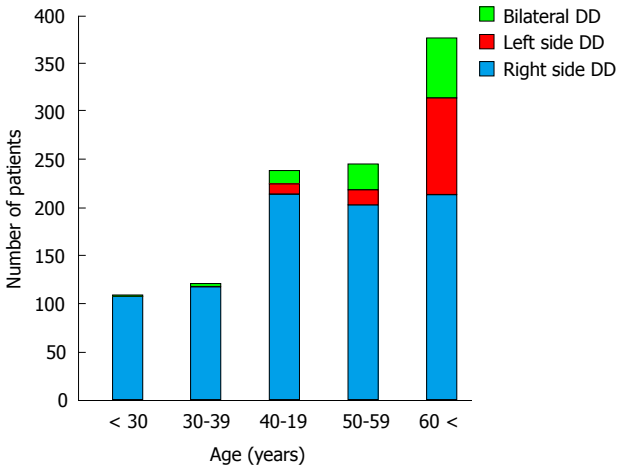

Figure 6 Relationship between the site of and age at onset for diverticular disease. DD: Diverticular disease.

was found to increase with age, likely due to increased vulnerability of the muscle layer over a person's lifespan. In adults under 29 years of age, 100% of the DD cases involved the right side. In Japan, pediatric DD between the ages of 7-15 years is not rare: These cases of pediatric DD occur in cecum and ascending colon[85,86]. This finding cannot be explained merely by age-related vulnerability of the muscle layer and may indicate factors related to childhood. It has been reported that up to 86% of Japanese children develop lactose intolerance by the age of 6 (30% in 3-year-old, 36% in 4-year-old, 58% in 5-year-old)[76]. The time that it takes for gas to increase in the intestines after lactose intake is 1-2 h[87-89], which is shorter than the times required for any of the other constituents of a high-FODMAP diet. Thus, lactose intake may induce a large amount of gas and liquid in the right colon of Japanese children, especially those with lactose intolerance. The physical pressure brought on by the increased gas and fluid will affect the mucous membrane, presumably pushing it outward into the physiological cleft that exists from birth. This may explain why diverticulum in young Japanese tends to be generated only on the right side.

Yet, many Europeans and Americans experience DD of the left colon LDD. This phenomenon may be related to the higher ingestion of wheat[90], compared to Asian societies historically. The fructan component of wheat is a part of the high-FODMAP. The time required for fermentation of fructan in the gut is relatively long, between 2 and 6 h[91,92], so that the high pressure would occur in the left colon. In Japan, however, consumption of wheat has increased since World War Ⅱ, and this change in dietary pattern - towards one that more closely resembles the European and American diets - has been accompanied by an increase in colonic DD. For example, DD was reportedly 2% in the 1960s[93] but had increased to 20% by the 1980s[94]. Additionally, the cases of LDD have increased in Japan as well[94-97]. This trend is similar to that reported in South Koreans[79].

Besides the change in eating habits, the increased longevity of the Japanese population in recent decades may also have contributed to the observed rise in LDD. In addition, the prevalence of IBS has remarkable increased in Japan; according to the various revised definitions of IBS made by the Rome diagnostic criteria, incidence was 3.6% in 1996 (Rome Ⅰ)[98], 10.7% in 2006 (Rome Ⅱ)[99], 14.0% in 2010 (Rome Ⅲ)[100]. Thus, not only may DD and IBS be correlated but they also may share an etiologic component of diet.

Several reports have addressed the potential correlation between IBS and DD[54,101-103]. Symptoms consistent with IBS are common among patients with DD, and this symptomology has been reported as significantly higher in the DD patients when compared to non-DD controls[103-105]. However, this overlap of symptoms can cause diverticulitis to be misdiagnosed as IBS[104].

IBS and DD are distinct conditions, the former having demonstrated characteristics of inflammation as a distinguishing feature; as such, it may be inappropriate to adapt the diagnostic criteria of IBS to patients with DD. Yet, the two share common symptoms of abdominal bloating accompanied by abdominal pain, which are presumed to be consequent to internal pressure in the

digestive tract. Regardless, if the cause of symptoms in either is an excess volume of gas and liquid content in the colon, reduction of either or both might help to prevent the chronic symptoms of diverticulosis.

IBS is classified as a functional disorder, while DD is classified as an organic disease. The most obvious difference between the two is that including a case having homeostatic stenosis after inflammation and/or inflammation among the group of DD. Shape change and inflammation is the result, the cause may be the same. Patient with DD will sustain severe symptoms than IBS without diverticulum. Cuomo *et al*[105] suggested that these symptoms may be used to differentiate the patients with DD from those with IBS. However, their study design was based upon a patient population presenting with fever and requiring hospitalization and treatment, so that cases of diverticulum without inflammation were not considered.

It is possible that inflammation related to diverticulitis may lead to excessive contraction and support development of IBS[106]. In both conditions, Bernoulli's principle may be at play, namely production of non-uniform pressure in the intestinal tract caused by any variety of factors. It is also possible that in patients with asymptomatic DD without stenosis, a high-fiber diet with high FODMAPs may be lead to IBS. In such a diet, the inulin and oligosaccharides may produce short-chain fatty acids and gases by fermentation at 6 to 48 h after ingestion, and pH of feces is reduced from 7 to 6[107,108].

It has been demonstrated that IBS patients have reduced colonic intraluminal pH, compared to healthy controls[109,110]; the lower pH is suggestive of higher colonic fermentation. Specifically, these studies used a wireless motility capsule (SmartPill™) to show that IBS patients had a pH of 6.8 in the colon (*vs* healthy controls who had a pH of 7.3) and showed that colonic low-pH levels were correlated with IBS symptom severity scores and abdominal pain. IBS is characterized by excessive contraction of the descending colon, starting from the sigmoid colon[111]. Interestingly, when another study found low-pH of the cecum in IBS patients, it was correlated with a reduction in right colon contraction[112]. These collective findings indicate that IBS is likely subject to the Bernoulli's principle, which is also inferred for the pathogenesis of DD.

CONCLUSION

The high fiber hypothesis represents a logical contradiction. A high-fiber diet is most likely not suitable for long-term management of diverticulitis. The anatomical clefts that are present in the musculature of the large intestine are prone to diverticula caused by gas and fluid-related force pressures following Bernoulli's principle. RDD, however, may also be related to lactose intolerance. The currently recommended diet of high fiber with high FODMAPs may bring about substantial amounts of gas in the colon and a low pH, which is linked with IBS symptoms. Theoretically, then, a low FODMAP diet will be valid for the prevention of recurrent diverticulitis.

REFERENCES

1 **Painter NS**. Diverticulosis of the colon and diet. *Br Med J* 1969; **2**: 764-765 [PMID: 5786772 DOI: 10.1136/bmj.2.5659.764-b]
2 **Painter NS**. Pressures in the colon related to diverticular disease. *Proc R Soc Med* 1970; **63** Suppl: 144-145 [PMID: 5525488]
3 **Painter NS**, Burkitt DP. Diverticular disease of the colon: a deficiency disease of Western civilization. *Br Med J* 1971; **2**: 450-454 [PMID: 4930390 DOI: 10.1136/bmj.2.5759.450]
4 **Burkitt DP**, Walker AR, Painter NS. Effect of dietary fibre on stools and the transit-times, and its role in the causation of disease. *Lancet* 1972; **2**: 1408-1412 [PMID: 4118696 DOI: 10.1016/S0140-6736(72)92974-1]
5 **Spiller GA**, Chernoff MC, Shipley EA, Beigler MA, Briggs GM. Can fecal weight be used to establish a recommended intake of dietary fiber (plantix) *Am J Clin Nutr* 1977; **30**: 659-661 [PMID: 855809]
6 **Saito T**, Hayakawa T, Nakamura K, Takita T, Suzuki K, Innami S. Fecal output, gastrointestinal transit time, frequency of evacuation and apparent excretion rate of dietary fiber in young men given diets containing different levels of dietary fiber. *J Nutr Sci Vitaminol* (Tokyo) 1991; **37**: 493-508 [PMID: 1666410 DOI: 10.3177/jnsv.37.493]
7 **Painter NS**. Diverticular disease of the colon: A deficiency disease of western civilization. USA: William Heinemann Medical Books Ltd, 1975 [DOI: 10.1016/b978-0-12-144750-2.50015-2]
8 **Yazici C**, Arslan DC, Abraham R, Cushing K, Keshavarzian A, Mutlu EA. Breath Methane Levels Are Increased Among Patients with Diverticulosis. *Dig Dis Sci* 2016; **61**: 2648-2654 [PMID: 27129485 DOI: 10.1007/s10620-016-4174-6]
9 **Robert C**, Bernalier-Donadille A. The cellulolytic microflora of the human colon: evidence of microcrystalline cellulose-degrading bacteria in methane-excreting subjects. *FEMS Microbiol Ecol* 2003; **46**: 81-89 [PMID: 19719585 DOI: 10.1016/S0168-6496(03)00207-1]
10 **Kalantar-Zadeh K**, Yao CK, Berean KJ, Ha N, Ou JZ, Ward SA, Pillai N, Hill J, Cottrell JJ, Dunshea FR, McSweeney C, Muir JG, Gibson PR. Intestinal Gas Capsules: A Proof-of-Concept Demonstration. *Gastroenterology* 2016; **150**: 37-39 [PMID: 26518389 DOI: 10.1053/j.gastro.2015.07.072]
11 **American Gastroenterological Association**. A Patient Guide: Managing Diverticulitis. *Gastroenterology* 2015; **149**: 1977-1978 [PMID: 26453775 DOI: 10.1053/j.gastro.2015.10.002]
12 **Tan KY**, Seow-Choen F. Fiber and colorectal diseases: separating fact from fiction. *World J Gastroenterol* 2007; **13**: 4161-4167 [PMID: 17696243 DOI: 10.3748/wjg.v13.i31.4161]
13 **Ünlü C**, Daniels L, Vrouenraets BC, Boermeester MA. A systematic review of high-fibre dietary therapy in diverticular disease. *Int J Colorectal Dis* 2012; **27**: 419-427 [PMID: 21922199 DOI: 10.1007/s00384-011-1308-3]
14 **Peery AF**, Sandler RS. Diverticular disease: reconsidering conventional wisdom. *Clin Gastroenterol Hepatol* 2013; **11**: 1532-1537 [PMID: 23669306 DOI: 10.1016/j.cgh.2013.04.048]
15 **Tursi A**, Papa A, Danese S. Review article: the pathophysiology and medical management of diverticulosis and diverticular disease of the colon. *Aliment Pharmacol Ther* 2015; **42**: 664-684 [PMID: 26202723 DOI: 10.1111/apt.13322]
16 **Elisei W**, Tursi A. Recent advances in the treatment of colonic diverticular disease and prevention of acute diverticulitis. *Ann Gastroenterol* 2016; **29**: 24-32 [PMID: 26752946]
17 **Peery AF**, Barrett PR, Park D, Rogers AJ, Galanko JA, Martin CF, Sandler RS. A high-fiber diet does not protect against asymptomatic diverticulosis. *Gastroenterology* 2012; **142**: 266-272.e1 [PMID: 22062360 DOI: 10.1053/j.gastro.2011.10.035]
18 **Peery AF**, Sandler RS, Ahnen DJ, Galanko JA, Holm AN, Shaukat A, Mott LA, Barry EL, Fried DA, Baron JA. Constipation and a low-fiber diet are not associated with diverticulosis. *Clin Gastroenterol Hepatol* 2013; **11**: 1622-1627 [PMID: 23891924 DOI: 10.1016/

j.cgh.2013.06.033]
19 **Braunschmid T**, Stift A, Mittlböck M, Lord A, Weiser FA, Riss S. Constipation is not associated with diverticular disease - Analysis of 976 patients. *Int J Surg* 2015; **19**: 42-45 [PMID: 25980396 DOI: 10.1016/j.ijsu.2015.04.045]
20 **Meyers MA**, Volberg F, Katzen B, Abbott G. Haustral anatomy and pathology: a new look. I. Roentgen identification of normal patterns and relationships. *Radiology* 1973; **108**: 497-504 [PMID: 4723648 DOI: 10.1148/108.3.497]
21 **Whiteway J**, Morson BC. Elastosis in diverticular disease of the sigmoid colon. *Gut* 1985; **26**: 258-266 [PMID: 3972272 DOI: 10.1136/gut.26.3.258]
22 **Almerie MQ**, Simpson J. Diagnosing and treating diverticular disease. *Practitioner* 2015; **259**: 29-33, 3 [PMID: 26591654]
23 **Watters DA**, Smith AN, Eastwood MA, Anderson KC, Elton RA, Mugerwa JW. Mechanical properties of the colon: comparison of the features of the African and European colon in vitro. *Gut* 1985; **26**: 384-392 [PMID: 3920126 DOI: 10.1136/gut.26.4.384]
24 **Iwasaki T**. Study on the strength of human intestinal walls. *J Kyoto Prefectural Univ Med* 1953; **52**: 673-702
25 **Uno Y**, Morita T. Colonic perforation and serosal tears associated with colonoscopy. *Lancet* 1997; **349**: 1888 [PMID: 9217768 DOI: 10.1016/S0140-6736(05)63879-2]
26 **Stevens CE**, Hume ID. Contributions of microbes in vertebrate gastrointestinal tract to production and conservation of nutrients. *Physiol Rev* 1998; **78**: 393-427 [PMID: 9562034]
27 **Hodgson J**. Animal models in the study of diverticular disease. Part I: aetiology and treatment. *Clin Gastroenterol* 1975; **4**: 201-219 [PMID: 1109816]
28 **Hare WC**. Observations On A Case Of A Diverticulum In The Small Colon Of The Horse. *Can J Comp Med Vet Sci* 1959; **23**: 272-273 [PMID: 17649171]
29 **Bunton TE**, Bacmeister CX. Diverticulosis and colonic leiomyosarcoma in an aged rhesus macaque. *Vet Pathol* 1989; **26**: 351-352 [PMID: 2763427 DOI: 10.1177/030098588902600415]
30 **Hayama SI**, Rika Akamatsu R, Kishimoto M, Suzuki M, Hideo Nigi H. A case of diverticular disease of the colon in a Japanese monkey (Macaca fuscata). *Primates* 1988; **29**: 423-426 [DOI: 10.1007/BF02380966]
31 **Meyers MA**, Volberg F, Katzen B, Abbott G. Haustral anatomy and pathology: a new look. II. Roentgen interpretation of pathological alterations. *Radiology* 1973; **108**: 505-512 [PMID: 4723649 DOI: 10.1148/108.3.505]
32 **Griffiths JD**. Extramural and intramural blood-supply of colon. *Br Med J* 1961; **1**: 323-326 [PMID: 13708616 DOI: 10.1136/bmj.1.5222.323]
33 **Slack WW**. The anatomy, pathology, and some clinical features of divericulitis of the colon. *Br J Surg* 1962; **50**: 185-190 [PMID: 13913774 DOI: 10.1002/bjs.18005022012]
34 **Hughes LE**. Postmortem survey of diverticular disease of the colon. I. Diverticulosis and diverticulitis. *Gut* 1969; **10**: 336-344 [PMID: 5771665]
35 **Maykel JA**, Opelka FG. Colonic diverticulosis and diverticular hemorrhage. *Clin Colon Rectal Surg* 2004; **17**: 195-204 [PMID: 20011276 DOI: 10.1055/s-2004-832702]
36 **Lineback PE**. Studies on the musculature of the human colon, with special reference to the taeniae. *Am J Anat* 1925; **36**: 357-383 [DOI: 10.1002/aja.1000360207]
37 **Sheth AA**, Longo W, Floch MH. Diverticular disease and diverticulitis. *Am J Gastroenterol* 2008; **103**: 1550-1556 [PMID: 18479497 DOI: 10.1111/j.1572-0241.2008.01879.x]
38 **Fenoglio-Preiser CM**, Lantz PL, Listrom MB, Davis M, Rike FO. Gastrointestinal pathology: An atlas and text. Raven Press, New York, 1989: 632-634
39 **Bassotti G**, Battaglia E, Spinozzi F, Pelli MA, Tonini M. Twenty-four hour recordings of colonic motility in patients with diverticular disease: evidence for abnormal motility and propulsive activity. *Dis Colon Rectum* 2001; **44**: 1814-1820 [PMID: 11742167 DOI: 10.1007/BF02234460]
40 **Viebig RG**, Pontes JF, Michelsohn NH. Electromanometry of the rectosigmoid in colonic diverticulosis. *Arq Gastroenterol* 1994; **31**: 135-144 [PMID: 7575173]
41 **Sosna J**, Bar-Ziv J, Libson E, Eligulashvili M, Blachar A. CT colonography: positioning order and intracolonic pressure. *AJR Am J Roentgenol* 2008; **191**: 1100 [PMID: 18806149 DOI: 10.2214/AJR.07.3303]
42 **Painter NS**. The aetiology of diverticulosis of the colon with special reference to the action of certain drugs on the behaviour of the colon. *Ann R Coll Surg Engl* 1964; **34**: 98-119 [PMID: 14120557]
43 **Noveroske RJ**. Intracolonic pressures during barium enema examination. *Am J Roentgenol Radium Ther Nucl Med* 1964; **91**: 852-863 [PMID: 14139913]
44 **Wojtalik RS**, Lindenauer SM, Kahn SS. Perforation of the colon associated with adynamic ileus. *Am J Surg* 1973; **125**: 601-606 [PMID: 4699203 DOI: 10.1016/0002-9610(73)90146-3]
45 **Kozarek RA**, Earnest DL, Silverstein ME, Smith RG. Air-pressure-induced colon injury during diagnostic colonoscopy. *Gastroenterology* 1980; **78**: 7-14 [PMID: 7350038]
46 **Sandhu KS**, Cohen H. Cecal perforation following fiberoptic flexible sigmoidoscopy. *Am J Med* 1987; **82**: 546-548 [PMID: 3826107 DOI: 10.1016/0002-9343(87)90459-1]
47 **Luchette FA**, Doerr RJ, Kelly K, Kulaylat M, Stephan RM, Hassett JM. Colonoscopic impaction in left colon strictures resulting in right colon pneumatic perforation. *Surg Endosc* 1992; **6**: 273-276 [PMID: 1448745 DOI: 10.1007/BF02498858]
48 **Foliente RL**, Chang AC, Youssef AI, Ford LJ, Condon SC, Chen YK. Endoscopic cecal perforation: mechanisms of injury. *Am J Gastroenterol* 1996; **91**: 705-708 [PMID: 8677933]
49 **Tzelepis GE**, Nasiff L, McCool FD, Hammond J. Transmission of pressure within the abdomen. *J Appl Physiol* (1985) 1996; **81**: 1111-1114 [PMID: 8889741]
50 **Woltjen JA**. A retrospective analysis of cecal barotrauma caused by colonoscope air flow and pressure. *Gastrointest Endosc* 2005; **61**: 37-45 [PMID: 15672054 DOI: 10.1016/S0016-5107(04)02453-8]
51 **Brayko CM**, Kozarek RA, Sanowski RA, Howells T. Diverticular rupture during colonoscopy. Fact or fancy? *Dig Dis Sci* 1984; **29**: 427-431 [PMID: 6425026 DOI: 10.1007/BF01296218]
52 **Leandro PA**, Cecconello I, Habr-Gama A, de Olivereira e Silva A, Pontes JF. Gastrointestinal motility in normal subjects and patients with diverticulosis of the colon. *Arq Gastroenterol* 1984; **21**: 157-163 [PMID: 6536253]
53 **Reeders JW**, Rodenbush G. Clinical radiology and endoscopy of the colon. New York: Thieme Medical Publishers, Inc., 1994
54 **Spiller R**. Editorial: new thoughts on the association between diverticulosis and irritable bowel syndrome. *Am J Gastroenterol* 2014; **109**: 1906-1908 [PMID: 25470583 DOI: 10.1038/ajg.2014.367]
55 **Sugihara K**, Muto T, Morioka Y. Motility study in right sided diverticular disease of the colon. *Gut* 1983; **24**: 1130-1134 [PMID: 6642277 DOI: 10.1136/gut.24.12.1130]
56 **Harder H**, Serra J, Azpiroz F, Passos MC, Aguadé S, Malagelada JR. Intestinal gas distribution determines abdominal symptoms. *Gut* 2003; **52**: 1708-1713 [PMID: 14633947 DOI: 10.1136/gut.52.12.1708]
57 **Marthinsen D**, Fleming SE. Excretion of breath and flatus gases by humans consuming high-fiber diets. *J Nutr* 1982; **112**: 1133-1143 [PMID: 6283045]
58 **Muir JG**, Shepherd SJ, Rosella O, Rose R, Barrett JS, Gibson PR. Fructan and free fructose content of common Australian vegetables and fruit. *J Agric Food Chem* 2007; **55**: 6619-6627 [PMID: 17625872 DOI: 10.1021/jf070623x]
59 **Suarez FL**, Springfield J, Furne JK, Lohrmann TT, Kerr PS, Levitt MD. Gas production in human ingesting a soybean flour derived from beans naturally low in oligosaccharides. *Am J Clin Nutr* 1999; **69**: 135-139 [PMID: 9925135]
60 **Cummings JH**, Macfarlane GT, Englyst HN. Prebiotic digestion and fermentation. *Am J Clin Nutr* 2001; **73**: 415S-420S [PMID: 11157351]
61 **Choi YK**, Kraft N, Zimmerman B, Jackson M, Rao SS. Fructose intolerance in IBS and utility of fructose-restricted diet. *J Clin Gastroenterol* 2008; **42**: 233-238 [PMID: 18223504 DOI: 10.1097/MCG.0b013e31802cbc2f]
62 **Gibson PR**, Shepherd SJ. Personal view: food for thought--western

lifestyle and susceptibility to Crohn's disease. The FODMAP hypothesis. *Aliment Pharmacol Ther* 2005; **21**: 1399-1409 [PMID: 15948806 DOI: 10.1111/j.1365-2036.2005.02506.x]
63 **Gibson PR**, Shepherd SJ. Food choice as a key management strategy for functional gastrointestinal symptoms. *Am J Gastroenterol* 2012; **107**: 657-666; quiz 667 [PMID: 22488077 DOI: 10.1038/ajg.2012.49]
64 **Halmos EP**, Power VA, Shepherd SJ, Gibson PR, Muir JG. A diet low in FODMAPs reduces symptoms of irritable bowel syndrome. *Gastroenterology* 2014; **146**: 67-75.e5 [PMID: 24076059 DOI: 10.1053/j.gastro.2013.09.046]
65 **Chumpitazi BP**, Cope JL, Hollister EB, Tsai CM, McMeans AR, Luna RA, Versalovic J, Shulman RJ. Randomised clinical trial: gut microbiome biomarkers are associated with clinical response to a low FODMAP diet in children with the irritable bowel syndrome. *Aliment Pharmacol Ther* 2015; **42**: 418-427 [PMID: 26104013 DOI: 10.1111/apt.13286]
66 **Clausen MR**, Jørgensen J, Mortensen PB. Comparison of diarrhea induced by ingestion of fructooligosaccharide Idolax and disaccharide lactulose: role of osmolarity versus fermentation of malabsorbed carbohydrate. *Dig Dis Sci* 1998; **43**: 2696-2707 [PMID: 9881502 DOI: 10.1023/A:1026659512786]
67 **Undseth R**, Berstad A, Kløw NE, Arnljot K, Moi KS, Valeur J. Abnormal accumulation of intestinal fluid following ingestion of an unabsorbable carbohydrate in patients with irritable bowel syndrome: an MRI study. *Neurogastroenterol Motil* 2014; **26**: 1686-1693 [PMID: 25271767 DOI: 10.1111/nmo.12449]
68 **Uno Y**. Pilot Study on Gas Patterns of Irritable Bowel Syndrome and Small Intestinal Bacterial Overgrowth Following Ingestion of Lactulose. *Open J Gastroenterol* 2015; **5**: 155-163 [DOI: 10.4236/ojgas.2015.511025]
69 **Jang SI**, Kim JH, Youn YH, Park H, Lee SI, Conklin JL. Relationship between intestinal gas and the development of right colonic diverticula. *J Neurogastroenterol Motil* 2010; **16**: 418-423 [PMID: 21103424 DOI: 10.5056/jnm.2010.16.4.418]
70 **Deng Y**, Misselwitz B, Dai N, Fox M. Lactose Intolerance in Adults: Biological Mechanism and Dietary Management. *Nutrients* 2015; **7**: 8020-8035 [PMID: 26393648 DOI: 10.3390/nu7095380]
71 **Bolin TD**, Davis AE, Seah CS, Chua KL, Yong V, Kho KM, Siak CL, Jacob E. Lactose intolerance in Singapore. *Gastroenterology* 1970; **59**: 76-84 [PMID: 5468089]
72 **Wong SK**, Ho YH, Leong AP, Seow-Choen F. Clinical behavior of complicated right-sided and left-sided diverticulosis. *Dis Colon Rectum* 1997; **40**: 344-348 [PMID: 9118752 DOI: 10.1007/BF02050427]
73 **Silanikove N**, Leitner G, Merin U. The Interrelationships between Lactose Intolerance and the Modern Dairy Industry: Global Perspectives in Evolutional and Historical Backgrounds. *Nutrients* 2015; **7**: 7312-7331 [PMID: 26404364 DOI: 10.3390/nu7095340]
74 **Rungjiratananon S**, Sirinthornpunya S. Prevalence of colonic diverticulosis in irritable bowel syndrome patients compared to other patients in Rajavithi hospital prevalence of colonic diverticulosis. *Thai J Gastroenterol* 2009; **10**: 148-151
75 **Chan CC**, Lo KK, Chung EC, Lo SS, Hon TY. Colonic diverticulosis in Hong Kong: distribution pattern and clinical significance. *Clin Radiol* 1998; **53**: 842-844 [PMID: 9833789 DOI: 10.1016/S0009-9260(98)80197-9]
76 **Nose O**, Iida Y, Kai H, Harada T, Ogawa M, Yabuuchi H. Breath hydrogen test for detecting lactose malabsorption in infants and children. Prevalence of lactose malabsorption in Japanese children and adults. *Arch Dis Child* 1979; **54**: 436-440 [PMID: 475426 DOI: 10.1136/adc.54.6.436]
77 **Kubo A**, Ishiwata J, Maeda Y, Kida T, Yamabe K, Shimosegawa T. Clinical studies on diverticular disease of the colon. *Jpn J Med* 1983; **22**: 185-189 [PMID: 6620706 DOI: 10.2169/internalmedicine1962.22.185]
78 **Park SH**, Chang YW, Kim SJ, Lee MH, Nam JH, Oh CH, Kim JW, Jang JY, Yang JO, Yoo JA, Chung JY. [Efficacy of Lactose-free Milk in Korean Adults with Lactose Intolerance]. *Korean J Gastroenterol* 2016; **67**: 22-27 [PMID: 26809628 DOI: 10.4166/kjg.2016.67.1.22]
79 **Oh HK**, Han EC, Ha HK, Choe EK, Moon SH, Ryoo SB, Jeong SY, Park KJ. Surgical management of colonic diverticular disease: discrepancy between right- and left-sided diseases. *World J Gastroenterol* 2014; **20**: 10115-10120 [PMID: 25110438 DOI: 10.3748/wjg.v20.i29.10115]
80 **Pawłowska K**, Umławska W, Iwańczak B. Prevalence of Lactose Malabsorption and Lactose Intolerance in Pediatric Patients with Selected Gastrointestinal Diseases. *Adv Clin Exp Med* 2015; **24**: 863-871 [PMID: 26768639 DOI: 10.17219/acem/26337]
81 **Blachut K**, Paradowski L, Garcarek J. Prevalence and distribution of the colonic diverticulosis. Review of 417 cases from Lower Silesia in Poland. *Rom J Gastroenterol* 2004; **13**: 281-285 [PMID: 15624024]
82 **Goenka MK**, Nagi B, Kochhar R, Bhasin DK, Singh A, Mehta SK. Colonic diverticulosis in India: the changing scene. *Indian J Gastroenterol* 1994; **13**: 86-88 [PMID: 8076987]
83 **Smith GD**, Lawlor DA, Timpson NJ, Baban J, Kiessling M, Day IN, Ebrahim S. Lactase persistence-related genetic variant: population substructure and health outcomes. *Eur J Hum Genet* 2009; **17**: 357-367 [PMID: 18797476 DOI: 10.1038/ejhg.2008.156]
84 **Golder M**, Ster IC, Babu P, Sharma A, Bayat M, Farah A. Demographic determinants of risk, colon distribution and density scores of diverticular disease. *World J Gastroenterol* 2011; **17**: 1009-1017 [PMID: 21448352 DOI: 10.3748/wjg.v17.i8.1009]
85 **Yoshimoto K**, Terakura H, Hiki S, Ogata H. Diverticulitis in children. *Stomach and Intestine* (Tokyo) 2012; **47**: 1135-1140 [DOI: 10.11477/mf.1403113527]
86 **Watanuki H**, Minota N, Takahara M, et al. Study on diverticulitis of the large intestine in children. *J Jpn Red Cross Himeji Hosp* 2015; **39**: 71-74
87 **Mummah S**, Oelrich B, Hope J, Vu Q, Gardner CD. Effect of raw milk on lactose intolerance: a randomized controlled pilot study. *Ann Fam Med* 2014; **12**: 134-141 [PMID: 24615309 DOI: 10.1370/afm.1618]
88 **Yang JF**, Fox M, Chu H, Zheng X, Long YQ, Pohl D, Fried M, Dai N. Four-sample lactose hydrogen breath test for diagnosis of lactose malabsorption in irritable bowel syndrome patients with diarrhea. *World J Gastroenterol* 2015; **21**: 7563-7570 [PMID: 26140004 DOI: 10.3748/wjg.v21.i24.7563]
89 **Schommer K**, Reljic D, Bärtsch P, Sauer P. Gastrointestinal complaints in runners are not due to small intestinal bacterial overgrowth. *J Negat Results Biomed* 2011; **10**: 8 [PMID: 21794099 DOI: 10.1186/1477-5751-10-8]
90 **De Giorgio R**, Volta U, Gibson PR. Sensitivity to wheat, gluten and FODMAPs in IBS: facts or fiction? *Gut* 2016; **65**: 169-178 [PMID: 26078292 DOI: 10.1136/gutjnl-2015-309757]
91 **Murray K**, Wilkinson-Smith V, Hoad C, Costigan C, Cox E, Lam C, Marciani L, Gowland P, Spiller RC. Differential effects of FODMAPs (fermentable oligo-, di-, mono-saccharides and polyols) on small and large intestinal contents in healthy subjects shown by MRI. *Am J Gastroenterol* 2014; **109**: 110-119 [PMID: 24247211 DOI: 10.1038/ajg.2013.386]
92 **Fedewa A**, Rao SS. Dietary fructose intolerance, fructan intolerance and FODMAPs. *Curr Gastroenterol Rep* 2014; **16**: 370 [PMID: 24357350 DOI: 10.1007/s11894-013-0370-0]
93 **Inoue M**. The epidemiologic and clinical features of diverticular disease of the colon. *J Jpn Soc Coloproctol* 1992; **45**: 904-913 [DOI: 10.3862/jcoloproctology.45.6_904]
94 **Miura S**, Kodaira S, Aoki H, Hosoda Y. Bilateral type diverticular disease of the colon. *Int J Colorectal Dis* 1996; **11**: 71-75 [PMID: 8739830 DOI: 10.1007/BF00342463]
95 **Takano M**, Yamada K, Sato K. An analysis of the development of colonic diverticulosis in the Japanese. *Dis Colon Rectum* 2005; **48**: 2111-2116 [PMID: 16228844 DOI: 10.1007/s10350-005-0111-z]
96 **Yamada E**, Inamori M, Uchida E, Tanida E, Izumi M, Takeshita K, Fujii T, Komatsu K, Hamanaka J, Maeda S, Kanesaki A, Matsuhashi N, Nakajima A. Association between the location of diverticular disease and the irritable bowel syndrome: a multicenter study in Japan. *Am J Gastroenterol* 2014; **109**: 1900-1905 [PMID: 25331347 DOI: 10.1038/ajg.2014.323]
97 **Tarao K**, Sekino Y, Nonaka T, Iida H, Inamori M, Nakajima A,

Maeda S, Natsumeda Y, Ikegami T, Ohshige K. Recent trends in colonic diverticulosis in Yokohama City: a possibility of changing to a more Western profile. *Intern Med* 2015; **54**: 2545-2550 [PMID: 26466687 DOI: 10.2169/internalmedicine.54.4813]

98 **Itasaka T**, Takahashi T. Epidemiological study of irritable bowel syndrome using population screening. *Ther Res* 1996; **17**: 4059-4063

99 **Shiotani A**, Miyanishi T, Takahashi T. Sex differences in irritable bowel syndrome in Japanese university students. *J Gastroenterol* 2006; **41**: 562-568 [PMID: 16868804]

100 **Miwa H**. Life style in persons with functional gastrointestinal disorders--large-scale internet survey of lifestyle in Japan. *Neurogastroenterol Motil* 2012; **24**: 464-471, e217 [PMID: 22292849 DOI: 10.1111/j.1365-2982.2011.01872.x]

101 **Annibale B**, Lahner E, Maconi G, Usai P, Marchi S, Bassotti G, Barbara G, Cuomo R. Clinical features of symptomatic uncomplicated diverticular disease: a multicenter Italian survey. *Int J Colorectal Dis* 2012; **27**: 1151-1159 [PMID: 22573184 DOI: 10.1007/s00384-012-1488-5]

102 **Sirinthornpunya S**, Rungjiratananon S. Association of colonic diverticular disease and irritable bowel syndrome in Thai patients. *J Med Assoc Thai* 2014; **97** Suppl 11: S18-S24 [PMID: 25509691]

103 **Jung HK**, Choung RS, Locke GR, Schleck CD, Zinsmeister AR, Talley NJ. Diarrhea-predominant irritable bowel syndrome is associated with diverticular disease: a population-based study. *Am J Gastroenterol* 2010; **105**: 652-661 [PMID: 19861955 DOI: 10.1038/ajg.2009.621]

104 **Longstreth GF**, Tieu RS. Clinically Diagnosed Acute Diverticulitis in Outpatients: Misdiagnosis in Patients with Irritable Bowel Syndrome. *Dig Dis Sci* 2016; **61**: 578-588 [PMID: 26441278 DOI: 10.1007/s10620-015-3892-5]

105 **Cuomo R**, Barbara G, Andreozzi P, Bassotti G, Casetti T, Grassini M, Ierardi E, Maconi G, Marchi S, Sarnelli G, Savarino V, Usai P, Vozzella L, Annibale B. Symptom patterns can distinguish diverticular disease from irritable bowel syndrome. *Eur J Clin Invest* 2013; **43**: 1147-1155 [PMID: 23992370 DOI: 10.1111/eci.12152]

106 **Cohen E**, Fuller G, Bolus R, Modi R, Vu M, Shahedi K, Shah R, Atia M, Kurzbard N, Sheen V, Agarwal N, Kaneshiro M, Yen L, Hodgkins P, Erder MH, Spiegel B. Increased risk for irritable bowel syndrome after acute diverticulitis. *Clin Gastroenterol Hepatol* 2013; **11**: 1614-1619 [PMID: 23524129 DOI: 10.1016/j.cgh.2013.03.007]

107 **Kaur A**, Rose DJ, Rumpagaporn P, Patterson JA, Hamaker BR. In vitro batch fecal fermentation comparison of gas and short-chain fatty acid production using "slowly fermentable" dietary fibers. *J Food Sci* 2011; **76**: H137-H142 [PMID: 22417432 DOI: 10.1111/j.1750-3841.2011.02172.x]

108 **Noack J**, Timm D, Hospattankar A, Slavin J. Fermentation profiles of wheat dextrin, inulin and partially hydrolyzed guar gum using an in vitro digestion pretreatment and in vitro batch fermentation system model. *Nutrients* 2013; **5**: 1500-1510 [PMID: 23645025 DOI: 10.3390/nu5051500]

109 **Choi CH**, Ringel-Kulka T, Temas DJ, Kim A, Scott K, Ringel Y. Altered colonic bacterial fermentation is a steadfast pathophysiological factor in irritable bowel syndrome. *Gastroenterology* 2014; **146**: S8 [DOI: 10.1016/S0016-5085(14)60028-7]

110 **Ringel-Kulka T**, Choi CH, Temas D, Kim A, Maier DM, Scott K, Galanko JA, Ringel Y. Altered Colonic Bacterial Fermentation as a Potential Pathophysiological Factor in Irritable Bowel Syndrome. *Am J Gastroenterol* 2015; **110**: 1339-1346 [PMID: 26303129 DOI: 10.1038/ajg.2015.220]

111 **Clemens CH**, Samsom M, Van Berge Henegouwen GP, Smout AJ. Abnormalities of left colonic motility in ambulant nonconstipated patients with irritable bowel syndrome. *Dig Dis Sci* 2003; **48**: 74-82 [PMID: 12645793]

112 **Farmer AD**, Mohammed SD, Dukes GE, Scott SM, Hobson AR. Caecal pH is a biomarker of excessive colonic fermentation. *World J Gastroenterol* 2014; **20**: 5000-5007 [PMID: 24803812 DOI: 10.3748/wjg.v20.i17.5000]

P- Reviewer: Horesh N, Montomoli J, Stam MAW
S- Editor: Gong ZM **L- Editor**: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i4.513

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 513-523
ISSN 2150-5349 (online)

REVIEW

Eosinophilic gastroenteritis: Approach to diagnosis and management

Antoine Abou Rached, Weam El Hajj

Antoine Abou Rached, Weam El Hajj, Division of Gastroenterology, Department of Internal Medicine, School of Medicine, Lebanese University, Hadath, Beirut 2903 1308, Lebanon

Author contributions: Abou Rached A and El Hajj W conceived and designed the study; El Hajj W performed the literature review and drafted the article; Abou Rached A critically revised the article for intellectual content and gave final approval of the manuscript.

Conflict-of-interest statement: Neither author has any personal or financial interests related to the publication of this study or its findings.

Manuscript source: Invited manuscript

Correspondence to: Antoine Abou Rached, MD, MBAIP, Division of Gastroenterology, Department of Internal Medicine, School of Medicine, Lebanese University, Hadath, Campus, PO Box #3, Hadath, Beirut 2903 1308, Lebanon. abourachedantoine@gmail.com
Telephone: +961-5-451100
Fax: +961-5-455131

Received: December 5, 2015
Peer-review started: December 7, 2015
First decision: February 15, 2016
Revised: July 23, 2016
Accepted: August 15, 2016
Article in press: August 17, 2016
Published online: November 6, 2016

Abstract

Eosinophilic gastroenteritis (EGE) is a rare and benign inflammatory disorder that predominantly affects the stomach and the small intestine. The disease is divided into three subtypes (mucosal, muscular and serosal) according to klein's classification, and its manifestations are protean, depending on the involved intestinal segments and layers. Hence, accurate diagnosis of EGE poses a significant challenge to clinicians, with evidence of the following three criteria required: Suspicious clinical symptoms, histologic evidence of eosinophilic infiltration in the bowel and exclusion of other pathologies with similar findings. In this review, we designed and applied an algorithm to clarify the steps to follow for diagnosis of EGE in clinical practice. The management of EGE represents another area of debate. Prednisone remains the mainstay of treatment; however the disease is recognized as a chronic disorder and one that most frequently follows a relapsing course that requires maintenance therapy. Since prolonged steroid treatment carries of risk of serious adverse effects, other options with better safety profiles have been proposed; these include budesonide, dietary restrictions and steroid-sparing agents, such as leukotriene inhibitors, azathioprine, anti-histamines and mast-cell stabilizers. Single cases or small case series have been reported in the literature for all of these options, and we provide in this review a summary of these various therapeutic modalities, placing them within the context of our novel algorithm for EGE management according to disease severity upon presentation.

Key words: Eosinophilic; Gastroenteritis; Diagnosis; Management; Algorithm; Review

Core tip: Eosinophilic gastroenteritis (EGE) is a heterogeneous inflammatory bowel disorder, which commonly follows a chronic and relapsing course. To date, only single cases or small case series provide insights into its diagnosis and management. This manuscript reviews the different diagnostic tools utilized in practice and provides an algorithm for diagnosis. It also provides a summary of

the therapeutic modalities applied in EGE management, which are placed within the context of an algorithm for systematic application of the different strategies according to the initial disease severity.

Abou Rached A, El Hajj W. Eosinophilic gastroenteritis: Approach to diagnosis and management. *World J Gastrointest Pharmacol Ther* 2016; 7(4): 513-523 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i4/513.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i4.513

INTRODUCTION

Eosinophilic gastroenteritis (EGE) is a rare inflammatory disorder characterized by eosinophilic infiltration of the intestinal wall. Since its first description, about 8 decades ago, reports of subsequent cases have revealed a widely variable and heterogeneous profile of physical manifestations. Studies from the United States have found a prevalence ranging between 8.4 and 28 per 100000[1,2], with a slightly increasing incidence over the past 50 years[3]; additionally, the disease is well known to be more common among the pediatric population, with afflicted adults typically between the 3rd and 5th decade of life[4]. Intriguingly, the more recent estimates of EGE in the United States have found a shift from male preponderance[4,5] to female predominance[2]. Higher socioeconomic status, Caucasian race and excess weight may be risk factors of EGE[3], and a possible hereditary component (genetic factor) is suggested by reports of familial cases[6].

Concomitant allergic disorders, including asthma, rhinitis, eczema and drug or food intolerances, are present in 45% to 63% of the reported EGE cases[1,3]; moreover, 64% of reported cases include a family history of atopic diseases[7]. Some studies have found an association with other autoimmune conditions, such as celiac disease[8], ulcerative colitis[9] and systemic lupus erythematosus[10]. These data collectively suggest that EGE may result from immune dysregulation in response to an allergic reaction; yet, a triggering allergen is not always identified. Indeed, about 50% of EGE cases involving the alimentary tract have been detected by allergy testing to address a suspected food allergy[3]. Other environmental factors, such as parasitic infestation and drugs, may act as predisposing agents as well[11].

Both immunoglobulin E (IgE) dependent and delayed TH2 cell-mediated allergic mechanisms have been demonstrated to be involved in the pathogenesis of EGE. Interleukin 5 (IL-5) has also been shown to play an essential role in the expansion of eosinophils and their recruitment to the gastrointestinal (GI) tract, the mechanism underlying the pathogenic hallmark of EGE. Chemokines, namely eotaxin 1 and $\alpha4\beta7$ integrin, are also known to contribute to eosinophilic homing inside the intestinal wall. Other mediators-most notably IL-3, IL-4, IL-13, leukotrienes and tumor necrosis factor (TNF)-alpha-act to enhance eosinophilic trafficking and have been proposed to help in prolonging lymphocytic and eosinophilic activity[11-13]. Many of these immune-related molecules are currently under consideration as potential targets for molecular therapy of EGE.

Once recruited to the GI tract, the activated eosinophils induce a significant inflammatory response by secreting a variety of mediators including the cytotoxic granules that lead to structural damage in the infiltrated intestinal layers[12]. Thus, EGE can affect any GI segment, but reports have shown that the small intestine and stomach are the most predominant areas[4]. In clinical practice, the Klein classification system[14] is used to categorize the disease type according to the involved intestinal layer; the 3 Klein categories are mucosal, muscular and serosal. The mucosal layer is the most commonly affected, as has been reported in the majority of case series in the literature, with prevalence ranging between 57% in older estimates[4] and 88% to 100% in more recent estimates[3,15]. Furthermore, the muscular and serosal types are commonly associated with concomitant mucosal eosinophilic infiltration, which raises the hypothesis of centrifugal disease progression from the deep mucosa toward the muscular and serosal layers[3].

DIAGNOSIS

Diagnosis of EGE requires three criteria, namely: (1) presence of GI symptoms; (2) histologic evidence of eosinophilic infiltration in one or more areas of the GI tract; and (3) exclusion of other causes of tissue eosinophilia[16] (Figure 1).

While EGE manifestations vary depending on the affected GI layer, abdominal pain is the predominant presenting symptom among all 3 of the disease types[5]. Involvement of the mucosal layer may cause diarrhea, vomiting, protein-losing enteropathy and malabsorption, which in turn can manifest as anemia, hypoalbuminemia and weight loss. Involvement of the muscular layer can lead to a partial or total intestinal obstruction. Involvement of the serosal layer may cause peritoneal irritation, which can lead to ascites, peritonitis and perforation in more severe cases; intestinal intussusception may occur in the serosal type as well[17]. An additional manifestation of the disease, peripapillary duodenal disease, which is secondary to the eosinophilic infiltration of the peripapillary duodenal region, might result in pancreatitis and biliary obstruction[18,19].

Some laboratory findings are sufficient to raise suspicion of EGE, although they are not adequate for an EGE diagnosis. About 70% of cases present with peripheral eosinophilia[4,20] and EGE cases with deep serosal involvement frequently have higher absolute eosinophilic counts (AECs)[20], the latter of which may also be associated with greater risk of relapse[20]. Elevated IgE is reportedly present in about two-thirds of EGE cases[5]

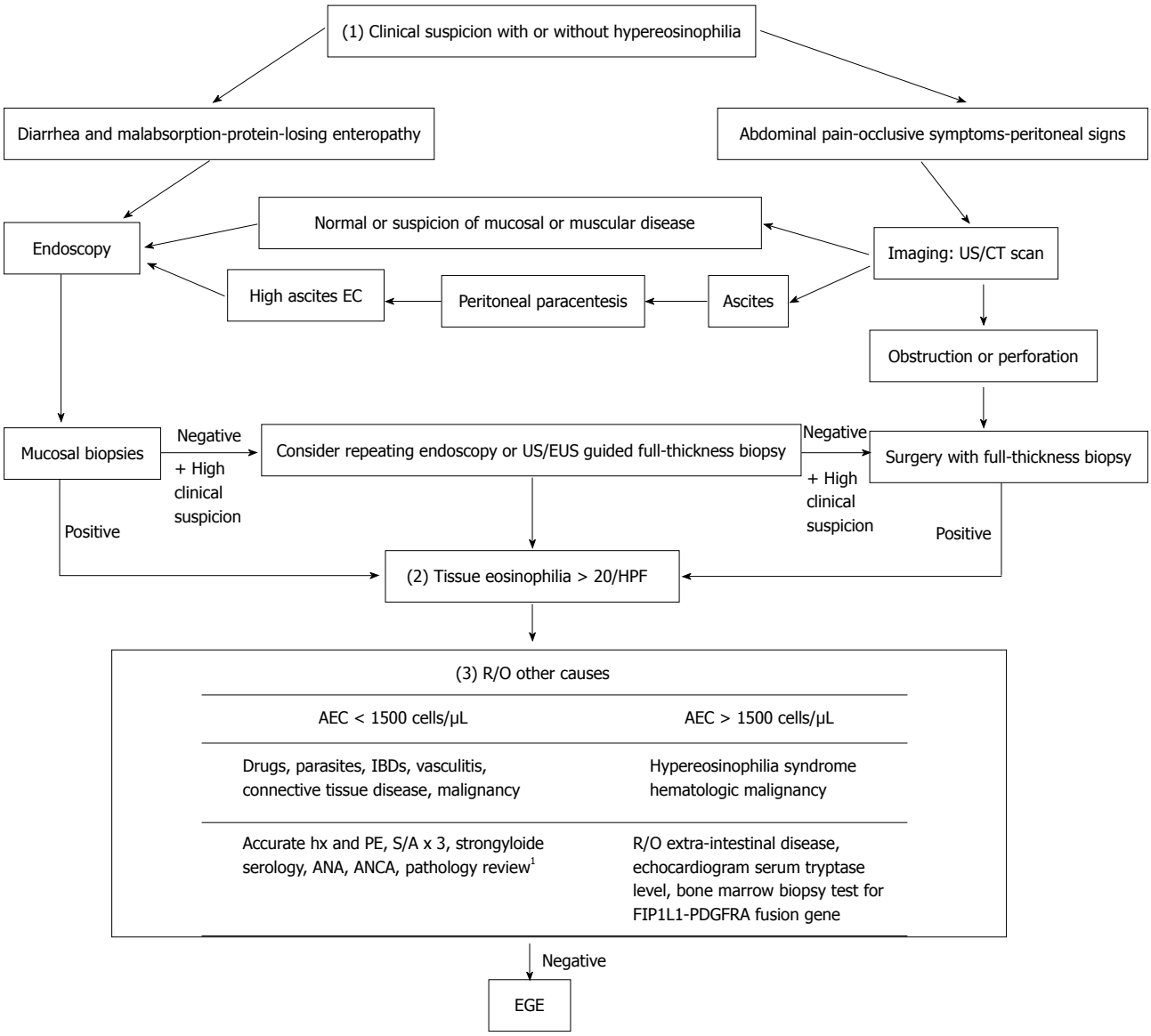

Figure 1 Algorithm for eosinophilic gastroenteritis diagnosis. [1]Histologic ascertainment for absence of malignant cells or findings suggestive of IBD, connective tissue diseases or vasculitis. AEC: Absolute eosinophilic count; ANA: Anti-nuclear antibody; ANCA: Anti-neutrophil cytoplasmic antibodies; EC: Eosinophilic count; EUS: Endoscopic ultrasound; Hx: History; IBD: Inflammatory bowel disease; PE: Physical examination; S/A: Stool analysis; US: Ultrasound; EGE: Eosinophilic gastroenteritis.

and a trend of increased erythrocyte sedimentation rate (ESR) values has been observed. Finally, some reports of EGE cases have demonstrated that peritoneal fluid analysis shows exudative fluid with a net eosinophilic predominance reaching about 90% of white blood cells (WBCs)[21].

Following assessment of the patient's initial presentation, the next step toward diagnosis will require either endoscopy or imaging studies (Figure 1). Endoscopic findings suggestive of EGE include normal aspect, erythematous friable mucosa, ulcers, pseudo-polyps and polyps[22,23], none of which are sensitive or specific for diagnosis of the disease. Thus, findings from endoscopic biopsies can play an essential role in diagnosis, as evidenced by the reported detection rate of 80% for this examination modality[24]. Unfortunately, however, the patchy distribution profile of the disease necessitates multiple biopsies, at least 5 or 6, be obtained from both endoscopically abnormal and normal mucosa, as the latter may mask about 60% of histologically proven disease[15]. Even in cases of negative initial biopsies, but with an otherwise high suspicion index, repeat endoscopy may be useful. Endoscopic ultrasound is also a useful tool for assessing muscular and sub-serosal involvement, as it facilitates access to these tissues for biopsy *via* fine needle aspiration[25,26].

Imaging studies are another diagnostic modality that has proven useful. In addition to guiding biopsy taking efforts, ultrasound can detect ascites and intestinal wall thickening[27]. Computed tomography (CT) scan can detect diffuse thickening of mucosal folds, intestinal wall thickening, ascites and obstruction. Two other

Table 1 Eosinophilic gastroenteritis severity upon presentation

Initial findings	Mild	Moderate	Severe	Complicated
Clinical				
Abdominal pain	Mild	Moderate	Severe	
Vomiting	Mild (< 3/d)	Moderate (3-7/d)	protracted (> 8/d)	
Diarrhea	< 6 BM/d	6-12 BM/d	> 12 BM/d	
Weight loss[1][35]	Non-significant	1 wk 1%-2% 1 mo 5% 3 mo 7.5% 6 mo 10%	1 wk > 2% 1 mo > 5% 3 mo > 7.5% 6 mo > 10%	
Laboratory				
Alb, g/dL	> 3	2.5-3	< 2.5	
HB, g/dL[36]	9.5-11	8-9.5	< 8	
AEC, cells/μL[37]	< 1500	1500-5000	> 5000	
Radiologic				
Ascites	None or mild	Moderate volume	Large volume	Perforation
Intestinal wall thickening[38]	Mild (1-2 cm) Focal (< 10 cm)	Marked (> 2 cm), segmental (10-30 cm)	Sub-occlusion, extensive (> 30 cm)	Occlusion Intussusception
Endoscopy				
Mucosal inflammation[39]	Normal or mild erythema	Moderate	Severe with pseudo-polyps/bleeding	GOO Pyloric stenosis
Histology				
Structural damage[2][34]	Minimal	Moderate	Severe	

[1]Percent weight change = [(usual weight - actual weight)/(usual weight)] × 100; [2]Subjective assessment by expert pathologist. AEC: Absolute eosinophilic count; Alb: Albumin; GOO: Gastric outlet obstruction; HB: Hemoglobin.

scanographic signs that may appear secondary to bowel wall layering are the "Halo sign" and the "araneid-limb-like sign", both of which can aid in differentiating between an inflammatory and a neoplastic lesion[28,29] and in ruling out extra-intestinal pathologies. The imaging modality of Tc-99m hexamethylpropyleneamineoxime (HMPAO)-labeled WBC scintigraphy provides a topographic description of the disease and allows for monitoring of therapeutic response[30]; however, this technology is not widely available and is not yet established as a reliable diagnostic tool for EGE.

While many tools can aid in obtainment of biopsies, the preferred method is still surgery, which provides a full thickness specimen for comprehensive pathology and the most accurate diagnosis, particularly for the muscular and serosal disease types[31].

Histologic examination remains the cornerstone of diagnosis. An absolute eosinophil count of at least 20 eosinophils/hpf has been set in most reports[7,23] as the threshold for fulfilling the second diagnostic criterion. The presence of intraepithelial eosinophils and eosinophils in the Peyer's patches[32], as well as of extracellular deposition of eosinophil major basic proteins (MBPs)[33], favor development of EGE. The latter finding, in particular, reflects the degree of degranulation in activated eosinophils, which is directly linked to greater structural damage[6]. Observation of villous atrophy, crypt hyperplasia or abscesses and epithelial degenerative/regenerative changes are also common findings of EGE. As such, some researchers have emphasized the importance of a subjective histological analysis, in addition to the eosinophilic count, as an important aspect for diagnosis[34].

Accordingly, we suggest dividing the disease into four classifications - mild, moderate, severe and complicated - based upon the initial clinical manifestations, initial laboratory findings, and severity of GI structural damage as assessed by radiologic, endoscopic and histologic examinations (Table 1)[34-39].

Following confirmation of eosinophilic infiltration to the GI tract, the exclusion of other possible causes of the initial clinical presentation is crucial for diagnosis of EGE (Figure 1). These other possible causes include parasitic infections (*i.e.*, *Strongyloides*, *Ascaris*, *Ancylostoma*, *Anisakis*, *Capillaria*, *Toxicara*, *Trichiura* and *Trichinella spp*), drugs, vasculitis (*i.e.,* Churg-Strauss syndrome, polyarteritis nodosa), connective tissue diseases, inflammatory bowel diseases (IBDs), celiac disease, lymphoma, leukemia and mastocytosis. Furthermore, ruling out of the hyper-eosinophilic syndrome is of special value as it is a myelo-proliferative disorder, characterized by idiopathic high peripheral eosinophilic count of > 1500 eos/hpf persisting for > 6 mo and having severe systemic implications due to its multisystem involvement, including heart, central nervous system, skin, lungs, liver and kidneys in addition to the GI tract[34,40].

It is also important to perform a food allergy evaluation in all patients with suspected EGE. Both IgE dependent (specific IgE and skin prick) and non-IgE TH2 dependent (skin patch) allergy tests may aid in identification of the specific allergen related to a case. However, these tests lack both sensitivity (missing about 40% of causative agents) and specificity (capable of overlapping detection of up to 14 allergens in some cases)[41]. A combination of both testing types, however, might enhance their overall predictive value for identifying the EGE-provoking

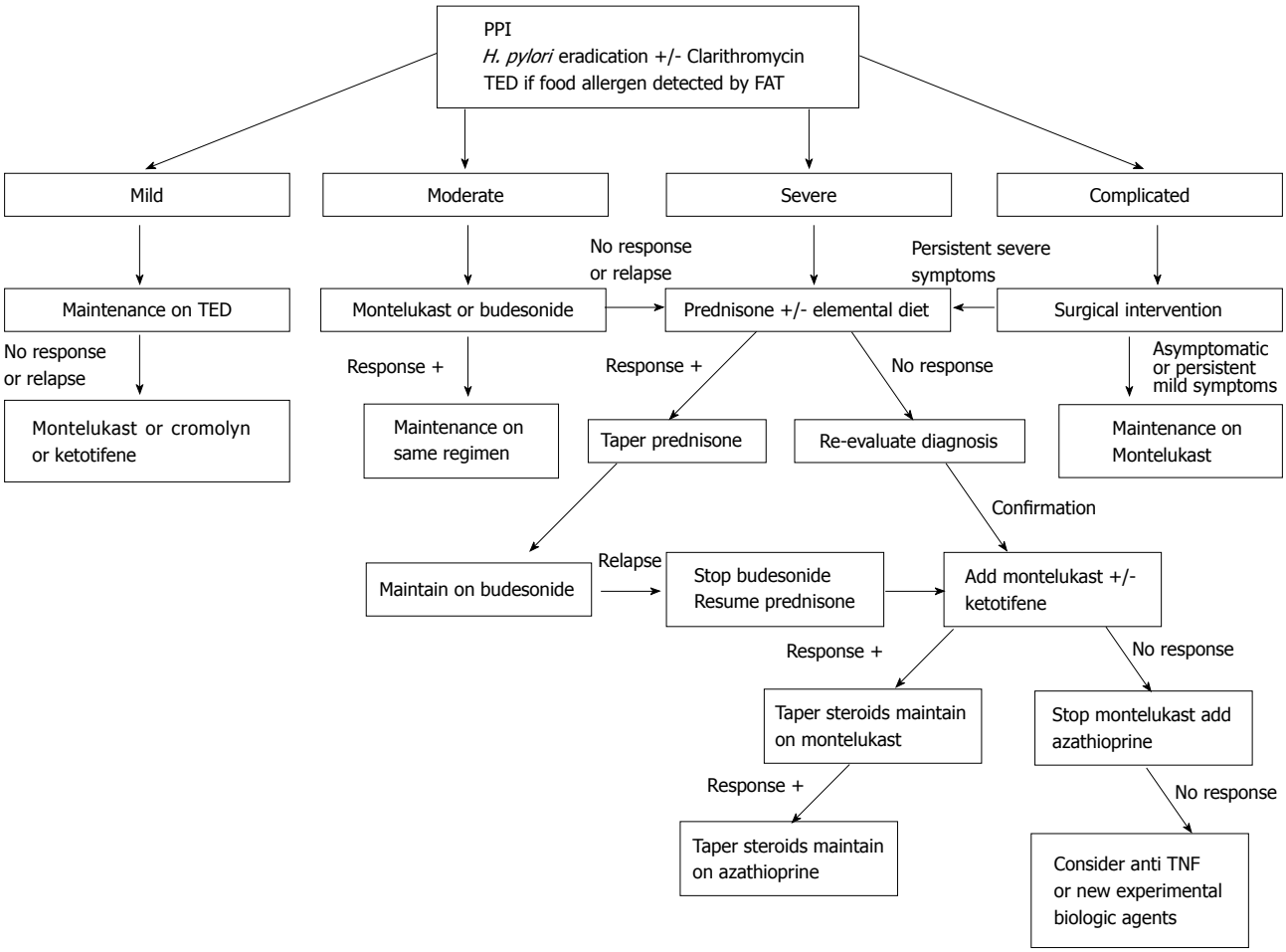

Figure 2 Eosinophilic gastroenteritis management based on initial disease severity. Anti-TNF: Anti-tumor necrosis factor; FAT: Food allergy testing; PPI: Proton pump inhibitor; TED: Targeted elimination diet.

agents[42].

MANAGEMENT

Although spontaneous remission reportedly occurs in around 30% to 40% of EGE cases[20,43], most patients require ongoing treatment. Many therapeutic options have been suggested, including dietary considerations, steroids, leukotrienes inhibitors and mast cells stabilizers. All of these treatment approaches have been described in small case series, but no randomized controlled or comparative trials have been published in the publicly available literature to describe the efficacies of different treatments or predictors of response to one or another option. Thus, no clear, systematic and practical strategy has been put forth for healthcare teams to follow in their management of EGE cases.

EGE is recognized as a chronic inflammatory disorder. Pineton de Chambrun *et al*[20] described three different long-term progression patterns: Non-relapsing disease (42%), commonly seen in patients with the serosal type; relapsing-remitting disease (37%), occurring primarily in patients with the muscular type; and chronic (persistent) disease (21%), predominantly observed in patients with the mucosal type. As mentioned above, a high AEC at diagnosis was found to be an independent predictor of relapses, as was extensive intestinal involvement. Some case series have found higher relapsing rates of 60% to 80%[7,26,44], while others have noted a possible association between younger age (under 20-year-old) and disease recurrence[24]. Unfortunately, research has not identified any other predictors of EGE disease evolution. Thus, it is worth contemplating maintenance treatment for patients after the initial induction phase has passed (Figure 2), taking into consideration the safety profile of the drug in use. It is important to remember, however, that the duration of such maintenance therapy cannot be predicted at this point.

TREATMENT MODALITIES

Proton pump inhibitor and Helicobacter pylori eradication

Proton pump inhibitor (PPI) treatment has been shown to improve the extent of duodenal eosinophilic infiltration in a patient with EGE, and the mechanism has been hypothesized to involve blockade of IL-4 and IL-13 activity[45]. *H. pylori* eradication has also been postulated as capable of inducing a cure of EGE disease[46]. The antibiotic clarithromycin, which is commonly used to

treat *H. pylori*-related ulcers, is also known to have immunomodulatory effect, whereby its actions cause inhibition of T cell proliferation and induction of eosinophil apoptosis; these mechanistic actions in the immune system have led to clarithromycin being applied as maintenance therapy for patients with steroid dependent EGE who are in remission[47].

Dietary therapy

Many dietary strategies have been proposed for management of EGE based on results from food allergy tests. In general, when a limited number of food allergens is detected, patients should be maintained on a "targeted elimination diet" (TED). When many or no allergens are identified, the more aggressive "empiric elimination diet" or "elemental diet" can be used. Lucendo *et al*[48] investigated dietary treatment efficacy in EGE through a systematic review and found significant improvement in most cases, especially in those who undertook the elemental diet, which induced clinical remission in > 75% of cases. However, the validity of such a high efficacy rate was questionable since no confirmation of histologic response was available for the majority of cases included in the review. On the other hand, the authors noted that dietary measures were predominantly considered in the setting of mucosal disease, which is well known to be associated with food allergy, while the efficacy in muscular and serosal types, which show weaker linkage to food allergy[4], was only rarely reported. In addition, patients' adherence and tolerability to such strategies remain an important drawback, especially when empiric elimination or elemental diets are used.

Thus, we suggest the TED for all EGE patients (Figure 2) who show few food allergens upon testing. The overall data in the literature is insufficient to recommend empiric and total elimination diets in routine management; however, an elemental diet can be used initially as adjunct treatment for severe cases.

Prednisone

Prednisone remains the mainstay for induction of remission of EGE. While most of the case series reported have shown a response rate to prednisone (up to 90%)[3,49], the most recent reports showed remarkably lower values (only 50%)[7]. This steroid acts by inducing eosinophil apoptosis and inhibiting chemotaxis. The recommended initial dose of 0.5 to 1 mg/kg usually induces remission within a 2 wk period, with the most dramatic response occurring in patients with the serosal type[50]. Thereafter, tapering dosage over a 6 to 8 wk period is recommended. Re-evaluation of the EGE diagnosis (and type) must be considered in cases of initial unresponsiveness[51]. Steroid dependent disease reportedly accounts for about 20% of cases[7] and, consequently, low doses of prednisone may be needed to maintain remission. Unfortunately, long-term steroid treatment predisposes some patients to serious side effects; in such cases, steroid-sparing agents can be of benefit.

Budesonide

Budesonide, a common steroid treatment of Crohn's disease and ulcerative colitis, has a high affinity for steroid receptors and produces fewer side effects due to its lower systemic impact. It has also been demonstrated as effective for induction and maintenance of remission in the majority of reported cases (Table 2)[15,26,52-59]. The usual dose is 9 mg/d, which can be tapered to 6 mg/d for use as prolonged maintenance therapy. The better safety profile of budesonide, compared to other steroid drugs, is of particular benefit for management of EGE cases over the long term, especially in the setting of steroid dependent disease.

Azathioprine

Azathioprine, a common immunosuppressive agent used in organ transplant and patients with autoimmune diseases, is an immunomodulator that induces apoptosis of T and B cells. The efficacy of this steroid-sparing agent has been demonstrated in patients with steroid dependent and refractory EGE disease. The usual dose for EGE patients is similar to that used in patients with IBD (2-2.5 mg/kg)[9,60,61]; lower doses may not be effective[62].

Montelukast sodium

Montelukast sodium, commonly used to treat asthma, is a selective leukotriene (LTD4) inhibitor with demonstrated efficacy for various eosinophilic disorders, including EGE. The majority of reports in the literature concerning its use in EGE (Table 3)[5,9,15,21,26,63-70] have shown significant clinical response in patients, either when the drug is used alone or in combination with steroids for induction and maintenance of remission in steroid dependent or refractory disease. The usual dose is 5-10 mg/d.

Oral cromolyn sodium

Oral cromolyn sodium is a mast cell stabilizer that blocks the release of immune mediators and the subsequent activation of eosinophils. While it has been shown to have significant efficacy in many of the reported cases of EGE, its effect was only modest in others, for unknown reasons (Table 4)[4,52,71-77]. The usual dose is 200 mg *tid* or *qid*.

Ketotifene

Ketotifene is a 2nd-generation H1-antihistamine agent that also modulates the release of mast cell mediators. Melamed *et al*[78] described 6 patients with EGE who responded clinically and histologically to ketotifen; however, Freeman *et al*[79] reported a single case in which the drug failed to maintain disease remission. This agent has also been proposed as an adjunct to steroids and montelukast for treating refractory EGE[5]. The usual dose is 1-2 mg twice daily.

Biologic agents

Biologic agents have also been reported in some case

Table 2 Published cases of eosinophilic gastroenteritis treated with budesonide

Ref.	Patient no.	Intestinal layer	Location	Previous treatment	Response
Russel *et al*[52], 1994	1	Mucosal	Ileum and cecum	Intolerant to steroids Failure of cromolyn sodium and mesalazine	Efficacy comparable to steroids over 5 mo
Tan *et al*[53], 2001	1	Full thickness with ascites	Antrum	Steroid dependent	Remission (+) over 2 yr
Siewert *et al*[54], 2006	1	Mucosal	Duodenum to ileum	None	Response (+)
Lombardi *et al*[55], 2007	1	Mucosal + submucosal	Ileum	Relapse after stopping budesonide and cromolyn sodium	Remission (+) on budesonide alone over 4 mo
Elsing *et al*[56], 2007	1	Muscular	Jejunum	Surgery + steroids for relapse	Remission (+) over 3 mo
Shahzad *et al*[57], 2011	1	Mucosal	Antrum + colon	None	Response (+)
Busoni *et al*[58], 2011	5	Mucosal	Lower + upper GI tract	Prednisone/methylprednisolone	Remission (+)
Lombardi *et al*[59], 2011	1	Muscular	Pyloric stenosis	Methylprednisolone	Remission (+) over 6 mo
Müller *et al*[26], 2014	1	Mucosal	Duodenum + colon + ileum	None	50% response (combined with 6-food elimination diet)
Wong *et al*[15], 2015	1	Mucosal +/- serosal or muscular	-	None	Recurrent symptoms

GI: Gastrointestinal.

Table 3 Published cases of eosinophilic gastroenteritis treated with montelukast

Ref.	Patient no.	Intestinal layer	Location	Previous treatment	Response
Neustrom *et al*[63], 1999	1	Mucosal	Esophagus + stomach + small intestine	Failure of response to elimination diet, cromolyn sodium, ranitidine and hydroxyzine	Clinical and histologic response (+)
Schwartz *et al*[64], 2001	1	Serosal	Duodenum	Steroid dependent	Remission (+) over 4 wk
Lu *et al*[65], 2003	2	Mucosal	-	Steroid dependent	1 → Not effective 2 → Partial response with tapering of prednisone to 10 mg/d
Vanderhoof *et al*[66], 2003	8	Mucosal	Esophagus (n = 4) Duodenum (n = 2) Colon (n = 2)	Failure of standard therapies	Clinical response (+) within 1 mo
Copeland *et al*[9], 2004	1	Mucosal	Stomach	Steroid refractory EGE (also receiving 6MP and 5ASA for UC)	Not effective
Friesen *et al*[67], 2004	40	Mucosal	Duodenum	None	Response (+) within 2 wk
Quack *et al*[68], 2005	1	Serosal	Ileum	Steroid dependent	Remission (+) over 2 yr
Urek *et al*[21], 2006	1	Serosal	Ileum	Steroid dependent	Response (+) within 4 wk
De Maeyer *et al*[69], 2011	1	-	-	Steroid dependent	Response (+)
Tien *et al*[5], 2011	12	Mucosal	Stomach + duodenum + colon + esophagus	4 → None 8 → Steroid dependent	Remission (+) over 12 mo 4/8 → Successful steroid tapering 3/8 → Not effective 1/8 → Lost to follow-up
Selva Kumar *et al*[70], 2011	1	Mucosal	Small intestine	Unresponsive to standard therapy	Response (+)
Müller *et al*[26], 2014	2	Mucosal (+/- serosal or muscular)	Stomach + small intestine	1 and 2 → Steroid dependent	1 → Remission (+) in combination with low-dose prednisone 2 → Remission (+) (off steroids)
Wong *et al*[15], 2015	2	Mucosal (+/- serosal or muscular)	-	1: Steroid dependent 2: None	Remission (+) for 36 mo (in combination with prednisone) Asymptomatic for 10 mo

5ASA: 5-Aminosalicylic acid; 6MP: 6 Mercaptopurine; UC: Ulcerative colitis.

studies of EGE. Mepolizumab (anti-IL5) was reported to have improved tissue and peripheral eosinophilia, but without relieving symptoms, in 4 patients with EGE[80]; unfortunately, another report associated its use

Table 4 Published cases of eosinophilic gastroenteritis treated with cromolyn sodium

Ref.	Patient no.	Intestinal layer	Location	Previous treatment	Response
Moots *et al*[71], 1988	1	Mucosal +/- muscular	Small intestine + colon	Prednisone, cyclophosphamide	Response (+) in 10 wk Maintenance over 2.5 yr
Talley *et al*[4], 1990	3	Mucosal	-	None	1 → Response (+) 2 → No response
Di Gioacchino *et al*[72], 1990	2	Mucosal	Stomach + duodenum	None	Clinical and histologic response (+) after 4-5 mo
Beishuizen *et al*[73], 1993	2	Mucosal	Upper gastrointestinal tract	Steroids	Prolonged response (+)
Van Dellen *et al*[74], 1994	1	Mucosal	Stomach + duodenum	Elemental diet (poorly tolerated)	Response (+)
Russel *et al*[52], 1994	1	Mucosal	Ileum + colon	Steroid dependent	None (failure to taper steroids)
Pérez-Millán *et al*[75], 1997	1	Serosal	Duodenum	None	Response (+) over 6 mo
Suzuki *et al*[76], 2003	1	Mucosal	Stomach + duodenum	Targeted elimination diet (poorly tolerated)	Response (+) (in combination with ketotifene)
Sheikh *et al*[77], 2009	3	Mucosal	Esophagus + stomach + duodenum	Steroid refractory	Not effective
		Mucosal	Stomach + duodenum + colon	None	Partial response
		Mucosal +/- muscular	Esophagus + stomach + duodenum + colon	Steroid dependent	Response (+) with tapering of prednisone over 6 mo

with rebound hypereosinophilia[81]. Omalizumab (anti-IgE) was reported to similarly result in a significant histologic response[82] but to be unlikely to efficiently treat EGE patients with a serum IgE level > 700 kIU/L[83]. Infliximab (anti-TNF) was reported as highly effective for inducing remission in refractory EGE, but its use is limited by the development of resistance and secondary loss of response, both of which can be managed by switching to adalimumab[84].

Surgery

Surgery is indicated in cases of severe disease that are complicated by perforation, intussusception or intestinal occlusion. It has been reported that about 40% of EGE patients may need surgery during the course of their disease, and about half of those may experience persistent symptoms postoperatively[85].

Other modalities

Other modalities include intravenous immunoglobulin and interferon-alpha, both of which appear to be effective in treating severe refractory and steroid dependent cases[10,65]. Suplatast tosilate, a TH2 cytokine inhibitor, can be beneficial as well[86]. Finally, fecal microbiota transplantation has also been reported to improve diarrhea in a patient with EGE, even before its application in combination with steroids[87].

FOLLOW UP AND TREATMENT END-POINTS

While most reported treatments of EGE aim to achieve clinical remission[48,67], histologic improvement remains the optimal way to assess a patient's response, even though it does not always correlate with clinical amelioration[79]. Biopsies can be obtained either endoscopically or under ultrasound guidance[27]. Other less invasive parameters may also be useful in monitoring of treatment response, such as reduction in peripheral eosinophilia[5] and improved radiologic aspects[88]. The choice of appropriate follow-up modality should always be individualized.

CONCLUSION

EGE is a chronic GI disease, having protean manifestations that mimic many other GI disorders. Its diagnosis requires a combination of clinical and pathologic criteria that are evaluated upon suspicious laboratory, radiologic and endoscopic findings. According to the disease severity at initial presentation, many therapeutic modalities can be applied, all of which have been reported in single and case series and have shown variable efficacy. A maintenance regimen is often needed, preferably based upon a safe steroid-sparing drug. Further studies are needed to compare the efficacy and safety profiles of the various treatments available as well as to select predictors of relapses, which might guide decision-making for the kind and duration of maintenance therapy.

REFERENCES

1 **Jensen ET**, Martin CF, Kappelman MD, Dellon ES. Prevalence of Eosinophilic Gastritis, Gastroenteritis, and Colitis: Estimates From a National Administrative Database. *J Pediatr Gastroenterol Nutr* 2016; **62**: 36-42 [PMID: 25988554 DOI: 10.1097/MPG.0000000000000865]

2 **Spergel JM**, Book WM, Mays E, Song L, Shah SS, Talley NJ, Bonis PA. Variation in prevalence, diagnostic criteria, and initial management options for eosinophilic gastrointestinal diseases in the United States. *J Pediatr Gastroenterol Nutr* 2011; **52**: 300-306 [PMID: 21057327 DOI: 10.1097/MPG.0b013e3181eb5a9f]

3 **Chang JY**, Choung RS, Lee RM, Locke GR, Schleck CD, Zinsmeister AR, Smyrk TC, Talley NJ. A shift in the clinical spectrum of eosinophilic gastroenteritis toward the mucosal disease type. *Clin Gastroenterol Hepatol* 2010; **8**: 669-675; quiz e88 [PMID: 20451664 DOI: 10.1016/j.cgh.2010.04.022]

4 **Talley NJ**, Shorter RG, Phillips SF, Zinsmeister AR. Eosinophilic gastroenteritis: a clinicopathological study of patients with disease of the mucosa, muscle layer, and subserosal tissues. *Gut* 1990; **31**: 54-58 [PMID: 2318432 DOI: 10.1136/gut.31.1.54]

5 **Tien FM**, Wu JF, Jeng YM, Hsu HY, Ni YH, Chang MH, Lin DT, Chen HL. Clinical features and treatment responses of children with eosinophilic gastroenteritis. *Pediatr Neonatol* 2011; **52**: 272-278 [PMID: 22036223 DOI: 10.1016/j.pedneo.2011.06.006]

6 **Keshavarzian A**, Saverymuttu SH, Tai PC, Thompson M, Barter S, Spry CJ, Chadwick VS. Activated eosinophils in familial eosinophilic gastroenteritis. *Gastroenterology* 1985; **88**: 1041-1049 [PMID: 3918913]

7 **Reed C**, Woosley JT, Dellon ES. Clinical characteristics, treatment outcomes, and resource utilization in children and adults with eosinophilic gastroenteritis. *Dig Liver Dis* 2015; **47**: 197-201 [PMID: 25547198 DOI: 10.1016/j.dld.2014.11.009]

8 **Butterfield JH**, Murray JA. Eosinophilic gastroenteritis and gluten-sensitive enteropathy in the same patient. *J Clin Gastroenterol* 2002; **34**: 552-553 [PMID: 11960068 DOI: 10.1097/00004836-200205000-00014]

9 **Copeland BH**, Aramide OO, Wehbe SA, Fitzgerald SM, Krishnaswamy G. Eosinophilia in a patient with cyclical vomiting: a case report. *Clin Mol Allergy* 2004; **2**: 7 [PMID: 15144561 DOI: 10.1186/1476-7961-2-7]

10 **Ciccia F**, Giardina AR, Alessi N, Rodolico V, Galia M, Ferrante A, Triolo G. Successful intravenous immunoglobulin treatment for steroid-resistant eosinophilic enteritis in a patient with systemic lupus erythematosus. *Clin Exp Rheumatol* 2011; **29**: 1018-1020 [PMID: 22132839]

11 **Daneshjoo R**, J Talley N. Eosinophilic gastroenteritis. *Curr Gastroenterol Rep* 2002; **4**: 366-372 [PMID: 12228038 DOI: 10.1007/s11894-002-0006-2]

12 **Rothenberg ME**. Eosinophilic gastrointestinal disorders (EGID). *J Allergy Clin Immunol* 2004; **113**: 11-28; quiz 29 [PMID: 14713902 DOI: 10.1016/j.jaci.2003.10.047]

13 **Jaffe JS**, James SP, Mullins GE, Braun-Elwert L, Lubensky I, Metcalfe DD. Evidence for an abnormal profile of interleukin-4 (IL-4), IL-5, and gamma-interferon (gamma-IFN) in peripheral blood T cells from patients with allergic eosinophilic gastroenteritis. *J Clin Immunol* 1994; **14**: 299-309 [PMID: 7814459 DOI: 10.1007/BF01540983]

14 **Klein NC**, Hargrove RL, Sleisenger MH, Jeffries GH. Eosinophilic gastroenteritis. *Medicine* (Baltimore) 1970; **49**: 299-319 [PMID: 5426746 DOI: 10.1097/00005792-197007000-00003]

15 **Wong GW**, Lim KH, Wan WK, Low SC, Kong SC. Eosinophilic gastroenteritis: Clinical profiles and treatment outcomes, a retrospective study of 18 adult patients in a Singapore Tertiary Hospital. *Med J Malaysia* 2015; **70**: 232-237 [PMID: 26358020]

16 **Cello JP**. Eosinophilic gastroenteritis--a complex disease entity. *Am J Med* 1979; **67**: 1097-1104 [PMID: 517550 DOI: 10.1016/0002-9343(79)90652-1]

17 **Shin WG**, Park CH, Lee YS, Kim KO, Yoo KS, Kim JH, Park CK. Eosinophilic enteritis presenting as intussusception in adult. *Korean J Intern Med* 2007; **22**: 13-17 [PMID: 17427639 DOI: 10.3904/kjim.2007.22.1.13]

18 **Maeshima A**, Murakami H, Sadakata H, Saitoh T, Matsushima T, Tamura J, Karasawa M, Naruse T. Eosinophilic gastroenteritis presenting with acute pancreatitis. *J Med* 1997; **28**: 265-272 [PMID: 9355030]

19 **Madhotra R**, Eloubeidi MA, Cunningham JT, Lewin D, Hoffman B. Eosinophilic gastroenteritis masquerading as ampullary adenoma. *J Clin Gastroenterol* 2002; **34**: 240-242 [PMID: 11873104 DOI: 10.1097/00004836-200203000-00009]

20 **Pineton de Chambrun G**, Gonzalez F, Canva JY, Gonzalez S, Houssin L, Desreumaux P, Cortot A, Colombel JF. Natural history of eosinophilic gastroenteritis. *Clin Gastroenterol Hepatol* 2011; **9**: 950-956.e1 [PMID: 21806952 DOI: 10.1016/j.cgh.2011.07.017]

21 **Urek MC**, Kujundzić M, Banić M, Urek R, Veic TS, Kardum D. Leukotriene receptor antagonists as potential steroid sparing agents in a patient with serosal eosinophilic gastroenteritis. *Gut* 2006; **55**: 1363-1364 [PMID: 16905705 DOI: 10.1136/gut.2006.099465]

22 **Chehade M**, Sicherer SH, Magid MS, Rosenberg HK, Morotti RA. Multiple exudative ulcers and pseudopolyps in allergic eosinophilic gastroenteritis that responded to dietary therapy. *J Pediatr Gastroenterol Nutr* 2007; **45**: 354-357 [PMID: 17873749 DOI: 10.1097/MPG.0b013e31803219d5]

23 **Manatsathit W**, Sermsathanasawadi R, Pongpaiboon A, Pongprasobchai S. Mucosal-type eosinophilic gastroenteritis in Thailand: 12-year retrospective study. *J Med Assoc Thai* 2013; **96** Suppl 2: S194-S202 [PMID: 23590042]

24 **Chen MJ**, Chu CH, Lin SC, Shih SC, Wang TE. Eosinophilic gastroenteritis: clinical experience with 15 patients. *World J Gastroenterol* 2003; **9**: 2813-2816 [PMID: 14669340 DOI: 10.3748/wjg.v9.i12.2813]

25 **Alnaser S**, Aljebreen AM. Endoscopic ultrasound and hisopathologic correlates in eosinophilic gastroenteritis. *Saudi J Gastroenterol* 2007; **13**: 91-94 [PMID: 19858621 DOI: 10.4103/1319-3767.32185]

26 **Müller M**, Keller K, Stallmann S, Eckardt AJ. Clinicopathologic Findings in Eosinophilic Gastroenteritis: A German Case Series. *J Genet Syndr Gene Ther* 2014; **5**: 1 [DOI: 10.4172/2157-7412.1000230]

27 **Marco-Doménech SF**, Gil-Sánchez S, Jornet-Fayos J, Ambit-Capdevila S, Gonzalez-Añón M. Eosinophilic gastroenteritis: percutaneous biopsy under ultrasound guidance. *Abdom Imaging* 1998; **23**: 286-288 [PMID: 9569298 DOI: 10.1007/s002619900341]

28 **Anuradha C**, Mittal R, Yacob M, Manipadam MT, Kurian S, Eapen A. Eosinophilic disorders of the gastrointestinal tract: imaging features. *Diagn Interv Radiol* 2012; **18**: 183-188 [PMID: 21948696]

29 **Zheng X**, Cheng J, Pan K, Yang K, Wang H, Wu E. Eosinophilic enteritis: CT features. *Abdom Imaging* 2008; **33**: 191-195 [PMID: 17387538 DOI: 10.1007/s00261-007-9209-1]

30 **Lee KJ**, Hahm KB, Kim YS, Kim JH, Cho SW, Jie H, Park CH, Yim H. The usefulness of Tc-99m HMPAO labeled WBC SPECT in eosinophilic gastroenteritis. *Clin Nucl Med* 1997; **22**: 536-541 [PMID: 9262899 DOI: 10.1097/00003072-199708000-00005]

31 **Solis-Herruzo JA**, de Cuenca B, Muñoz-Yagüe MT. Laparoscopic findings in serosal eosinophilic gastroenteritis. Report of two cases. *Endoscopy* 1988; **20**: 152-153 [PMID: 2972537 DOI: 10.1055/s-2007-1018162]

32 **Rothenberg ME**, Mishra A, Brandt EB, Hogan SP. Gastrointestinal eosinophils. *Immunol Rev* 2001; **179**: 139-155 [PMID: 11292017 DOI: 10.1034/j.1600-065X.2001.790114.x]

33 **Torpier G**, Colombel JF, Mathieu-Chandelier C, Capron M, Dessaint JP, Cortot A, Paris JC, Capron A. Eosinophilic gastroenteritis: ultrastructural evidence for a selective release of eosinophil major basic protein. *Clin Exp Immunol* 1988; **74**: 404-408 [PMID: 3233790]

34 **Hurrell JM**, Genta RM, Melton SD. Histopathologic diagnosis of eosinophilic conditions in the gastrointestinal tract. *Adv Anat Pathol* 2011; **18**: 335-348 [PMID: 21841404 DOI: 10.1097/PAP.0b013e318229bfe2]

35 **Blackburn GL**, Bistrian BR, Maini BS, Schlamm HT, Smith MF. Nutritional and metabolic assessment of the hospitalized patient. *JPEN J Parenter Enteral Nutr* 1977; **1**:11-22 [PMID: 98649]

36 **Carley A**. Anemia: when is it not iron deficiency? *Pediatr Nurs* 2003; **29**: 205-211 [PMID: 12836997]

37 **Tefferi A**. Blood eosinophilia: a new paradigm in disease classification, diagnosis, and treatment. *Mayo Clin Proc* 2005; **80**: 75-83 [PMID: 15667033 DOI: 10.4065/80.1.75]

38 **Macari M**, Balthazar EJ. CT of bowel wall thickening: significance and pitfalls of interpretation. *AJR Am J Roentgenol* 2001; **176**: 1105-1116 [PMID: 11312162 DOI: 10.2214/ajr.176.5.1761105]

39 **Weinstein WM**, Wiggett SD. The spectrum of endoscopic and histologic findings in eosinophilic gastroenteritis. A video capsule and push enteroscopic study. *Gastroenterology* 2003; **124**: A191 [DOI: 10.1016/s0016-5085(03)80957-5]

40 **Prussin C**. Eosinophilic gastroenteritis and related eosinophilic disorders. *Gastroenterol Clin North Am* 2014; **43**: 317-327 [PMID: 24813518 DOI: 10.1016/j.gtc.2014.02.013]

41 **Guajardo JR**, Plotnick LM, Fende JM, Collins MH, Putnam PE, Rothenberg ME. Eosinophil-associated gastrointestinal disorders: a world-wide-web based registry. *J Pediatr* 2002; **141**: 576-581 [PMID: 12378201 DOI: 10.1067/mpd.2002.127663]

42 **Spergel JM**, Brown-Whitehorn T, Beausoleil JL, Shuker M,

Liacouras CA. Predictive values for skin prick test and atopy patch test for eosinophilic esophagitis. *J Allergy Clin Immunol* 2007; **119**: 509-511 [PMID: 17291865 DOI: 10.1016/j.jaci.2006.11.016]
43 **Liao WH**, Wei KL, Po-Yen-Lin CS. A rare case of spontaneous resolution of eosinophilic ascites in a patient with primary eosinophilic gastroenteritis. *Chang Gung Med J* 2012; **35**: 354-359 [PMID: 22913863]
44 **Venkataraman S**, Ramakrishna BS, Mathan M, Chacko A, Chandy G, Kurian G, Mathan VI. Eosinophilic gastroenteritis--an Indian experience. *Indian J Gastroenterol* 1998; **17**: 148-149 [PMID: 9795503]
45 **Yamada Y**, Toki F, Yamamoto H, Nishi A, Kato M. Proton pump inhibitor treatment decreased duodenal and esophageal eosinophilia in a case of eosinophilic gastroenteritis. *Allergol Int* 2015; **64** Suppl: S83-S85 [PMID: 26344087 DOI: 10.1016/j.alit.2015.06.002]
46 **Soavi C**, Caselli M, Sioulis F, Cassol F, Lanza G, Zuliani G. Eosinophilic gastroenteritis cured with Helicobacter pylori eradication: case report and review of literature. *Helicobacter* 2014; **19**: 237-238 [PMID: 24650300 DOI: 10.1111/hel.12122]
47 **Ohe M**, Hashino S. Successful treatment of eosinophilic gastroenteritis with clarithromycin. *Korean J Intern Med* 2012; **27**: 451-454 [PMID: 23269887 DOI: 10.3904/kjim.2012.27.4.451]
48 **Lucendo AJ**, Serrano-Montalbán B, Arias Á, Redondo O, Tenias JM. Efficacy of Dietary Treatment for Inducing Disease Remission in Eosinophilic Gastroenteritis. *J Pediatr Gastroenterol Nutr* 2015; **61**: 56-64 [PMID: 25699593 DOI: 10.1097/MPG.0000000000000766]
49 **Khan S**. Eosinophilic gastroenteritis. *Best Pract Res Clin Gastroenterol* 2005; **19**: 177-198 [PMID: 15833687 DOI: 10.1016/j.bpg.2005.01.009]
50 **Zhang L**, Duan L, Ding S, Lu J, Jin Z, Cui R, McNutt M, Wang A. Eosinophilic gastroenteritis: clinical manifestations and morphological characteristics, a retrospective study of 42 patients. *Scand J Gastroenterol* 2011; **46**: 1074-1080 [PMID: 21623674 DOI: 10.3109/00365521.2011.579998]
51 **Stone KD**, Prussin C. Immunomodulatory therapy of eosinophil-associated gastrointestinal diseases. *Clin Exp Allergy* 2008; **38**: 1858-1865 [PMID: 19037962 DOI: 10.1111/j.1365-2222.2008.03122.x]
52 **Russel MG**, Zeijen RN, Brummer RJ, de Bruine AP, van Kroonenburgh MJ, Stockbrügger RW. Eosinophilic enterocolitis diagnosed by means of technetium-99m albumin scintigraphy and treated with budesonide (CIR). *Gut* 1994; **35**: 1490-1492 [PMID: 7959211 DOI: 10.1136/gut.35.10.1490]
53 **Tan AC**, Kruimel JW, Naber TH. Eosinophilic gastroenteritis treated with non-enteric-coated budesonide tablets. *Eur J Gastroenterol Hepatol* 2001; **13**: 425-427 [PMID: 11338074]
54 **Siewert E**, Lammert F, Koppitz P, Schmidt T, Matern S. Eosinophilic gastroenteritis with severe protein-losing enteropathy: successful treatment with budesonide. *Dig Liver Dis* 2006; **38**: 55-59 [PMID: 16326154]
55 **Lombardi C**, Salmi A, Savio A, Passalacqua G. Localized eosinophilic ileitis with mastocytosis successfully treated with oral budesonide. *Allergy* 2007; **62**: 1343-1345 [PMID: 17919149]
56 **Elsing C**, Placke J, Gross-Weege W. Budesonide for the treatment of obstructive eosinophilic jejunitis. *Z Gastroenterol* 2007; **45**: 187-189 [PMID: 17304405]
57 **Shahzad G**, Moise D, Lipka S, Rizvon K, Mustacchia PJ. Eosinophilic enterocolitis diagnosed by means of upper endoscopy and colonoscopy with random biopsies treated with budenoside: a case report and review of the literature. *ISRN Gastroenterol* 2011; **2011**: 608901 [PMID: 21991521 DOI: 10.5402/2011/608901]
58 **Busoni VB**, Lifschitz C, Christiansen S, G de Davila MT, Orsi M. [Eosinophilic gastroenteropathy: a pediatric series]. *Arch Argent Pediatr* 2011; **109**: 68-73 [PMID: 21283948 DOI: 10.1590/S0325-00752011000100019]
59 **Lombardi C**, Salmi A, Passalacqua G. An adult case of eosinophilic pyloric stenosis maintained on remission with oral budesonide. *Eur Ann Allergy Clin Immunol* 2011; **43**: 29-30 [PMID: 21409859]
60 **Redondo-Cerezo E**, Cabello MJ, González Y, Gómez M, García-Montero M, de Teresa J. Eosinophilic gastroenteritis: our recent experience: one-year experience of atypical onset of an uncommon disease. *Scand J Gastroenterol* 2001; **36**: 1358-1360 [PMID: 11761030]
61 **Netzer P**, Gschossmann JM, Straumann A, Sendensky A, Weimann R, Schoepfer AM. Corticosteroid-dependent eosinophilic oesophagitis: azathioprine and 6-mercaptopurine can induce and maintain long-term remission. *Eur J Gastroenterol Hepatol* 2007; **19**: 865-869 [PMID: 17873610 DOI: 10.1097/MEG.0b013e32825a6ab4]
62 **Kim YJ**, Chung WC, Kim Y, Chung YY, Lee KM, Paik CN, Chin HM, Hyun Choi HJ. A Case of Steroid Dependent Eosinophilic Gastroenteritis Presenting as a Huge Gastric Ulcer. *Korean J Helicobacter Up Gastrointest Res* 2012; **12**:103-107 [DOI: 10.7704/kjhugr.2012.12.2.103]
63 **Neustrom MR**, Friesen C. Treatment of eosinophilic gastroenteritis with montelukast. *J Allergy Clin Immunol* 1999; **104**: 506 [PMID: 10452782]
64 **Schwartz DA**, Pardi DS, Murray JA. Use of montelukast as steroid-sparing agent for recurrent eosinophilic gastroenteritis. *Dig Dis Sci* 2001; **46**: 1787-1790 [PMID: 11508684]
65 **Lu E**, Ballas ZK. Immuno-modulation in the treatment of eosinophilic gastroenteritis. *J Allergy Clin Immunol* 2003, **111**: S262 [DOI: 10.1016/S0091-6749(03)80936-3]
66 **Vanderhoof JA**, Young RJ, Hanner TL, Kettlehut B. Montelukast: use in pediatric patients with eosinophilic gastrointestinal disease. *J Pediatr Gastroenterol Nutr* 2003; **36**: 293-294 [PMID: 12548071]
67 **Friesen CA**, Kearns GL, Andre L, Neustrom M, Roberts CC, Abdel-Rahman SM. Clinical efficacy and pharmacokinetics of montelukast in dyspeptic children with duodenal eosinophilia. *J Pediatr Gastroenterol Nutr* 2004; **38**: 343-351 [PMID: 15076638]
68 **Quack I**, Sellin L, Buchner NJ, Theegarten D, Rump LC, Henning BF. Eosinophilic gastroenteritis in a young girl--long term remission under Montelukast. *BMC Gastroenterol* 2005; **5**: 24 [PMID: 16026609]
69 **De Maeyer N**, Kochuyt AM, Van Moerkercke W, Hiele M, Montelukast as a treatment modality for eosinophilic gastroenteritis. *Acta Gastroenterol Belg* 2011; **74**:570-575 [PMID: 22319970]
70 **Selva Kumar C**, Das RR, Balakrishnan CD, Balagurunathan K, Chaudhuri K. Malabsorption syndrome and leukotriene inhibitor. *J Trop Pediatr* 2011; **57**: 135-137 [PMID: 20571153]
71 **Moots RJ**, Prouse P, Gumpel JM. Near fatal eosinophilic gastroenteritis responding to oral sodium chromoglycate. *Gut* 1988; **29**: 1282-1285 [PMID: 3143628]
72 **Di Gioacchino M**, Pizzicannella G, Fini N, Falasca F, Antinucci R, Masci S, Mezzetti A, Marzio L, Cuccurullo F. Sodium cromoglycate in the treatment of eosinophilic gastroenteritis. *Allergy* 1990; **45**: 161-166 [PMID: 2109547]
73 **Beishuizen A**, van Bodegraven AA, Bronsveld W, Sindram JW. Eosinophilic gastroenteritis--a disease with a wide clinical spectrum. *Neth J Med* 1993; **42**: 212-217 [PMID: 8377880]
74 **Van Dellen RG**, Lewis JC. Oral administration of cromolyn in a patient with protein-losing enteropathy, food allergy, and eosinophilic gastroenteritis. *Mayo Clin Proc* 1994; **69**: 441-444 [PMID: 8170195 DOI: 10.1016/S0025-6196(12)61640-1]
75 **Pérez-Millán A**, Martín-Lorente JL, López-Morante A, Yuguero L, Sáez-Royuela F. Subserosal eosinophilic gastroenteritis treated efficaciously with sodium cromoglycate. *Dig Dis Sci* 1997; **42**: 342-344 [PMID: 9052516]
76 **Suzuki J**, Kawasaki Y, Nozawa R, Isome M, Suzuki S, Takahashi A, Suzuki H. Oral disodium cromoglycate and ketotifen for a patient with eosinophilic gastroenteritis, food allergy and protein-losing enteropathy. *Asian Pac J Allergy Immunol* 2003; **21**: 193-197 [PMID: 15032404]
77 **Sheikh RA**, Prindiville TP, Pecha RE, Ruebner BH. Unusual presentations of eosinophilic gastroenteritis: case series and review of literature. *World J Gastroenterol* 2009; **15**: 2156-2161 [PMID: 19418590 DOI: 10.3748/wjg.15.2156]
78 **Melamed I**, Feanny SJ, Sherman PM, Roifman CM. Benefit of ketotifen in patients with eosinophilic gastroenteritis. *Am J Med* 1991; **90**: 310-314 [PMID: 2003512 DOI: 10.1016/0002-9343(91)80010-J]
79 **Freeman HJ**. Longstanding eosinophilic gastroenteritis of more

than 20 years. *Can J Gastroenterol* 2009; **23**: 632-634 [PMID: 19816628]
80 **Prussin C**, James SP, Huber MM, Klion AD, Metcalfe DD. Pilot study of anti-IL-5 in eosinophilic gastroenteritis. *J Allergy Clin Immunol* 2003; **111**: S275 [DOI: 10.1016/S0091-6749(03)80986-7]
81 **Kim YJ**, Prussin C, Martin B, Law MA, Haverty TP, Nutman TB, Klion AD. Rebound eosinophilia after treatment of hypereosinophilic syndrome and eosinophilic gastroenteritis with monoclonal anti-IL-5 antibody SCH55700. *J Allergy Clin Immunol* 2004; **114**: 1449-1455 [PMID: 15577851 DOI: 10.1016/j.jaci.2004.08.027]
82 **Foroughi S**, Foster B, Kim N, Bernardino LB, Scott LM, Hamilton RG, Metcalfe DD, Mannon PJ, Prussin C. Anti-IgE treatment of eosinophil-associated gastrointestinal disorders. *J Allergy Clin Immunol* 2007; **120**: 594-601 [PMID: 17765756 DOI: 10.1016/j.jaci.2007.06.015]
83 **Hochhaus G**, Brookman L, Fox H, Johnson C, Matthews J, Ren S, Deniz Y. Pharmacodynamics of omalizumab: implications for optimised dosing strategies and clinical efficacy in the treatment of allergic asthma. *Curr Med Res Opin* 2003; **19**: 491-498 [PMID: 14594521 DOI: 10.1185/030079903125002171]
84 **Turner D**, Wolters VM, Russell RK, Shakhnovich V, Muise AM, Ledder O, Ngan B, Friesen C. Anti-TNF, infliximab, and adalimumab can be effective in eosinophilic bowel disease. *J Pediatr Gastroenterol Nutr* 2013; **56**: 492-497 [PMID: 23221994]
85 **Naylor AR**. Eosinophilic gastroenteritis. *Scott Med J* 1990; **35**: 163-165 [PMID: 2077646]
86 **Shirai T**, Hashimoto D, Suzuki K, Osawa S, Aonahata M, Chida K, Nakamura H. Successful treatment of eosinophilic gastroenteritis with suplatast tosilate. *J Allergy Clin Immunol* 2001; **107**: 924-925 [PMID: 11344364]
87 **Dai YX**, Shi CB, Cui BT, Wang M, Ji GZ, Zhang FM. Fecal microbiota transplantation and prednisone for severe eosinophilic gastroenteritis. *World J Gastroenterol* 2014; **20**: 16368-16371 [PMID: 25473198 DOI: 10.3748/wjg.v20.i43.16368]
88 **Buljevac M**, Urek MC, Stoos-Veić T. Sonography in diagnosis and follow-up of serosal eosinophilic gastroenteritis treated with corticosteroid. *J Clin Ultrasound* 2005; **33**: 43-46 [PMID: 15690448]

P- Reviewer: Capasso R, Gupta N, Liu F **S- Editor**: Gong ZM
L- Editor: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i4.524

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 524-530
ISSN 2150-5349 (online)

MINIREVIEWS

How I treat my inflammatory bowel disease-patients with thiopurines?

Berrie Meijer, Chris JJ Mulder, Adriaan A van Bodegraven, Nanne KH de Boer

Berrie Meijer, Chris JJ Mulder, Nanne KH de Boer, Department of Gastroenterology and Hepatology, VU University Medical Center, 1007 MB, Amsterdam, The Netherlands

Adriaan A van Bodegraven, Department of Gastroenterology, Geriatrics, Internal and Intensive Care Medicine (Co-MIK), Zuyderland Medical Center, 6130 MB, Sittard-Geleen, The Netherlands

Author contributions: de Boer NKH was the guarantor of the article; Meijer B completed the first draft of the manuscript; Mulder CJJ, van Bodegraven AA and de Boer NKH critically revised the manuscript; Meijer B finalized the article.

Conflict-of-interest statement: There is no conflict of interest associated with any of the senior author or other coauthors contributed their efforts in this manuscript.

Manuscript source: Invited manuscript

Correspondence to: Nanne KH de Boer, MD, PhD, Department of Gastroenterology and Hepatology, VU University Medical Center, PO Box 7057, 1007 MB, Amsterdam, The Netherlands. khn.deboer@vumc.nl
Telephone: +31-20-4440613
Fax: +31-20-4440554

Received: July 21, 2016
Peer-review started: July 21, 2016
First decision: September 5, 2016
Revised: September 12, 2016
Accepted: October 5, 2016
Article in press: October 7, 2016
Published online: November 6, 2016

Abstract

Thiopurines are essential drugs to maintain remission in patients with inflammatory bowel disease (IBD). Thiopurines used in IBD are azathioprine (2.0-2.5 mg/kg), mercaptopurine (1.0-1.5 mg/kg) and thioguanine (0.2-0.3 mg/kg). However, mainly due to numerous adverse events associated with thiopurine use, almost 50% of the patients have to discontinue conventional thiopurine treatment. Extensive monitoring and the application of several treatment strategies, such as split-dose administration, co-administration with allopurinol or dose reduction/increase, may increase the chance of successful therapy. With this review, we provide practical information on how thiopurines are initiated and maintained in two thiopurine research centers in The Netherlands. We provide clinical information concerning safety issues, indications and management of therapy that may serve as a guide for the administration of thiopurines in IBD patients in daily practice.

Key words: Thiopurines; Azathioprine; Mercaptopurine; Thioguanine; Inflammatory bowel disease; Therapeutic drug monitoring; Pregnancy; Metabolites

Core tip: Conventional thiopurine therapy with azathioprine and mercaptopurine in inflammatory bowel disease is associated with several adverse events causing cessation of therapy in up to half of the patients. On the contrary, thiopurine therapy is often unnecessarily discontinued. In this practical review, we provide information on how thiopurine therapy is initiated and maintained using periodical laboratory tests and the application of various treatment strategies (including the administration of a third thiopurine; thioguanine), based on the experience in the two expert thiopurine centers in The Netherlands.

Meijer B, Mulder CJJ, van Bodegraven AA, de Boer NKH. How I treat my inflammatory bowel disease-patients with thiopurines? *World J Gastrointest Pharmacol Ther* 2016; 7(4): 524-530 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i4/524.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i4.524

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic condition of the gastrointestinal tract which is characterized by episodes of remission and relapses and encompasses both Crohn's disease (CD) and ulcerative colitis (UC). In the management of IBD, thiopurines [*i.e.*, azathioprine (AZA), mercaptopurine (MP) and thioguanine (TG)] play an important role in clinical practice, mainly in order to maintain remission[1-4]. Over the past decades, extensive research has been performed to elucidate the complex metabolism of thiopurine derivatives[5-7]. In this article, we demonstrate and discuss the way we use thiopurine therapy in the treatment of adult IBD patients in two referral centers in The Netherlands in daily practice. For information about thiopurine therapy in pediatric IBD, we refer to reviews focused on this patient group[8-10].

DISCOVERY OF THIOPURINES

Thiopurines were firstly described in the early 1950s by Gertrude Elion and George Hitchings, primarily as antimetabolite therapy[11]. Initially, thiopurines were used in the treatment of acute lymphatic leukemia in children and in the prevention of organ transplant rejection. The first IBD patient treated with thiopurines has been described by Dr. Bean[12] in 1962. At this moment, thiopurines are used in a variety of autoimmune disorders and hematologic malignancies[13,14].

PHARMACOLOGY OF THIOPURINES

The thiopurine derivatives AZA, MP and TG are all pro-drugs which are subsequently converted into the allegedly most important pharmacologically active end-metabolites, 6-thioguanine nucleotides (6-TGN)[5]. AZA is converted into MP by the enzyme glutathione S-transferase, after which MP is metabolized by three competing enzymatic systems. First, a part of the concentration MP is withdrawn from bioavailability by xanthine oxidase (XO) and thiopurine-S-methyltransferase (TPMT), converting MP into 6-thiouric acid (6-TUA) and 6-methylmercaptopurine (6-MMP), respectively. The remaining concentration of MP is metabolized *via* the purine salvage pathway into 6-TGN by a cascade of hypoxanthine-guanine phosphoribosyl transferase (HGPRT), inosine monophosphate dehydrogenase (IMPD) and guanosine monophosphate synthetase (GMPS). The 6-TGN can be incorporated in the DNA (thus achieving an anti-metabolic effect), but also account for inhibition of anti-apoptotic effects and down-regulation of pro-inflammatory cytokines. In daily practice, we measure 6-TGN and 6-MMP in red blood cells (RBC), which is mainly due to the fact that in patients with leukemia, the original indication for thiopurine therapy, successful treatment with thiopurines leads to the unavailability of leukocytes[11,15].

In contrast to AZA and MP, the metabolism of TG is less extensive as TG is directly converted into 6-TGN by HGPRT. Whether TG is also withdrawn from bioavailability by the effect of TPMT and XO, this effect is relatively smaller than in AZA and MP, leaving a larger portion of TG available for (direct) conversion into 6-TGN (Figure 1)[5,7,16,17].

INDICATIONS OF THIOPURINE THERAPY

When treating IBD patients in our centers, we apply the therapeutic approach known as accelerated step up care in both CD and UC[18]. Conventional thiopurines (*i.e.*, AZA and MP) do not play a standard role in the active phase of CD and UC as such, however it may be added to induction course therapy with corticosteroids in those patients who are suspected of having a more severe or prolonged disease course. In patients with only mildly active disease with good reaction on initial induction therapy, thiopurines do not have to be initiated straight away[19]. However, in those patients with a relapse of disease despite two induction courses of corticosteroids, thiopurines are required to maintain remission[20]. Furthermore, thiopurines are co-administered as a routine to treatment with anti-TNF therapy in our centers, in line with recent observations from the SONIC trial[2-4,21]. In those patients receiving vedolizumab (Entyvio®) evidence is scarce whether to (dis)continue simultaneous thiopurine therapy[22,23]. In our centers, we continue thiopurine therapy in the majority of patients, since patients receiving vedolizumab are likely to have highly complex disease in which vedolizumab is often initiated as rescue drug. Additionally, in line with the observations in the SONIC trial in patients receiving infliximab and adalimumab, we presume that thiopurines might have a protecting effect on the development of antibodies against vedolizumab[24,25]. Finally, thiopurines are administered in surgical CD patients to prevent post-surgical recurrence, especially in complex patients with fistulizing disease or multiple surgical interventions[3].

Thiopurine therapy is initiated in a dosage of 2.0-2.5 mg/kg for AZA or 1.0-1.5 mg/kg for MP, starting with 50 mg/d in the first week and increasing to full-dose when patients experience no adverse effects on low-dose therapy[1]. In those patients in whom thiopurines were co-administered next to induction corticosteroid therapy, the steroids are tapered down in 2-3 mo.

In our center, we prefer to initiate thiopurine therapy using MP, based on results of several rechallenge studies[26-31]. Furthermore, in those patients with mild adverse events (*i.e.*, no severe myelotoxicity or pancreatitis) on MP therapy, we rechallenge these patients with MP with low threshold.

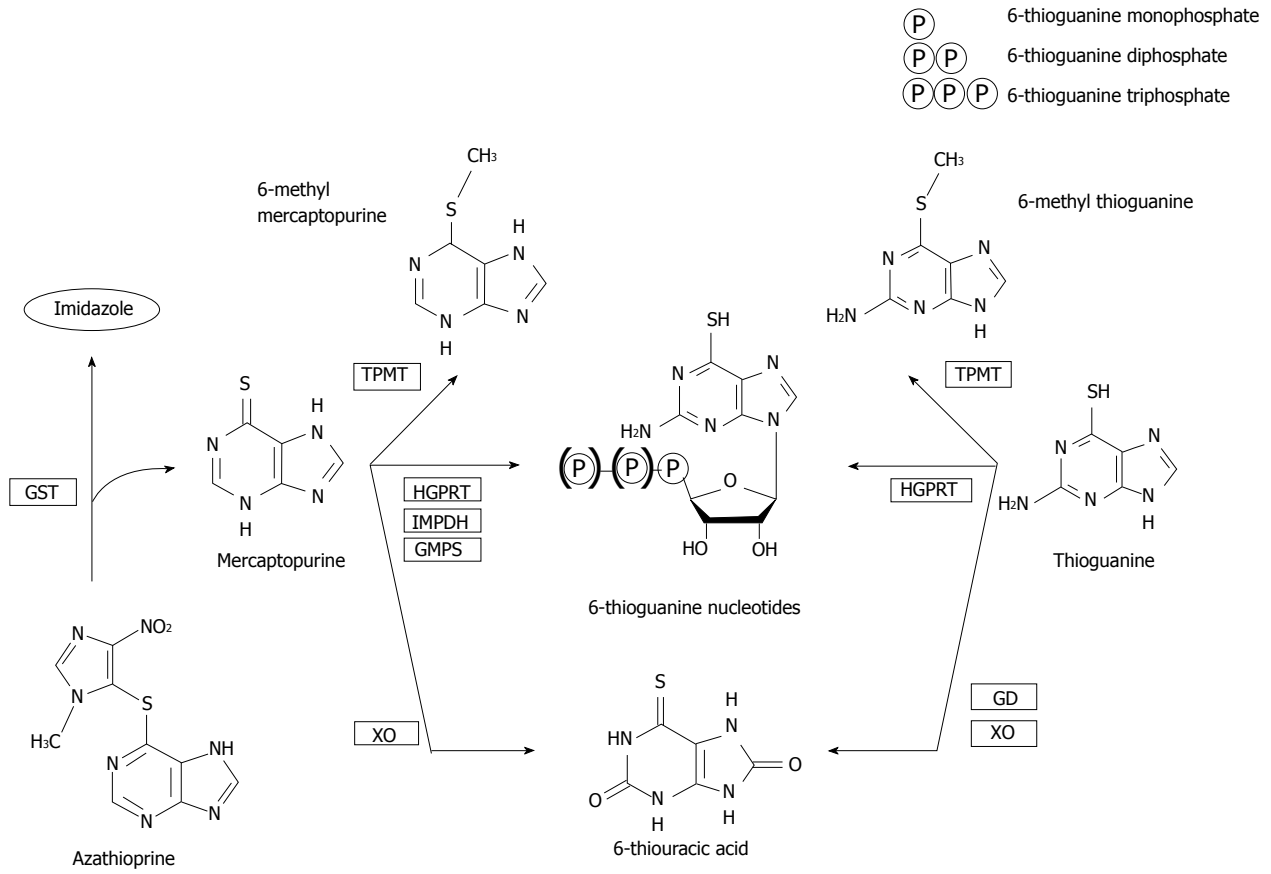

Figure 1 Simplified scheme of thiopurine metabolism. Azathioprine is converted to mercaptopurine by the enzyme glutathione S-transferase (GST), by separating the imidazole-group. 6-Mercaptopurine is enzymatically converted into 6-methylmercaptopurine (6-MMP) by thiopurine-S-methyltransferase (TPMT) and into 6-thiouracic acid (6-TUA) by xanthine oxidase (XO). The remaining portion of mercaptopurine is converted into the biochemically active end-metabolites 6-thioguaninenucleotides (6-TGN, consisting of 6-thioguanine monophosphate, 6-thioguanine diphosphate and 6-thioguanine triphosphate) by a pathway of hypoxanthine-guanine phosphoribosyl transferase (HGPRT), inosine monophosphate dehydrogenase (IMPDH) and guanosine monophosphate synthetase (GMPS). Thioguanine is metabolized by TPMT into 6-methylthioguanine (6-MTG) and into 6-TUA by guanine deaminase (GD) and XO. The remaining portion of thioguanine is directly converted into 6-TGN by HGPRT. Squared abbreviations display enzymatic conversions. Adapted from van Asseldonk *et al*[5].

TOXICITY OF THIOPURINE THERAPY

As thiopurines are associated with a broad spectrum of adverse events (*i.e.*, flu-like symptoms, arthralgia, gastrointestinal complaints, rash, pancreatitis, hepatotoxicity and myelotoxicity), one of the applied strategies to reduce the risk of developing adverse events is to measure TPMT activity before initiating thiopurine therapy, to identify patients at risk of developing adverse events based on an aberrant thiopurine metabolism[7,16,32,33]. Literature reports show that 1:300 (Caucasian) individuals have TPMT deficiency, making them at risk for developing (severe) myelosuppression due to preferential 6-TGN formation[34]. In our centers, however, TPMT activity is not determined as we initiate thiopurine therapy with a low-dose start up scheme. Furthermore, many patients with normal TPMT activity could still develop adverse events of thiopurine therapy[32,35,36]. For this reason amongst others, we choose to extensively monitor laboratory and clinical parameters in the first three months after initiation of thiopurine therapy. At week 0, 1, 2, 4, 8 and 12, hematologic and hepatic parameters are being measured, as well as creatinine and C-reactive protein (Table 1). After initiation, these parameters are determined each 3-4 mo during thiopurine maintenance therapy.

Table 1 Laboratory tests to determine risk of myelotoxicity and hepatotoxicity during initiation of thiopurine therapy

Hematologic parameters
Hemoglobin
White blood cell count
Platelet count
Hepatic parameters
Alkalic phosphatase
Gamma glutamyl transpeptidase
Alanine aminotransferase
Other parameters
Creatinine
C-reactive protein

Measuring of above mentioned parameters: Induction phase: week 0, 1, 2, 4, 8 and 12; Maintenance phase: Each 3-4 mo.

MEASURING METABOLITES

Measurement of thiopurine metabolites (6-TGN and 6-MMP) is not performed routinely in clinical practice at our centers. In those patients who experience adverse

Table 2 Interpretation of metabolite levels in patients with inflammatory bowel disease treated with azathioprine or mercaptopurine

6-TGN (pmol/8 × 10^8 RBC)	6-MMP (pmol/8 × 10^8 RBC)	Non-response	Adverse event (dose-dependent)	Recommendation
<< 230	<< 5700	Non-compliance	Not expected	Gain compliance
< 230	< 5700	Non-compliance/under dosing	Not expected	Gain compliance/increase dose[1]
230-400	< 5700	Possible resistance to thiopurine therapy	Not expected	Increase dose1 or change therapy[2]
> 400	< 5700	Therapy resistance	Myelotoxicity	Change therapy[2]
< 230	>> 5700	Shunting	Myelotoxicity	Consider allopurinol[3] or switch to TG[4]
< 230	> 5700	Shunting	Hepatotoxicity	Consider allopurinol, 5-ASA or switch to TG
230-400	> 5700	Possible resistance to thiopurine therapy	Hepatotoxicity	Consider allopurinol[3] or 5-ASA
> 400	> 5700	Therapy resistance	Hepatotoxicity	Change therapy[2]
			Myelotoxicity	Decrease dose[5]

Therapeutic target of therapy: 6-TGN: 230-400 pmol/8 × 10^8 RBC; 6-MMP: < 5700 pmol/8 × 10^8 RBC. [1]AZA: Increase with 50 mg, MP: Increase with 25 mg; [2]In case of thiopurine refractoriness, consider switch of therapy to non-thiopurine therapy; [3]Allopurinol co-treatment with 100 mg daily requires dose-adjustment of thiopurine therapy to approximately 25%-33% of original daily dose[38,60]; [4]Treatment with thioguanine in low dose (*i.e.*, 0.3 mg/kg) bypasses the formation of 6-MMP; [5]In case of adverse events. 6-TGN: 6-thioguanine nucleotides; 6-MMP: 6-methylmercaptopurine; RBC: Red blood cells; <: Lower than; <<: Much lower than; >: Higher than; >>: Much higher than; AZA: Azathioprine; MP: Mercaptopurine; TG: Thioguanine; 5-ASA: 5-aminosalicylic acid (mesalazine).

events or non-response to treatment, metabolite levels may explain why these patients are intolerant or resistant to therapy (Table 2). Based on these results, individual treatment strategies (*i.e.*, split-dose administration, dose reduction/increase, the addition of mesalazine or allopurinol to a 25%-33% dose of the original thiopurine in patients with an altered thiopurine metabolism, so-called "skewers") may be applied[37-39].

TG THERAPY

In those patients with either idiosyncratic (*e.g.*, pancreatitis) adverse events on conventional thiopurine therapy or adverse events based on elevated 6-MMP concentrations, these patients can be switched to TG. In some countries, TG is considered as rescue drug when conventional therapy fails, however, in The Netherlands this drug is officially registered as treatment option for IBD since March 2016. One of the feared complications of TG treatment is the development of nodular regenerative hyperplasia (NRH), a condition of the liver in which patients might develop non-cirrhotic portal hypertension. However, in contrary to earlier observations[40-42], the development of NRH is seldomly witnessed in those patients treated with low-dose TG therapy (*i.e.*, 0.3 mg/kg)[43-47] and furthermore not associated with clinically relevant liver disease[48]. In our patients treated with TG, liver biopsies are only performed in patients with symptoms of portal hypertension or persisting liver test abnormalities and no longer as a routine[48].

CANCER RISK

The use of thiopurines is associated with a three- to fivefold higher risk of the development of lymphoproliferative disorders, in particular non-Hodgkin lymphoma, as well as hepatosplenic T-cell lymphoma, especially in patients without prior Epstein-Barr virus (EBV) exposure[49]. We do not systematically test EBV seroprevalence in patients starting with thiopurines, since over 90% of Dutch inhabitants are exposed to EBV during childhood[50].

Furthermore, there is a clinically significant elevated risk of developing non-melanoma skin cancer, such as squamous cell carcinoma and basal cell carcinoma[49,51]. In our centers, we inform our patients of this higher risk and instruct them to, for example, apply sunscreen to unprotected skin and mention newly developed skin lesions directly to the treating physician or IBD-nurse. However, since the absolute incidence of these malignancies in thiopurine-using IBD patients is still low, we do not systematically screen our patients for the existence of these tumors.

PREGNANCY

According to recent literature reviews, conventional thiopurine use during pregnancy is not associated with a higher risk of preterm birth, congenital disorders or children with low birth weight[52-54]. For this reason amongst others, we do not cessate thiopurine therapy in patients that become pregnant, but we refer patients during pregnancy to a dedicated team of gynecologists with interest in IBD. Furthermore, after a successful pregnancy, there is insufficient evidence to discourage patients to give breastfeeding; however, this should always be adjusted to the individual patient wishes[55-57]. Evidence concerning the use of TG during pregnancy is scarce and further prospective trials are needed to confirm the safety of this thiopurine derivative in pregnant women[58].

WHEN TO STOP THIOPURINE THERAPY?

Whether patients achieving a deep remission may successfully stop thiopurines is not known[19,59,60]. In our centers, we continue thiopurine therapy with low threshold, especially in patients with a predicted complex

course (*i.e.*, severe or difficult to manage disease). An exception is the patient with deep prolonged (*i.e.*, ⩾ 2-3 years) remission on thiopurine therapy with no signs of active disease on clinical, biochemical, endoscopic, histological and radiologic evaluation. In these patients, thiopurine therapy could be ceased with a good probability of relapse-free disease.

CONCLUSION

Whereas treatment with thiopurine therapy in IBD patients is hampered by a high number of discontinuations, mostly due to adverse events, several treatment strategies may be applied to maximize effectiveness and optimize safety. With this article, we provided a practical overview on how thiopurine therapy is being prescribed in two of the thiopurine research expert centers in Europe. We provided information concerning pharmacotherapy, indications of thiopurine treatment, toxicity of thiopurines and how to optimize treatment in individual patients using different treatment strategies.

REFERENCES

1 **Dignass A**, Lindsay JO, Sturm A, Windsor A, Colombel JF, Allez M, D'Haens G, D'Hoore A, Mantzaris G, Novacek G, Oresland T, Reinisch W, Sans M, Stange E, Vermeire S, Travis S, Van Assche G. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 2: current management. *J Crohns Colitis* 2012; **6**: 991-1030 [PMID: 23040451 DOI: 10.1016/j.crohns.2012.09.002]

2 **Dignass A**, Van Assche G, Lindsay JO, Lémann M, Söderholm J, Colombel JF, Danese S, D'Hoore A, Gassull M, Gomollón F, Hommes DW, Michetti P, O'Morain C, Oresland T, Windsor A, Stange EF, Travis SP. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Current management. *J Crohns Colitis* 2010; **4**: 28-62 [PMID: 21122489 DOI: 10.1016/j.crohns.2009.12.002]

3 **Chande N**, Patton PH, Tsoulis DJ, Thomas BS, MacDonald JK. Azathioprine or 6-mercaptopurine for maintenance of remission in Crohn's disease. *Cochrane Database Syst Rev* 2015; **10**: CD000067 [PMID: 26517527 DOI: 10.1002/14651858.CD000067.pub3]

4 **Chande N**, Tsoulis DJ, MacDonald JK. Azathioprine or 6-mercaptopurine for induction of remission in Crohn's disease. *Cochrane Database Syst Rev* 2013; **4**: CD000545 [PMID: 23633304 DOI: 10.1002/14651858.CD000545.pub4]

5 **Van Asseldonk DP**, de Boer NK, Peters GJ, Veldkamp AI, Mulder CJ, Van Bodegraven AA. On therapeutic drug monitoring of thiopurines in inflammatory bowel disease; pharmacology, pharmacogenomics, drug intolerance and clinical relevance. *Curr Drug Metab* 2009; **10**: 981-997 [PMID: 20214590]

6 **Al Hadithy AF**, de Boer NK, Derijks LJ, Escher JC, Mulder CJ, Brouwers JR. Thiopurines in inflammatory bowel disease: pharmacogenetics, therapeutic drug monitoring and clinical recommendations. *Dig Liver Dis* 2005; **37**: 282-297 [PMID: 15788214 DOI: 10.1016/j.dld.2004.09.029]

7 **de Boer NK**, van Bodegraven AA, Jharap B, de Graaf P, Mulder CJ. Drug Insight: pharmacology and toxicity of thiopurine therapy in patients with IBD. *Nat Clin Pract Gastroenterol Hepatol* 2007; **4**: 686-694 [PMID: 18043678 DOI: 10.1038/ncpgasthep1000]

8 **Ruemmele FM**, Turner D. Differences in the management of pediatric and adult onset ulcerative colitis--lessons from the joint ECCO and ESPGHAN consensus guidelines for the management of pediatric ulcerative colitis. *J Crohns Colitis* 2014; **8**: 1-4 [PMID: 24230969 DOI: 10.1016/j.crohns.2013.10.006]

9 **Ruemmele FM**, Veres G, Kolho KL, Griffiths A, Levine A, Escher JC, Amil Dias J, Barabino A, Braegger CP, Bronsky J, Buderus S, Martín-de-Carpi J, De Ridder L, Fagerberg UL, Hugot JP, Kierkus J, Kolacek S, Koletzko S, Lionetti P, Miele E, Navas López VM, Paerregaard A, Russell RK, Serban DE, Shaoul R, Van Rheenen P, Veereman G, Weiss B, Wilson D, Dignass A, Eliakim A, Winter H, Turner D. Consensus guidelines of ECCO/ESPGHAN on the medical management of pediatric Crohn's disease. *J Crohns Colitis* 2014; **8**: 1179-1207 [PMID: 24909831 DOI: 10.1016/j.crohns.2014.04.005]

10 **Rosen MJ**, Dhawan A, Saeed SA. Inflammatory Bowel Disease in Children and Adolescents. *JAMA Pediatr* 2015; **169**: 1053-1060 [PMID: 26414706 DOI: 10.1001/jamapediatrics.2015.1982]

11 **Elion GB**. The purine path to chemotherapy. *Science* 1989; **244**: 41-47 [PMID: 2649979]

12 **Bean RH**. The treatment of chronic ulcerative colitis with 6-mercaptopurine. *Med J Aust* 1962; **49**: 592-593 [PMID: 13969929]

13 **Karran P**, Attard N. Thiopurines in current medical practice: molecular mechanisms and contributions to therapy-related cancer. *Nat Rev Cancer* 2008; **8**: 24-36 [PMID: 18097462 DOI: 10.1038/nrc2292]

14 **Fong SC**, Blaker PA, Arenas-Hernandez M, Marinaki AM, Sanderson JD. Getting the best out of thiopurine therapy: thiopurine S-methyltransferase and beyond. *Biomark Med* 2015; **9**: 51-65 [PMID: 25605455 DOI: 10.2217/bmm.14.97]

15 **Dervieux T**, Chu Y, Su Y, Pui CH, Evans WE, Relling MV. HPLC determination of thiopurine nucleosides and nucleotides in vivo in lymphoblasts following mercaptopurine therapy. *Clin Chem* 2002; **48**: 61-68 [PMID: 11751539]

16 **Teml A**, Schaeffeler E, Herrlinger KR, Klotz U, Schwab M. Thiopurine treatment in inflammatory bowel disease: clinical pharmacology and implication of pharmacogenetically guided dosing. *Clin Pharmacokinet* 2007; **46**: 187-208 [PMID: 17328579 DOI: 10.2165/00003088-200746030-00001]

17 **Zaza G**, Cheok M, Krynetskaia N, Thorn C, Stocco G, Hebert JM, McLeod H, Weinshilboum RM, Relling MV, Evans WE, Klein TE, Altman RB. Thiopurine pathway. *Pharmacogenet Genomics* 2010; **20**: 573-574 [PMID: 19952870 DOI: 10.1097/FPC.0b013e328334338f]

18 **van Bodegraven AA**, van Everdingen JJ, Dijkstra G, de Jong DJ, Oldenburg B, Hommes DW. [Guideline 'Diagnosis and treatment of inflammatory bowel disease in adults'. I. Diagnosis and treatment]. *Ned Tijdschr Geneeskd* 2010; **154**: A1899 [PMID: 21029496]

19 **Frei P**, Biedermann L, Nielsen OH, Rogler G. Use of thiopurines in inflammatory bowel disease. *World J Gastroenterol* 2013; **19**: 1040-1048 [PMID: 23467510 DOI: 10.3748/wjg.v19.i7.1040]

20 **Goel RM**, Blaker P, Mentzer A, Fong SC, Marinaki AM, Sanderson JD. Optimizing the use of thiopurines in inflammatory bowel disease. *Ther Adv Chronic Dis* 2015; **6**: 138-146 [PMID: 25954498 DOI: 10.1177/2040622315579063]

21 **Colombel JF**, Reinisch W, Mantzaris GJ, Kornbluth A, Rutgeerts P, Tang KL, Oortwijn A, Bevelander GS, Cornillie FJ, Sandborn WJ. Randomised clinical trial: deep remission in biologic and immunomodulator naïve patients with Crohn's disease - a SONIC post hoc analysis. *Aliment Pharmacol Ther* 2015; **41**: 734-746 [PMID: 25728587 DOI: 10.1111/apt.13139]

22 **Bressler B**, Marshall JK, Bernstein CN, Bitton A, Jones J, Leontiadis GI, Panaccione R, Steinhart AH, Tse F, Feagan B. Clinical practice guidelines for the medical management of nonhospitalized ulcerative colitis: the Toronto consensus. *Gastroenterology* 2015; **148**: 1035-1058 .e3 [PMID: 25747596 DOI: 10.1053/j.gastro.2015.03.001]

23 **Côté-Daigneault J**, Bouin M, Lahaie R, Colombel JF, Poitras P. Biologics in inflammatory bowel disease: what are the data? *United European Gastroenterol J* 2015; **3**: 419-428 [PMID: 26535119 DOI: 10.1177/2050640615590302]

24 **Colombel JF**, Sandborn WJ, Reinisch W, Mantzaris GJ, Kornbluth A, Rachmilewitz D, Lichtiger S, D'Haens G, Diamond RH, Broussard DL, Tang KL, van der Woude CJ, Rutgeerts P. Infliximab, azathioprine, or combination therapy for Crohn's disease. *N Engl*

J Med 2010; **362**: 1383-1395 [PMID: 20393175 DOI: 10.1056/NEJMoa0904492]
25 **Lam MC**, Bressler B. Vedolizumab for ulcerative colitis and Crohn's disease: results and implications of GEMINI studies. *Immunotherapy* 2014; **6**: 963-971 [PMID: 25341118 DOI: 10.2217/imt.14.66]
26 **Meijer B**, Seinen ML, Leijte NN, Mulder CJ, van Bodegraven AA, de Boer NK. Clinical Value of Mercaptopurine After Failing Azathioprine Therapy in Patients With Inflammatory Bowel Disease. *Ther Drug Monit* 2016; **38**: 463-470 [PMID: 27158876 DOI: 10.1097/FTD.0000000000000312]
27 **Bowen DG**, Selby WS. Use of 6-mercaptopurine in patients with inflammatory bowel disease previously intolerant of azathioprine. *Dig Dis Sci* 2000; **45**: 1810-1813 [PMID: 11052324 DOI: 10.1023/A:1005569808947]
28 **Boulton-Jones JR**, Pritchard K, Mahmoud AA. The use of 6-mercaptopurine in patients with inflammatory bowel disease after failure of azathioprine therapy. *Aliment Pharmacol Ther* 2000; **14**: 1561-1565 [PMID: 11121903 DOI: 10.1046/j.1365-2036.2000.00872.x]
29 **Actis GC**, Pellicano R, Rosina F. 6-Mercaptopurine for Azathioprine Intolerant Inflammatory Bowel Disease: Literature Search and Reappraisal of Own Data. *Inflamm Allergy Drug Targets* 2015; **14**: 133-137 [PMID: 26728773 DOI: 10.2174/1871528114666160105112915]
30 **Domènech E**, Nos P, Papo M, López-San Román A, Garcia-Planella E, Gassull MA. 6-mercaptopurine in patients with inflammatory bowel disease and previous digestive intolerance of azathioprine. *Scand J Gastroenterol* 2005; **40**: 52-55 [PMID: 15841714 DOI: 10.1080/00365520410009492]
31 **Hindorf U**, Johansson M, Eriksson A, Kvifors E, Almer SH. Mercaptopurine treatment should be considered in azathioprine intolerant patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2009; **29**: 654-661 [PMID: 19183142 DOI: 10.1111/j.1365-2036.2008.03925.x]
32 **Chouchana L**, Narjoz C, Roche D, Golmard JL, Pineau B, Chatellier G, Beaune P, Loriot MA. Interindividual variability in TPMT enzyme activity: 10 years of experience with thiopurine pharmacogenetics and therapeutic drug monitoring. *Pharmacogenomics* 2014; **15**: 745-757 [PMID: 24897283 DOI: 10.2217/pgs.14.32]
33 **Gearry RB**, Barclay ML. Azathioprine and 6-mercaptopurine pharmacogenetics and metabolite monitoring in inflammatory bowel disease. *J Gastroenterol Hepatol* 2005; **20**: 1149-1157 [PMID: 16048561 DOI: 10.1111/j.1440-1746.2005.03832.x]
34 **Weinshilboum RM**, Sladek SL. Mercaptopurine pharmacogenetics: monogenic inheritance of erythrocyte thiopurine methyltransferase activity. *Am J Hum Genet* 1980; **32**: 651-662 [PMID: 7191632]
35 **Higgs JE**, Payne K, Roberts C, Newman WG. Are patients with intermediate TPMT activity at increased risk of myelosuppression when taking thiopurine medications? *Pharmacogenomics* 2010; **11**: 177-188 [PMID: 20136357 DOI: 10.2217/pgs.09.155]
36 **McGovern DP**, Travis SP, Duley J, Shobowale-Bakre el M, Dalton HR. Azathioprine intolerance in patients with IBD may be imidazole-related and is independent of TPMT activity. *Gastroenterology* 2002; **122**: 838-839 [PMID: 11878296]
37 **Seinen ML**, de Boer NK, Smid K, van Asseldonk DP, Bouma G, van Bodegraven AA, Peters GJ. Allopurinol enhances the activity of hypoxanthine-guanine phosphoribosyltransferase in inflammatory bowel disease patients during low-dose thiopurine therapy: preliminary data of an ongoing series. *Nucleosides Nucleotides Nucleic Acids* 2011; **30**: 1085-1090 [PMID: 22132961 DOI: 10.1080/15257770.2011.597371]
38 **Seinen ML**, van Asseldonk DP, de Boer NK, Losekoot N, Smid K, Mulder CJ, Bouma G, Peters GJ, van Bodegraven AA. The effect of allopurinol and low-dose thiopurine combination therapy on the activity of three pivotal thiopurine metabolizing enzymes: results from a prospective pharmacological study. *J Crohns Colitis* 2013; **7**: 812-819 [PMID: 23317929 DOI: 10.1016/j.crohns.2012.12.006]
39 **Meijer B**, Bouma G, de Boer NK. Optimize Thiopurine Therapy in Autoimmune Hepatitis. *Clin Gastroenterol Hepatol* 2016; **14**: 1062-1063 [PMID: 26872401 DOI: 10.1016/j.cgh.2016.02.006]
40 **Dubinsky MC**, Vasiliauskas EA, Singh H, Abreu MT, Papadakis KA, Tran T, Martin P, Vierling JM, Geller SA, Targan SR, Poordad FF. 6-thioguanine can cause serious liver injury in inflammatory bowel disease patients. *Gastroenterology* 2003; **125**: 298-303 [PMID: 12891528 DOI: 10.1016/S0016-5085(03)00938-7]
41 **Seiderer J**, Zech CJ, Reinisch W, Lukas M, Diebold J, Wrba F, Teml A, Chalupna P, Stritesky J, Schoenberg SO, Schima W, Göke B, Ochsenkühn T. A multicenter assessment of liver toxicity by MRI and biopsy in IBD patients on 6-thioguanine. *J Hepatol* 2005; **43**: 303-309 [PMID: 15964660 DOI: 10.1016/j.jhep.2005.02.051]
42 **Teml A**, Schwab M, Hommes DW, Almer S, Lukas M, Feichtenschlager T, Florin T, Seiderer J, Petritsch W, Bokemeyer B, Kreisel W, Herrlinger KR, Knoflach P, Bonaz B, Klugmann T, Herfarth H, Pedarnig N, Reinisch W. A systematic survey evaluating 6-thioguanine-related hepatotoxicity in patients with inflammatory bowel disease. *Wien Klin Wochenschr* 2007; **119**: 519-526 [PMID: 17943403 DOI: 10.1007/s00508-007-0841-0]
43 **Ansari A**, Elliott T, Fong F, Arenas-Hernandez M, Rottenberg G, Portmann B, Lucas S, Marinaki A, Sanderson J. Further experience with the use of 6-thioguanine in patients with Crohn's disease. *Inflamm Bowel Dis* 2008; **14**: 1399-1405 [PMID: 18521912 DOI: 10.1002/ibd.20492]
44 **van Asseldonk DP**, Jharap B, Kuik DJ, de Boer NK, Westerveld BD, Russel MG, Kubben FJ, van Bodegraven AA, Mulder CJ. Prolonged thioguanine therapy is well tolerated and safe in the treatment of ulcerative colitis. *Dig Liver Dis* 2011; **43**: 110-115 [PMID: 20739231 DOI: 10.1016/j.dld.2010.07.004]
45 **de Boer NK**, Zondervan PE, Gilissen LP, den Hartog G, Westerveld BD, Derijks LJ, Bloemena E, Engels LG, van Bodegraven AA, Mulder CJ. Absence of nodular regenerative hyperplasia after low-dose 6-thioguanine maintenance therapy in inflammatory bowel disease patients. *Dig Liver Dis* 2008; **40**: 108-113 [PMID: 18083079 DOI: 10.1016/j.dld.2007.10.013]
46 **Gilissen LP**, Derijks LJ, Driessen A, Bos LP, Hooymans PM, Stockbrügger RW, Engels LG. Toxicity of 6-thioguanine: no hepatotoxicity in a series of IBD patients treated with long-term, low dose 6-thioguanine. Some evidence for dose or metabolite level dependent effects? *Dig Liver Dis* 2007; **39**: 156-159 [PMID: 17188950 DOI: 10.1016/j.dld.2006.10.007]
47 **Seinen ML**, van Asseldonk DP, Mulder CJ, de Boer NK. Dosing 6-thioguanine in inflammatory bowel disease: expert-based guidelines for daily practice. *J Gastrointestin Liver Dis* 2010; **19**: 291-294 [PMID: 20922194]
48 **van Asseldonk DP**, Jharap B, Verheij J, den Hartog G, Westerveld DB, Becx MC, Russel MG, Engels LG, de Jong DJ, Witte BI, Mulder CJ, van Nieuwkerk CM, Bloemena E, de Boer NK, van Bodegraven AA. The Prevalence of Nodular Regenerative Hyperplasia in Inflammatory Bowel Disease Patients Treated with Thioguanine Is Not Associated with Clinically Significant Liver Disease. *Inflamm Bowel Dis* 2016; **22**: 2112-2120 [PMID: 27482972 DOI: 10.1097/MIB.0000000000000869]
49 **Magro F**, Peyrin-Biroulet L, Sokol H, Aldeger X, Costa A, Higgins PD, Joyce JC, Katsanos KH, Lopez A, de Xaxars TM, Toader E, Beaugerie L. Extra-intestinal malignancies in inflammatory bowel disease: results of the 3rd ECCO Pathogenesis Scientific Workshop (III). *J Crohns Colitis* 2014; **8**: 31-44 [PMID: 23721759 DOI: 10.1016/j.crohns.2013.04.006]
50 **National Institute for Public Health and the Environment (RIVM)**. LCI-guideline Pfeiffer's disease, 2011. [accessed 2016 Sept 12]. Available from: URL: http://www.rivm.nl/Documenten_en_publicaties/Professioneel_Praktisch/Richtlijnen/Infectieziekten/LCI_richtlijnen/LCI_richtlijn_Pfeiffer_ziekte_van
51 **Biancone L**, Onali S, Petruzziello C, Calabrese E, Pallone F. Cancer and immunomodulators in inflammatory bowel diseases. *Inflamm Bowel Dis* 2015; **21**: 674-698 [PMID: 25545375 DOI: 10.1097/MIB.0000000000000243]
52 **van der Woude CJ**, Ardizzone S, Bengtson MB, Fiorino G, Fraser G, Katsanos K, Kolacek S, Juillerat P, Mulders AG, Pedersen N, Selinger C, Sebastian S, Sturm A, Zelinkova Z, Magro F. The second European evidenced-based consensus on reproduction and

pregnancy in inflammatory bowel disease. *J Crohns Colitis* 2015; **9**: 107-124 [PMID: 25602023 DOI: 10.1093/ecco-jcc/jju006]

53 **Damas OM**, Deshpande AR, Avalos DJ, Abreu MT. Treating Inflammatory Bowel Disease in Pregnancy: The Issues We Face Today. *J Crohns Colitis* 2015; **9**: 928-936 [PMID: 26129693 DOI: 10.1093/ecco-jcc/jjv118]

54 **Jharap B**, de Boer NK, Stokkers P, Hommes DW, Oldenburg B, Dijkstra G, van der Woude CJ, de Jong DJ, Mulder CJ, van Elburg RM, van Bodegraven AA. Intrauterine exposure and pharmacology of conventional thiopurine therapy in pregnant patients with inflammatory bowel disease. *Gut* 2014; **63**: 451-457 [PMID: 23424097 DOI: 10.1136/gutjnl-2012-303615]

55 **de Meij TG**, Jharap B, Kneepkens CM, van Bodegraven AA, de Boer NK. Long-term follow-up of children exposed intrauterine to maternal thiopurine therapy during pregnancy in females with inflammatory bowel disease. *Aliment Pharmacol Ther* 2013; **38**: 38-43 [PMID: 23675854 DOI: 10.1111/apt.12334]

56 **Gardiner SJ**, Gearry RB, Roberts RL, Zhang M, Barclay ML, Begg EJ. Exposure to thiopurine drugs through breast milk is low based on metabolite concentrations in mother-infant pairs. *Br J Clin Pharmacol* 2006; **62**: 453-456 [PMID: 16995866 DOI: 10.1111/j.1365-2125.2006.02639.x]

57 **Van Assche G**, Dignass A, Reinisch W, van der Woude CJ, Sturm A, De Vos M, Guslandi M, Oldenburg B, Dotan I, Marteau P, Ardizzone A, Baumgart DC, D'Haens G, Gionchetti P, Portela F, Vucelic B, Söderholm J, Escher J, Koletzko S, Kolho KL, Lukas M, Mottet C, Tilg H, Vermeire S, Carbonnel F, Cole A, Novacek G, Reinshagen M, Tsianos E, Herrlinger K, Oldenburg B, Bouhnik Y, Kiesslich R, Stange E, Travis S, Lindsay J. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Special situations. *J Crohns Colitis* 2010; **4**: 63-101 [PMID: 21122490 DOI: 10.1016/j.crohns.2009.09.009]

58 **de Boer NK**, Van Elburg RM, Wilhelm AJ, Remmink AJ, Van Vugt JM, Mulder CJ, Van Bodegraven AA. 6-Thioguanine for Crohn's disease during pregnancy: thiopurine metabolite measurements in both mother and child. *Scand J Gastroenterol* 2005; **40**: 1374-1377 [PMID: 16334449]

59 **Clarke K**, Regueiro M. Stopping immunomodulators and biologics in inflammatory bowel disease patients in remission. *Inflamm Bowel Dis* 2012; **18**: 174-179 [PMID: 21674731 DOI: 10.1002/ibd.21792]

60 **Chocair P**, Duley J, Simmonds HA, Cameron JS, Ianhez L, Arap S, Sabbaga E. Low-dose allopurinol plus azathioprine/cyclosporin/prednisolone, a novel immunosuppressive regimen. *Lancet* 1993; **342**: 83-84 [PMID: 8100914]

P- Reviewer: Actis GC, Capasso R, Serban DE **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i4.531

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 531-539
ISSN 2150-5349 (online)

MINIREVIEWS

Widespread use of gastric acid inhibitors in infants: Are they needed? Are they safe?

Mark Safe, Wei H Chan, Steven T Leach, Lee Sutton, Kei Lui, Usha Krishnan

Mark Safe, Lee Sutton, Usha Krishnan, Department of Paediatric Gastroenterology, Sydney Children's Hospital, Randwick NSW 2031, Australia

Wei H Chan, Steven T Leach, Kei Lui, Usha Krishnan, School of Women's and Children's Health, University of New South Wales, Sydney NSW 2052, Australia

Lee Sutton, Department of Newborn Care, Royal Hospital for Women, Randwick NSW 2031, Australia

Author contributions: All authors contributed to the conception of the work, interpretation of data, and drafting and/or revision of final manuscript.

Conflict-of-interest statement: No potential conflicts of interest relevant to this article were reported.

Manuscript source: Invited manuscript

Correspondence to: Dr. Usha Krishnan, Department of Paediatric Gastroenterology, Sydney Children's Hospital, High Street, Randwick NSW 2031, Australia. usha.krishnan@sesiahs.health.nsw.gov.au
Telephone: +61-2-93821752
Fax: +61-2-93821787

Received: April 6, 2016
Peer-review started: April 7, 2016
First decision: June 6, 2016
Revised: July 16, 2016
Accepted: August 6, 2016
Article in press: August 8, 2016
Published online: November 6, 2016

Abstract

Gastroesophageal reflux is a common phenomenon in infants, but the differentiation between gastro-esophageal reflux and gastroesophageal reflux disease can be difficult. Symptoms are non-specific and there is increasing evidence that the majority of symptoms may not be acid-related. Despite this, gastric acid inhibitors such as proton pump inhibitors are widely and increasingly used, often without objective evidence or investigations to guide treatment. Several studies have shown that these medications are ineffective at treating symptoms associated with reflux in the absence of endoscopically proven oesophagitis. With a lack of evidence for efficacy, attention is now being turned to the potential risks of gastric acid suppression. Previously assumed safety of these medications is being challenged with evidence of potential side effects including GI and respiratory infections, bacterial overgrowth, adverse bone health, food allergy and drug interactions.

Key words: Gastroesophageal reflux; Infants; Proton pump inhibitors; Ranitidine; Safety; Adverse events

Core tip: Gastroesophageal reflux is a common phenomenon in infants, but the differentiation between gastroesophageal reflux and gastroesophageal reflux disease can be difficult. Symptoms are non-specific and there is increasing evidence that the majority of symptoms may not be acid-related. Despite this, gastric acid inhibitors such as proton pump inhibitors are widely and increasingly used, often without objective evidence or investigations to guide treatment. Several studies have shown that these medications are ineffective at treating symptoms associated with reflux in the absence of endoscopically proven oesophagitis. With a lack of evidence for efficacy, attention is now being turned to the potential risks of gastric acid suppression. Previously

assumed safety of these medications is being challenged with evidence of potential side effects including GI and respiratory infections, bacterial overgrowth, adverse bone health, food allergy and drug interactions.

Safe M, Chan WH, Leach ST, Sutton L, Lui K, Krishnan U. Widespread use of gastric acid inhibitors in infants: Are they needed? Are they safe? *World J Gastrointest Pharmacol Ther* 2016; 7(4): 531-539 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i4/531.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i4.531

INTRODUCTION

Gastro-oesophageal reflux (GOR) is the physiologic process involving the passage of gastric contents into the oesophagus which is often accompanied by postprandial regurgitation or vomiting[1]. The term gastro-oesophageal reflux disease (GORD) applies to persistent reflux that causes troublesome symptoms and/or complications, and is therefore, considered pathologic[1]. This distinction remains a challenge in infant care.

Infants are physiologically predisposed to GOR because of their shorter intra-abdominal oesophagus, frequent liquid feeds that distend the stomach, and supine position[2]. Infants with GOR have been found to have frequent transient lower oesophageal sphincter relaxations, which are thought to be the pathophysiological basis of the condition. Fifty-percent of infants reportedly experience daily regurgitation in the first 3 mo of life, which resolve by 12-14 mo in most healthy infants[3]. The pathogenic mechanism leading infant GOR to develop into GORD is unclear, although decreased neural protective reflexes and delayed gastric emptying are thought to play a role[1].

Since infant GORD has been linked to significant clinical morbidity in some patients, including worsening lung disease, aspiration and oesophagitis, medical intervention is frequently sought[4]. Common and non-specific symptoms attributed to GOR are often considered troublesome enough to justify treatment, especially in the neonatal intensive care setting[5]. This has led to the widespread usage of gastric acid inhibitors (GAI), in the form of proton pump inhibitors (PPIs) and/or histamine-2 receptor antagonists (H2RAs) in infants, despite uncertainty as to their efficacy and risks. This report will review recent evidence on the suitability of PPIs as an effective therapy for GORD in symptomatic infants and their potential for short- and long-term side effects.

GASTRIC ACID INHIBITOR USE IN INFANTS

GAI use for infants with symptoms attributed to GORD has risen dramatically despite only very limited approval for their use in this age group[6,7]. From 2000 to 2003, there was a 4-fold increase in off-label PPI prescriptions in this age-group, despite less than 10% of patients being investigated for GORD by diagnostic procedure[8]. There has also been a concerning rise in the frequency of GAI use in preterm infants, despite the lack of published evidence regarding pharmacological management of GOR or the safety and efficacy of GAI in preterm infants. According to a survey of neonatologists across 77 secondary and tertiary NICUs, GORD is perceived to affect more than one-fifth of infants born before 34 wk, and this perception may be leading to increased prescribing[9].

Symptoms described in infants with GORD include frequent regurgitation and vomiting, chronic cough, irritability, feeding resistance, failure to thrive, apnoea, bronchospasm and back-arching[2]. However, GORD diagnosis based on these symptoms is unreliable and non-specific. Regurgitation, irritability and vomiting thought to be secondary to GORD, are indistinguishable from the symptoms of food allergy, colic and other disorders[1]. Poor association between symptoms and pathologic acid exposure in oesophageal pH monitoring and histological scores, make symptoms unreliable in the diagnosis of GORD in infants[10]. GAI therapy in infants is largely extrapolated from studies of adults and older children, in whom symptoms are more reliably associated with acid exposure. In infants, significant recent data point to the possibility that the majority of symptoms are associated either with non-acid reflux or with no reflux at all[11]. In adults, there have been moves to even more potent acid suppression with the novel potassium competitive acid blockers such as vonoprazan. There is no safety data in children for this therapy, and considering that acid suppression has not been shown to affect symptoms in the majority of cases, there is likely to be very limited role for this drug.

Studies have also failed to find any association between GOR and cardiorespiratory events including apnoea, bradycardia, and oxygen desaturation in preterm infants[12,13]. Even so, two thirds of neonatologists have reported using GOR medications to treat apnoeas[14]. Overall, it has been widely recommended that GAI treatment in infants should be reserved for cases with evidence of pathological exposure to acid reflux episodes and/or oesophagitis[1]. Despite these recommendations, studies have found very poor adherence to guidelines and significant overtreatment with PPIs[15]. There is a concerning increase in the use of pharmacological intervention using acid suppression therapy using PPIs and H2RAs in preterm infants, with a presumed diagnosis of GORD based on symptoms alone in the absence of any objective measures for the diagnosis of GORD including pH and impedance monitoring or gastroscopy and biopsy[5]. Whilst there is no contemporary data outlining the relative frequency of H2RA and PPI use, the authors have observed a definite trend towards

PPI as the predominant medication prescribed or acid suppression.

Although, GAIs have previously been considered to be well tolerated by infants, emerging evidence suggests potential harmful associations between the use of GAIs and the development of infection and atopic disease in murine, adult and limited paediatric studies[16,17]. GAIs serve to protect the mucosa from excessive acid production, however giving such aggressive acid suppression at such a young age without evidence of oesophagitis remains controversial. Acid suppression is thought to interfere with natural defences against gastric bacterial colonization[18], and also protein digestion to trigger allergic sensitization of dietary peptides[19]. There is also mounting evidence that children are being exposed to unnecessarily high doses of PPI with doses of 1 mg/kg per day up to as high as 4 mg/kg per day used in clinical practice. Recent randomised trials have shown that although there is a dose-dependant reduction in acid production, for the treatment of erosive esophagitis there is no significant difference in healing between 5 mg/d and 10 mg/d for children < 20 kg[20,21].

ACTION AND EFFICACY OF PPI

PPIs bind irreversibly to the H^+-K^+-ATPase complex ("proton pump") of gastric parietal cells to prevent the reuptake of extracellular potassium in exchange with intracellular hydrogen, thus inhibiting acid secretion[22]. Their use in infants has been extrapolated from numerous adult studies, for whom PPIs are superior in healing erosive oesophagitis and providing symptom relief compared with H2RAs, which are more effective than placebo[1]. PPIs have been found to maintain intragastric pH > 4 for prolonged periods and to inhibit meal-induced acid secretion.

However, PPIs have consistently failed to show efficacy in reducing infant GORD symptoms compared with placebo. Chen *et al*[23] reviewed four randomised control trials (RCTs) of PPIs in treating symptomatic GORD infants < 12 mo, conducted by pharmaceutical companies under formal requests by the Food and Drug Administration. The results of independent studies such as Moore *et al*[24] have corroborated with their results, which are summarised in Table 1[23-28]. Notably, Moore *et al*[24] enrolled infants with endoscopically confirmed GORD and found omeprazole significantly reduced the reflux index (percentage of total duration pH < 4) in these infants compared with placebo, but irritability improved regardless of treatment. In the most recent randomised controlled trial of PPI (Esomeprazole) for the treatment of symptomatic GORD, without endoscopy, all children were initially treated with PPI and then randomised to continuation of PPI or placebo[25]. It found no statistically significant difference in apparent treatment failure between the PPI or placebo group.

SAFETY OF GASTRIC ACID INHIBITORS

With any pharmacological agent, there is potential for side effects. Headache, diarrhoea, constipation and nausea are idiosyncratic effects of PPIs that occur in 14% of children[1]. Acute interstitial nephritis, a rare, idiosyncratic hypersensitivity reaction to medications including PPIs, has also been reported in observational adult studies[29]. Increased risk of infection, for example, Clostridium Difficile, is increasingly being recognised[30]. Side effects related to the direct inhibition of gastric acid and reflex hypergastrinaemia, immunosuppression and drug metabolism have also been suggested (Table 2).

Bacterial overgrowth

The human stomach has a median pH of 1.4, and a pH < 4 has a powerful bactericidal effect on ingested acid-sensitive bacteria[18]. PPIs often cause a gastric environment with pH > 4, inducing a state of hypochlorhydria which allows the overgrowth of bacteria in the stomach[18]. Recently, Kanno *et al*[31] observed the effect of gastric acid inhibition in altering lower-intestinal microflora in PPI treated rats and asymptomatic humans with achlorhydria. The authors showed a significant dose-dependent increase in Lactobacillus and Veillonella populations (bacteria of oropharyngeal origin) in both rats and humans and in rats, potent gastric acid inhibition also led to a marked and significant increase of intestinal bacteria, including the Bacteroides fragilis group[31]. Modern genomic techniques have confirmed these PPI-related changes through 16S sequencing[32]. These microbial changes are thought to be due to the lack of the gastric acid barrier allowing bacteria to enter the intestine and also the effect of impaired protein digestion providing nutrients to facilitate bacterial growth[31]. Links have previously been made between these and similar changes to intestinal microbiome and the pathogenesis of inflammatory and malignant conditions of the bowel[33].

Risk of infections

The pathogenic mechanism that allows enteric bacteria to cause gastrointestinal infections is multi-factorial. Gastric acid inhibition reduces the gastric microbiocidal barrier, delays gastric emptying, reduces gastric mucus viscosity thereby increasing the risk of bacterial translocation in addition to increasing the risk of colonisation by bacterial agents. Gastric acid inhibition also has an adverse effect on leukocyte function by decreasing adhesion to endothelial cells, reducing chemotactic response to bacterial proteins and inhibiting neutrophil phagocytosis by phagosome acidification[16]. This is potentially important in neonates and infants, who have immature humoral immunity[16]. A study on the numbers and type of bacteria in nasogastric tubes of patients receiving GAI demonstrated increased numbers of bacteria including Streptococcus, a known cause of community acquired pneumonia[34]. It is possible that the risk of pneumonia is

Table 1 Summary randomised control trials examining proton pump inhibitors efficacy in reducing symptoms in infants with gastro-oesophageal reflux disease

Parameter	Esomeprazole	Lansoprazole	Pantoprazole	Omeprazole	Omeprazole (independent)	Esomeprazole
Control group	Placebo	Placebo	Placebo	Dosing range	Placebo	Placebo
Blinding	Double	Double	Double	Single	Double	Double
Trial of conservative measures	No	Yes	Yes	Yes	Yes[1]	No
Antacids allowed as rescue	Yes	No	Yes	Yes	No	Yes
Open-label phase to identify PPI responders	Yes (2 wk)	No	Yes (4 wk)	No	No	Yes (2 wk)
Randomised withdrawal from PPI	Yes	No	Yes	No	Yes	Yes
Length of randomised phase (wk)	4	4	4	8	4	4
Age in months	1-12	1-12	1-12	0-24[3]	3-12	1-11
n	40	81	50	35	30	80
GORD symptoms for clinical diagnosis	Vomiting; Regurgitation; Irritability; Supra-oesophageal disturbances; Respiratory Disturbance; Feeding difficulty	Crying; Fussiness; Irritability	Vomiting; Regurgitation; Spitting up; Irritability; Fussiness; Feeding Refusal; Choking; Gagging	Vomiting Regurgitation	[4]Frequent spilling Irritability/crying	Vomiting, regurgitation, irritability, cough, wheezing, stridor, labored breathing, resp symptoms triggered by feeding, food refusal, gagging, choking, hiccups for > 1 h/d
Primary endpoints	Time from randomisation to discontinuation because of symptom worsening perceived by parent or physician on symptom severity scale	Proportion with ≤ 50% reduction in PGA of symptoms	Proportion of infants who withdrew due to the "lack of efficacy" including worsening of symptoms, and/or antacid use for 7 consecutive days and/or oesophagitis and/or physician judgements	Change from baseline in daily symptoms based on PGA and parent perception	Reflux index from baseline	Time from randomization to discontinuation owing to symptom worsening in the double-blind phase
Primary end point efficacy result	Hazard ratio = 0.69 (PPI/Placebo); 95%CI: 0.35-1.35; $P = 0.275$	Responder rate: 54% (44/81) PPI *vs* 54% (44/81) Placebo; $P = 1.000$	Responder rate: 12% PPI *vs* 11% Placebo; $P = 1.000$	Mean daily vomiting/regurgitation episodes decreased by 4.34/d (0.5 mg/kg; 2.97/d – 1.0 mg/kg 4.35/d – 1.5 mg/kg; $P > 0.50$ in all group comparisons	Change from baseline of parent-recorded 24 h crying and fussing time and visual analogue scores of parental impression of the intensity of irritability Reflux index; -8.9% ± 5.6% PPI; -1.9% ± 2.0% Placebo $P < 0.001$ Cry/fuss times (min/24 h); 191 ± 120 (PPI); 201 ± 100 (Placebo) $P = 0.400$ Combined PPI and Placebo groups total cry fuss time[2] Baseline *vs* 2 wk $P = 0.040$ Baseline *vs* 4 wk $P = 0.008$ VA Score 5.0 ± 3.1 (PPI); 5.9 ± 2.1 (Placebo) $P = 0.214$	Discontinuation rates owing to symptom worsening were 48.8% (20/41) for placebo-treated *vs* 38.5% (15/39) for esomeprazole-treated patients (hazard ratio 0.69; $P = 0.28$)
Limitations of studies	Small sample size Symptom-based diagnosis Subjective assessment	Small sample size; Symptom-based diagnosis; Subjective assessment	Small sample size Symptom-based diagnosis Subjective assessment	Single blinded; Not placebo-controlled; Small sample size; Symptom-based diagnosis; Subjective assessment	Small sample size; Subjective assessment	Small sample size; Symptom-based diagnosis; Subjective assessment

[1]All infants were given empirical pharmacologic treatment (excluding PPIs) including cisapride (87%), H2 receptor antagonists (73%), antacid (67%) and thickening agent (20%); [2]Significant decrease in cry-fuss time independent of treatment; [3]Ninty percent of patients were younger than 12 mo; [4]Entry into study required a reflux index of > 5% or endoscopic biopsy evidence of oesophagitis. Data adapted from Chen *et al*[23]; Moore *et al*[24]; Orenstein *et al*[27]; Shakhnovich *et al*[28]. PPI: Proton pump inhibitor; GORD: Gastro-oesophageal reflux disease; PGA: Physician global assessment; VA: Visual analogue.

Table 2 Outline of the proposed side effects associated with proton pump inhibitors use, and the evidence supporting the association

Potential side effects	Level of evidence showing an association with PPI use
Acute Interstitial Nephritis	Level Ⅲ
Bacterial overgrowth in the stomach, small and large intestine	Murine models
Bacterial enteric infections	Level Ⅰ
Causative agents:	
Clostridium difficile	
Salmonella species	
Campylobacter species	
Pneumonia (Community-acquired)	Level Ⅰ
Necrotizing enterocolitis	Level Ⅲ[1]
Blood stream infections, including candidemia	Level Ⅲ[1]
Allergic sensitization in adults and in children with *in utero exposure*	Level Ⅲ Study and Murine Models
Parietal and Enterochromaffin-like cell hyperplasia	Level Ⅱ
Fundic gland polyps	Level Ⅲ
Vitamin B12 deficiency	Level Ⅲ
Fractures (osteoporotic and non-osteoporotic)	Level Ⅲ
Hypomagnesemia	Level Ⅳ and one level Ⅲ study
Reduced Antiplatelet effect of Clopidogrel	Level Ⅱ
Adverse Cardiovascular outcomes due to Clopidogrel interactions	Level Ⅲ[2]

[1]Only single reports showing an association with acid inhibition induced by H2RA treatment; [2]RCTs (level II) not shown an increase risk of adverse outcomes.

increased as result of reflux aspiration of gastrointestinal contents into the lungs. PPIs may also directly inhibit the H^+-K^+-ATPase present in the respiratory tract, altering the pH of its seromucinous secretions[35].

Adult studies

A meta-analysis of 26 observational studies found a significant association between PPI/H2RA use and Clostridium difficile infections (pooled OR = 1.95, 95%CI: 1.48-2.58), and "other" enteric infections (Salmonella or Campylobactor) (OR = 2.55, 95%CI: 1.53-4.26)[36]. Salmonella, Campylobacter and the vegetative form of C. difficile are acid-sensitive bacteria but are able to survive with PPI-induced acid suppression[36]. Experimental studies have shown that pretreatment with gastric acid inhibitors in a mouse model prior to *C. difficile* inoculation resulted in similar rates of infection, toxin production and colon injury compared with a group of mice pretreated with ampicillin[36]. Spore germination was also favoured by high pH levels and the presence of potassium chloride. Blockage of potassium pumps in the stomach could potentially lead to increased potassium as the proton pumps exchange potassium for hydrogen ions.

In a systematic review, Bavishi and Dupont[18] found that while it was difficult to establish causation in some studies due to other contributing factors such as advanced age and hospital exposure, patients on PPIs demonstrated a greater-than 4-fold risk for recurrent C. difficile infection[37].

A meta-analysis by Eom *et al*[35] also found significant association between PPIs and pneumonia (adjusted OR = 1.27, 95%CI: 1.11-1.46), with an even greater risk for community-acquired pneumonia (OR = 1.34, 95%CI: 1.14-1.57). This risk of pneumonia was markedly higher within the first week of PPI use (OR = 3.95, 95%CI: 2.86-5.45) suggesting that patients who were already susceptible to pneumonia would become ill soon after PPI treatment. With a small number of studies investigating the relationship between PPIs and hospital-acquired pneumonia, only an increased risk of hospital-acquired pneumonia was observed with H2RA therapy[35].

Paediatric studies

The few paediatric studies available have made similar conclusions. Notably, a prospective study of 93 paediatric patients (4-36 mo) with endoscopically diagnosed GORD, showed that children treated with either ranitidine or omeprazole for 8 wk were 3.58 and 6.39 times more likely to develop acute gastroenteritis and community-acquired pneumonia respectively, compared with healthy children during the 4 mo follow-up[17]. Comparing 4 mo before and after enrolment, a significant increase in the incidence of acute gastroenteritis and pneumonia was found only in the treatment group, demonstrating that infection susceptibility could continue even after therapy cessation[17].

The results of safety studies on the use of gastric acid inhibiting drugs in infants, particularly in intensive care, where hospital-acquired pathogens are responsible for significant morbidity and mortality are concerning[38]. A case-control study of very low birth weight infants showed H2RA use was associated with higher rates of necrotizing enterocolitis (OR = 1.71, 95%CI: 1.34-2.19)[39]. Stoll *et al*[40] also observed an increased risk of sepsis and meningitis with H2RAs given at 2 wk of age as a secondary outcome of their RCT comparing dexamethasone exposure. Beck-Sague *et al*[41] also reported H2RAs as a significant risk factor for bloodstream infections (RR = 4.2) in level Ⅲ neonatal intensive care, including Candida species; and the risk of candidemia

(OR = 2.44) was shown again by Saiman *et al*[42]. Very few studies have explored the risk of infections in the preterm infant population, but of these, Guillet *et al*[39] showed H2RA use was associated with higher rates of necrotising enterocolitis (NEC) (OR = 1.71) in large cohort study of 11072 very low birth weight infants. H2RAs have also been found to be a significant risk factor for blood stream infections in a level Ⅲ NICU[41], and candidemia[39]. The pathogenic mechanism of GAIs to cause infection is thought to be a result of reducing the gastric acid barrier against gastrointestinal tract colonisation with acid-sensitive bacteria such as Clostridium difficile[18]. Carrion and Egan[43] conducted a small prospective double-blind trial in 68 preterm infants (< 1250 g) supplemented with either HCl or water with feeds, and found that increased gastric bacterial colony counts were strongly correlated with gastric pH > 4 (P < 0.001), and acidification significantly reduced the incidences of NEC.

Allergic sensitization

Elevation of gastric pH also interferes with protein digestion, and it is hypothesised that normally digestible dietary peptides are preserved and recognised by the immune system as allergens[19]. Schöll *et al*[19] showed that omeprazole with hazelnut-extract treatment induced hazelnut-specific IgG1 in 3 of 5 mice (P = 0.754); and in the human study, 3.3% of patients receiving 3 mo of H2RA/PPI treatment also developed de novo allergic sensitization, which was higher than the reported prevalence of all tree nut allergies in the general US population (0.2%-0.7%). Schöll *et al*[44] also proposed that an allergic status induced in mothers had the potential to transfer (*via* placenta or breast milk) to the child. A study in pregnant mice demonstrated that increasing the gastric pH with sucralfate induced higher levels of codfish-specific IgG1 in mothers and offspring[44]. In offspring splenocytes, there was also a suppressed production of IFN-γ (Th1-cytokine), allowing the Th2-cytokine response to dominate (a phenotype predisposed to allergy); and T-regulatory cytokine IL-10, which regulates the allergic response[44]. A Swedish population register-based study found a significantly increased risk of developing childhood asthmas (51%), or any allergy (43%) in children exposed to PPIs/H2RAs in utero, irrespective of the drug type, trimester of exposure or maternal history of allergy[45].

HYPERGASTRINAEMIA AND MUCOSA CHANGES

Increasing gastric pH leads to hypergastrinemia, which has growth-promoting effects on several epithelial types[46]. Consequently, long-term PPI therapy is associated with parietal and enterochromaffin-like cell hyperplasia, as demonstrated by a RCT between esomeprazole treatment for 5 years compared with laparoscopic antireflux procedures for GORD[47]. Despite the proliferative drive of chronically elevated gastrin, no dysplastic changes were found.

Jalving *et al*[48] also found that PPI use > 1 year was associated with an increased risk of benign fundic gland polyps (OR = 2.8, 95%CI: 1.8-4.5), believed to arise from parietal cell protrusions and hyperplasia. One low-grade dysplastic polyp was found in a patient already predisposed with familial adenomatous polyposis, and did not appear to be PPI-related[48].

Vitamin and mineral deficiencies

By reducing gastric acidity, PPIs may interfere with the absorption of dietary protein-bound vitamin B12 and ionised calcium from dietary salts[22]. However, evidence of an effect of long-term PPI use in the elderly (over 65) on vitamin B12 has shown conflicting results. One case-control study (n = 53) found a 4.45 times increased risk for vitamin B12 deficiency in patients (> 12 mo of H2RAs/PPIs)[49]. However, a more recent cross-sectional study of 125 chronic (> 3 years) PPI users found no difference in serum vitamin B12 levels compared with controls[50].

PPIs have also been associated with an increased risk of fracture, as impaired calcium absorption is thought to cause a compensatory state of hyperparathyroidism to stimulate osteoclasts and bone resorption[51], but, there is also significant heterogeneity among these studies[52]. However, case-control studies have demonstrated significantly increased fracture risk in those with recent or current PPI use and at least one other risk factor for fracture[53,54].

During 2006-2012, there were 26 reported cases of hypomagnesaemia associated with PPIs in literature, with symptoms including electrocardiogram abnormalities and neuroexcitability, including tetanus and seizures, which resolved following withdrawal of PPI[52]. The mechanism of PPI-induced hypomagnesaemia is unknown, however, monitoring of serum magnesium levels has been recommended for susceptible patients, including patients using diuretics concurrently[55,56].

Drug interactions

In vitro studies have demonstrated a theoretical potential for PPIs and clopidogrel to interact through competitive binding at the cytochrome (CYP) 450 isoform CYP2C19, an enzyme involved in PPI metabolism[52]. Consequently, a significant reduction in the antiplatelet effect of clopidogrel has been reported. Although there have been no RCTs demonstrating increased cardiovascular risk, a recent propensity score analysis of a very large cohort showed an increased risk of myocardial infarction for adults taking PPI with an adjusted hazard ratio of 1.58[52].

CONCLUSION

This review highlights the issues regarding PPIs as treatment for infants with a presumed diagnosis of GORD based on symptomatology alone. For many clinicians, concern regarding the theoretical risk of tissue injury and

secondary morbidities, seem to outweigh any concern for the risks of PPI use. Currently, several RCTs of PPIs have shown a consistent lack of efficacy in relieving "distressed" GORD behaviours thought to be indicative of painful stimuli, suggesting they may have other underlying causes. Nonetheless, there is a need for more sizeable RCTs, standardised diagnostic procedures and better end-points in treatment in this population. Symptom assessments are clinically relevant but there is a lack of validated symptom-reported questionnaires for GORD in infants.

The safety of PPIs in infants also requires more prospective RCTs to remove the effect of confounders and bias. Irritable infants with uncomplicated GORD are hence recommended to continue lifestyle modifications, such as changing feeding techniques or formula composition, and avoid acid suppression. If PPIs are to be prescribed, only the minimal effective dose should be used, and should be weaned as soon as possible. There is no direct evidence to suggest increased safety of H2RA medication compared with PPI and in situations where acid suppression is indicated (*e.g.,* esophagitis) they have decreased potency. Attention should be paid to the substantial epidemiological evidence of increased infection risk with PPIs, especially in the vulnerable population group of preterm infants.

REFERENCES

1 **Vandenplas Y**, Rudolph CD, Di Lorenzo C, Hassall E, Liptak G, Mazur L, Sondheimer J, Staiano A, Thomson M, Veereman-Wauters G, Wenzl TG. Pediatric gastroesophageal reflux clinical practice guidelines: joint recommendations of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN). *J Pediatr Gastroenterol Nutr* 2009; **49**: 498-547 [PMID: 19745761 DOI: 10.1097/MPG.0b013e3181b7f563]

2 **Blanco FC**, Davenport KP, Kane TD. Pediatric gastroesophageal reflux disease. *Surg Clin North Am* 2012; **92**: 541-558, viii [PMID: 22595708 DOI: 10.1016/j.suc.2012.03.009]

3 **Nelson SP**, Chen EH, Syniar GM, Christoffel KK. Prevalence of symptoms of gastroesophageal reflux during infancy. A pediatric practice-based survey. Pediatric Practice Research Group. *Arch Pediatr Adolesc Med* 1997; **151**: 569-572 [PMID: 9193240 DOI: 10.1001/archpedi.1997.02170430035007]

4 **Birch JL**, Newell SJ. Gastrooesophageal reflux disease in preterm infants: current management and diagnostic dilemmas. *Arch Dis Child Fetal Neonatal Ed* 2009; **94**: F379-F383 [PMID: 19307225 DOI: 10.1136/adc.2008.149112]

5 **Malcolm WF**, Gantz M, Martin RJ, Goldstein RF, Goldberg RN, Cotten CM. Use of medications for gastroesophageal reflux at discharge among extremely low birth weight infants. *Pediatrics* 2008; **121**: 22-27 [PMID: 18166553 DOI: 10.1542/peds.2007-0381]

6 **Sandström M**, Davidson G, Tolia V, Sullivan JE, Långström G, Lundborg P, Brown K. Phase I, multicenter, randomized, open-label study evaluating the pharmacokinetics and safety profile of repeated once-daily doses of intravenous esomeprazole in children 0 to 17 years of age. *Clin Ther* 2012; **34**: 1828-1838 [PMID: 22832034 DOI: 10.1016/j.clinthera.2012.06.028]

7 **Illueca M**, Alemayehu B, Shoetan N, Yang H. Proton pump inhibitor prescribing patterns in newborns and infants. *J Pediatr Pharmacol Ther* 2014; **19**: 283-287 [PMID: 25762873 DOI: 10.5863/1551-6776-19.4.283]

8 **Barron JJ**, Tan H, Spalding J, Bakst AW, Singer J. Proton pump inhibitor utilization patterns in infants. *J Pediatr Gastroenterol Nutr* 2007; **45**: 421-427 [PMID: 18030207 DOI: 10.1097/MPG.0b013e31812e0149]

9 **Dhillon AS**, Ewer AK. Diagnosis and management of gastro-oesophageal reflux in preterm infants in neonatal intensive care units. *Acta Paediatr* 2004; **93**: 88-93 [PMID: 14989446 DOI: 10.1080/08035250310007934]

10 **Salvatore S**, Hauser B, Vandemaele K, Novario R, Vandenplas Y. Gastroesophageal reflux disease in infants: how much is predictable with questionnaires, pH-metry, endoscopy and histology? *J Pediatr Gastroenterol Nutr* 2005; **40**: 210-215 [PMID: 15699699 DOI: 10.1016/s8756-5005(08)70322-6]

11 **Orenstein SR**. Infant GERD: symptoms, reflux episodes & amp; reflux disease, acid & amp; non-acid reflllux--implications for treatment with PPIs. *Curr Gastroenterol Rep* 2013; **15**: 353 [PMID: 24197743 DOI: 10.1007/s11894-013-0353-1]

12 **Di Fiore J**, Arko M, Herynk B, Martin R, Hibbs AM. Characterization of cardiorespiratory events following gastroesophageal reflux in preterm infants. *J Perinatol* 2010; **30**: 683-687 [PMID: 20220760 DOI: 10.1038/jp.2010.27]

13 **Smits MJ**, van Wijk MP, Langendam MW, Benninga MA, Tabbers MM. Association between gastroesophageal reflux and pathologic apneas in infants: a systematic review. *Neurogastroenterol Motil* 2014; **26**: 1527-1538 [PMID: 25080836 DOI: 10.1111/nmo.12405]

14 **Abu Jawdeh EG**, Martin RJ. Neonatal apnea and gastroesophageal reflux (GER): is there a problem? *Early Hum Dev* 2013; **89** Suppl 1: S14-S16 [PMID: 23809340 DOI: 10.1016/S0378-3782(13)70005-7]

15 **Quitadamo P**, Papadopoulou A, Wenzl T, Urbonas V, Kneepkens CM, Roman E, Orel R, Pavkov DJ, Dias JA, Vandenplas Y, Kostovski A, Miele E, Villani A, Staiano A. European pediatricians' approach to children with GER symptoms: survey of the implementation of 2009 NASPGHAN-ESPGHAN guidelines. *J Pediatr Gastroenterol Nutr* 2014; **58**: 505-509 [PMID: 24663035 DOI: 0.1097/MPG.0b013e3182a69912]

16 **Canani RB**, Terrin G. Gastric acidity inhibitors and the risk of intestinal infections. *Curr Opin Gastroenterol* 2010; **26**: 31-35 [PMID: 19907324 DOI: 10.1097/MOG.0b013e328333d781]

17 **Canani RB**, Cirillo P, Roggero P, Romano C, Malamisura B, Terrin G, Passariello A, Manguso F, Morelli L, Guarino A. Therapy with gastric acidity inhibitors increases the risk of acute gastroenteritis and community-acquired pneumonia in children. *Pediatrics* 2006; **117**: e817-e820 [PMID: 16651285 DOI: 10.1542/peds.2005-1655]

18 **Bavishi C**, Dupont HL. Systematic review: the use of proton pump inhibitors and increased susceptibility to enteric infection. *Aliment Pharmacol Ther* 2011; **34**: 1269-1281 [PMID: 21999643 DOI: 10.1111/j.1365-2036.2011.04874.x]

19 **Schöll I**, Untersmayr E, Bakos N, Roth-Walter F, Gleiss A, Boltz-Nitulescu G, Scheiner O, Jensen-Jarolim E. Antiulcer drugs promote oral sensitization and hypersensitivity to hazelnut allergens in BALB/c mice and humans. *Am J Clin Nutr* 2005; **81**: 154-160 [PMID: 15640475]

20 **Omari T**, Davidson G, Bondarov P, Naucler E, Nilsson C, Lundborg P. Pharmacokinetics and Acid-suppressive Effects of Esomeprazole in Infants 1-24 Months Old With Symptoms of Gastroesophageal Reflux Disease. *J Pediatric Gastroenterol Nutr* 2015; **60**: S2-8 [DOI: 10.1097/01.mpg.0000469415.50085.f7]

21 **Tolia V,** Youssef NN, Gilger MA, Traxler B, Illueca M. Esomeprazole for the Treatment of Erosive Esophagitis in Children: An International, Multicenter, Randomized, Parallel-Group, Double-Blind (for Dose) Study. *J Pediatric Gastroenterol Nutr* 2015; **60**: S24-30 [DOI: 10.1097/01.mpg.0000469419.29000.94]

22 **Yang YX**, Metz DC. Safety of proton pump inhibitor exposure. *Gastroenterology* 2010; **139**: 1115-1127 [PMID: 20727892 DOI: 10.1053/j.gastro.2010.08.023]

23 **Chen IL**, Gao WY, Johnson AP, Niak A, Troiani J, Korvick J, Snow N, Estes K, Taylor A, Griebel D. Proton pump inhibitor

use in infants: FDA reviewer experience. *J Pediatr Gastroenterol Nutr* 2012; **54**: 8-14 [PMID: 21946832 DOI: 10.1097/MPG.0b013e31823890b4]
24 **Moore DJ**, Tao BS, Lines DR, Hirte C, Heddle ML, Davidson GP. Double-blind placebo-controlled trial of omeprazole in irritable infants with gastroesophageal reflux. *J Pediatr* 2003; **143**: 219-223 [PMID: 12970637 DOI: 10.1067/S0022-3476(03)00207-5]
25 **Winter H**, Gunasekaran T, Tolia V, Gottrand F, Barker PN, Illueca M. Esomeprazole for the Treatment of GERD in Infants Ages 1-11 Months. *J Pediatr Gastroenterol Nutr* 2015; **60** Suppl 1: S9-15 [PMID: 26422097 DOI: 10.1097/MPG.0b013e3182496b35]
26 **Omari T**, Davidson G, Bondarov P, Nauclér E, Nilsson C, Lundborg P. Pharmacokinetics and acid-suppressive effects of esomeprazole in infants 1-24 months old with symptoms of gastroesophageal reflux disease. *J Pediatr Gastroenterol Nutr* 2007; **45**: 530-537 [PMID: 18030229 DOI: 10.1097/MPG.0b013e31812e012f]
27 **Orenstein SR**, Hassall E, Furmaga-Jablonska W, Atkinson S, Raanan M. Multicenter, double-blind, randomized, placebo-controlled trial assessing the efficacy and safety of proton pump inhibitor lansoprazole in infants with symptoms of gastroesophageal reflux disease. *J Pediatr* 2009; **154**: 514-520.e4 [PMID: 19054529 DOI: 10.1016/j.jpeds.2008.09.054]
28 **Shakhnovich V**, Ward RM, Kearns GL. Failure of proton pump inhibitors to treat GERD in neonates and infants: a question of drug, diagnosis, or design. *Clin Pharmacol Ther* 2012; **92**: 388-392 [PMID: 22805424 DOI: 10.1038/clpt.2012.86]
29 **Sierra F,** Suarez M, Rey M, Vela MF. Systematic review: proton pump inhibitor-associated acute interstitial nephritis. *Alimentary Pharmacol Ther* 2007; **26**: 545-553 [PMID: 17661758 DOI: 10.1111/j.1365-2036.2007.03407.x]
30 **Turco R**, Martinelli M, Miele E, Roscetto E, Del Pezzo M, Greco L, Staiano A. Proton pump inhibitors as a risk factor for paediatric Clostridium difficile infection. *Aliment Pharmacol Ther* 2010; **31**: 754-759 [PMID: 20047577 DOI: 10.1111/j.1365-2036.2009.04229.x]
31 **Kanno T**, Matsuki T, Oka M, Utsunomiya H, Inada K, Magari H, Inoue I, Maekita T, Ueda K, Enomoto S, Iguchi M, Yanaoka K, Tamai H, Akimoto S, Nomoto K, Tanaka R, Ichinose M. Gastric acid reduction leads to an alteration in lower intestinal microflora. *Biochem Biophys Res Commun* 2009; **381**: 666-670 [PMID: 19248769 DOI: 10.1016/j.bbrc.2009.02.109]
32 **Rosen R**, Hu L, Amirault J, Khatwa U, Ward DV, Onderdonk A. 16S community profiling identifies proton pump inhibitor related differences in gastric, lung, and oropharyngeal microflora. *J Pediatr* 2015; **166**: 917-923 [PMID: 25661411 DOI: 10.1016/j.jpeds.2014.12.067]
33 **Gagnière J**, Raisch J, Veziant J, Barnich N, Bonnet R, Buc E, Bringer MA, Pezet D, Bonnet M. Gut microbiota imbalance and colorectal cancer. *World J Gastroenterol* 2016; **22**: 501-518 [PMID: 26811603 DOI: 10.3748/wjg.v22.i2.501]
34 **Vakil N**. Acid inhibition and infections outside the gastrointestinal tract. *Am J Gastroenterol* 2009; **104** Suppl 2: S17-S20 [PMID: 19262541 DOI: 10.1038/ajg.2009.47]
35 **Eom CS**, Jeon CY, Lim JW, Cho EG, Park SM, Lee KS. Use of acid-suppressive drugs and risk of pneumonia: a systematic review and meta-analysis. *CMAJ* 2011; **183**: 310-319 [PMID: 21173070 DOI: 10.1503/cmaj.092129]
36 **Leonard J**, Marshall JK, Moayyedi P. Systematic review of the risk of enteric infection in patients taking acid suppression. *Am J Gastroenterol* 2007; **102**: 2047-2056; quiz 2057 [PMID: 17509031 DOI: 10.1111/j.1572-0241.2007.01275.x]
37 **Kim JW**, Lee KL, Jeong JB, Kim BG, Shin S, Kim JS, Jung HC, Song IS. Proton pump inhibitors as a risk factor for recurrence of Clostridium-difficile-associated diarrhea. *World J Gastroenterol* 2010; **16**: 3573-3577 [PMID: 20653067 DOI: 10.3748/wjg.v16.i28.3573]
38 **Saiman L**, Ludington E, Dawson JD, Patterson JE, Rangel-Frausto S, Wiblin RT, Blumberg HM, Pfaller M, Rinaldi M, Edwards JE, Wenzel RP, Jarvis W. Risk factors for Candida species colonization of neonatal intensive care unit patients. *Pediatr Infect Dis J* 2001; **20**: 1119-1124 [PMID: 11740316 DOI: 10.1097/00006454-200112000-00005]
39 **Guillet R**, Stoll BJ, Cotten CM, Gantz M, McDonald S, Poole WK, Phelps DL. Association of H2-blocker therapy and higher incidence of necrotizing enterocolitis in very low birth weight infants. *Pediatrics* 2006; **117**: e137-e142 [PMID: 16390920 DOI: 10.1542/peds.2005-1543]
40 **Stoll BJ**, Temprosa M, Tyson JE, Papile LA, Wright LL, Bauer CR, Donovan EF, Korones SB, Lemons JA, Fanaroff AA, Stevenson DK, Oh W, Ehrenkranz RA, Shankaran S, Verter J. Dexamethasone therapy increases infection in very low birth weight infants. *Pediatrics* 1999; **104**: e63 [PMID: 10545589 DOI: 10.1542/peds.104.5.e63]
41 **Beck-Sague CM**, Azimi P, Fonseca SN, Baltimore RS, Powell DA, Bland LA, Arduino MJ, McAllister SK, Huberman RS, Sinkowitz RL. Bloodstream infections in neonatal intensive care unit patients: results of a multicenter study. *Pediatr Infect Dis J* 1994; **13**: 1110-1116 [PMID: 7892080 DOI: 10.1097/00006454-199412000-00008]
42 **Saiman L**, Ludington E, Pfaller M, Rangel-Frausto S, Wiblin RT, Dawson J, Blumberg HM, Patterson JE, Rinaldi M, Edwards JE, Wenzel RP, Jarvis W. Risk factors for candidemia in Neonatal Intensive Care Unit patients. The National Epidemiology of Mycosis Survey study group. *Pediatr Infect Dis J* 2000; **19**: 319-324 [PMID: 10783022 DOI: 10.1097/00006454-200004000-00011]
43 **Carrion V**, Egan EA. Prevention of neonatal necrotizing enterocolitis. *J Pediatr Gastroenterol Nutr* 1990; **11**: 317-323 [PMID: 2246712 DOI: 10.1097/00005176-199010000-00006]
44 **Schöll I**, Ackermann U, Ozdemir C, Blümer N, Dicke T, Sel S, Sel S, Wegmann M, Szalai K, Knittelfelder R, Untersmayr E, Scheiner O, Garn H, Jensen-Jarolim E, Renz H. Anti-ulcer treatment during pregnancy induces food allergy in mouse mothers and a Th2-bias in their offspring. *FASEB J* 2007; **21**: 1264-1270 [PMID: 17227952 DOI: 10.1096/fj.06-7223com]
45 **Dehlink E**, Yen E, Leichtner AM, Hait EJ, Fiebiger E. First evidence of a possible association between gastric acid suppression during pregnancy and childhood asthma: a population-based register study. *Clin Exp Allergy* 2009; **39**: 246-253 [PMID: 19134022 DOI: 10.1111/j.1365-2222.2008.03125.x]
46 **Heidelbaugh JJ**, Metz DC, Yang YX. Proton pump inhibitors: are they overutilised in clinical practice and do they pose significant risk? *Int J Clin Pract* 2012; **66**: 582-591 [PMID: 22607510 DOI: 10.1111/j.1742-1241.2012.02921.x]
47 **Fiocca R**, Mastracci L, Attwood SE, Ell C, Galmiche JP, Hatlebakk J, Bärthel A, Långström G, Lind T, Lundell L. Gastric exocrine and endocrine cell morphology under prolonged acid inhibition therapy: results of a 5-year follow-up in the LOTUS trial. *Aliment Pharmacol Ther* 2012; **36**: 959-971 [PMID: 22998687 DOI: 10.1111/apt.12052]
48 **Jalving M**, Koornstra JJ, Wesseling J, Boezen HM, DE Jong S, Kleibeuker JH. Increased risk of fundic gland polyps during long-term proton pump inhibitor therapy. *Aliment Pharmacol Ther* 2006; **24**: 1341-1348 [PMID: 17059515 DOI: 10.1111/j.1365-2036.2006.03127.x]
49 **Valuck RJ**, Ruscin JM. A case-control study on adverse effects: H2 blocker or proton pump inhibitor use and risk of vitamin B12 deficiency in older adults. *J Clin Epidemiol* 2004; **57**: 422-428 [PMID: 15135846 DOI: 10.1016/j.jclinepi.2003.08.015]
50 **den Elzen WP**, Groeneveld Y, de Ruijter W, Souverijn JH, le Cessie S, Assendelft WJ, Gussekloo J. Long-term use of proton pump inhibitors and vitamin B12 status in elderly individuals. *Aliment Pharmacol Ther* 2008; **27**: 491-497 [PMID: 18194503]
51 **Insogna KL**. The effect of proton pump-inhibiting drugs on mineral metabolism. *Am J Gastroenterol* 2009; **104** Suppl 2: S2-S4 [PMID: 19262542]
52 **Chen J**, Yuan YC, Leontiadis GI, Howden CW. Recent safety concerns with proton pump inhibitors. *J Clin Gastroenterol* 2012; **46**: 93-114 [PMID: 22227731]
53 **Kaye JA**, Jick H. Proton pump inhibitor use and risk of hip fractures in patients without major risk factors. *Pharmacotherapy* 2008; **28**: 951-959 [PMID: 18657011 DOI: 10.1592/phco.28.8.951]
54 **Corley DA**, Kubo A, Zhao W, Quesenberry C. Proton pump inhibitors and histamine-2 receptor antagonists are associated with

hip fractures among at-risk patients. *Gastroenterology* 2010; **139**: 93-101 [PMID: 20353792 DOI: 10.1053/j.gastro.2010.03.055]

55 **Danziger J**, William JH, Scott DJ, Lee J, Lehman LW, Mark RG, Howell MD, Celi LA, Mukamal KJ. Proton-pump inhibitor use is associated with low serum magnesium concentrations. *Kidney Int* 2013; **83**: 692-699 [PMID: 23325090]

56 **Shih CJ**, Chen YT, Ou SM, Li SY, Chen TJ, Wang SJ. Proton pump inhibitor use represents an independent risk factor for myocardial infarction. *Int J Cardiol* 2014; **177**: 292-297 [PMID: 25499395 DOI: 10.1016/j.ijcard.2014.09.036]

P- Reviewer: Hatta W **S- Editor**: Qi Y **L- Editor**: A
E- Editor: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i4.540

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 540-549
ISSN 2150-5349 (online)

ORIGINAL ARTICLE

Basic Study

A20 inhibits lipopolysaccharide-induced inflammation in enterocytes

Cui-Fang Zheng, Jie-Ru Shi, Ying Huang, Sheng-Nan Wang

Cui-Fang Zheng, Jie-Ru Shi, Ying Huang, Sheng-Nan Wang, Department of Gastroenterology, Children's Hospital of Fudan University, Shanghai 201102, China

Author contributions: Zheng CF and Huang Y designed the study; Zheng CF and Wang SN performed the research; Zheng CF and Shi JR analysed the data; Zheng CF, Shi JR and Huang Y wrote the paper.

Supported by The National Natural Science Foundation of China, No. 81300291.

Institutional review board statement: The publication of this manuscript has been reviewed and approved by the Children's Hospital of Fudan University Review Board.

Conflict-of-interest statement: All authors declare no conflicts of interest.

Data sharing statement: The technical appendix, statistical code, and dataset are available from the corresponding author at yhuang815@163.com. The study participants provided their informed consent for data sharing. No additional data are available.

Manuscript source: Unsolicited manuscript

Correspondence to: Ying Huang, MD, PhD, Department of Gastroenterology, Children's Hospital of Fudan University, No. 399 Wan-Yuan Road, Minhang District, Shanghai 201102, China. yhuang815@163.com
Telephone: +86-21-64931727
Fax: +86-21-64931901

Received: May 1, 2016
Peer-review started: May 2, 2016
First decision: July 4, 2016
Revised: July 14, 2016
Accepted: August 15, 2016
Article in press: August 17, 2016
Published online: November 6, 2016

Abstract

AIM

To examine the role of A20 in the regulation of intestinal epithelial cells (IECs) inflammation.

METHODS

Using gene transfection, both stable overexpression and knockdown A20-expressed HT-29 cell lines were established. Accordingly, the cells were divided into the following groups: The control group, the A20 over-expression group, the A20 knockdown group and the respective controls. A20 was stimulated with lipo-polysaccharide (LPS) in a dose- and time-dependent manner and was detected using western blotting and real-time polymerase chain reaction (PCR) analyses. Immunofluorescence and western blotting analyses were performed to investigate the role of A20 in the regulation of nuclear factor (NF)-κB activation and translocation into the nucleus. ELISA and real-time PCR were performed to examine A20 in regulating the release of the following inflammatory cytokines: Tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6 and IL-8.

RESULTS

The expression of A20 in IECs was inducible. When intestinal epithelial cells were subjected to the stimulation of LPS, the expression of A20 was increased, and the expression of A20 was induced in a dose- and time-dependent manner. The expression of A20 was very low in HT-29 cells without LPS stimulation but rapidly increased and was maintained at a high level 2-4 h after stimulation

with LPS. These levels gradually declined with a change in time-course, and the expression of A20 increased with increasing LPS stimulation. Western blotting and immunofluorescence revealed that overexpression of A20 can inhibit NF-κB activation and its translocation to the nucleus. The overexpression of A20 can reduce the levels of proinflammatory cytokines involved in the pathophysiology of inflammatory bowel disease. There was no significant difference in the expression of IL-8 mRNA in the control group, A20 overexpression group or A20 knockdown group without LPS stimulation (P > 0.05); however, while after 2 h, 4 h and 8 h stimulation with LPS, the expression of IL-8 in the A20 overexpression group was lower than the control group and the A20 knockdown group (P < 0.05 or P < 0.01). The expression of TNF-α was different at different time points after 8 h of LPS stimulation (F = 31.33, DF = 5, P < 0.001), and the expression of TNF-α increased as the LPS stimulation time increased. Upon LPS stimulation, lower levels of TNF-α were detected in the A20 overexpression cell lines (P < 0.05). There were no significant differences in the induction of IL-6 and IL-1β among the control group, A20 overexpression group and A20 knockdown group (P > 0.05).

CONCLUSION

A20 plays an important role in limiting inflammation by inhibiting LPS-induced NF-κB responses in the gut luminal. A20 may be a potential therapeutic tool for inflammatory diseases.

Key words: A20 (TNFAIP3); Lipopolysaccharide; Nuclear factor-κB; Inflammatory bowel disease

Core tip: The use of A20-deficient mice and RNA interference technologies has revealed that mice or enterocytes lacking A20 showed hyper-responsiveness to stimulation. However, studies on whether the overexpression of A20 can extenuate enterocyte inflammation are limited. Our present results demonstrated that the expression of A20 was increased in a dose- and time-dependent manner upon lipopolysaccharide (LPS) stimulation in intestinal epithelial cells. More importantly, the overexpression of A20 suppressed the activation of nuclear factor-κB and the induction of pro-inflammatory molecules, such as Tumor necrosis factor-α and IL-8. Taken together, these findings indicate that A20 plays a critical role in limiting LPS-induced inflammation in the gut luminal and may be a potential therapeutic tool for immune and inflammatory diseases.

Zheng CF, Shi JR, Huang Y, Wang SN. A20 inhibits lipopolysaccharide-induced inflammation in enterocytes. *World J Gastrointest Pharmacol Ther* 2016; 7(4): 540-549 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i4/540.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i4.540

INTRODUCTION

Inflammatory bowel disease (IBD) has been proposed to be caused by an inappropriate inflammatory response to normal components of the intestinal micro-biota in genetically predisposed individuals[1-3]. Bacterial wall products play an important role in the activation of the immune and non-immune cells of the intestinal mucosa, and intestinal epithelial cells (IECs) represent a unique population of cells that exist in direct contact with a dense and complex milieu of commensal microorganisms. As a primary interface between pathogens and the intestinal tract, the epithelial cells lining the gut luminal play a key role in defence against microbial pathogens[4]. Rather than functioning as a passive barrier, IECs are an active participant in the mucosal immune response. The interaction between IECs and intestinal micro-biota results in the activation of multiple intracellular signalling events, including the activation of nuclear factor (NF)-κB. The inappropriate activation of NF-κB is central to the pathogenesis of IBD. On the one hand, NF-κB regulates the expression of various cytokines and other modulators of the inflammatory processes in IBD[5,6]. On the other hand, the inhibition of NF-κB activity has been suggested as a major component of the anti-inflammatory activity of glucocorticoids that are frequently used for treatment of IBD[7,8]. Thus, a tight regulation of the NF-κB signalling pathway and the genes induced is an absolute requirement. A20 (also known as Tumour Necrosis Factor Alpha-Induced Protein 3 or TNFAIP3) is a widely expressed and inducible cytoplasmic protein that plays a key role in the negative regulation of inflammation and immunity[9,10]. Several studies have shown that the ubiquitin-editing protein A20 is a key player in the negative feedback regulation of NF-κB signalling in response to multiple stimulants[11]. An essential role of A20 in the regulation of NF-κB signalling was clearly demonstrated with the generation of A20-deficient mice and RNA interference technologies. Mice deficient for A20 are extremely susceptible to sub-lethal doses of TNF and die prematurely due to severe multi-organ inflammation and cachexia[12].

More specifically related to IBD, a recent genome-wide association study identified A20 as a Crohn's disease (CD) susceptibility gene[13]. Specific deletion of A20 in enterocytes increased the susceptibility of mice to dextran sodium sulphate (DSS)-induced colitis and prevented the recovery from acute DSS-induced inflammation[14]. Finally, mucosal biopsies from 69 CD patients were analysed and confirmed a consistent down-regulation of mucosal A20 expression[15]. Our previous work found that there is an excessive inflammatory response but insufficient up-regulation of A20 expression in IBD patients[16]. These studies indicate that defective A20 expression or activity could be a risk factor for IBD.

The use of A20-deficient mice and RNA interference technologies has revealed that mice or enterocytes lacking A20 showed hyper-responsiveness to stimulation.

Based on the knowledge that A20 plays a central role in inflammation and immunity, this study aimed to determine whether A20 has a potential therapeutic value and whether overexpression of A20 could extenuate enterocyte inflammation. Previous studies have confirmed that the overexpression of A20 can attenuate allergic airway inflammation in mice[17] and protect kidneys from ischaemia/reperfusion injury[18]. However, studies on whether the overexpression of A20 may reduce intestinal inflammation are limited. Thus, we performed this study to examine the effectiveness of A20 in reducing IECs inflammatory reaction and NF-κB activation. In this study, we recapitulated the response of epithelial cells to LPS to mimic the *in vivo* response of intestinal epithelial cells to infection of pathogenic and/or commensal microbes, so as to explore the role of A20 in the regulation of intestinal epithelial cell inflammation.

MATERIALS AND METHODS

Reagents

The main reagents and antibodies used in our experiments are as follows: Unpurified LPS from *Escherichia coli* 0127:B8 (Sigma, St. Louis, MO, United States, L4516), anti-A20 polyclonal antibody (Abcam, Cambridge, United Kingdom, ab45366), anti-NF-κB p65 monoclonal antibody (Epitomics, California, United States, E379), anti-β-actin antibody (Sigma, St. Louis, MO, United States), TNF-α and IL-1β ELISA kit (Senxiong Technology Industrial Company, Shanghai, China), TRIzol (Invitrogen, Carlsbad, CA, United States, 15596-018), SYBR green PCR reagent kits (Toyobo Co, Osaka, Japan, QPK-201), and Lipofectamine 2000 (Invitrogen, Carlsbad, CA, United States).

Cells and cell culture

The human intestinal epithelial cell line HT-29 was purchased from the Chinese Academy of Sciences (Shang hai) and grown in Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen, C11965), supplemented with 10% foetal bovine serum (Invitrogen) and maintained at 37 ℃ in a humidified incubator under an atmosphere of 5% CO_2.

Plasmids and transfection

Human A20 cDNA was amplified with the following primers: F1: 5'-AGAGGTGTTGGAGAGCACAATGG-3' and R1: 5'-CACCTGTTTCCGGTTAGCCATACA-3', and HA-tagged A20 was cloned into the lentiviral vector pWPI. Lentiviral particles were produced by transfecting HEK-293T cells with pWPI.1-HA-A20, psPAX2 and pMD2.G. After incubation with HT-29 cells for 1 wk, the cells were screened using a flow cytometer.

To silence A20, we employed the lentiviral silencing system. ShRNA oligos against A20 were cloned into the lentiviral vector pLKO.1-TRC cloning vector (Addgene Plasmid 10878). The 21-bp target sequences of A20 were CACTGGAAGAAATACACATAT and GCACCGATACACACTGGAAAT. To produce lentiviral particles, the shRNA construct was co-transfected with psPAX2 and pMD2.G into HEK-293T cells. The media was harvested to obtain lentiviral particles. The target cells with lentiviral particles were infected for 24 h and selected with puromycin at a concentration of 2 μg/mL. The day before transfection, the cells were plated in growth medium at a density of approximately 70%. The shRNA was transfected at a concentration of 50 nmol/L using Lipofectamine 2000 according to the manufacturer's instructions.

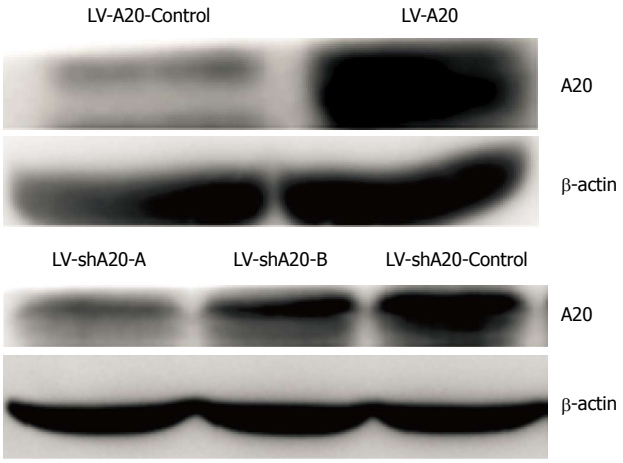

Figure 1 Expression of A20 in different groups. HT-29 cells were transduced with LV-A20, LV-shA20, or LV-controls. Cytosolic lysates were tested by Western blotting using anti-A20 antibodies or anti-β-actin antibodies to normalize protein levels. In A20 overexpression cell lines, the expression of A20 was much higher than that of the control. Two shRNA sequences, A and B, were obtained, and the expression of A20 in both of the A20 knockdown groups were much lower than the control. The effect of A was more pronounced than B. Thus, sequence A was selected for subsequent experiments.

HT-29 cells were transduced with LV-A20, LV-shA20, or LV-controls, respectively. Accordingly, the cells were divided into the following groups: The control group (HT-29 cells without transfection), A20 overexpression group, A20 knockdown group, A20 overexpression-control group and A20 knockdown-control group. Overexpression and silencing of A20 were validated by Western blotting analysis prior to the experimentation. The presence of the 90-kDa bands of extracts from transfected cell populations were significantly higher or lower than the controls (Figure 1). We have constructed two shRNA (A and B) sequences to silence A20, as shown in Figure 1. The silencing effect of A was more pronounced than B. Thus, sequence A was selected for subsequent experiments.

Real time-polymerase chain reaction

Total RNA was isolated from cells using TRIzol reagent, and equal amounts of RNA were reverse-transcribed into cDNA using a quantitative PCR cDNA kit (TaKaRa, Biotechnology, Dalian, China, DRR037Ab). Specific primers (Table 1) used in the real-time PCR studies were designed and generated by Takara Biotechnology Company (Dalian, China). Quantitative real-time PCR was performed using SYBR green PCR reagent kits according

Table 1 Primer sequences

Gene	Sequences(5′-3′)
A20	Forward: AAAGCCCTCATCGACAGAAA
	Reverse: CAGTTGCCAGCGGAATTTA
IL-6	Forward: AAGCCAGAGCTGTGCAGATGAGTA
	Reverse: TGTCCTGCAGCCACTGGTTC
IL-8	Forward: ACACTGCGCCAACACAGAAATTA
	Reverse: TTTGCTTGAAGTTTCACTGGCATC
GAPDH	Forward: GCACCGTCAAGGCTGAGAAC
	Reverse: TGGTGAAGACGCCAGTGGA

to the manufacturer's instructions. Data were recorded and analysed using the real-time PCR analysis software Bio-Rad iQ5. The A20, IL-6 and IL-8 mRNA levels were normalized against GAPDH levels in the cells. The relative gene expression was calculated by comparing the number of thermal cycles that were necessary to generate the threshold amounts of product (CT).

Western blotting analysis and enzyme-linked immunosorbent assay

HT-29 cells were homogenized with protease and phosphatase inhibitors and prepared in protein extraction solution (Thermo, 78835). Protein lysates, quantified using a BCA assay (Sangon Biotech Company, Ltd., Shanghai, China), were separated on reducing SDS-polyacrylamide gels and transferred onto polyvinyldifluoride membranes (PVDF, Millipore). The membranes were blocked with 5% non-fat milk TBS buffer for 2 h at room temperature and incubated with primary antibodies overnight at 4 ℃. β-actin levels were used to normalize loading. The first antibody exposure was followed by incubation with an anti-rabbit IgG, HRP-linked secondary antibody (Cell Signaling, Beverly, MA, United States). Antigen-antibody complexes were visualized using the enhanced chemiluminescence detection method (ECL kit, Thermo, Waltham, MA, United States).

The concentrations of TNF-α and IL-1β in the culture supernatants were measured using a commercially available enzyme-linked immunosorbent assay kit (Senxiong Technology Industrial Company, Shanghai, China), according to the manufacturer's instructions.

Immunofluorescence

The immunofluorescence of cultured cells was performed as recommended by the antibody manufacturers. Horseradish peroxidase-conjugated anti-rabbit IgG was used as a secondary antibody. Images were obtained on a BX51 microscope equipped with a colour camera using Picture Frame software (Olympus).

Statistical analysis

For all statistical analysis, data were expressed as the mean ± standard deviations (SD) or standard error of the mean. Student's *t*-test was used for comparisons between groups for continuous variables. One-way analysis of variance (ANOVA) was used to compare differences between groups. *P* values < 0.05 were accepted as significant.

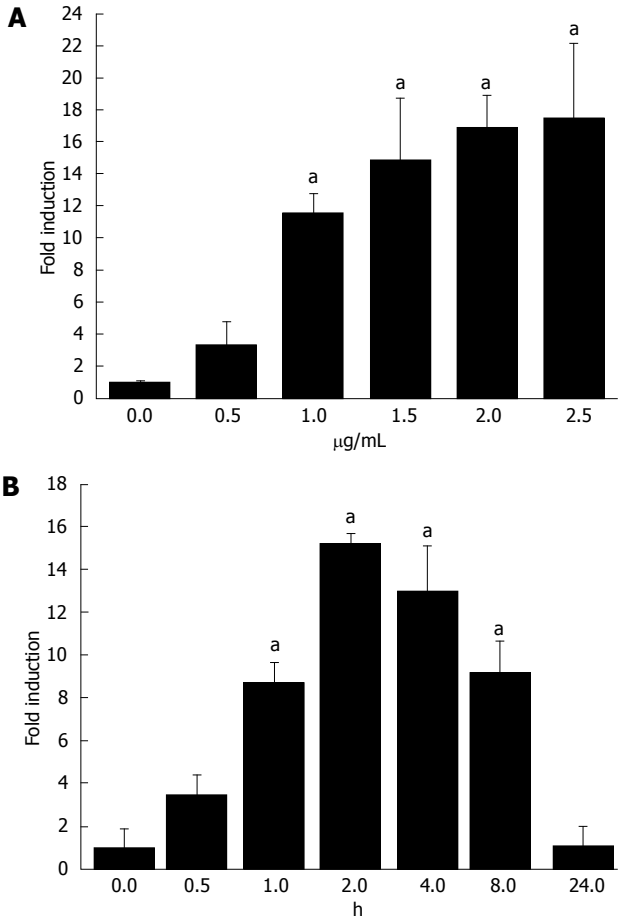

Figure 2 Relative A20 mRNA ratio in HT-29 cells after lipopolysaccharide stimulation with different doses or different time points. CT was calculated for the genes of interest and for the housekeeping gene GAPDH. For each cDNA sample, the CT for GAPDH was subtracted from the CT for each gene of interest to obtain the parameter $^{\Delta}$Ct, thereby normalizing the initial amount of RNA used. The amount of each target was calculated as $2^{\Delta\Delta}$Ct, where $^{\Delta\Delta}$CT is the difference between the $^{\Delta}$CT of the two cDNA samples to be compared. A: Cultured HT-29 cells were stimulated with LPS at different doses for 1 h, and the results showed that the expression of A20 was increased with increasing amounts of LPS stimulation. $^{a}P < 0.05$ *vs* non-stimulated cells; B: HT-29 cells were treated with 1 μg/mL LPS at various time points (0-8 h). The expression of A20 was very low in HT-29 cells without LPS stimulation but was rapidly increased and peaked at 2 h. $^{a}P < 0.05$ *vs* non-stimulated cells and cells stimulated for 24 h. LPS: Lipopolysaccharide.

RESULTS

A20 expression was rapidly increased upon stimulation with LPS in cultured enterocytes

Initially, we aimed to determine whether LPS could induce the expression of A20 in a dose-dependent manner. Cultured HT-29 cells were stimulated with LPS at different doses for 1 h and harvested for the analysis of A20 using real-time PCR and Western blotting analyses. As shown in Figures 2A and 3A, the expression of A20 increased with increasing LPS stimulation. Furthermore, to gain insight into the time-course changes of A20

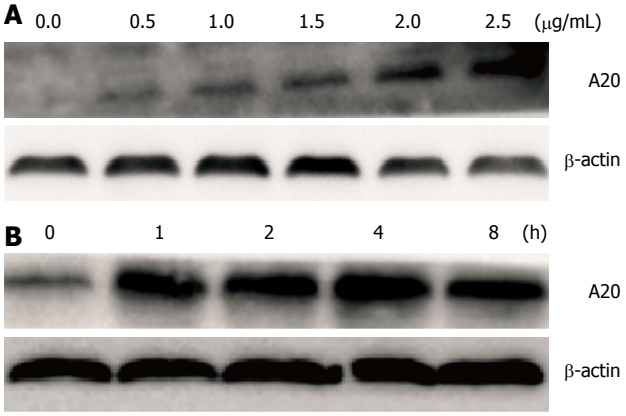

Figure 3 Expression of A20 in HT-29 cells after lipopolysaccharide stimulation with different doses or different time points. A: Cultured HT-29 cells were stimulated with lipopolysaccharide (LPS) at different doses for 1 h. Western blotting analysis showed that the expression of A20 was increased with increasing amounts of LPS stimulation; B: HT-29 cells were treated with 1 μg/mL LPS at various time points (0-8 h). Western blotting analysis showed that the expression of A20 was rapidly increased and maintained at a high level for 1-4 h after stimulation with LPS.

levels when enterocytes were subjected to infection, the expression of A20 protein and mRNA was detected at various time points after 1 μg/mL LPS stimulation using Western blotting and real-time PCR analyses. We found that the expression of A20 increased in a time-dependent manner after LPS stimulation. The expression of A20 was very low in HT-29 cells without LPS stimulation but rapidly increased and maintained a high level at 2-4 h after stimulation with LPS. These levels then declined gradually with a change in time-course (Figures 2B and 3B).

Overexpression of A20 inhibited NF-κB translocation to the nucleus

It is known that NF-κB is kept inactive by binding to the inhibitor of κB (IκB) proteins in resting cells. Upon stimulation with a wide variety of agonists, IκB is phosphorylated and subsequently polyubiquitinated and degraded by the proteasome, thereby releasing NF-κB, which then accumulates in the nucleus and activates the transcription of its target genes. As a negative regulator of NF-κB signalling pathway, we confirmed whether overexpression of A20 will alter the expression of NF-κB and its translocation to the nucleus in HT-29 cells. After a 2-h stimulation with LPS (1 μg/mL), Western blotting was performed to detect the expression of NF-κB p65 in the control group, A20 overexpression group and A20 knockdown group. As shown in Figure 4, the expression of NF-κB p65 in A20 overexpression cell lines was much lower than the control group and A20 knockdown group. In addition, immunofluorescence was performed to detect the location of NF-κB p65 in the different groups, and the results demonstrated that overexpression of A20 reduced NF-κB p65 translocation to the nucleus (Figure 5). Taken together, these data indicated that A20 inhibits the translocation of NF-κB to the nucleus in intestinal epithelial cells.

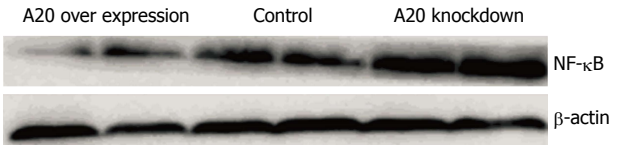

Figure 4 Overexpression of A20 decreased the level of nuclear factor-κB. Control group, A20 overexpression group and A20 knockdown group cells were treated with LPS (1 μg/mL) for 2 h. Western blotting showed that overexpression of A20 significantly decreased the level of NF-κB p65, whereas down-regulation of A20 increased NF-κ p65 expression. LPS: Lipopolysaccharide; NF: Nuclear factor.

Overexpression of A20 reduces the levels of proinflammatory cytokines involved in the pathophysiology of IBD

To determine whether the overexpression of A20 would decrease proinflammatory cytokines, such as TNF-α, IL-1β, IL-6 and IL-8, in IECs *via* modulation of NF-κB activation, the total RNA was isolated from IECs that were subjected to LPS. IL-6 and IL-8 expression were analysed using RT-PCR, while the expression of TNF-α and IL-1β in the culture supernatants were assayed by ELISA. The expression of TNF-α was different at different time points during an 8 h stimulation with LPS ($F = 31.33$, DF = 5, $P < 0.001$), and the expression of TNF-α was increased as the LPS stimulation time increased ($F = 111.435$, DF = 1, $P < 0.001$). At different time points during the 8 h stimulation with LPS, there was a significant difference in the expression of TNF-α between the A20 knockdown and the A20 overexpression ($P < 0.05$). In addition, there was a significant difference in the expression of TNF-α between the control group and the A20 knockdown group during the 8 h stimulation ($P < 0.05$, Figure 6A). Similar to the expression of TNF-α, the expression of IL-1β was different at different time points during the 8 h stimulation with LPS ($F = 9.216$, DF = 5, $P < 0.001$), and the expression of IL-1β increased with increasing LPS stimulation time ($F = 80.829$, DF = 1, $P < 0.001$). However, there was no significant difference in the expression of IL-1β in the control group, A20 knockdown group or A20 overexpression group during the 8 h stimulation of LPS ($F = 2.456$, DF = 2, $P = 0.166$, Figure 6B).

As shown in Figure 7, there was no significant difference in the expression of IL-8 mRNA in the control group, A20 overexpression-control group and A20 knockdown-control group. After 2 h, 4 h and 8 h stimulation with LPS, the expression of IL-8 mRNA in the A20 overexpression group was lower than the control group and A20 knockdown group, and the expression of IL-8 in the A20 knockdown group was higher than both the control group and the A20 overexpression group. However, there was no significant difference in the expression of IL-6 mRNA in the control group, A20 knockdown group or A20 overexpression group after 8 h of stimulation with LPS. These results demonstrated that

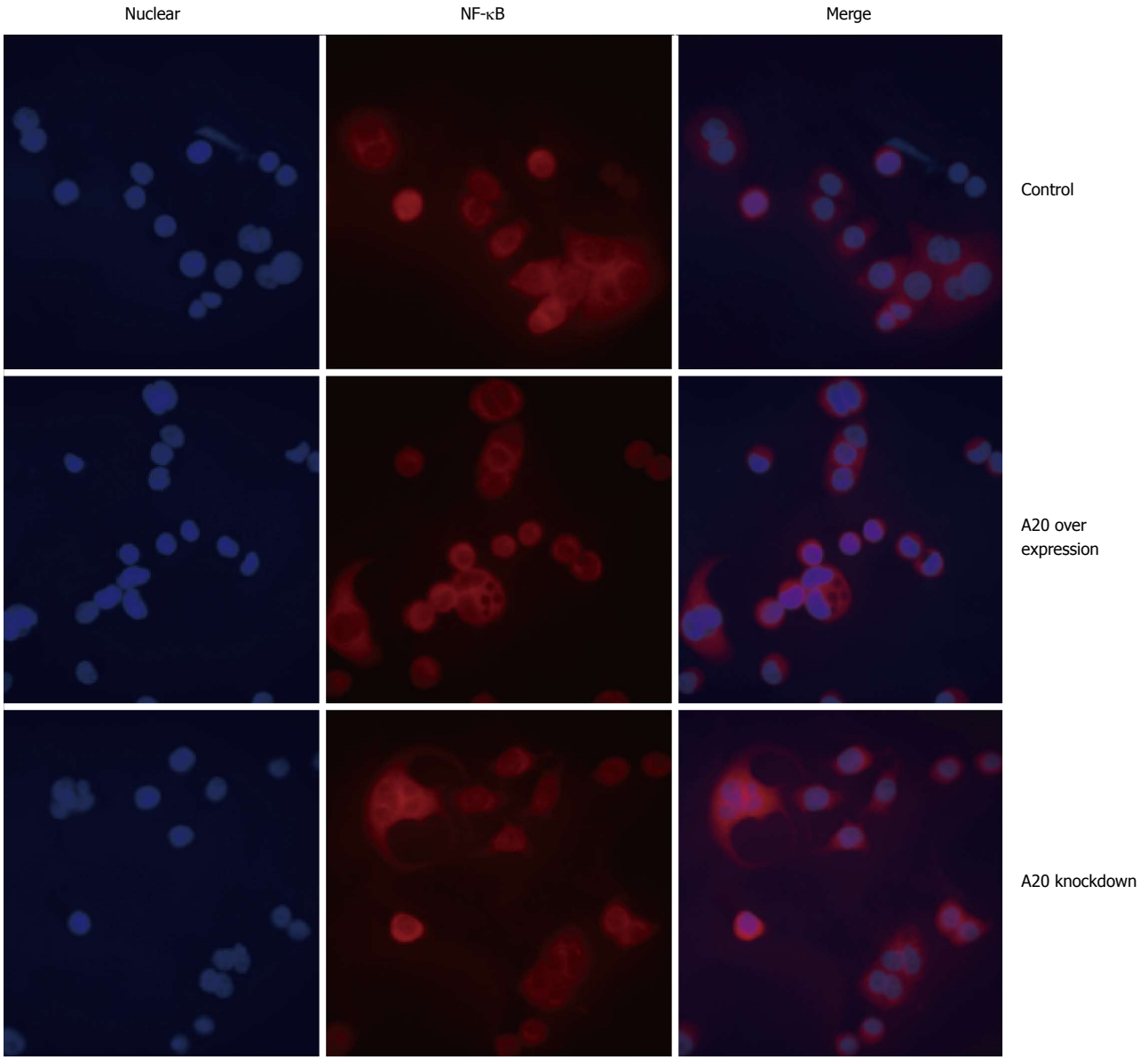

Figure 5 A20 inhibited nuclear factor-κB activation and translocation to the nucleus. Immunofluorescence was performed to detect the intracellular localization of NF-κB using anti-NF-κB p65 primary antibodies and fluorescent secondary antibodies (red) followed by confocal microscopy. DAPI-stained nuclei appear in blue. Weaker fluorescence intensity in the nucleus was detected in A20 overexpression cell lines.

the overexpression of A20 can significantly reduce the levels of pro-inflammatory cytokines.

DISCUSSION

TNF-α is regarded as a critical contributor to IBD. Its functions include the recruitment of circulating inflammatory cells to local tissue sites of inflammation, induction of oedema, the activation of the coagulation cascade, and the initiation of the formation of granuloma[19]. Clinical trials demonstrating symptomatic improvement and remission of IBD by suppressing TNF-α have provided additional evidence of the role of TNF-α in the pathogenesis of IBD[20,21]. Thus, TNF-α is considered an attractive target for the treatment of IBD. The introduction of anti-TNF-α agents (*e.g.*, Infliximab) has greatly advanced the therapeutic armamentarium of IBD, but the clinical application of infliximab is limited due to the occurrence of neutralizing antibodies[22,23], which is associated with allergic reactions and a loss of response. Thus, the development of novel therapeutic strategies for IBD is needed.

There is accumulating evidence to support the therapeutic potential of A20 in autoimmune diseases. The specific deletion of A20 in enterocytes increased the susceptibility of mice to dextran sodium sulphate (DSS)-induced colitis and prevented recovery from DSS-induced inflammation[14], whereas the expression of A20 by dendritic cells protects mice from LPS-induced mortality and DSS-induced colitis[24,25]. Not limited to IBD, A20 was also identified to be associated with numerous autoimmune diseases[11,26]. It was previously reported

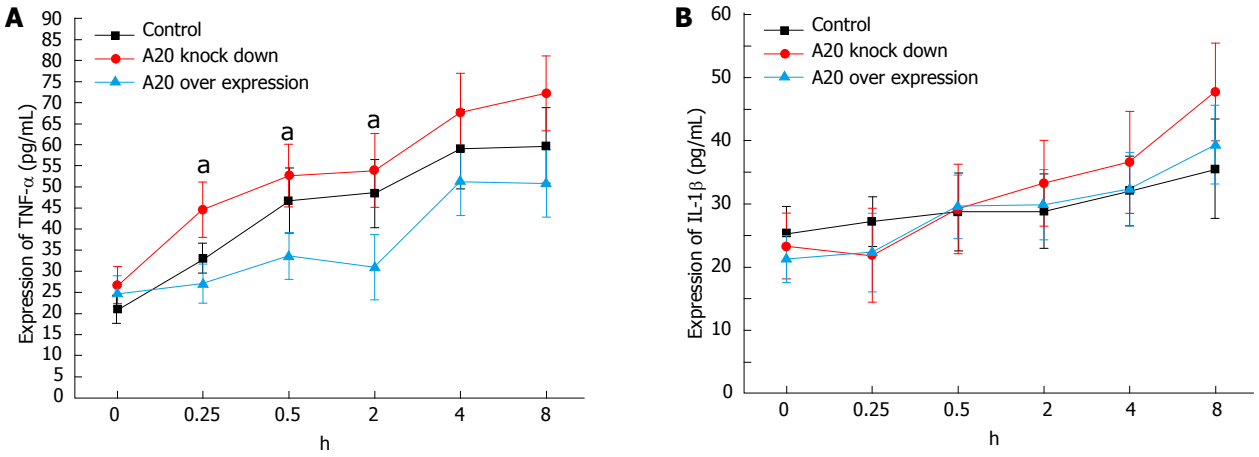

Figure 6 Expression of tumor necrosis factor-α and interleukin-1β in different groups. A: There was no significant difference in the expression of TNF-α among the control group, A20 knockdown-control group and A20 overexpression-control group. At 0.25 h, 0.5 h and 2 h, the expression of TNF-α in the A20 overexpression group was lower than the control group and A20 knockdown group, and the expression of TNF-α in the A20 knockdown group was higher than the control group; B: There was no significant difference in the expression of IL-1β in the control group, A20 knockdown group and A20 overexpression group after 8 h of LPS stimulation. $^{a}P < 0.05$. IL: Interleukin; TNF: Tumor necrosis factor; LPS: Lipopolysaccharide.

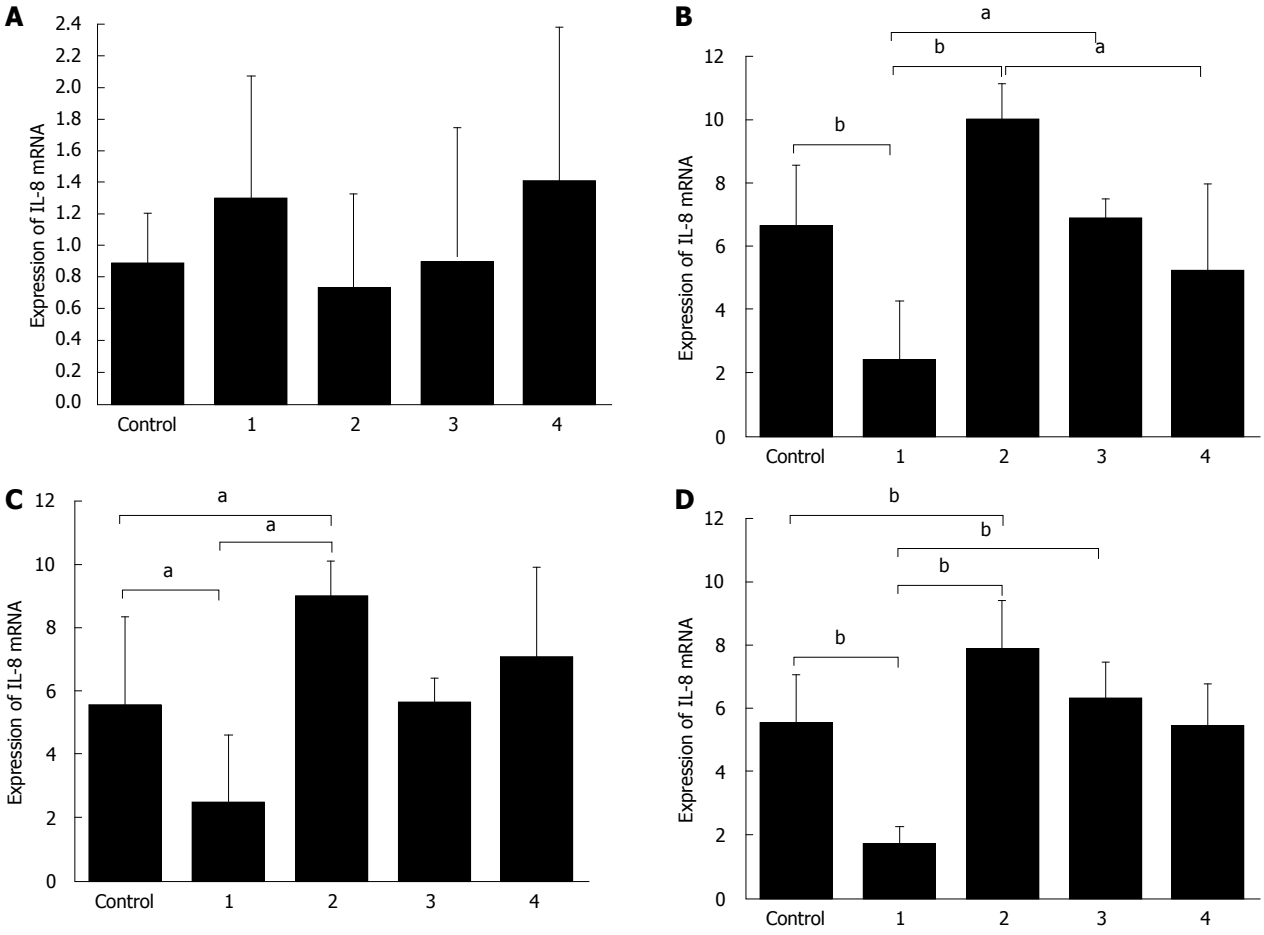

Figure 7 Interleukin-8 mRNA expression in different cell groups. 1: A20 overexpression group; 3: A20 overexpression-control group; 2: A20 knockdown group; 4: A20 knockdown-control group; A: Expression of IL-8 mRNA in different groups without LPS stimulation; B: Expression of IL-8 mRNA in different groups with LPS stimulation for 2 h; C: Expression of IL-8 mRNA in different groups with LPS stimulation for 4 h; D: Expression of IL-8 mRNA in different groups with LPS for 8 h. There were no significant differences in the IL-8 mRNA among the different groups without LPS stimulation. At some time points, the expression of IL-8 in the A20 overexpression group was lower than the control group and A20 knockdown group, and the expression of IL-8 in A20 knockdown group was higher than both the control group and A20 overexpression group. $^{a}P < 0.05$, $^{b}P < 0.01$. IL: Interleukin; TNF: Tumor necrosis factor; LPS: Lipopolysaccharide.

that prior injection with adenovirus containing A20 cDNA significantly diminished OVA challenge-mediated TNF-α production and attenuated allergic airway inflammation in mice[17]. In this study, we showed that the NF-κB responsive gene A20 is a major protective factor in intestinal epithelium cells, and the overexpression of A20 can significantly reduce the level of TNF-α in IECs. Taken together, these findings may provide some useful clues towards the development of novel anti- TNF-α strategies.

A20 has been described as a central gatekeeper in inflammation and immunity[9]. Emerging evidence indicates that LPS is a pathogenic factor that induces several inflammatory disorders, including necrotizing enterocolitis and IBD[27]. The tendency of A20-deficient mice to develop severe inflammation and their hyper-responsive nature to LPS[12] suggests that A20 may act as an endogenous regulatory system in controlling the inflammatory response to gram-negative bacteria. Oshima *et al*[28] showed that the gene expression of A20 is rapidly increased after ligand stimulation. In the present study, our results also confirmed that A20 expression was markedly up-regulated at both the mRNA and protein level upon stimulation with LPS in IECs. In addition, our experiments also found that LPS-induced A20 expression not only in a time dependent manner but also in a dose-dependent manner. The expression of A20 was very low in HT-29 cells without LPS stimulation but rapidly increased and was maintained at a high level 2-4 h after stimulation with LPS. This expression gradually declined with a change in time-course. Furthermore, the expression of A20 increased with increasing LPS stimulation.

Wang *et al*[29] found that A20 is necessary and sufficient for the development of LPS tolerance in enterocytes. Given that the human gut harbours a large collection of commensal bacteria, LPS released by gut microbes has a large effect on gut homeostasis, and the expression of A20 was rapidly increased in a dose- and time-dependent manner upon LPS stimulation. Taken together, these findings indicate the importance of A20 in the IEC response to inflammatory stimuli.

The transcription factor NF-κB has served as a standard for inducible transcription factors for more than 20 years. NF-κB can be found in the cytoplasm of most cells as an inactive complex with unprocessed precursor proteins (*e.g.*, p105) or IκB (*e.g.*, IκBa) proteins. Upon activation, NF-κB translocates into the nucleus and binds to DNA. The initiation of NF-κB signalling is tightly regulated because prolonged and excessive activation of NF-κB can lead to uncontrolled inflammation that is detrimental to the host, and thus, the inducible regulation of gene expression is a central element of normal physiology and is key to the ability of multicellular organisms to adapt to environmental, mechanical, chemical, and microbiological stresses[30]. In this study, RNA interference technology was utilized to down-regulate the expression of A20. Consistent with these findings, our results proved that the lack of A20 increased LPS-induced activation of NF-κB and its translocation to the nucleus. Furthermore, we also showed that overexpression of A20 resulted in a dramatic decrease in LPS-induced activation of NF-κB. Immunofluorescence results revealed that NF-κB translocation to the nucleus was reduced in the A20 overexpression cell group. These data indicated that the overexpression of A20 can inhibit LPS-induced NF-κB activation and translocation to the nucleus.

By far, the gastrointestinal tract is the most susceptible tissue to inflammatory responses due to its constant exposure to various antigenic, mutagenic and toxic factors. Deregulated cytokine production and signalling mechanisms by epithelial cells, mucosal lymphocytes and macrophages have been implicated in the pathogenesis of IBD. Numerous studies have identified altered proinflammatory cytokines in IBD[31]. A previous study showed that the overexpression of A20 significantly inhibited the activation of IL-8 in airway epithelial cells[32]. Furthermore, the present study demonstrates that overexpression of A20 can significantly reduce LPS-induced expression of TNF-α, IL-6 and IL-8, while the lack of A20 increased the level of TNF-α, IL-6 and IL-8 in IECs. These results demonstrated that A20 plays an important role in ameliorating the production of inflammatory cytokines.

Taken together, these findings underscore the importance of A20 in controlling inflammatory responses and indicate that A20 may be a potential therapeutic tool for the treatment of inflammatory diseases. However, its function remains poorly understood and additional investigations are necessary to elucidate the precise role of A20 in inflammatory diseases.

ACKNOWLEDGMENTS

We wish to express our deepest gratitude to the team of Professor Qing-Hai Ye, Zhongshan Hospital, the Liver Cancer Institute, Fudan University, Shanghai, for their generous help throughout the entire course of this project, without which it would not have been possible for us to complete this work.

COMMENTS

Background

A20 is regarded as the central gatekeeper in inflammation and immunity and is associated with numerous autoimmune diseases. The use of A20-deficient mice and RNA interference technologies has revealed that mice or enterocytes lacking A20 showed hyper-responsiveness to stimulations. Previous studies have confirmed that overexpression of A20 can attenuate allergic airway inflammation in mice and protects kidneys from ischaemia/reperfusion injury. However, studies on whether the overexpression of A20 extenuates enterocyte inflammation are rare.

Research frontiers

The inappropriate activation of nuclear factor (NF)-κB is central to the pathogenesis of inflammatory bowel disease (IBD). Thus, tight regulation of the NF-κB signalling pathway and the genes induced is an absolute requirement. Several studies have shown that the ubiquitin-editing protein A20 is a key player in the negative feedback regulation of NF-κB signalling in response to multiple stimulants.

Innovations and breakthroughs

A20 may be a potential therapeutic tool for immune and inflammatory diseases. Previous studies have identified that defective A20 expression or activity could be a risk factor for IBD. However, research studies related to the protective effect of A20 on intestinal epithelial cells are limited. These results demonstrated that the overexpression of A20 suppressed the activation of NF-κB and induction of proinflammatory molecules, such as TNF-α and IL-8. These data indicate that A20 plays an important role in limiting lipopolysaccharide (LPS)-induced inflammation in the gut luminal.

Applications

The introduction of infliximab has greatly advanced the therapeutic armamentarium of inflammatory bowel diseases, but the clinical application of infliximab is limited due to the occurrence of neutralizing antibodies. Thus, the development of novel therapeutic strategies for IBD is always needed. The experiments showed that A20 is critical for the inhibition of LPS-induced inflammation in enterocytes. These findings underscore the importance of A20 in controlling inflammatory responses and indicate that A20 may be a potential therapeutic tool for the treatment of inflammatory diseases.

Terminology

LPS is the major component of the outer membrane of gram-negative bacteria. The lentiviral vector is a type of retroviral vector that has become an ideal vector for target gene transfer due to its high efficiency of transfection, ability of transfection into dividing or non-dividing cells and its capacity for large target gene fragments. RNA interference technology is a type of technology that can be used to eliminate a specific gene or to suppress the expression of a specific gene.

Peer-review

This paper in interesting and clear way shows the negative regulation of inflammation under the influence of bacterial lipopolysaccharide. The experiments are well designed and the results demonstrate that A20 is able to limit the intestinal inflammation associated to NF-κB.

REFERENCES

1 **Xavier RJ**, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. *Nature* 2007; **448**: 427-434 [PMID: 17653185 DOI: 10.1038/nature06005]

2 **Artis D**. Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut. *Nat Rev Immunol* 2008; **8**: 411-420 [PMID: 18469830 DOI: 10.1038/nri2316]

3 **Round JL**, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol* 2009; **9**: 313-323 [PMID: 19343057 DOI: 10.1038/nri2515]

4 **Cario E**. Bacterial interactions with cells of the intestinal mucosa: Toll-like receptors and NOD2. *Gut* 2005; **54**: 1182-1193 [PMID: 15840688 DOI: 10.1136/gut.2004.062794]

5 **Atreya I**, Atreya R, Neurath MF. NF-kappaB in inflammatory bowel disease. *J Intern Med* 2008; **263**: 591-596 [PMID: 18479258 DOI: 10.1111/j.1365-2796.2008.01953.x]

6 **Kumar A**, Takada Y, Boriek AM, Aggarwal BB. Nuclear factor-kappaB: its role in health and disease. *J Mol Med* (Berl) 2004; **82**: 434-448 [PMID: 15175863 DOI: 10.1007/s00109-004-0555-y]

7 **Auphan N**, DiDonato JA, Rosette C, Helmberg A, Karin M. Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. *Science* 1995; **270**: 286-290 [PMID: 7569976 DOI: 10.1126/science.270.5234.286]

8 **Thiele K**, Bierhaus A, Autschbach F, Hofmann M, Stremmel W, Thiele H, Ziegler R, Nawroth PP. Cell specific effects of glucocorticoid treatment on the NF-kappaBp65/IkappaBalpha system in patients with Crohn's disease. *Gut* 1999; **45**: 693-704 [PMID: 10517905 DOI: 10.1136/gut.45.5.693]

9 **Coornaert B**, Carpentier I, Beyaert R. A20: central gatekeeper in inflammation and immunity. *J Biol Chem* 2009; **284**: 8217-8221 [PMID: 19008218 DOI: 10.1074/jbc.R800032200]

10 **Verstrepen L**, Verhelst K, van Loo G, Carpentier I, Ley SC, Beyaert R. Expression, biological activities and mechanisms of action of A20 (TNFAIP3). *Biochem Pharmacol* 2010; **80**: 2009-2020 [PMID: 20599425 DOI: 10.1016/j.bcp.2010.06.044]

11 **Vereecke L**, Beyaert R, van Loo G. The ubiquitin-editing enzyme A20 (TNFAIP3) is a central regulator of immunopathology. *Trends Immunol* 2009; **30**: 383-391 [PMID: 19643665 DOI: 10.1016/j.it.2009.05.007]

12 **Lee EG**, Boone DL, Chai S, Libby SL, Chien M, Lodolce JP, Ma A. Failure to regulate TNF-induced NF-kappaB and cell death responses in A20-deficient mice. *Science* 2000; **289**: 2350-2354 [PMID: 11009421 DOI: 10.1126/science.289.5488.2350]

13 **Wellcome Trust Case Control Consortium**. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007; **447**: 661-678 [PMID: 17554300 DOI: 10.1038/nature05911]

14 **Vereecke L**, Sze M, Mc Guire C, Rogiers B, Chu Y, Schmidt-Supprian M, Pasparakis M, Beyaert R, van Loo G. Enterocyte-specific A20 deficiency sensitizes to tumor necrosis factor-induced toxicity and experimental colitis. *J Exp Med* 2010; **207**: 1513-1523 [PMID: 20530205 DOI: 10.1084/jem.20092474]

15 **Arsenescu R**, Bruno ME, Rogier EW, Stefka AT, McMahan AE, Wright TB, Nasser MS, de Villiers WJ, Kaetzel CS. Signature biomarkers in Crohn's disease: toward a molecular classification. *Mucosal Immunol* 2008; **1**: 399-411 [PMID: 19079204 DOI: 10.1038/mi.2008.32]

16 **Zheng CF**, Huang Y. [Expression of zinc finger protein A20 in pediatric inflammatory bowel disease]. *Zhonghua Erke Zazhi* 2011; **49**: 261-265 [PMID: 21624200]

17 **Kang NI**, Yoon HY, Lee YR, Won M, Chung MJ, Park JW, Hur GM, Lee HK, Park BH. A20 attenuates allergic airway inflammation in mice. *J Immunol* 2009; **183**: 1488-1495 [PMID: 19553520 DOI: 10.4049/jimmunol.0900163]

18 **Lutz J**, Luong le A, Strobl M, Deng M, Huang H, Anton M, Zakkar M, Enesa K, Chaudhury H, Haskard DO, Baumann M, Boyle J, Harten S, Maxwell PH, Pusey C, Heemann U, Evans PC. The A20 gene protects kidneys from ischaemia/reperfusion injury by suppressing pro-inflammatory activation. *J Mol Med* (Berl) 2008; **86**: 1329-1339 [PMID: 18813897 DOI: 10.1007/s00109-008-0405-4]

19 **Van Deventer SJ**. Tumour necrosis factor and Crohn's disease. *Gut* 1997; **40**: 443-448 [PMID: 9176068 DOI: 10.1136/gut.40.4.443]

20 **Nakamura K**, Honda K, Mizutani T, Akiho H, Harada N. Novel strategies for the treatment of inflammatory bowel disease: Selective inhibition of cytokines and adhesion molecules. *World J Gastroenterol* 2006; **12**: 4628-4635 [PMID: 16937430 DOI: 10.3748/wjg.v12.i29.4628]

21 **Hyams J**, Crandall W, Kugathasan S, Griffiths A, Olson A, Johanns J, Liu G, Travers S, Heuschkel R, Markowitz J, Cohen S, Winter H, Veereman-Wauters G, Ferry G, Baldassano R. Induction and maintenance infliximab therapy for the treatment of moderate-to-severe Crohn's disease in children. *Gastroenterology* 2007; **132**: 863-873; quiz 1165-1166 [PMID: 17324398 DOI: 10.1053/j.gastro.2006.12.003]

22 **Vande Casteele N**, Khanna R, Levesque BG, Stitt L, Zou GY, Singh S, Lockton S, Hauenstein S, Ohrmund L, Greenberg GR, Rutgeerts PJ, Gils A, Sandborn WJ, Vermeire S, Feagan BG. The relationship between infliximab concentrations, antibodies to infliximab and disease activity in Crohn's disease. *Gut* 2015; **64**: 1539-1545 [PMID: 25336114 DOI: 10.1136/gutjnl-2014-307883]

23 **O'Meara S**, Nanda KS, Moss AC. Antibodies to infliximab and risk of infusion reactions in patients with inflammatory bowel disease: a systematic review and meta-analysis. *Inflamm Bowel Dis* 2014; **20**: 1-6 [PMID: 24280879 DOI: 10.1097/01.MIB.0000436951]

24 **Hammer GE**, Turer EE, Taylor KE, Fang CJ, Advincula R, Oshima S, Barrera J, Huang EJ, Hou B, Malynn BA, Reizis B, DeFranco A, Criswell LA, Nakamura MC, Ma A. Expression of A20 by dendritic cells preserves immune homeostasis and prevents colitis and spondyloarthritis. *Nat Immunol* 2011; **12**: 1184-1193 [PMID: 22019834 DOI: 10.1038/ni.2135]

25 **Xuan NT**, Wang X, Nishanth G, Waisman A, Borucki K, Isermann B, Naumann M, Deckert M, Schlüter D. A20 expression in dendritic cells protects mice from LPS-induced mortality. *Eur J Immunol*

2015; **45**: 818-828 [PMID: 25472594 DOI: 10.1002/eji.201444795]
26 **Zhang M**, Peng LL, Wang Y, Wang JS, Liu J, Liu MM, Hu J, Song B, Yang HB. Roles of A20 in autoimmune diseases. *Immunol Res* 2016; **64**: 337-344 [PMID: 26135958 DOI: 10.1007/s12026-015-8677-6]
27 **Rhee SH**. Lipopolysaccharide: basic biochemistry, intracellular signaling, and physiological impacts in the gut. *Intest Res* 2014; **12**: 90-95 [PMID: 25349574 DOI: 10.5217/ir.2014.12.2.90]
28 **Oshima N**, Ishihara S, Rumi MA, Aziz MM, Mishima Y, Kadota C, Moriyama I, Ishimura N, Amano Y, Kinoshita Y. A20 is an early responding negative regulator of Toll-like receptor 5 signalling in intestinal epithelial cells during inflammation. *Clin Exp Immunol* 2010; **159**: 185-198 [PMID: 19912257 DOI: 10.1111/j.1365-2249.2009.04048.x]
29 **Wang J**, Ouyang Y, Guner Y, Ford HR, Grishin AV. Ubiquitin-editing enzyme A20 promotes tolerance to lipopolysaccharide in enterocytes. *J Immunol* 2009; **183**: 1384-1392 [PMID: 19570823 DOI: 10.4049/jimmunol.0803987]
30 **Hayden MS**, Ghosh S. Shared principles in NF-kappaB signaling. *Cell* 2008; **132**: 344-362 [PMID: 18267068 DOI: 10.1016/j.cell.2008.01.020]
31 **Neurath MF**. Cytokines in inflammatory bowel disease. *Nat Rev Immunol* 2014; **14**: 329-342 [PMID: 24751956 DOI: 10.1038/nri3661]
32 **Gon Y**, Asai Y, Hashimoto S, Mizumura K, Jibiki I, Machino T, Ra C, Horie T. A20 inhibits toll-like receptor 2- and 4-mediated interleukin-8 synthesis in airway epithelial cells. *Am J Respir Cell Mol Biol* 2004; **31**: 330-336 [PMID: 15142865 DOI: 10.1165/rcmb.2003-0438OC]

P- Reviewer: Futoma-Koloch B, Kamada N, Vivas S
S- Editor: Gong ZM **L- Editor**: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i4.550

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 550-555
ISSN 2150-5349 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Usefulness of vonoprazan, a potassium ion-competitive acid blocker, for primary eradication of *Helicobacter pylori*

Shinya Yamada, Takumi Kawakami, Yoshikazu Nakatsugawa, Takahiro Suzuki, Hideki Fujii, Naoya Tomatsuri, Hideki Nakamura, Hideki Sato, Yusuke Okuyama, Hiroyuki Kimura, Norimasa Yoshida

Shinya Yamada, Takumi Kawakami, Yoshikazu Nakatsugawa, Takahiro Suzuki, Hideki Fujii, Naoya Tomatsuri, Hideki Nakamura, Hideki Sato, Yusuke Okuyama, Hiroyuki Kimura, Norimasa Yoshida, Department of Gastroenterology, Japanese Red Cross Kyoto Daiichi Hospital, Kyoto 605-0981, Japan

Author contributions: Yamada S and Yoshida N contributed to study conception and design; they also contributed to writing, editing and final approval of article; Kawakami T, Nakatsugawa Y, Suzuki T, Fujii H, Tomatsuri N, Nakamura H, Sato H, Okuyama Y and Kimura H performed the research.

Institutional review board statement: The study protocol was approved by the Ethics Committee of Japanese Red Cross Kyoto Daiichi Hospital.

Informed consent statement: All study participants, or their legal guardian, provided informed verbal consent prior to study enrollment.

Conflict-of-interest statement: There is no conflict of interest.

Data sharing statement: No additional data are available.

Manuscript source: Unsolicited manuscript

Correspondence to: Dr. Shinya Yamada, Department of Gastroenterology, Japanese Red Cross Kyoto Daiichi Hospital, Hon-machi 15-749, Kyoto 605-0981, Japan. syo335@koto.kpu-m.ac.jp
Telephone: +81-75-5611121
Fax: +81-75-5616308

Received: June 15, 2016
Peer-review started: June 17, 2016
First decision: July 20, 2016
Revised: July 28, 2016
Accepted: August 27, 2016
Article in press: August 29, 2016
Published online: November 6, 2016

Abstract

AIM

To investigate usefulness of triple therapy with vonoprazan, a potassium ion-competitive acid blocker and antibiotics, for *Helicobacter pylori* (*H. pylori*) eradication.

METHODS

The *H. pylori* eradication rate was examined in 2507 patients (2055 undergoing primary eradication and 452 undergoing secondary eradication, excluding patients with subtotal gastrectomy) at the Japanese Red Cross Kyoto Daiichi Hospital from March 2013 to September 2015. For patients treated from March 2013 to February 2015, a proton pump inhibitor (PPI) was used to reduce acid secretion, while vonoprazan was used after March 2015. The success rates of the 2 regimens (PPI + amoxicillin + clarithromycin/metronidazole, or vonoprazan + amoxicillin + clarithromycin/metronidazole) were compared.

RESULTS

The success rate of primary *H. pylori* eradication was significantly higher in the vonoprazan group. When stratified by the underlying disease, a significant increase of the *H. pylori* eradication rate was observed in patients with chronic gastritis. A significantly lower *H. pylori* eradication rate was observed in younger patients compared to older patients in the PPI group, but there was no difference according to age in the vonoprazan group. On the other

hand, the success rate of secondary eradication was similar at approximately 90% in both groups.

CONCLUSION

Vonoprazan is very useful for primary eradication of *H. pylori*, and may become a first-line acid secretion inhibitor instead of PPIs.

Key words: *Helicobacter pylori*; Eradication; Vonoprazan; Chronic gastritis; Potassium ion-competitive acid blocker

Core tip: Use of vonoprazan, a potassium ion-competitive acid blocker, is expected to achieve a higher eradication rate than conventional triple therapy. The success rates of the 2 regimens (use of proton pump inhibitor *vs* vonoprazan) were compared. The success rate of primary *Helicobacter pylori* (*H. pylori*) eradication was significantly higher in the vonoprazan group. When stratified by the underlying disease, a significant increase of the *H. pylori* eradication rate was observed in patients with chronic gastritis. Vonoprazan is very useful for primary eradication of *H. pylori*, and may become a first-line acid secretion inhibitor instead of proton pump inhibitors.

Yamada S, Kawakami T, Nakatsugawa Y, Suzuki T, Fujii H, Tomatsuri N, Nakamura H, Sato H, Okuyama Y, Kimura H, Yoshida N. Usefulness of vonoprazan, a potassium ion-competitive acid blocker, for primary eradication of *Helicobacter pylori*. *World J Gastrointest Pharmacol Ther* 2016; 7(4): 550-555 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i4/550.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i4.550

INTRODUCTION

Since *Helicobacter pylori* (*H. pylori*) eradication therapy for chronic gastritis was approved for insurance cover in February 2013 in Japan, the number of patients undergoing eradication of *H. pylori* has greatly increased. In September 2014, the International Agency for Research of Cancer (IARC) reported that 80% of stomach cancer is caused by *H. pylori* infection, and the incidence of stomach cancer can be reduced by 30%-40% through *H. pylori* eradication[1]. However, the success rate of bacterial eradication by conventional primary triple therapy, involving the administration of a proton pump inhibitor (PPI) + amoxicillin (AMPC) + clarithromycin (CAM) for 1 wk, has steadily declined due to an increase of CAM resistance[2]. On the other hand, it is reported that secondary eradication therapy using metronidazole (MNZ) has a success rate exceeding 90%[3-7]. Reports about carcinogenicity of MNZ have appeared (although the risk is low)[8], and there is the possibility of resistance increasing due to its increased use in the near future. Consequently, development of new primary eradication therapy is desired.

Vonoprazan, a potassium ion-competitive acid blocker (P-CAB), was launched in Japan in February 2015 before its release on the world market[9]. Use of vonoprazan is expected to achieve a higher eradication rate than conventional triple therapy due to its strong inhibitory effect on gastric acid secretion[10,11]. Against this background, the current study aimed at evaluating the usefulness of triple therapy containing P-CAB compared with 7-d PPI-based triple therapy.

MATERIALS AND METHODS

This study is a retrospective analysis of prospectively collected data comparing outcomes of patients received *H. pylori* eradiation therapy by vonoprazan from March to September 2015 against a historical cohort of patient by a proton pump inhibitor (PPI) carried out from March 2013 to February 2015.

The subjects were 2507 patients (2055 undergoing primary eradication and 452 undergoing secondary eradication, excluding patients with subtotal gastrectomy) who tested positive for *H. pylori* at the Gastroenterology Department of the Japanese Red Cross Kyoto Daiichi Hospital from March 2013 to September 2015. In patients treated from March 2013 to February 2015, a PPI was used to inhibit gastric acid secretion, while vonoprazan was used for patients treated after March 2015. Patients were received 7-d course of triple therapy with amoxicillin 1500 mg and clarithromycin 400 mg plus lansoprazole 60 mg, esomeprazole 40 mg, rabeprazole 20 mg, or vonoprazan 40 mg as first-line treatment, and 7-d course of triple therapy with amoxicillin 1500 mg and metronidazole 500 mg plus lansoprazole 60 mg, esomeprazole 40 mg, rabeprazole 20 mg, or vonoprazan 40 mg as second-line treatment. Success rate was compared between the 2 *H. pylori* eradication methods.

Before starting the eradication therapy, patients underwent a medical interview concerning their drug allergy. Adverse effect was evaluated after eradication therapy by a medical interview.

The presence of *H. pylori* infection was confirmed by a positive result in any of the following tests: Urea breath test (n = 52), rapid urease test (n = 668), serum *H. pylori* IgG antibody (n = 1074), fecal *H. pylori* antigen (n = 7), and microscopy (n = 254). Eradication effect was confirmed by performing the urea breath test at two months after treatment, using a cut-off of 2.5‰. We did not investigate the strains and levels of resistance of *H. pylori* to the antimicrobial drugs planned to administer.

Patients who received eradication therapy were divided into a group treated with a PPI (lansoprazole, omeprazole, rabeprazole, or esomeprazole) and a group treated with vonoprazan, and success rates were compared, including the success rates stratified according to gender, age, and underlying disease.

The study protocol was approved by the Ethics Committee of Japanese Red Cross Kyoto Daiichi Hospital and was conducted in compliance with the Helsinki Declaration.

Table 1 Patient profile of primary eradication therapy

	PPI group	Vonoprazan group
Average age	62.8 (18-94)	62.7 (22-89)
Gender (male:female)	902:818	170:165
PPI		
Lansoprazole	1206	
Omeprazole	0	
Rabeprazole	62	
Esomeprazole	452	
Underlying disease		
Chronic gastritis	1362	255
Peptic ulcer	248	55
After endoscopic therapy for early gastric cancer	107	25
Other	3	0

PPI: Proton pump inhibitor.

Table 2 Patient profile of secondary eradication therapy

	PPI group	Vonoprazan group
Average age	62.3 (19-96)	63.6 (39-89)
Gender(male:female)	182:204	28:38
PPI		
Lansoprazole	266	
Omeprazole	1	
Rabeprazole	32	
Esomeprazole	87	
Underlying disease		
Chronic gastritis	313	48
Peptic ulcer	44	8
After endoscopic therapy for early gastric cancer	28	10
Other	1	0

PPI: Proton pump inhibitor.

For statistical analysis, the χ^2 test and Fischer's exact probability test were used, and significance was accepted at $P < 0.05$. All analyses were performed using the program GraphPad Prism 4 (GraphPad Soft ware, San Diego, CA).

RESULTS

Patient profile

Among the 2055 patients receiving primary eradication therapy, a PPI was used in 1720 and vonoprazan was used in 335. Lansoprazole was the PPI most commonly used to inhibit acid secretion (1206 patients), followed by esomeprazole and rabeprazole. Omeprazole was not used. In both the PPI group and the vonoprazan group, chronic gastritis was the underlying disease in more than 75% of the patients, followed by peptic ulcer and endoscopic therapy (Table 1).

Among the 452 patients receiving secondary eradication therapy, a PPI was used in 386 and vonoprazan was used in 66. The trends for PPI use and underlying cause were similar to those in the primary eradication group (Table 2).

Table 3 Results of eradication therapy

	PPI group	Vonoprazan group	*P* value
Primary eradication therapy			
ITT analysis	73.2% (1259/1720)	85.7% (287/335)	< 0.0001
PP analysis	76.4% (1259/1647)	90.3% (287/318)	< 0.0001
Secondary eradication therapy			
ITT analysis	89.9% (347/386)	89.4% (59/66)	0.87
PP analysis	92.8% (347/374)	96.7% (59/61)	0.4

PPI: Proton pump inhibitor.

Success rate of H. pylori eradication therapy

Regarding the success rate of primary eradication therapy in the PPI group, it was 73.2% by ITT analysis and 76.4% by PP analysis, while it was 85.7% by ITT analysis and 90.3% by PP analysis in the vonoprazan group. The *H. pylori* eradication success rate was significantly higher in the vonoprazan group by both ITT analysis and PP analysis.

Regarding the success rate of secondary eradication therapy in the PPI group, it was 89.9% by ITT analysis and 92.8% by PP analysis, while it was 89.4% by ITT analysis and 96.7% by PP analysis in the vonoprazan group, with no significant difference between the 2 groups (Table 3).

Success rate and underlying disease

In patients with chronic gastritis undergoing primary eradication therapy, a significantly higher success rate was observed in the vonoprazan group than the PPI group by both ITT analysis (86.7%) and PP analysis (90.6%). In patients with peptic ulcer undergoing primary eradication, the success rate was also higher in the vonoprazan group, but no significant difference was observed compared to the PPI group. In patients undergoing primary eradication after endoscopic therapy for early gastric cancer, there was little difference of the success rate between the PPI group and the vonoprazan group (Table 4). In patients undergoing secondary eradication, the PPI group and the vonoprazan group showed no differences of the success rate in relation to underlying diseases (Table 5).

Success rate and gender or age

No difference of the success rate according to gender was observed in either the PPI group or the vonoprazan group. Patients younger than 50 years were defined as younger and those older than 50 years were defined as older, and the success rates in both age groups were examined for PPI and vonoprazan therapy. In the PPI group, the success rate was significantly lower in younger patients, but there was no difference of the success rate based on age in the vonoprazan group (Table 6).

Table 4 Success rate and underlying disease: Primary eradication therapy

		PPI group	Vonoprazan group	*P* value
Chronic gastritis	ITT analysis	72.5% (988/1362)	86.7% (221/255)	< 0.0001
	PP analysis	75.1% (988/1316)	90.6% (221/244)	< 0.0001
Peptic ulcer	ITT analysis	75.4% (187/248)	83.6% (46/55)	0.22
	PP analysis	82.7% (187/226)	93.9% (46/49)	0.051
After endoscopic therapy for early gastric cancer	ITT analysis	76.6% (82/107)	80% (20/25)	0.8
	PP analysis	78.1% (82/105)	80% (20/25)	1

PPI: Proton pump inhibitor.

Table 5 Success rate and underlying disease: Secondary eradication therapy

		PPI group	Vonoprazan group	*P* value
Chronic gastritis	ITT analysis	91.5% (292/319)	89.6% (43/48)	0.59
	PP analysis	93.9% (292/311)	100% (43/43)	0.15
Peptic ulcer	ITT analysis	86.4% (38/44)	100% (8/8)	0.57
	PP analysis	95.0% (38/40)	100% (8/8)	1
After endoscopic therapy for early gastric cancer	ITT analysis	78.6% (22/28)	80% (8/10)	1
	PP analysis	78.6% (22/28)	80% (8/10)	1

PPI: Proton pump inhibitor.

Table 6 Success rate and age: Primary eradication therapy

		Younger than 50 yr	Older than 50 yr	*P* value
PPI group	ITT analysis	67.8% (185/273)	74.3% (1074/1446)	0.03
	PP analysis	72.3% (185/256)	77.2% (1074/1391)	0.09
Vonoprazan group	ITT analysis	84.8% (50/59)	86.2% (238/276)	0.84
	PP analysis	92.6% (50/54)	90.2% (238/264)	0.8

PPI: Proton pump inhibitor.

Adverse events

In the PPI group, 7 patients (0.4%) discontinued treatment due to adverse events during primary eradication therapy, including 2 cases of diarrhea, 4 cases of rash, and 1 other event. Two patients (0.5%) from the PPI group discontinued secondary eradication therapy, including 1 case of diarrhea and 1 other event. No cases of discontinuation of treatment due to adverse events were observed in the vonoprazan group. The incidence of major adverse effect such as diarrhea, dysgeusia and skin rash showed no difference between the two groups. No specific adverse effect was observed in the vonoprazan group.

DISCUSSION

In this investigation, the success rate of primary *H. pylori* eradication therapy was significantly higher in the vonoprazan group, and vonoprazan treatment achieved a significantly higher success rate in patients with chronic gastritis. In the PPI group, the success rate of *H. pylori* eradication therapy was significantly lower for younger patients than for older patients, but no difference related to age was observed in the vonoprazan group. On the other hand, the success rate of secondary *H. pylori* eradication therapy was similar (Approximately 90%) in the PPI group and the vonoprazan group.

Vonoprazan is a new potassium ion-competitive acid blocker, which is stable in an acid environment, and shows rapid and potent inhibition of gastric acid secretion[9]. Vonoprazan is instantly protonated in an acidic and even in a neutral environment, and is suggested to bind to and inhibit H^+,K^+-ATPase in the

protonated form[9]. Insufficient inhibition of gastric acid secretion has previously been reported to cause failure of *H. pylori* eradication[12]. Vonoprazan has been reported to rapidly increase the gastric pH for an extended period from the initial day of administration[10,11]. In this study, the success rate for young patients was much higher than that for older patients in the vonoprazan group. From these results, it can be inferred that the success rate of primary *H. pylori* eradication therapy was increased in the vonoprazan group due to vonoprazan improving the antibacterial activity of the antibiotics used in combination. In addition, the enzyme involved in metabolism of vonoprazan is another possible reason. The gene polymorphism of the liver enzyme cytochrome P450 (CYP) 2C19 affects the metabolic rate and the acid inhibitory effect of PPIs. However, vonoprazan is mainly metabolized in the liver by CYP3A4[12]. Therefore, vonoprazan exerts a potent inhibitory activity regardless of CYP2C19 polymorphism. CAM, which is used in primary *H. pylori* eradication therapy, is also metabolized by CYP3A4[13], and the $AUC_{0\text{-}12}$ and Cmax of vonoprazan are reported to be respectively increased 1.5 times and 1.6 times during combined administration of AMPC, CAM, and vonoprazan compared with single-agent administration[14]. In the present study, the success rate showed a significant increase with primary *H. pylori* eradication therapy, suggesting that the interaction of vonoprazan and CAM promoted the action of both agents. However, a smaller population was examined for secondary eradication therapy, so collection of more cases is needed in the future.

Moreover, regarding the *H. pylori* eradication rate in relation to the underlying disease in the vonoprazan group, a significantly higher success rate was seen in patients with chronic gastritis. While a similar trend to that for chronic gastritis was also observed for peptic ulcer, an almost equal *H. pylori* eradication rate was observed in the vonoprazan and PPI groups among patients treated after endoscopic therapy. Further investigation is also needed to determine if the difference in the eradication rate by underlying disease was due to the small number of subjects, host factors, or bacterial factors. In this study, we demonstrated that vonoprazan was very useful for primary eradication of *H. pylori.* However, the success rate of secondary *H. pylori* eradication therapy had no difference in the PPI group and the vonoprazan group. MNZ based conventional triple therapy has sufficiently high eradication success rate in Japan[3-7]. It has been reported that sufficient acid inhibition during eradication was more important in CAM based regimen than MNZ[15]. Therefore, we could not show the difference in secondary eradication therapy between the PPI group and the vonoprazan group.

Our study has some limitations. Although we used historical controls in the same institute, this was a retrospective study at a single center. Second, adverse effect was not precisely evaluated because we could not track patients who were not confirmed the effect of eradication therapy. Therefore, important events during the eradication therapy might have been lost. Although Murakami *et al*[16] reported that no marked differences were observed in adverse effects between the vonoprazan and the PPI group, Suzuki *et al*[17] indicated that the incidence of skin rash was significantly higher with vonoprazan therapy than with PPI therapy. Further investigation will be needed to clarify the adverse effect of vonoparazan in *H. pylori* eradication therapy. Third, factors which may affect success rate of *H. pylori* eradication therapy, such as alcohol, smoking, or the use of other medications were not recorded in the patients.

Despite these limitations, the results obtained were comparable to the *H. pylori* eradication rate at the time when vonoprazan was approved for patients with healed gastroduodenal ulcers[16], and the incidence of adverse events was similar to that with conventional eradication therapy using PPIs. In the future, *H. pylori* eradication therapy using the three-agent combination of AMPC, CAM, and vonoprazan may possibly become a first-line option.

In conclusion, vonoprazan is considered to be useful for triple therapy aimed at primary *H. pylori* eradication, and the possibility of using vonoprazan as first-line treatment to inhibit acid secretion instead of PPIs was suggested. For secondary eradication therapy, further investigation is needed to determine if the success rate is higher than that achieved with PPIs.

COMMENTS

Background

The International Agency for Research of Cancer reported that 80% of stomach cancer is caused by *Helicobacter pylori* (*H. pylori*) infection, and the incidence of stomach cancer can be reduced by 30%-40% through *H. pylori* eradication. Consequently, development of new primary eradication therapy is desired.

Research frontiers

The authors' group pioneered a novel primary eradication therapy for *H. pylori*, using vonoprazan. The number of patients undergoing eradication of *H. pylori* has greatly increased. However, the success rate of bacterial eradication by conventional primary triple therapy has steadily declined due to an increase of clarithromycin (CAM) resistance.

Innovations and breakthroughs

The success rate of bacterial eradication by conventional primary triple therapy, involving the administration of a proton pump inhibitor (PPI) + amoxicillin (AMPC) + CAM for 1 wk, has steadily declined due to an increase of CAM resistance. However, use of vonoprazan achieved a higher eradication rate than conventional triple therapy due to its strong inhibitory effect on gastric acid secretion.

Applications

Vonoprazan is considered to be useful for triple therapy aimed at primary *H. pylori* eradication, and the possibility of using vonoprazan as first-line treatment to inhibit acid secretion instead of PPIs was suggested.

Terminology

Vonoprazan is a novel oral potassium-competitive acid blocker, which is stable in an acid environment, and shows rapid and potent inhibition of gastric acid secretion.

Peer-review

The authors concluded that vonoprazan therapy for *H. pylori* eradication may be advantageous over those utilizing PPIs. The paper is well written, and the possible advantageous mechanism of vonoprazan action is adequately explored in the Discussion. Therefore, the paper should be of interest to the readership of the Journal.

REFERENCES

1 **Herrero R**, Park JY, Forman D. The fight against gastric cancer - the IARC Working Group report. *Best Pract Res Clin Gastroenterol* 2014; **28**: 1107-1114 [PMID: 25439075 DOI: 10.1016/j.bpg.2014.10.003]

2 **Kobayashi I**, Murakami K, Kato M, Kato S, Azuma T, Takahashi S, Uemura N, Katsuyama T, Fukuda Y, Haruma K, Nasu M, Fujioka T. Changing antimicrobial susceptibility epidemiology of Helicobacter pylori strains in Japan between 2002 and 2005. *J Clin Microbiol* 2007; **45**: 4006-4010 [PMID: 17942652 DOI: 10.1128/JCM.00740-07]

3 **Nagahara A**, Miwa H, Ohkura R, Yamada T, Sato K, Hojo M, Sato N. Strategy for retreatment of therapeutic failure of eradication of Helicobacter pylori infection. *J Gastroenterol Hepatol* 2001; **16**: 613-618 [PMID: 11422612 DOI: 10.1046/j.1440-1746.2001.02491]

4 **Isomoto H**, Inoue K, Furusu H, Enjoji A, Fujimoto C, Yamakawa M, Hirakata Y, Omagari K, Mizuta Y, Murase K, Shimada S, Murata I, Kohno S. High-dose rabeprazole-amoxicillin versus rabeprazole-amoxicillin-metronidazole as second-line treatment after failure of the Japanese standard regimen for Helicobacter pylori infection. *Aliment Pharmacol Ther* 2003; **18**: 101-107 [PMID: 12848631 DOI: 10.1046/j.0269-2813.2003.01659.x]

5 **Murakami K**, Sato R, Okimoto T, Nasu M, Fujioka T, Kodama M, Kagawa J. Efficacy of triple therapy comprising rabeprazole, amoxicillin and metronidazole for second-line Helicobacter pylori eradication in Japan, and the influence of metronidazole resistance. *Aliment Pharmacol Ther* 2003; **17**: 119-123 [PMID: 12492740 DOI: 10.1046/j.0269-2813.2003.01401.x]

6 **Miwa H**, Nagahara A, Kurosawa A, Ohkusa T, Ohkura R, Hojo M, Enomoto N, Sato N. Is antimicrobial susceptibility testing necessary before second-line treatment for Helicobacter pylori infection? *Aliment Pharmacol Ther* 2003; **17**: 1545-1551 [PMID: 12823158 DOI: 10.1046/j.1365-2036.2003.01541.x]

7 **Shimoyama T**, Fukuda S, Mikami T, Fukushi M, Munakata A. Efficacy of metronidazole for the treatment of clarithromycin-resistant Helicobacter pylori infection in a Japanese population. *J Gastroenterol* 2004; **39**: 927-930 [PMID: 15549444]

8 **Beard CM**, Noller KL, O'Fallon WM, Kurland LT, Dahlin DC. Cancer after exposure to metronidazole. *Mayo Clin Proc* 1988; **63**: 147-153 [PMID: 3339906 DOI: 10.1016/S0025-6196(12)64947-7]

9 **Hori Y**, Imanishi A, Matsukawa J, Tsukimi Y, Nishida H, Arikawa Y, Hirase K, Kajino M, Inatomi N. 1-[5-(2-Fluorophenyl)-1-(pyridin-3-ylsulfonyl)-1H-pyrrol-3-yl]-N-methylmethanamine monofumarate (TAK-438), a novel and potent potassium-competitive acid blocker for the treatment of acid-related diseases. *J Pharmacol Exp Ther* 2010; **335**: 231-238 [PMID: 20624992 DOI: 10.1124/jpet.110.170274]

10 **Jenkins H**, Sakurai Y, Nishimura A, Okamoto H, Hibberd M, Jenkins R, Yoneyama T, Ashida K, Ogama Y, Warrington S. Randomised clinical trial: safety, tolerability, pharmacokinetics and pharmacodynamics of repeated doses of TAK-438 (vonoprazan), a novel potassium-competitive acid blocker, in healthy male subjects. *Aliment Pharmacol Ther* 2015; **41**: 636-648 [PMID: 25707624 DOI: 10.1111/apt.13121]

11 **Sakurai Y**, Mori Y, Okamoto H, Nishimura A, Komura E, Araki T, Shiramoto M. Acid-inhibitory effects of vonoprazan 20 mg compared with esomeprazole 20 mg or rabeprazole 10 mg in healthy adult male subjects--a randomised open-label cross-over study. *Aliment Pharmacol Ther* 2015; **42**: 719-730 [PMID: 26193978 DOI: 10.1111/apt.13325]

12 **Cederbrant G**, Kahlmeter G, Schalén C, Kamme C. Additive effect of clarithromycin combined with 14-hydroxy clarithromycin, erythromycin, amoxycillin, metronidazole or omeprazole against Helicobacter pylori. *J Antimicrob Chemother* 1994; **34**: 1025-1029 [PMID: 7730216 DOI: 10.1093/jac/34.6.1025]

13 The interview form for vonoprazan fumarate (Takecab® tablets 10 mg and 20 mg) ver. 6, (in Japanese). Takeda Pharmaceutical Company Limited. 2015. Available from: URL: http://www.takeda.co.jp/

14 **Rodrigues AD**, Roberts EM, Mulford DJ, Yao Y, Ouellet D. Oxidative metabolism of clarithromycin in the presence of human liver microsomes. Major role for the cytochrome P4503A (CYP3A) subfamily. *Drug Metab Dispos* 1997; **25**: 623-630 [PMID: 9152603]

15 **Sugimoto M**, Uotani T, Sahara S, Ichikawa H, Yamade M, Sugimoto K, Furuta T. Efficacy of tailored Helicobacter pylori eradication treatment based on clarithromycin susceptibility and maintenance of acid secretion. *Helicobacter* 2014; **19**: 312-318 [PMID: 24690010 DOI: 10.1111/hel.12128]

16 **Murakami K**, Sakurai Y, Shiino M, Funao N, Nishimura A, Asaka M. Vonoprazan, a novel potassium-competitive acid blocker, as a component of first-line and second-line triple therapy for Helicobacter pylori eradication: a phase III, randomised, double-blind study. *Gut* 2016; 65: 1439-1446 [PMID: 26935876 DOI: 10.1136/gutjnl-2015-311304]

17 **Suzuki S**, Gotoda T, Kusano C, Iwatsuka K, Moriyama M. The Efficacy and Tolerability of a Triple Therapy Containing a Potassium-Competitive Acid Blocker Compared With a 7-Day PPI-Based Low-Dose Clarithromycin Triple Therapy. *Am J Gastroenterol* 2016; **111**: 949-956 [PMID: 27185079 DOI: 10.1038/ajg.2016.182]

P- Reviewer: Abadi ATB, Engin AB, Kadayifci A, Murakami K, Slomiany BL, Siavoshi F **S- Editor**: Qi Y **L- Editor**: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i4.556

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 556-563
ISSN 2150-5349 (online)

ORIGINAL ARTICLE

Observational Study

Family history and disease outcomes in patients with Crohn's disease: A comparison between China and the United States

Pei-Qi Wang, Jun Hu, Elie S Al Kazzi, Eboselume Akhuemonkhan, Min Zhi, Xiang Gao, Raquel Holand de Paula Pessoa, Sami Ghazaleh, Tuhina Cornelius, Suhel Abbas Sabunwala, Shadi Ghadermarzi, Kartikeya Tripathi, Mark Lazarev, Pin-Jin Hu, Susan Hutfless

Pei-Qi Wang, Eboselume Akhuemonkhan, Shadi Ghadermarzi, Mark Lazarev, Susan Hutfless, Department of Medicine, Division of Gastroenterology and Hepatology, Johns Hopkins University, Baltimore, MD 21287, United States

Jun Hu, Guangdong provincial Key Laboratory of Colorectal and Pelvic Floor Diseases, the Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou 510655, Guangdong Province, China

Jun Hu, Min Zhi, Xiang Gao, Pin-Jin Hu, Department of Gastroenterology, the Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou 510655, Guangdong Province, China

Elie S Al Kazzi, Department of Medicine, MedStar Health Internal Medicine, Baltimore, MD 21287, United States

Raquel Holand de Paula Pessoa, Department of Health and Sport Sciences, Federal University of Acre, Rio Branco, Acre 69915-900, Brazil

Sami Ghazaleh, Department of Medicine, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus 00972, Palestine

Tuhina Cornelius, Suhel Abbas Sabunwala, Ravindra Nath Tagore Medical College, Udaipur, Rajasthan 313001, India

Kartikeya Tripathi, St Vincent Hospital, Worcester, MA 01608, United States

Susan Hutfless, Department of Epidemiology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD 21205, United States

Author contributions: Wang PQ participated in study design, analyzed and interpreted the data, and drafted the initial manuscript; Hutfless S as the principle investigator, supervised and advised the study; Hu J, Al Kazzi ES, Akhuemonkhan E, Zhi M, Gao X, de Paula Pessoa RH, Ghazaleh S, Cornelius T, Sabunwala SA, Ghadermarzi S and Tripathi K participated in recruitment of study participants; all authors participated in reviewing and revising the manuscript.

Supported by (in part) Johns Hopkins Institute for Clinical and Translational Research, No. UL1TR001079.

Institutional review board statement: The study was reviewed and approved by the Institutional Review Boards at Johns Hopkins Medicine and the Institutional Review Boards at the Sixth Affiliated Hospital of Sun Yat-sen University.

Informed consent statement: All study participants provided consent.

Conflict-of-interest statement: There are no conflicts of interest to report.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

Correspondence to: Susan Hutfless, PhD, Assistant Professor, Director, Department of Medicine, Division of Gastroenterology and Hepatology, Johns Hopkins University, 600 N Wolfe Street, Blalock 449, Baltimore, MD 21287, United States. shutfle1@jhmi.edu
Telephone: +1-410-5020194

Received: June 6, 2016
Peer-review started: June 12, 2016
First decision: July 20, 2016
Revised: August 5, 2016
Accepted: September 13, 2016

Article in press: September 15, 2016
Published online: November 6, 2016

Abstract

AIM

To investigate the differences in family history of inflammatory bowel disease (IBD) and clinical outcomes among individuals with Crohn's disease (CD) residing in China and the United States.

METHODS

We performed a survey-based cross-sectional study of participants with CD recruited from China and the United States. We compared the prevalence of IBD family history and history of ileal involvement, CD-related surgeries and IBD medications in China and the United States, adjusting for potential confounders.

RESULTS

We recruited 49 participants from China and 145 from the United States. The prevalence of family history of IBD was significantly lower in China compared with the United States (China: 4.1%, United States: 39.3%). The three most commonly affected types of relatives were cousin, sibling, and parent in the United States compared with child and sibling in China. Ileal involvement (China: 63.3%, United States: 63.5%) and surgery for CD (China: 51.0%, United States: 49.7%) were nearly equivalent in the two countries.

CONCLUSION

The lower prevalence of familial clustering of IBD in China may suggest that the etiology of CD is less attributed to genetic background or a family-shared environment compared with the United States. Despite the potential difference in etiology, surgery and ileal involvement were similar in the two countries. Examining the changes in family history during the continuing rise in IBD may provide further insight into the etiology of CD.

Key words: Crohn's disease; Family history; Disease outcome; Inflammatory bowel disease; Epidemiology; genetics; Environment; Medication; Surgery

Core tip: Crohn's disease (CD) diagnoses are increasing in Asia. While family history of inflammatory bowel disease (IBD) is recognized as the strongest independent risk factor for CD in western populations, it is unknown if family history plays a role in Asians. This study compares the prevalence of IBD family history, the relationships of affected relatives, and CD-related outcomes such as ileal involvement, surgery, and medication use between China and the United States.

Wang PQ, Hu J, Al Kazzi ES, Akhuemonkhan E, Zhi M, Gao X, de Paula Pessoa RH, Ghazaleh S, Cornelius T, Sabunwala SA, Ghadermarzi S, Tripathi K, Lazarev M, Hu PJ, Hutfless S. Family history and disease outcomes in patients with Crohn's disease: A comparison between China and the United States. *World J Gastrointest Pharmacol Ther* 2016; 7(4): 556-563 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i4/556.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i4.556

INTRODUCTION

Crohn's disease (CD) is a form of inflammatory bowel disease (IBD), featured by chronic inflammation, most frequently affecting the terminal ileum and colon, but can also involve other portions of the gastrointestinal (GI) tract from mouth to anus[1]. The first mention of CD appeared in the literature in 1932 in a publication by Burrill Crohn reporting on a subacute or chronic necrotizing and cicatrizing inflammatory disease of the terminal ileum affecting mainly young adults in New York city[2]. Incidence rates of CD were first published in 1965, reporting an estimated 1.3 cases per 100000 person-years during 1955 to 1963 in Scotland[3]. Since then, the incidence rate of CD in western countries has shown a significant increasing trend, and the annual incidence rates between 1991 to 2008 were estimated to range from 10.6 to 29.3 per 100000 in North America, Europe, and Australia[4]. In contrast, the CD incidence rate in Asia was first published in 1974, reporting 0.04 cases per 100000 person-years in Singapore during 1965-1970[5]. Similar to western countries, the incidence of CD has increased over time. The estimated annual CD incidence rate (per 100000 person-years) based on population-based longitudinal cohorts in Hong Kong[6], Japan[7], and South Korea[8] has increased from 0.1-0.6 in 1986-1992 to 1.0-1.3 in 1998-2005.

In China, CD was first documented in the literature in 1960s and the incidence is assumed to be increasing based on single hospital-based studies. The current estimated incidence is 0.1 to 1.3 cases per 100000 person-years[9]. The proposed increase in incidence has been attributed to environmental exposures, such as industrialization and urbanization of the society, westernization of lifestyle and dietary habits, because of the parallelism of their occurrence[4,10,11].

The lower incidence of CD may be due in part to differences in genetic risk factors. Polymorphisms of the *NOD2/CARD15* gene have been found to account for up to 20% of CD in Caucasian[12] populations[13], but there have not been consistent findings of such association in China[14-17].

Family history is a window to both the genetic background and shared environment. Having a relative with IBD is the strongest known risk factor for developing CD in western countries[18]. However, family history of IBD among Chinese CD patients has not been studied in detail. Thirteen hospital-based studies reported the prevalence of family history of IBD (any affected family

member) among Chinese CD patients to be 0% to 4.5%, although the details on the relations of family members are not known[6,19-29]. In contrast, 8% to 25% of CD patients in western countries have a blood relative with IBD[30,31], and 8% to 14.5% of CD patients have a first-degree relative with IBD[18,31].

Clinical characteristics and complication rates differ among Asians with CD. A systematic review of 151 studies suggested Asian CD patients are more likely to be male, with a higher prevalence of ileocolonic involvement and lower surgical rates than Western estimates[29,32].

We hypothesized that there would be differences in family history of IBD and clinical outcomes between CD patients in China and the United States. We targeted three aims to address our hypothesis. The first was to investigate and compare the prevalence of family history of IBD among CD patients in China and the United States. The second was to examine the distribution of relationships of the affected relatives. Third, we aimed to study the difference in prevalence of CD outcomes including ileal involvement, CD-related surgeries and use of medications.

MATERIALS AND METHODS

Study population

Patients were recruited from one source in China and two sources in the United States. In China, patients with CD seen by gastroenterologists at the IBD clinic of the 6th Affiliated Hospital of Sun Yat-sen University (SYSU), Guangdong, China, during a clinic visit from May 2014 to December 2015. This hospital is a referral center for IBD patients in the Guangdong area. In the United States, patients were recruited at Johns Hopkins University-affiliated clinics or through the participant recruitment website ResearchMatch (RM). RM is a disease-neutral, institution-neutral, online volunteer recruitment platform designed to match volunteers with researchers[33]. The Johns Hopkins participants were recruited by email, letter, flyer, or during a clinic visit between May 2015 and February 2016. Individuals who indicated that they had IBD on their ResearchMatch profiles were recruited *via* email from May 2015 to February 2016.

In China, participants were administered a survey by face-to-face interview with a healthcare professional. In the United States participants completed the survey online (bit.ly/IBD-MIMAS). The survey was created and administered using the REDCap[34] project hosted at Johns Hopkins University. The survey included questions about demographics, timing of CD diagnosis, disease location and treatment history, family history of IBD and relationship, age at diagnosis of IBD type of the affected family members (Appendix tables). Participants were included in the analysis only if they completed the family history and disease outcome modules of the survey and were aged 18 years or older at the time of survey completion. Individuals with unknown family history were excluded (1 individual from the United States who was adopted).

IBD family history assessment

Family history of IBD was classified using self-reported information on the survey. Questions were asked regarding knowledge of any family history, details on the number of relatives affected, as well as the relationship to the participant (Appendix Table 2). Any family history of IBD was defined as having one or more blood relatives diagnosed with any type of IBD. For participants who had any family history of IBD, they were further subdivided as having first-degree relative family history if they had at least one parent, sibling or child with IBD.

Crohn's disease related outcomes assessment

The primary clinical outcome was ileal involvement. History of ileal involvement was determined by self-report (Appendix Table 3). Secondary outcomes included self-reported history of CD-related surgery, and ever use of IBD-related medications including steroids, immunomodulators, and biologics (Appendix Table 4).

Potential confounding factors

Participants self-reported sex, date of birth, date of CD diagnosis, number of siblings, number of children, and smoking status at CD diagnosis (Appendix Tables 1 and 2). Duration of disease was defined as the period of time from the date of clinical diagnosis to the date of survey completion. Smoking status at the time of diagnosis was categorized as current, former, and never smoker. Participants with more family members and longer disease duration are more likely to have a family member with IBD and the family size and duration of disease differed between countries making these factors potential confounders. Similarly, individuals with longer disease duration had greater potential to have ileal disease detected or medication changes.

Statistical analysis

We compared the demographic differences between China and the United States using frequencies for categorical variables and median and range for continuous variables. Frequency distributions were compared using Fisher's exact tests and medians were compared with Mood's median tests. We created a figure similar to a pedigree but for the entire population to examine the familial relationships between countries. We used multivariable logistic regression models to compare the prevalence of family history between China and the United States, adjusting for the total number of siblings and children and the duration of disease at the time of survey. We used separate multivariable logistic regression models to compare the difference in the outcomes between countries adjusting for sex, smoking status at CD diagnosis, age at diagnosis older than 40 years *vs* less, and disease duration. We conducted all the statistical analysis in SAS® 9.4 (SAS Institute Inc., Cary, NC, United States).

Table 1 Demographics of Crohn's disease patients in China and the United States recruited 2014-2016

	China (n = 49)	United States (n = 145)	P value
Age at clinical diagnosis (yr)	26.6 (13.1-46.7)	25.9 (5.1-73.1)	0.87
Age at survey completion (yr)	29.5 (19.2-49.9)	43.4 (18.3-82.7)	< 0.0001
Duration of disease at time of survey (yr)	2.2 (0.0-12.7)	12.4 (0.0-55.4)	< 0.0001
Calendar year of diagnosis	2013 (2003-2015)	2003 (1960-2015)	< 0.0001
Before 1969	0	1.4%	
1970-1979	0	6.2%	
1980-1989	0	9.7%	
1990-1999	0	25.5%	
2000-2009	12.2%	30.3%	
2010-2015	87.8%	26.9%	
Female (%)	42.9%	58.6%	0.06
Smoking status at diagnosis (%)			0.5
Current smoker	12.2%	16.5%[1]	
Former smoker	6.1% (n = 3)	10.80%	
Never smoker	81.6%	72.7%	
Number of siblings	2 (0-7)	2 (0-9)	0.2
0	8.2% (n = 4)	6.9%	
1	12.2%	29.7%	
2	44.9%	30.3%	
3	12.2%	18.6%	
4+	22.5%	14.5%	
Number of children	0 (0-3)	0 (0-7)	0.81
0	53.1%	51.0%	
1	26.5%	15.9%	
2	18.4%	22.8%	
3	2.0% (n = 1)	9.0%	
4+	0	1.3%	

[1]Six participants recruited in the United States did not report smoking status at diagnosis.

RESULTS

Patient demographics

We included 194 individuals with CD, 49 from China and 145 from the United States (Table 1). In the United States, 111 were recruited from the academic center and 34 from ResearchMatch. Similarities between the two countries included a median age of approximately 26 years at diagnosis and a similar median number of siblings and children. Differences between the two countries included those from China were more likely to be male; had a younger age at diagnosis and age at the time of survey completion; and were diagnosed more recently.

Family history of IBD

After adjusting for duration of disease at the time of survey and the total number of relatives, the difference in family history was statistically significant. The prevalence of any family history of IBD was markedly lower in China than the United States (China: 4.1% *vs* United States: 39.3%, adjusted P = 0.0008, Table 2). Family history of IBD in first-degree relatives was also less prevalent in China than in the United States (China: 4.1% *vs* United States: 23.5%, adjusted P = 0.01). Only two participants in China had a family history of IBD. Both had a first-degree relative affected (Figure 1).

For participants who reported any family history of IBD, the distribution of relationships of affected relatives was significantly different (Figure 1). For the two patients who had family history of IBD in China, one had a sibling affected and the other a child. In the United States, the mean number of affected relatives was 1.6 (median 1, maximum 5). The most commonly affected types of relatives were cousin (41.1%), sibling (37.5%), and parent (28.6%).

Disease outcomes

Participants from the two countries had equally high prevalence of ileal involvement (China: 63.3% *vs* United States: 63.5%, adjusted P = 0.74) and surgery (China: 51.0% *vs* United States: 49.7%, adjusted P = 0.19; Table 3). Compared with the United States, the percentage of participants that had ever used steroids or a biologic was significantly lower in China. The difference in the use of immunomodulators was of borderline statistical significance with China having greater use than the United States (China: 73.5%, United States: 61.4%, adjusted P = 0.08).

DISCUSSION

The prevalence of family history of IBD and CD related clinical outcomes were different in China and the United States. The probability that CD patients from China had any blood relative affected with IBD was only 1/10 that of the United States CD patients, despite both patient populations coming from academic medical centers with dedicated IBD clinics. The percentage of CD patients in China who had first-degree relative affected with

Table 2 Prevalence and odds ratio of having family history of inflammatory bowel disease in China *vs* the United States

	China (n = 49)	United States (n = 145)	Unadjusted OR (95%CI) P value (Reference = US)	Adjusted[1] OR (95% CI) P value (Reference = US)
Any family history of IBD (%)	4.1% (n = 2)	39.3%	0.07 (0.02-0.28) P = 0.0002	0.08 (0.02-0.34) P = 0.0008
First-degree family history of IBD (%)	4.1% (n = 2)	23.5%	0.14 (0.03-0.60) P = 0.008	0.14 (0.03-0.65) P = 0.01

[1]Adjusted for the total number of siblings and children, and duration of disease at survey completion. IBD: Inflammatory bowel disease; OR: Odds ratio.

Table 3 Prevalence and odds ratio of Crohn's disease outcomes in China *vs* the United States

Outcome	China (n = 49)	United States (n = 145)	Unadjusted OR (95%CI), P value (Reference = US)	Adjusted[1] OR (95%CI), P value (Reference = US)
Ileal involvement	63.3%	63.5%	0.99 (0.50-1.94) P = 0.98	1.14 (0.51-2.55) P = 0.74
Surgery for IBD	51.0%	49.7%	1.06 (0.55-2.02) P = 0.87	1.70 (0.77-3.75) P = 0.19
Ever steroids use	46.9%	91.0%	0.09 (0.04-0.19) P < 0.0001	0.19 (0.07-0.50) P = 0.0007
Steroids use within 3 mo of diagnosis	24.5%	46.2%	0.38 (0.18-0.78) P = 0.009	0.53 (0.22-1.25) P = 0.15
Ever immunomodulators[2]	73.5% (n = 36)	61.4% (n = 89)	1.74 (0.85-3.57) P = 0.13	2.13 (0.92-4.91) P = 0.08
6-MP/Azathioprine	88.9%	89.9%		
Methotrexate	19.4%	23.6%		
Cyclosporine	0%	6.7%		
Tacrolimus	0%	3.4%		
Ever biologics use	34.7%	73.8%	0.19 (0.09-0.38) P < 0.0001	0.09 (0.04-0.24) P < 0.0001
Ever TPN use	8.2%	21.4%	0.33 (0.11-0.98) P = 0.05	0.67 (0.19-2.38) P = 0.54
Antibiotics use within 30 d before time of survey	18.8%	15.9%	1.22 (0.52-2.87) P = 0.64	1.29 (0.47-3.55) P = 0.62

[1]Adjusted for sex, smoking status at diagnosis, age at diagnosis older than 40 years or less, and duration of disease at survey completion; [2]For ever users of immunomodulators, the percentage of use of each type of immunomodulators was calculated. IBD: Inflammatory bowel disease; 6-MP: Mercaptopurine; TPN: Total parenteral nutrition; OR: Odds ratio.

IBD was 1/6 that of the United States. Despite the differences in family history, the two countries shared high prevalence of ileal involvement and surgical history for IBD. However, CD patients in China were less likely to have ever used steroids and biologics but more likely to use immunomodulators, which might reflect differences in access to medications or practice variation.

Both the Chinese and the US estimates of family history prevalence are high compared with other studies. One possible reason is that the recruitment sites have dedicated IBD clinics with large numbers of patients referred by outside providers often because of greater disease severity, which might be associated with genetic predisposition. The higher estimates could also be due to the difference in ascertainment methods of family history. We ascertained family history through self-report on a questionnaire instead of obtaining information from physicians' notes in medical records. We believe that self-report might be able to capture more accurate information on detailed family history than medical records.

There has been much interest in investigating the characteristics of CD in Asian and western countries, but CD patients in China and the US have rarely been compared. Luo *et al*[29] compared family history of 85 Chinese and 68 American patients based on information obtained from medical records. They concluded Chinese CD patients had lower prevalence of CD family history than Americans (China 1% *vs* United States 12%, P = 0.016). One strength of our study was that we used the same questionnaire in both settings (available in English and Mandarin after back-translation confirmation) to ascertain family history during the same time period. In the comparison of family history prevalence, we adjusted for confounders such as patient's disease duration and family size.

We analyzed the distribution of relationships of affected relatives, which was rarely reported in previous studies in China. Although no individuals from China reported a cousin with IBD, this was the most commonly affected relative type in the United States. This finding

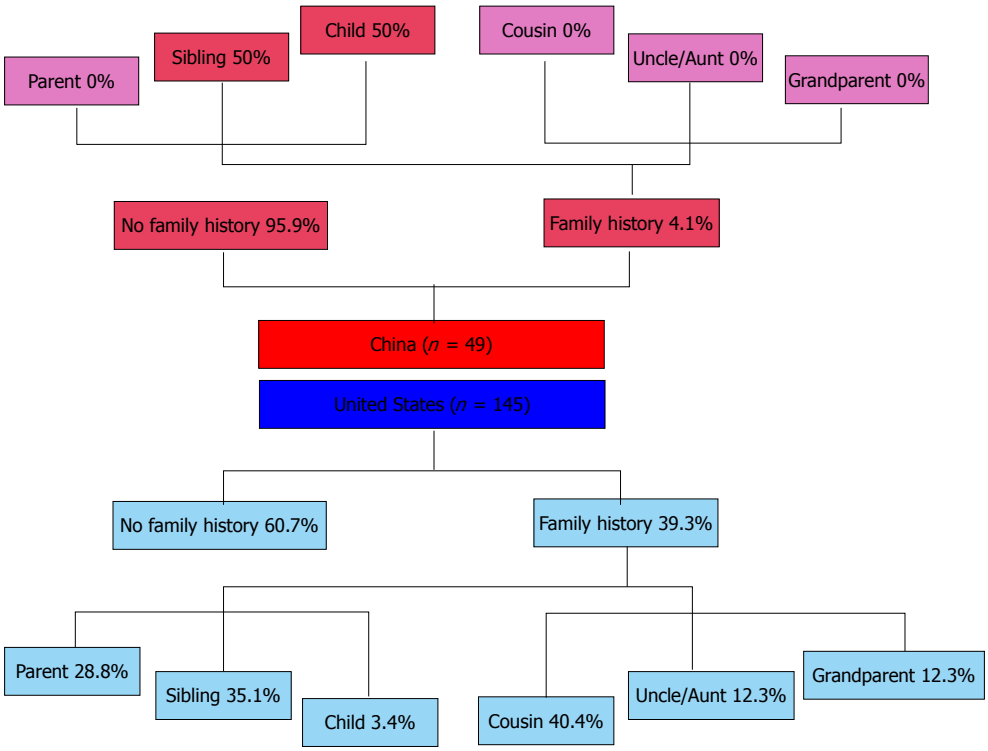

Figure 1 Relationship map of relatives with inflammatory bowel disease to patients with Crohn's disease in China and the United States.

could point to shared environmental risk between cousins and cases that could be explored further, especially among the Chinese CD participants as diagnoses of IBD are expected to increase in the coming years.

More than half of CD patients in both China and the United States had history of ileal involvement and about half had undergone surgeries to treat CD. This implies that CD patients of the two countries had similar severity of disease despite differences in family history and duration of disease. In contrast, Luo *et al*[29] found Chinese CD patients had significantly lower odds of having ileocaecum involvement compared with Americans. We were not able to examine the association of IBD family history and CD outcome by country in the present study, because the small number of individuals with IBD family history in China (n = 2) limited this analysis.

CD patients in China were less likely to have used steroids or biologics than those in the United States. Prideaux *et al*[23] had a similar finding when comparing steroid use at diagnosis and ever use of biologics between CD patients in Melbourne *vs* those in Hong Kong (steroids: Hong Kong 37.0% *vs* Melbourne 61.2%, $P < 0.001$; anti-tumor necrosis factor therapy: Hong Kong 11.0% *vs* Melbourne 39.9%, $P < 0.001$). This could be due to either real difference in the needs for steroids treatment or difference in physicians' practicing patterns. These same reasons could explain the differences in biologic use with the additional facts that biologics were introduced into China market later than the United States (2007 *vs* 1999), that only infliximab is currently available, and that most Chinese patients pay out of pocket at the United States equivalent rates limiting the population that has access to these drugs.

In conclusion, the lower prevalence of familial clustering of IBD among CD patients in China may suggest that the etiology of CD in Chinese population is to a lesser extent attributed to genetic background or family-shared environment compared with the US population. Despite the potential difference in etiology, CD patients from China were as likely to have a history of ileal involvement or have a history of surgery for IBD.

ACKNOWLEDGMENTS

We acknowledge Ludwig-Bayless Science Award for overall support of the study. We also acknowledge ResearchMatch.org. Part of the recruitment for the study included was done *via* ResearchMatch, a national health volunteer registry that was created by several academic institutions and supported by the United States National Institutes of Health as part of the Clinical Translational Science Award (CTSA) program. ResearchMatch has a large population of volunteers who have consented to be contacted by researchers about health studies for which they may be eligible.

COMMENTS

Background

Family history of inflammatory bowel disease (IBD) is the strongest risk factor for developing Crohn's disease (CD) among Western population, and it is also potentially associated with higher probability of having ileal involvement and demand for surgical treatment for CD. However, the prevalence of IBD family

history and its role in CD among Asian population has not been well studied.

Research frontiers

CD has a complex and multifactorial etiology. The roles of and interactions among genetic background, gut microbiota, diet, and autoimmunity are at the frontiers of research. Although the incidence rate of CD in Asian countries is relatively low compared with Western countries, CD incidence is increasing. This epidemiological change gives us a unique opportunity to explore the driving factors of the disease, which could increase the authors understanding of CD and ultimately lead to novel therapeutics.

Innovations and breakthroughs

Few studies compare family history and disease outcomes of Western and Asian CD patients. The few available studies are mostly based on chart review of patients' medical records from different countries and in different settings, which may be biased by the level of detail recorded within the different medical systems. The current study is innovative in that CD patients from China and United States were investigated with the same survey during the same time period Patients reported information on family history and smoking history themselves, rather than relying on medical notes.

Applications

The results of this study suggest lower prevalence of IBD familial clustering among CD patients in China as compared with the United States. This may suggest that the etiology of CD in Chinese population is to a lesser extent attributed to genetic background or family-shared environment compared with the United States population. Despite the potential difference in etiology, CD patients from China were just as likely to have a history of ileal involvement or have a history of IBD-related surgery as those from United States.

Terminology

CD is a form of IBD, featured by chronic inflammation, most frequently affecting the terminal ileum and colon, but can also involve other portions of the gastrointestinal tract from mouth to anus.

Peer-review

In the presented study the differences in family history and clinical outcomes among individuals residing in China and the United States were investigated with a survey-based cross-sectional study. The prevalence of IBD family history was significantly lower in China. It will be interesting to see if the results change as the Chinese study population is studied for a longer period of time, including both longer follow-up of the Chinese population and a larger sample size.

REFERENCES

1 **Abraham C**, Cho JH. Inflammatory bowel disease. *N Engl J Med* 2009; **361**: 2066-2078 [PMID: 19923578 DOI: 10.1056/NEJMra0804647]

2 **Crohn BB**, Ginzburg L, Oppenheimer GD. Regional ileitis: a pathologic and clinical entity. 1932. *Mt Sinai J Med* 2000; **67**: 263-268 [PMID: 10828911 DOI: 10.1001/jama.1932.02740680019005]

3 **Kyle J**, Blair DW. Epidemiology of regional enteritis in North-East Scotland. *Br J Surg* 1965; **52**: 215-217 [PMID: 14261132 DOI: 10.1002/bjs.1800520318]

4 **Molodecky NA**, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, Benchimol EI, Panaccione R, Ghosh S, Barkema HW, Kaplan GG. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology* 2012; **142**: 46-54.e42; quiz e30 [PMID: 22001864 DOI: 10.1053/j.gastro.2011.10.001]

5 **Lee SK**. Crohn's disease in Singapore. *Med J Aust* 1974; **1**: 266-269 [PMID: 4821537 DOI: 10.1016/s0016-5085(98)80100-5]

6 **Leong RW**, Lau JY, Sung JJ. The epidemiology and phenotype of Crohn's disease in the Chinese population. *Inflamm Bowel Dis* 2004; **10**: 646-651 [PMID: 15472528 DOI: 10.1097/00054725-200409000-00022]

7 **Morita N**, Toki S, Hirohashi T, Minoda T, Ogawa K, Kono S, Tamakoshi A, Ohno Y, Sawada T, Muto T. Incidence and prevalence of inflammatory bowel disease in Japan: nationwide epidemiological survey during the year 1991. *J Gastroenterol* 1995; **30** Suppl 8: 1-4 [PMID: 8563866]

8 **Yang SK**, Yun S, Kim JH, Park JY, Kim HY, Kim YH, Chang DK, Kim JS, Song IS, Park JB, Park ER, Kim KJ, Moon G, Yang SH. Epidemiology of inflammatory bowel disease in the Songpa-Kangdong district, Seoul, Korea, 1986-2005: a KASID study. *Inflamm Bowel Dis* 2008; **14**: 542-549 [PMID: 17941073 DOI: 10.1002/ibd.20310]

9 **Ye L**, Cao Q, Cheng J. Review of inflammatory bowel disease in China. *ScientificWorldJournal* 2013; **2013**: 296470 [PMID: 24348149 DOI: 10.1155/2013/296470]

10 **Ng SC**, Bernstein CN, Vatn MH, Lakatos PL, Loftus EV, Tysk C, O'Morain C, Moum B, Colombel JF. Geographical variability and environmental risk factors in inflammatory bowel disease. *Gut* 2013; **62**: 630-649 [PMID: 23335431 DOI: 10.1007/s11894-013-0313-9]

11 **Molodecky NA**, Kaplan GG. Environmental risk factors for inflammatory bowel disease. *Gastroenterol Hepatol* (N Y) 2010; **6**: 339-346 [PMID: 20567592]

12 **Hugot JP**, Chamaillard M, Zouali H, Lesage S, Cézard JP, Belaiche J, Almer S, Tysk C, O'Morain CA, Gassull M, Binder V, Finkel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel JF, Sahbatou M, Thomas G. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001; **411**: 599-603 [PMID: 11385576 DOI: 10.1038/35079107]

13 **Ogura Y**, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr RH, Achkar JP, Brant SR, Bayless TM, Kirschner BS, Hanauer SB, Nuñez G, Cho JH. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 2001; **411**: 603-606 [PMID: 11385577 DOI: 10.1038/35079114]

14 **Guo QS**, Xia B, Jiang Y, Qu Y, Li J. NOD2 3020insC frameshift mutation is not associated with inflammatory bowel disease in Chinese patients of Han nationality. *World J Gastroenterol* 2004; **10**: 1069-1071 [PMID: 15052696 DOI: 10.3748/wjg.v10.i7.1069]

15 **Lv C**, Yang X, Zhang Y, Zhao X, Chen Z, Long J, Zhang Y, Zhong C, Zhi J, Yao G, Jiang B, Zhi F. Confirmation of three inflammatory bowel disease susceptibility loci in a Chinese cohort. *Int J Colorectal Dis* 2012; **27**: 1465-1472 [PMID: 22426692 DOI: 10.1007/s00384-012-1450-6]

16 **Wang ZW**, Ji F, Teng WJ, Yuan XG, Ye XM. Risk factors and gene polymorphisms of inflammatory bowel disease in population of Zhejiang, China. *World J Gastroenterol* 2011; **17**: 118-122 [PMID: 21218092 DOI: 10.3748/wjg.v17.i1.118]

17 **Li M**, Gao X, Guo CC, Wu KC, Zhang X, Hu PJ. OCTN and CARD15 gene polymorphism in Chinese patients with inflammatory bowel disease. *World J Gastroenterol* 2008; **14**: 4923-4927 [PMID: 18756601 DOI: 10.3748/wjg.14.4923]

18 **Peeters M**, Nevens H, Baert F, Hiele M, de Meyer AM, Vlietinck R, Rutgeerts P. Familial aggregation in Crohn's disease: increased age-adjusted risk and concordance in clinical characteristics. *Gastroenterology* 1996; **111**: 597-603 [PMID: 8780562 DOI: 10.1053/gast.1996.v111.pm8780562]

19 **Li Y**, Ren J, Wang G, Gu G, Wu X, Ren H, Hong Z, Hu D, Wu Q, Li G, Liu S, Anjum N, Li J. Diagnostic delay in Crohn's disease is associated with increased rate of abdominal surgery: A retrospective study in Chinese patients. *Dig Liver Dis* 2015; **47**: 544-548 [PMID: 25840874 DOI: 10.1016/j.dld.2015.03.004]

20 **Wang XQ**, Zhang Y, Xu CD, Jiang LR, Huang Y, Du HM, Wang XJ. Inflammatory bowel disease in Chinese children: a multicenter analysis over a decade from Shanghai. *Inflamm Bowel Dis* 2013; **19**: 423-428 [PMID: 23340680 DOI: 10.1097/MIB.0b013e318286f9f2]

21 **Yi F**, Chen M, Huang M, Li J, Zhao J, Li L, Xia B. The trend in newly diagnosed Crohn's disease and extraintestinal manifestations of Crohn's disease in central China: a retrospective study of a single center. *Eur J Gastroenterol Hepatol* 2012; **24**: 1424-1429 [PMID: 22895389 DOI: 10.1097/MEG.0b013e3283583e5c]

22 **Li Y**, Zhu W, Zuo L, Zhang W, Gong J, Gu L, Cao L, Li N, Li J.

Frequency and risk factors of postoperative recurrence of Crohn's disease after intestinal resection in the Chinese population. *J Gastrointest Surg* 2012; **16**: 1539-1547 [PMID: 22555673 DOI: 10.1007/s11605-012-1902-x]

23 **Prideaux L**, Kamm MA, De Cruz P, Williams J, Bell SJ, Connell WR, Brown SJ, Lust M, Desmond PV, Chan H, Chow DK, Wu JC, Leong RW, Sung JJ, Chan FK, Ng SC. Comparison of clinical characteristics and management of inflammatory bowel disease in Hong Kong versus Melbourne. *J Gastroenterol Hepatol* 2012; **27**: 919-927 [PMID: 22098103 DOI: 10.1111/j.1440-1746.2011.06984.x]

24 **Song XM**, Gao X, Li MZ, Chen ZH, Chen SC, Hu PJ, He YL, Zhan WH, Chen MH. Clinical features and risk factors for primary surgery in 205 patients with Crohn's disease: analysis of a South China cohort. *Dis Colon Rectum* 2011; **54**: 1147-1154 [PMID: 21825896 DOI: 10.1097/DCR.0b013e318222ddc3]

25 **Chow DK**, Sung JJ, Tsoi KK, Wong VW, Wu JC, Leong RW, Chan FK. Predictors of corticosteroid-dependent and corticosteroid-refractory inflammatory bowel disease: analysis of a Chinese cohort study. *Aliment Pharmacol Ther* 2009; **29**: 843-854 [PMID: 19154567 DOI: 10.1111/j.1365-2036.2009.03944.x]

26 **Chow DK**, Sung JJ, Wu JC, Tsoi KK, Leong RW, Chan FK. Upper gastrointestinal tract phenotype of Crohn's disease is associated with early surgery and further hospitalization. *Inflamm Bowel Dis* 2009; **15**: 551-557 [PMID: 19067420 DOI: 10.1002/ibd.20804]

27 **Leong RW**, Lawrance IC, Chow DK, To KF, Lau JY, Wu J, Leung WK, Chan FK, Sung JJ. Association of intestinal granulomas with smoking, phenotype, and serology in Chinese patients with Crohn's disease. *Am J Gastroenterol* 2006; **101**: 1024-1029 [PMID: 16573779 DOI: 10.1111/j.1572-0241.2006.00503.x]

28 **Leong RW**, Armuzzi A, Ahmad T, Wong ML, Tse P, Jewell DP, Sung JJ. NOD2/CARD15 gene polymorphisms and Crohn's disease in the Chinese population. *Aliment Pharmacol Ther* 2003; **17**: 1465-1470 [PMID: 12823148 DOI: 10.1046/j.1365-2036.2003.01607.x]

29 **Luo CH**, Wexner SD, Liu QS, Li L, Weiss E, Zhao RH. The differences between American and Chinese patients with Crohn's disease. *Colorectal Dis* 2011; **13**: 166-170 [PMID: 19878519 DOI: 10.1111/j.1463-1318.2009.02094.x]

30 **Roth MP**, Petersen GM, McElree C, Vadheim CM, Panish JF, Rotter JI. Familial empiric risk estimates of inflammatory bowel disease in Ashkenazi Jews. *Gastroenterology* 1989; **96**: 1016-1020 [PMID: 2925048 DOI: 10.1016/0016-5085(89)91618-1]

31 **Weterman IT**, Peña AS. Familial incidence of Crohn's disease in The Netherlands and a review of the literature. *Gastroenterology* 1984; **86**: 449-452 [PMID: 6693011]

32 **Thia KT**, Loftus EV, Sandborn WJ, Yang SK. An update on the epidemiology of inflammatory bowel disease in Asia. *Am J Gastroenterol* 2008; **103**: 3167-3182 [PMID: 19086963 DOI: 10.1111/j.1572-0241.2008.02158.x]

33 **Harris PA**, Scott KW, Lebo L, Hassan N, Lightner C, Pulley J. ResearchMatch: a national registry to recruit volunteers for clinical research. *Acad Med* 2012; **87**: 66-73 [PMID: 22104055 DOI: 10.1097/ACM.0b013e31823ab7d2]

34 **Harris PA**, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009; **42**: 377-381 [PMID: 18929686 DOI: 10.1016/j.jbi.2008.08.010]

P- Reviewer: Ozen H, Vradelis S **S- Editor**: Gong ZM **L- Editor**: A
E- Editor: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i4.564

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 564-571
ISSN 2150-5349 (online)

ORIGINAL ARTICLE

Observational Study

Good adherence to mediterranean diet can prevent gastrointestinal symptoms: A survey from Southern Italy

Francesco Paolo Zito, Barbara Polese, Letizia Vozzella, Antonietta Gala, Daniela Genovese, Viviana Verlezza, Fabiana Medugno, Antonello Santini, Luigi Barrea, Martina Cargiolli, Paolo Andreozzi, Giovanni Sarnelli, Rosario Cuomo

Francesco Paolo Zito, Barbara Polese, Letizia Vozzella, Antonietta Gala, Daniela Genovese, Viviana Verlezza, Fabiana Medugno, Martina Cargiolli, Paolo Andreozzi, Giovanni Sarnelli, Rosario Cuomo, Department of Surgery, Gastroenterology and Endocrinology, University of Naples "Federico II", Naples, 80131 Campania, Italy

Antonello Santini, Department of Pharmacy, University of Naples "Federico II", Naples, 80131 Campania, Italy

Luigi Barrea, IOS s.r.l and COLEMAN S.p.A., Naples, 80131 Campania, Italy

Author contributions: Sarnelli G and Cuomo R ideated the project; Polese B, Gala A, Genovese D, Verlezza V and Medugno F collected the data; Zito FP and Vozzella L wrote the first draft of the manuscript; Santini A, Barrea L, Cargiolli M and Andreozzi P critically reviewed the manuscript; all authors approved the final version of the manuscript.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: There are no conflicts of interest to report.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

Correspondence to: Rosario Cuomo, MD, Associate Professor, Department of Surgery, Gastroenterology and Endocrinology, University of Naples "Federico II", Via S. Pansini 5, Building 6, Naples, 80131 Campania, Italy. rcuomo@unina.it
Telephone: +39-81-7463892
Fax: +39-339-7221830

Received: June 1, 2016
Peer-review started: June 2, 2016
First decision: July 4, 2016
Revised: July 19, 2016
Accepted: September 13, 2016
Article in press: September 15, 2016
Published online: November 6, 2016

Abstract

AIM

To evaluate how different levels of adherence to a mediterranean diet (MD) correlate with the onset of functional gastrointestinal disorders.

METHODS

As many as 1134 subjects (598 M and 536 F; age range 17-83 years) were prospectively investigated in relation to their dietary habits and the presence of functional gastrointestinal symptoms. Patients with relevant chronic organic disease were excluded from the study. The Mediterranean Diet Quality index for children and adolescents (KIDMED) and the Short Mediterranean Diet Questionnaire were administered. All subjects were grouped into five categories according to their ages: 14-24 years; 25-34; 35-49; 50-64; above 64.

RESULTS

On the basis of the Rome Ⅲ criteria, our population consisted of 719 (63.4%) individuals who did not meet the criteria for any functional disorder and were classified as controls (CNT), 172 (13.3%) patients meeting

criteria for prevalent irritable bowel syndrome (IBS), and 243 (23.3%) meeting criteria for prevalent functional dyspepsia (FD). A significantly lower adherence score in IBS (0.57 ± 0.23, $P < 0.001$) and FD (0.56 ± 0.24, $P < 0.05$) was found compared to CNT (0.62 ± 0.21). Females with FD and IBS exhibited significantly lower adherence scores (respectively 0.58 ± 0.24, $P < 0.05$ and 0.56 ± 0.22, $P < 0.05$) whereas males were significantly lower only for FD (0.53 ± 0.25, $P < 0.05$). Age cluster analyses showed a significantly lower score in the 17-24 years and 25-34 year categories for FD (17-24 years: 0.44 ± 0.21, $P < 0.001$; 25-34 years: 0.48 ± 0.22, $P < 0.05$) and IBS (17-24 years: 0.45 ± 0.20, $P < 0.05$; 24-34 years: 0.44 ± 0.21, $P < 0.001$) compared to CNT (17-24 years: 0.56 ± 0.21; 25-34 years: 0.69 ± 0.20).

CONCLUSION

Low adherence to MD may trigger functional gastrointestinal symptoms, mainly in younger subjects. Moreover, with increasing age, patients tend to adopt dietary regimens closer to MD.

Key words: Mediterranean diet; Irritable bowel syndrome; Dietary regimen; Functional gastro-intestinal disorders; Functional dyspepsia

Core tip: Diet seems to be one of the most important triggering factor for functional gastrointestinal disorders (FGID). In fact, patients suffering from irritable bowel syndrome or functional dyspepsia frequently report the onset of symptoms after a meal or the consumption of certain types of foods. The mediterranean (MD) diet is universally considered a health-promoting dietary regimen, since populations adopting this type of diet exhibit a lower rate of major cardiovascular, neoplastic, metabolic morbidity and mortality. Emerging evidence supports a beneficial effect of MD on the gastrointestinal tract, although the association between a high adherence to MD and FGID symptoms is still unclear.

Zito FP, Polese B, Vozzella L, Gala A, Genovese D, Verlezza V, Medugno F, Santini A, Barrea L, Cargiolli M, Andreozzi P, Sarnelli G, Cuomo R. Good adherence to mediterranean diet can prevent gastrointestinal symptoms: A survey from Southern Italy. *World J Gastrointest Pharmacol Ther* 2016; 7(4): 564-571 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i4/564.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i4.564

INTRODUCTION

The Mediterranean diet (MD) is considered as a complex set of eating habits adopted by peoples in countries bordering the Mediterranean Sea. It includes high consumption of olive oil, fiber-rich foods, milk or dairy products and low consumption of meat or meat-based products[1]. In the last few years, this dietary regimen has been universally proposed as a health-protective diet since populations who have adopted it show a remarkable reduction in all-cause mortality[2], especially from cardiovascular diseases and cancer, compared to the United States or Northern European countries[3]. The beneficial effects of MD may be attributed to the large consumption of antioxidants contained in raw fruit and vegetables or to a reduced consumption of saturated fats.

A healthy diet is also important to preserve gastrointestinal balance. Indeed, some alimentary regimens or even some meals are able to trigger symptoms in individuals with functional gastrointestinal disorders (FGIDs), such as functional dyspepsia (FD) or irritable bowel syndrome (IBS). FGIDs are highly prevalent chronic disorders occurring in the absence of any organic etiology[4]. They are often associated with psychological co-morbidities, such as depression or anxiety, which negatively influence the quality of life and cause absence from work or school with a consequently relevant economic burden. The etiology of FGID is thought to be multifactorial, and includes altered brain-gut interactions, genetic predispositions, and/or environmental factors, such as diet[5,6]. Actually, food - especially some types of foods - seems to be the most important triggering factor. Up to 75% of adults with IBS report that diets high in carbohydrates, fatty foods, coffee, alcohol, and hot spices worsen their GI symptoms[7]. Dyspeptic patients usually report that meal size, eating patterns, caloric intake as well as nutrient composition-lipid content in particular-strongly influence the onset of dyspeptic symptoms. Several mechanisms have been hypothesized to account for the association between food and gastrointestinal symptoms, *i.e.,* influence of food on microbiota composition; luminal distension related to gas production from bacterial fermentation; direct effects of some nutrients on GI sensitivity and motility[8,9].

Emerging evidence[4,10] supports the hypothesis that MD may be beneficial also for functional gastrointestinal disorders. Against this background, the aim of our study was to evaluate how different levels of adherence to MD correlate with the onset of functional gastrointestinal disorders, such as irritable bowel syndrome or functional dyspepsia.

MATERIALS AND METHODS

Population features

Our study was performed in Campania, a region of Southern Italy between May 2011 and April 2012. As many as 1134 subjects (598 M and 536 F; age range 17-83 years) without a prior abdominal surgery or relevant organic chronic disease, on the basis of an accurate history taking, were investigated in relation to their dietary habits; of these, 114 were outpatients of the "Federico Ⅱ" University Hospital (Naples), 401 had been surveyed during an open event for health prevention

Table 1 KidMed test to assess adherence to the Mediterranean diet

Scoring	
+1	Has one fruit or fruit juice every day
+1	Has a second fruit every day
+1	Has fresh or cooked vegetables regularly once a day
+1	Has fresh or cooked vegetables more than once a day
+1	Consumes fish regularly (at least 2-3 times per week)
-1	Goes more than once a week to a fast-food (hamburger) restaurant
+1	Likes pulses and eats them more than once a week
+1	Consumes pasta or rice almost every day (5 or more times per week)
+1	Has cereals or grains for breakfast
+1	Consumes nuts regularly (at least 2-3 times per week)
+1	Uses olive oil at home
-1	Skips breakfast
+1	Has a dairy product for breakfast (yoghurt, milk, *etc.*)
-1	Has commercially baked goods or pastries for breakfast
+1	Has two yoghurt and/or some cheese (40 g) daily
-1	Has sweets and candy several times every day

held in the city of Caserta; 619 were evaluated during a health care program in a secondary school in Naples.

Clinical questionnaire

All subjects were administered the Rome Ⅲ questionnaire to assess the presence of upper or lower gastrointestinal symptoms: 719 patients did not report any relevant gastrointestinal symptoms (controls), 172 met the criteria for prevalent irritable bowel syndrome and 243 for prevalent functional dyspepsia. Controls, IBS and FD patients were classified according to age: 14-24 years old, 25 to 34 years old, 35 to 49 years old, 50 to 64 years old and, finally, above 64 years of age.

Adherence to MD questionnaire

In addition, all subjects completed a standardized food frequency questionnaire (FFQ) evaluating their adherence to the Mediterranean Diet model. In order to explore the adherence to MD in different age categories, we used two different questionnaire: The Mediterranean Diet Quality index for children and adolescents (KIDMED) was administered to participants whose ages ranged from 17 to 24 years, and the Short Mediterranean Diet Questionnaire to those older than 24 years. Both questionnaires were developed according to the principles behind Mediterranean dietary patterns.

The KIDMED index is based on a 16-question test with scores ranging from 0 to 12; questions denoting a negative connotation compared to the Mediterranean diet model were assigned a value of -1, those with positive aspects were assigned +1 (Table 1). The score obtained was classified into three levels of adherence to the Mediterranean dietary model: > 8 optimal; 4-7 intermediate, ≤ 3 very low adherence[11,12].

The Short Mediterranean Diet Questionnaire derives from a larger validated FFQ including 136 items; it is based on a 9-question test assessing the frequency of consumption for nine typical food categories. A specific frequency score was assigned to each food to attribute +1 only when food consumption satisfied the criteria (Table 2). The final composite score ranged from 0 to 9 and, as for the KIDMED index, it was classified into three levels of adherence to the Mediterranean diet: > 7 optimal; 4-6 intermediate, ≤ 3 very low adherence[13].

Table 2 Short mediterranean diet questionnaire

Scoring	
+1	Olive Oil (> 1 spoon/d)
+1	Fruit (≥ 1 serving/d)
+1	Vegetables or Salads (≥ 1 serving/d)
+1	Fruit (≥ 1 serving/d) and vegetable (≥ 1 serving/d)
+1	Legumes (≥ 2 serving/d)
+1	Fish (≥ 3 serving/d)
+1	Wine (≥ 1 glass/d)
+1	Meat (≤ 1 serving/d)
+1	White bread (≤ 1 serving/d) and rice (≤ 1 serving/wk) or whole-grain bread (> 5/wk)

Since two different types of questionnaires were used, the final index ranged from 0 to 9 or from 0 to 12; for this reason, to equalize the score obtained for each patient, final scores were divided by the maximum result achievable depending on the questionnaire employed.

Statistical analysis

Data were evaluated using SPSS for Windows version 13 (SPSS Inc., Chicago, IL, United States). Results were analyzed using *t*-test and ANOVA. Differences were considered significant when the *P* value was below 0.05. A multinomial regression model was used to analyze whether the level of adherence to MD was associated with functional gastrointestinal disorders, age category, sex or BMI. Results are reported as adjusted odds ratio with 95%CI; *P*-values below 0.05 were considered as significant. The output of the multinomial logistic regression is presented as a set of two dichotomous logistic regressions that provide a pairwise comparison of the phenotypes, as follows: High *vs* low adherence to MD and high *vs* intermediate adherence to MD. The oldest group, controls, individuals presenting with third grade obesity, and females were used as reference category in the multinomial regression model.

RESULTS

Overall, 1134 subjects were investigated as to presence of both upper and lower gastrointestinal symptoms and were thus classified into the following groups: Controls, functional dyspepsia or irritable bowel syndrome. Thereafter, they were all stratified by level of adherence to the Mediterranean diet (low, intermediate and high adherence) and age category (17-24; 25-34; 35-49; 50-65; > 65 years) (Table 3).

Table 3 Distribution by level of adherence to the Mediterranean diet (low, intermediate and high adherence) and age category (17-24; 25-34; 35-49; 50-65; > 65 years) *n* (%)

		Level of adherence to MD		
	Age category (yr)	Low	Intermediate	High
CNT	17-24	61 (20.5)	158 (53.0)	79 (26.5)
	25-34	11 (11.8)	55 (59.1)	27 (29)
	35-49	14 (9.9)	91 (64.5)	36 (25.5)
	50-65	22 (16.4)	89 (66.4)	23 (17.2)
	> 65	3 (5.7)	37 (69.8)	13 (21.9)
IBS	17-24	10 (34.5)	17 (56.8)	2 (6.9)
	25-34	6 (25)	18 (75)	0
	35-49	13 (28.3)	27 (58.7)	6 (13)
	50-65	4 (9.8)	29 (70.7)	8 (19.5)
	> 65	5 (15.6)	20 (62.5)	7 (21.9)
FD	17-24	38 (39.2)	43 (44.3)	16 (16.5)
	25-34	7 (20.6)	25 (73.5)	2 (5.9)
	35-49	7 (14)	34 (68)	9 (18)
	50-65	7 (15.2)	25 (54.3)	14 (30.4)
	> 65	4 (25)	6 (37.5)	6 (37.5)

MD: Mediterranean diet; IBS: Irritable bowel syndrome; FD: Functional dyspepsia.

Univariate analyses

A significantly lower adherence score among FD (0.56 ± 0.24, $P < 0.001$) and IBS (0.57 ± 0.23, $P < 0.05$) was found compared to CNT (0.62 ± 0.21) (Figure 1A). When the data were stratified by gender, females with FD and IBS exhibited significantly lower adherence scores (respectively 0.58 ± 0.24, $P < 0.05$ and 0.56 ± 0.22, $P < 0.05$) as compared to CNT (0.64 ± 0.22), whereas in males they were significantly lower only for FD (0.53 ± 0.25, $P < 0.05$) compared to CNT (0.61 ± 0.21) (Figure 1B and C). Age cluster adherence scores were significantly lower in the 17-24 years and 25-34 years categories for FD (17-24 years: 0.44 ± 0.21, $P < 0.001$; 25-34 years 0.58 ± 0.22, $P < 0.05$) and IBS (17-24 years: 0.45 ± 0.20, $P < 0.05$; 25-34 years: 0.49 ± 0.21, $P < 0.001$) compared to CNT (17-24 years: 0.56 ± 0.21; 25-34 years: 0.69 ± 0.20). In the 35-49 year category, the adherence score was significantly lower only in the IBS group (0.56 ± 0.24, $P < 0.001$) compared to CNT (0.69 ± 0.19). No differences were observed between the other age clusters (Figure 2). However, when stratified by gender, in the 17-24 year category, a lower adherence score was confirmed for females with FD (0.48 ± 0.22, $P < 0.05$) and IBS (0.44 ± 0.17, $P < 0.05$) *vs* CNT (0.57 ± 0.22), whereas the male group presented a significantly lower adherence score only for FD (0.39 ± 0.19, $P < 0.001$) compared to CNT (0.55 ± 0.20). At the same time, stratifying the 25-34 year category by gender, only IBS males (0.46 ± 0.20, $P < 0.05$) and females (0.50 ± 0.21, $P < 0.05$) had a significantly lower mean adherence score compared to controls (0.70 ± 0.18 and 0.68 ± 0.21). Finally, stratifying other age categories by gender, only IBS females in the 35-49 year group (0.55 ± 0.24, $P < 0.05$) exhibited a significantly lower mean adherence score compared to controls (0.69 ± 0.19). No differences were observed for other gender groups (Table 4).

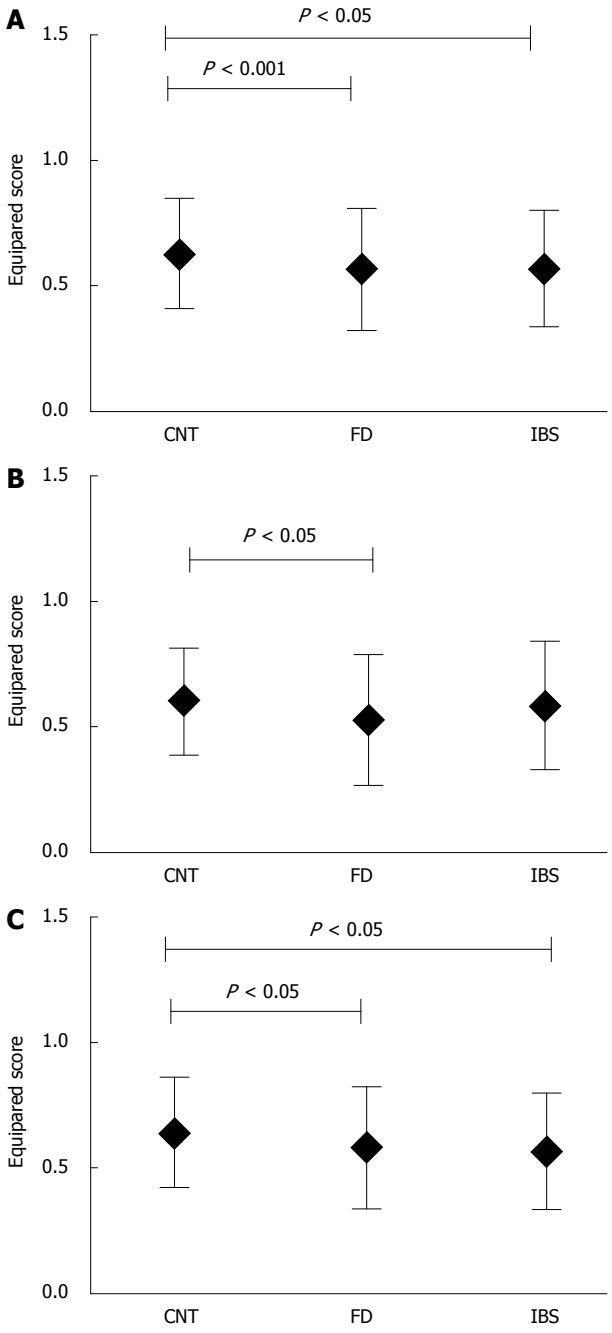

Figure 1 Equalized mean score to mediterranean diet in CNT, functional dyspepsia and irritable bowel syndrome patients. A: All subjects show a significantly lower adherence score in FD and irritable bowel syndrome (IBS) compared to CNT; B: In the male group only FD exhibited significantly lower adherence scores compared to CNT; C: In females a significant difference for FD and IBS compared to CNT was confirmed.

Multivariate analyses

Both low (OR = 3.24, 95%CI: 1.73-6.08, $P < 0.0001$) and intermediate (OR = 1.91, 95%CI: 1.14-3.22, $P < 0.05$) levels of adherence to MD were independently associated with the presence of IBS. However only FD

Table 4 Mean adherence score sorted by gender and cluster

Age range (yr)	Sex	CNT	FD	IBS
		Mean ± SD	Mean ± SD	Mean ± SD
17-24	Tot	0.56 ± 0.21	0.44[b] ± 0.21	0.45[a] ± 0.20
	M	0.55 ± 0.20	0.39[b] ± 0.19	0.46 ± 0.26
	F	0.57 ± 0.22	0.48[a] ± 0.22	0.44[a] ± 0.17
25-34	Tot	0.69 ± 0.20	0.58[a] ± 0.22	0.49[b] ± 0.21
	M	0.70 ± 0.18	0.60 ± 0.24	0.46[a] ± 0.20
	F	0.68 ± 0.21	0.56 ± 0.17	0.50[a] ± 0.21
35-49	Tot	0.69 ± 0.19	0.64 ± 0.20	0.56 ± 0.24
	M	0.70 ± 0.20	0.74 ± 0.15	0.65 ± 0.21
	F	0.69 ± 0.19	0.61 ± 0.21	0.55[a] ± 0.24
50-64	Tot	0.62 ± 0.20	0.69 ± 0.24	0.66 ± 0.18
	M	0.60 ± 0.19	0.65 ± 0.21	0.75 ± 0.26
	F	0.63 ± 0.20	0.70 ± 0.25	0.64 ± 0.19
> 64	Tot	0.71 ± 0.18	0.67 ± 0.28	0.64 ± 0.24
	M	0.74 ± 0.12	0.70 ± 0.32	0.69 ± 0.26
	F	0.69 ± 0.21	0.64 ± 0.25	0.62 ± 0.23

[a]$P < 0.05$ *vs* CNT; [b]$P < 0.001$ *vs* CNT. FD: Functional dyspepsia; IBS: Irritable bowel syndrome.

Table 5 Multivariate analyses

	Low adherence			Intermediate adherence		
	OR	95%CI	*P* value	OR	95%CI	*P* value
FD	2.42	1.47-3.99	< 0.0001	1.34	0.88-2.03	NS
IBS	3.24	1.73-6.08	< 0.0001	1.91	1.13-3.22	< 0.05
17-24 yr	4.65	2.00-10.81	< 0.0001	1.78	0.13-24.96	NS

IBS: Irritable bowel syndrome; FD: Functional dyspepsia.

(OR = 2.42, 95%CI: 1.47-3.99, $P < 0.0001$) and the youngest age category (OR = 4.65, 95%CI: 2.00-10.81, $P < 0.0001$) were associated with a low level of adherence to MD (Table 5, Figure 3A and B).

DISCUSSION

The present study provides evidence that the MD adherence score is significantly lower in subjects with GI symptoms than in asymptomatic subjects, showing an inverse relationship between adherence to MD and prevalence of gastrointestinal symptoms. Moreover, among FGID subjects, only younger age categories (17-24 and 25-34 years.) were associated with lower adherence to MD compared to controls, whereas for older people, no differences were observed between symptomatic and asymptomatic subjects.

Many factors, such as age, gender, nationality, socio-economic condition, may influence dietary adherence to MD[14]. Our results confirm the data from several studies exploring dietary habits in different European countries, widely demonstrating that young people exhibit the lowest level of adherence to MD[12,15,16]. In Italy, a lower adherence to MD has also been confirmed among young people, particularly for those coming from northern region. As shown by Noale *et al*[17] of the Italian adolescents examined in their study, only

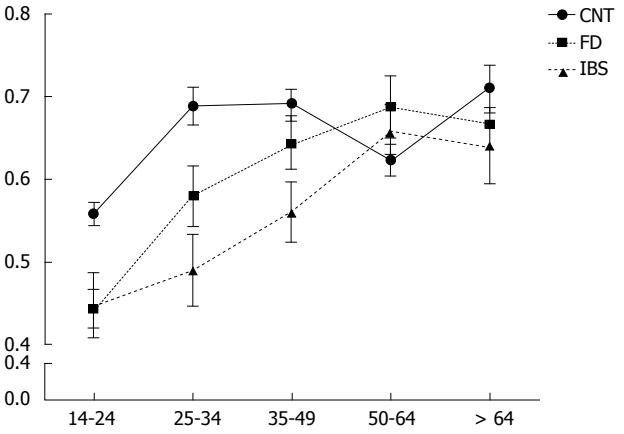

Figure 2 Distribution of equalized mean adherence score to mediterranean diet in CNT, functional dyspepsia and irritable bowel syndrome stratifying for age clusters. In the 17-24 years and 25-34 years groups both FD and IBS exhibited significantly lower mean adherence score compared to CNT. In the 35-49 years group only IBS patients exhibited a significantly lower mean score compared to CNT. No differences were observed in other age categories (data ± SEM are shown). FD: Functional dyspepsia; IBS: Irritable bowel syndrome.

14% exhibited a high adherence to the Mediterranean diet, and 47% a moderate adherence. In fact, not only did the mean daily calorie intake exceed the dietary requirements recommended by "Nutrient Intake Goal for the Italian population" or LARN tables, but they also observed an increase in the intake of saturated fats and proteins as compared to carbohydrates, fruit or vegetables. This may be a consequence of the frequent use of easy-to-prepare and ready-to-use products or fast foods, which are characterized by a lower nutritional quality due to the addition of sugar and saturated fats[18].

Unbalanced diets adopted by young people may contribute to the onset of gastrointestinal symptoms in patients suffering from functional dyspepsia or irritable bowel syndrome[6,8]. Patients with FGID exhibit gastrointestinal hypersensitivity and exaggerated reflex after ingestion of lipids. Moreover, fats influence gastric activity, by delaying gastric emptying and promoting relaxation of the fundus in healthy subjects as well as in dyspeptic patients[7,19,20]. However, fundus relaxation appears impaired in functional dyspepsia since, following lipid infusion, the discomfort threshold observed in FD patients appears to be lower than in controls[21]. In clinical practice, all these disturbances following lipid intake occur much more frequently in dyspeptic patients than in healthy individuals.

Similarly to FD, patients with IBS report abdominal bloating following the intake of foods rich in fatty acids. Fatty meals are also able to alter intestinal motility, increasing whole intestinal transit time and, in some cases, inducing a reflex stimulation of colonic motor activity (gastrocolonic reflex) which may explain the post-prandial defecation observed in IBS-D patients[7].

On the basis of such evidence, it has been hypothesized that since the Mediterranean diet is based only minimally on foods or eating habits able to trigger

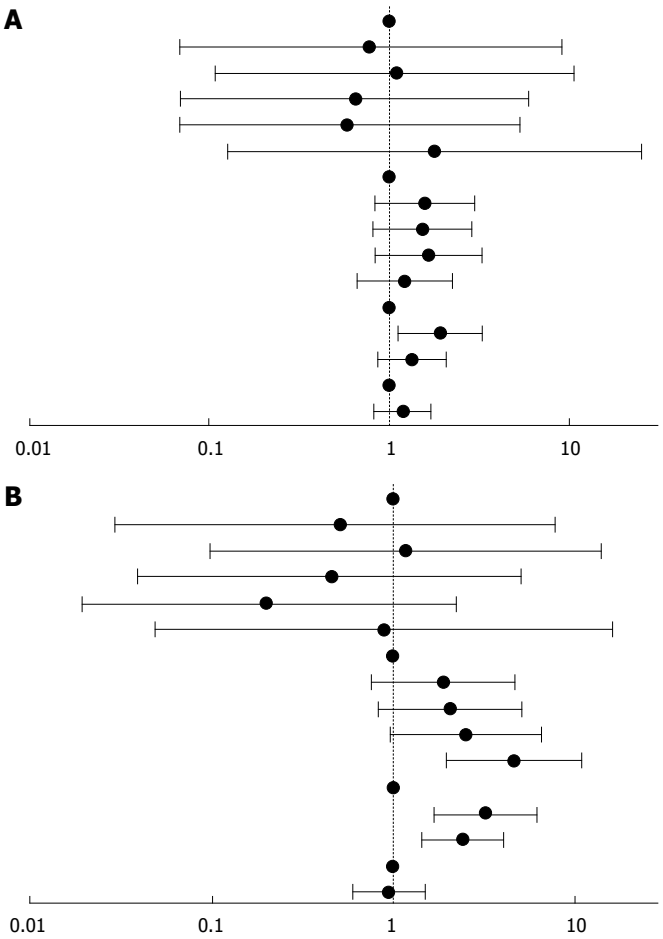

Figure 3 Multivariate analysis shows a pairwise comparison of the phenotypes (body mass index category, age category, clinical pattern and sex) as follows: A: Comparison between high *vs* intermediate adherence to MD: only IBS was significantly associated with Intermediate adherence to MD; B: Comparison between high *vs* low adherence to MD: both IBS and FD as well as the youngest age category were significantly associated with low adherence to MD. The oldest group, controls, individuals presenting with third grade obesity and females are used as reference category in the multinomial regression model. MD: Mediterranean diet; IBS: Irritable bowel syndrome; FD: Functional dyspepsia.

gastrointestinal symptoms, it represents a therapeutic dietary regimen for FGID patients. Indeed, MD is a restricted-calorie dietary model with fats providing less than 35% of total calories[2,22,23]. In addition, MD is characterized by small meals that may exert beneficial effects in both FD patients - by favoring easy gastric emptying, and in IBS-D patients - since it stimulates impaired gastro-colonic reflex only to a limited extent[4,7]. The use of olive oil, as main source of fatty acids, largely preferred to meat and meat-based foods, provides a high intake of mono-unsaturated and omega-3 fatty acids, which may alter gastric emptying less than saturated fats. At the same time, several studies have shown that these kinds of fatty acids may even reduce the microscopic inflammation of colonic mucosa occurring in IBS patients, providing long-term beneficial effects[24].

Noteworthy, our study has shown that subjects in older age categories show greater adherence to MD but nonetheless have persistent functional gastrointestinal symptoms; several factors have been taken into account to explain such evidence. Ageing is characterized by cellular senescence - with consequent reduction in cellular proliferation capability, enteric neuronal loss and low-grade systemic inflammation status called "inflammaging". Consequently, ageing itself may strongly influence gastrointestinal motility, sensitivity, nutrient absorption or gut microbiota composition. Moreover, the presence of several co-morbidities, both mild or severe, is associated with an increased use of medications that deeply influence gastrointestinal activity and its environment (pH, temperature or motility), with secondary effects on bacterial colonization[25,26]. Several research studies have shown that, in the elderly, the stability and variety of microbiota tend to diminish[27]. In fact, there is evidence that Bacteroides and Bifidobacteria become less abundant, mylolytic activity and the availability of short chain fatty acid (SCFA) decrease, with a concomitant increase in the presence of facultative anaerobes, fusobacteria and clostridia[28]. For these reasons, differently from younger individuals, many factors other than diet predispose elderly people to the onset of gastrointestinal problems, which explains the persistence of GI symptoms despite a higher adherence to MD.

The use of questionnaire, both KIDMED and Short Mediterranean one, to evaluate the adherence to a specific diet needs caution because a short score focuses just to a few foods, representative for that alimentary behavior, instead of the whole diet. Moreover, it would be very useful to expand the dietary analysis considering even the amount of food intake in order to evaluate the calories consumed in association with the adherence to MD. This study was performed on subjects living in the same region, therefore, since MD is a dietary regimen which involves several populations, a multicenter prospective study would clarify better the beneficial effect of this diet on functional gastrointestinal symptoms.

In conclusion, the association between food intake and FGID seems to be very complex since each food item may exert a specific effect on the gastrointestinal tract. As several studies have reported that food intake is associated with the onset of symptoms in these disorders, dietary intervention is strongly recommended in the management of FGID. Nutritional therapeutic measures may include different aspects of food intake such as meal size, calorie intake as well as nutrient composition or even meal viscosity. A wide range of dietary regimens, including the Mediterranean Diet, have been proposed for FGID, however the efficacy of such "therapeutic diets" on gastrointestinal symptoms needs further evaluation.

COMMENTS

Background

The mediterranean diet (MD) is universally considered as a health protecting dietary regimen since people who adopt it exhibit a lower incidence of cardiovascular, metabolic and neoplastic disease. However, this dietary pattern may have beneficial effects even on gastrointestinal functional disorders, such as functional dyspepsia or irritable bowel syndrome.

Research frontiers

As the beneficial effects of the MD are widely accepted, the author's aim is to evaluate how different levels of adherence to this type of dietary regimen may influence the onset of functional gastrointestinal symptoms.

Innovations and breakthroughs

The data show that low adherence to MD may trigger functional gastrointestinal symptoms, mainly in younger subjects. Moreover, with increasing age, patients attribute greater importance to their diet and, for this reason, tend to adopt eating habits closer to MD.

Applications

Dietary interventions are fundamental for a correct therapeutic approach of functional gastro-intestinal disorders, such as irritable bowel syndrome or functional dyspepsia. The data support the notion that the adoption of a diet very close to MD - instead of extremely restricted dietary regimens, should be proposed in individuals presenting these disorders.

Peer-review

The topic was interesting and this study was well conducted. The results could help to identify a new dietary habit to prevent gastrointestinal disorders.

REFERENCES

1 **Trichopoulou A**, Martínez-González MA, Tong TY, Forouhi NG, Khandelwal S, Prabhakaran D, Mozaffarian D, de Lorgeril M. Definitions and potential health benefits of the Mediterranean diet: views from experts around the world. *BMC Med* 2014; **12**: 112 [PMID: 25055810 DOI: 10.1186/1741-7015-12-112]

2 **Castro-Quezada I**, Román-Viñas B, Serra-Majem L. The Mediterranean diet and nutritional adequacy: a review. *Nutrients* 2014; **6**: 231-248 [PMID: 24394536 DOI: 10.3390/nu6010231]

3 **Lopez-Garcia E**, Rodriguez-Artalejo F, Li TY, Fung TT, Li S, Willett WC, Rimm EB, Hu FB. The Mediterranean-style dietary pattern and mortality among men and women with cardiovascular disease. *Am J Clin Nutr* 2014; **99**: 172-180 [PMID: 24172306 DOI: 10.3945/ajcn.113.068106]

4 **Azpiroz F**, Hernandez C, Guyonnet D, Accarino A, Santos J, Malagelada JR, Guarner F. Effect of a low-flatulogenic diet in patients with flatulence and functional digestive symptoms. *Neurogastroenterol Motil* 2014; **26**: 779-785 [PMID: 24548289 DOI: 10.1111/nmo.12324]

5 **Vanuytsel T**, Tack JF, Boeckxstaens GE. Treatment of abdominal pain in irritable bowel syndrome. *J Gastroenterol* 2014; **49**: 1193-1205 [PMID: 24845149 DOI: 10.1007/s00535-014-0966-7]

6 **Tack J**, Talley NJ. Functional dyspepsia--symptoms, definitions and validity of the Rome III criteria. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 134-141 [PMID: 23399526 DOI: 10.1038/nrgastro.2013.14]

7 **Gibson PR**, Shepherd SJ. Food choice as a key management strategy for functional gastrointestinal symptoms. *Am J Gastroenterol* 2012; **107**: 657-666; quiz 667 [PMID: 22488077 DOI: 10.1038/ajg.2012.49]

8 **Feinle-Bisset C**, Azpiroz F. Dietary and lifestyle factors in functional dyspepsia. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 150-157 [PMID: 23296252 DOI: 10.1038/nrgastro.2012.246]

9 **Lacy BE**, Talley NJ, Locke GR, Bouras EP, DiBaise JK, El-Serag HB, Abraham BP, Howden CW, Moayyedi P, Prather C. Review article: current treatment options and management of functional dyspepsia. *Aliment Pharmacol Ther* 2012; **36**: 3-15 [PMID: 22591037 DOI: 10.1111/j.1365-2036.2012.05128.x]

10 **Halmos EP**, Power VA, Shepherd SJ, Gibson PR, Muir JG. A diet low in FODMAPs reduces symptoms of irritable bowel syndrome. *Gastroenterology* 2014; **146**: 67-75.e5 [PMID: 24076059 DOI: 10.1053/j.gastro.2013.09.046]

11 **Sahingoz SA**, Sanlier N. Compliance with Mediterranean Diet Quality Index (KIDMED) and nutrition knowledge levels in adolescents. A case study from Turkey. *Appetite* 2011; **57**: 272-277 [PMID: 21624407 DOI: 10.1016/j.appet.2011.05.307]

12 **Antonogeorgos G**, Panagiotakos DB, Grigoropoulou D, Papadimitriou A, Anthracopoulos M, Nicolaidou P, Priftis KN. The mediating effect of parents' educational status on the association between adherence to the Mediterranean diet and childhood obesity: the PANACEA study. *Int J Public Health* 2013; **58**: 401-408 [PMID: 23128782 DOI: 10.1007/s00038-012-0424-3]

13 **Martínez-González MA**, Fernández-Jarne E, Serrano-Martínez M, Wright M, Gomez-Gracia E. Development of a short dietary intake questionnaire for the quantitative estimation of adherence to a cardioprotective Mediterranean diet. *Eur J Clin Nutr* 2004; **58**: 1550-1552 [PMID: 15162136 DOI: 10.1038/sj.ejcn.1602004]

14 **Arriscado D**, Muros JJ, Zabala M, Dalmau JM. Factors associated with low adherence to a Mediterranean diet in healthy children in northern Spain. *Appetite* 2014; **80**: 28-34 [PMID: 24798762 DOI: 10.1016/j.appet.2014.04.027]

15 **Tognon G**, Moreno LA, Mouratidou T, Veidebaum T, Molnár D, Russo P, Siani A, Akhandaf Y, Krogh V, Tornaritis M, Börnhorst C, Hebestreit A, Pigeot I, Lissner L. Adherence to a Mediterranean-like dietary pattern in children from eight European countries. The IDEFICS study. *Int J Obes* (Lond) 2014; **38** Suppl 2: S108-S114 [PMID: 25219407 DOI: 10.1038/ijo.2014.141]

16 **Santomauro F**, Lorini C, Tanini T, Indiani L, Lastrucci V, Comodo N, Bonaccorsi G. Adherence to Mediterranean diet in a sample of Tuscan adolescents. *Nutrition* 2014; **30**: 1379-1383 [PMID: 25280416 DOI: 10.1016/j.nut.2014.04.008]

17 **Noale M**, Nardi M, Limongi F, Siviero P, Caregaro L, Crepaldi G, Maggi S. Adolescents in southern regions of Italy adhere to the Mediterranean diet more than those in the northern regions. *Nutr Res* 2014; **34**: 771-779 [PMID: 25213381 DOI: 10.1016/j.nutres.2014.08.001]

18 **Serra-Majem L**, Ribas L, García A, Pérez-Rodrigo C, Aranceta J. Nutrient adequacy and Mediterranean Diet in Spanish school children and adolescents. *Eur J Clin Nutr* 2003; **57** Suppl 1: S35-S39 [PMID: 12947450]

19 **Tack J**, Caenepeel P, Piessevaux H, Cuomo R, Janssens J. Assessment of meal induced gastric accommodation by a satiety drinking test in health and in severe functional dyspepsia. *Gut* 2003; **52**: 1271-1277 [PMID: 12912857]

20 **Cuomo R**, Vandaele P, Coulie B, Peeters T, Depoortere I, Janssens J, Tack J. Influence of motilin on gastric fundus tone and on meal-induced satiety in man: role of cholinergic pathways. *Am J Gastroenterol* 2006; **101**: 804-811 [PMID: 16635226]

21 **Feinle C**, Meier O, Otto B, D'Amato M, Fried M. Role of duodenal lipid and cholecystokinin A receptors in the pathophysiology of functional dyspepsia. *Gut* 2001; **48**: 347-355 [PMID: 11171824]

22 **Sirtori CR**, Tremoli E, Gatti E, Montanari G, Sirtori M, Colli S, Gianfranceschi G, Maderna P, Dentone CZ, Testolin G. Controlled evaluation of fat intake in the Mediterranean diet: comparative activities of olive oil and corn oil on plasma lipids and platelets in high-risk patients. *Am J Clin Nutr* 1986; **44**: 635-642 [PMID: 3094360]

23 **Shai I**, Spence JD, Schwarzfuchs D, Henkin Y, Parraga G, Rudich A, Fenster A, Mallett C, Liel-Cohen N, Tirosh A, Bolotin A, Thiery J, Fiedler GM, Blüher M, Stumvoll M, Stampfer MJ. Dietary intervention to reverse carotid atherosclerosis. *Circulation* 2010; **121**: 1200-1208 [PMID: 20194883 DOI: 10.1161/CIRCULATIONAHA.109.879254]

24 **Santoro A**, Pini E, Scurti M, Palmas G, Berendsen A, Brzozowska A, Pietruszka B, Szczecinska A, Cano N, Meunier N, de Groot CP, Feskens E, Fairweather-Tait S, Salvioli S, Capri M, Brigidi P, Franceschi C. Combating inflammaging through a Mediterranean whole diet approach: the NU-AGE project's conceptual framework and design. *Mech Ageing Dev* 2014; **136-137**: 3-13 [PMID: 24342354 DOI: 10.1016/j.mad.2013.12.001]

25 **Biagi E**, Nylund L, Candela M, Ostan R, Bucci L, Pini E, Nikkïla J, Monti D, Satokari R, Franceschi C, Brigidi P, De Vos W. Through ageing, and beyond: gut microbiota and inflammatory status in seniors and centenarians. *PLoS One* 2010; **5**: e10667 [PMID: 20498852 DOI: 10.1371/journal.pone.0010667]

26 **Saffrey MJ**. Cellular changes in the enteric nervous system during ageing. *Dev Biol* 2013; **382**: 344-355 [PMID: 23537898 DOI: 10.1016/j.ydbio.2013.03.015]

27 **Claesson MJ**, Jeffery IB, Conde S, Power SE, O'Connor EM, Cusack S, Harris HM, Coakley M, Lakshminarayanan B, O'Sullivan O, Fitzgerald GF, Deane J, O'Connor M, Harnedy N, O'Connor K, O'Mahony D, van Sinderen D, Wallace M, Brennan L, Stanton C, Marchesi JR, Fitzgerald AP, Shanahan F, Hill C, Ross RP, O'Toole PW. Gut microbiota composition correlates with diet and health in the elderly. *Nature* 2012; **488**: 178-184 [PMID: 22797518 DOI: 10.1038/nature11319]

28 **Del Chierico F**, Vernocchi P, Dallapiccola B, Putignani L. Mediterranean diet and health: food effects on gut microbiota and disease control. *Int J Mol Sci* 2014; **15**: 11678-11699 [PMID: 24987952 DOI: 10.3390/ijms150711678]

P- Reviewer: Chiang TA, Sun XT, Tantau A **S- Editor**: Qi Y
L- Editor: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i4.572

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 572-578
ISSN 2150-5349 (online)

ORIGINAL ARTICLE

Randomized Clinical Trial

Efficacy of small-volume simethicone given at least 30 min before gastroscopy

Mingjun Song, Andrew Boon Eu Kwek, Ngai Moh Law, Jeannie Peng Lan Ong, Jessica Yi-Lyn Tan, Prem Harichander Thurairajah, Daphne Shih Wen Ang, Tiing Leong Ang

Mingjun Song, Andrew Boon Eu Kwek, Ngai Moh Law, Jeannie Peng Lan Ong, Jessica Yi-Lyn Tan, Prem Harichander Thurairajah, Daphne Shih Wen Ang, Tiing Leong Ang, Department of Gastroenterology and Hepatology, Changi General Hospital, Singapore 529889, Singapore

Author contributions: Song M, Kwek ABE and Ang TL were responsible for the study conception and design; Song M was responsible for data analysis and interpretation, and manuscript drafting; Kwek ABE and Ang TL critically revised the article for important intellectual content; all the authors reviewed and approved the final version to be published.

Supported by Changi General Hospital Research Grant, No. 2015[CHF2015.02-S].

Institutional review board statement: The study was reviewed and approved by the SingHealth Centralized Institutional Review Board (Ref: 2015/2519).

Clinical trial registration statement: The study is registered under clinicaltrials.gov (NCT02555228).

Informed consent statement: All study participants provided informed written consent prior to study enrollment.

Conflict-of-interest statement: All authors declare no potential conflicting interests related to this paper.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

Correspondence to: Tiing Leong Ang, Chief, Department of Gastroenterology and Hepatology, Changi General Hospital, 2 Simei Street 3, Singapore 529889, Singapore. tiing_leong_ang@cgh.com.sg
Telephone: +65-68503974
Fax: +65-67816202

Received: June 27, 2016
Peer-review started: June 28, 2016
First decision: August 10, 2016
Revised: August 16, 2016
Accepted: October 17, 2016
Article in press: October 19, 2016
Published online: November 6, 2016

Abstract

AIM

To evaluate the efficacy of 5 mL simethicone solution in decreasing gastric foam if given at least 30 min before gastroscopy.

METHODS

This was a randomized, placebo controlled, endoscopist blinded study performed at Changi General Hospital. Patients were at least 21 years old, had no prior surgical resection of the upper gastrointestinal tract, and scheduled for elective diagnostic gastroscopies. The primary outcome was the total mucosal visibility score (TMVS) which was evaluated using McNally score. The sample size was calculated to be 24 per group (SD 2.4, 80% power, $P < 0.05$, 2-sample t test).

RESULTS

Fifty-four patients were randomised to receive either simethicone [1 mL liquid simethicone (100 mg) in 5 mL of water] or placebo (5 mL of water) at least 30 min before their gastroscopy. Six accredited consultants conducted

the gastroscopy, and the interobserver agreement of scoring TMVS was good with a Kappa statistic of 0.73. The simethicone group had significantly better mean TMVS compared to placebo (5.78 ± SD 1.65 *vs* 8.89 ± SD 1.97, $P < 0.001$). The improvement was statistically significant for the duodenum and the gastric antrum, angularis, body, and fundus. Percent 51.9 of patients in the simethicone group had a TMVS of 4 (no bubbles at all) to 5 (only 1 area with minimal bubbles), while in the placebo group 3.7% of patients had TMVS of 4 or 5. The number needed to treat was 2.1 to avoid a TMVS of 6 and more. The simethicone group also had a significantly shorter procedure time with less volume of additional flushes required during gastroscopy to clear away obscuring gastric foam.

CONCLUSION

With a premedication time of at least 30 min, 5 mL simethicone can significantly decrease gastric foam, decrease the volume of additional flushes, and shorten gastroscopy time.

Key words: Simethicone; Premedication; Gastroscopy; Gastric foam

Core tip: This is the first study to evaluate the efficacy of a low volume (5 mL) simethicone solution compared to a placebo using the McNally score to calculate the total mucosal visibility for gastroscopy. Our study showed that although earlier studies had favored higher volumes (typically 100 mL), a low volume is still effective as long as adequate premedication time of at least 30 min is allowed. Such a small volume is more suitable for patients with swallowing difficulties and the formulation had excellent patient compliance with no adverse effects.

Song M, Kwek ABE, Law NM, Ong JPL, Tan JYL, Harichander Thurairajah P, Ang DSW, Ang TL. Efficacy of small-volume simethicone given at least 30 min before gastroscopy. *World J Gastrointest Pharmacol Ther* 2016; 7(4): 572-578 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i4/572.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i4.572

INTRODUCTION

Image-enhanced gastroscopy, such as narrow-band imaging and magnifying endoscopy, can detect subtle early gastric cancer or precancerous lesions and this technology is widely available in Singapore[1]. However, the presence of foam, bubbles and mucus can preclude the benefits of enhanced endoscopy, as subtle mucosal lesions could be covered. In the latest Singapore Cancer Registry, more than 50% of gastric cancers were diagnosed at stage Ⅳ of disease[2]. This suggests that improvement is required for endoscopic detection of early gastric cancer.

Many studies have proven that premedication before gastroscopy will improve the total mucosal visibility scores. However, there is significant heterogeneity between these studies in terms of premedication time, mucosal scoring systems, primary outcome measurements and the type of medications used. Pronase, N-acetylcysteine, dimethicone, dimethylpolysiloxane and simethicone have all been demonstrated by multiple studies to be effective mucolytic and anti-foaming agents[3-11]. All the studies suggested that the best premedication regime is a combination of premedication (a mucolytic and an anti-foaming agent) delivered at large volumes (typically with 100 mL of water). Only one study had a treatment arm looking at small volume premedication (100 mg simethicone in 5 mL water) but this was not compared against a placebo group and the study used a unique 3-grade scoring system[11].

In Singapore, pronase, dimethicone and dimethylpolysiloxane are not available. N-acetylcysteine is a prescription drug and simethicone is an over-the-counter drug for infant colic used off-label for flushes during endoscopy. Premedication before gastroscopy is not routinely given to patients at all endoscopy centers due to several reasons; the perception that premedication may slow down the endoscopy schedule; the worry of adverse reactions from the medications (such as allergic reactions to N-acetylcysteine); and the worry of aspiration from drinking premedication shortly before gastroscopy. As Singapore has an aging population[12], it is common now to perform endoscopy on elderly patients with swallowing dysfunction. However 100 mL premedication solution before gastroscopy puts these patients at risk for aspiration, especially in the setting of moderate sedation during the procedure[13].

We hypothesized that if the premedication time is extended to at least 30 min, 100 mg of simethicone added to 5 mL of water will be able to mix with gastric secretions and swallowed saliva to coat a larger surface area of the gastric mucosa, and significantly improve mucosal visibility compared to placebo.

MATERIALS AND METHODS

Patient selection

This study was conducted in Changi General Hospital in Singapore, from 14th August 2015 to 19th November 2015, at the outpatient gastroenterology clinics. All patients who were planned for gastroscopy as part of their management plan were asked by their respective clinic attending if they would permit a research coordinator to speak to them. If they agreed, the research coordinator would find the patient at the endoscopy listing room to obtain informed consent from the patient to participate in the study. Patients who were at least 21 years old, mentally competent to give informed consent, and scheduled for outpatient elective diagnostic gastroscopy were enrolled. Patients who were incarcerated; had prior history of surgical resection of the esophagus, stomach, or

duodenum; had known hypersensitivity to simethicone; or required gastroscopy for urgent indications such as suspected gastrointestinal bleeding were all excluded from the study.

Study design

This was a randomized, placebo-controlled, endoscopist-blinded study which was approved by the SingHealth Centralized Institutional Review Board (Ref: 2015/2519) and registered under clinicaltrials.gov (NCT02555228). The randomisation sequence (in blocks of 6) was computer generated by a statistician at Changi General Hospital's Clinical Trials and Research Unit (CTRU). The allocation sequence was written on separate cards as number codes and each card was placed inside a sealed opaque envelope. After a study participant registered for the elective gastroscopy, the research coordinator would open an opaque envelope outside the endoscopy suites and the patient would be allocated to either the simethicone group (100 mg of liquid simethicone added to 5 mL of water) or the placebo group (5 mL of water) based on the number written on a card.

Study participants underwent gastroscopy by 1 of the 6 accredited consultant endoscopists who were blinded to the premedication as well as the premedication time. The premedication was prepared by a research coordinator at a separate location, out of sight from the endoscopist or the endoscopy nurses inside the procedure suites. The patient was informed not to disclose the nature of the premedication to the endoscopy suite nurses or endoscopists. The research coordinator worked together with the scheduling nurse at the endoscopy center to ensure that the premedication was taken at least 30 min before the gastroscopy commenced. To confirm that the patient did not tell the endoscopist about the premedication's nature, the research coordinator followed the patient into the endoscopy suite and stood beside the patient until the procedure was over and the data collection form had been completed by the endoscopist. Premedication time was defined from the administration of the solution to the insertion of the tip of the gastroscope into the patient's mouth. All patients received topical analgesic xylocaine 10% spray to the back of their throat and intravenous midazolam with fentanyl to achieve moderate sedation during gastroscopy.

During the procedure, the endoscopist was allowed to flush additional diluted simethicone solution (1 to 3 drops of simethicone added to about 100 mL of water) down the gastroscope channel if there was obscuring gastric foam preventing a satisfactory view. The total volume of additional flushes was recorded by the endoscopy nurse assisting the procedure. After the endoscopist completed an adequate inspection of the mucosal surfaces, the endoscopist withdrew the tip of the gastroscopy up to the gastroesophageal junction and the research coordinator noted the time. The procedure time was defined as the period of time from insertion of the gastroscope to the withdrawal of the gastroscopy back to the gastroesophageal junction. After this, the endoscopist advanced the gastroscope back into the stomach and proceeded to do any interventions deemed necessary such as biopsies of detected lesions. This ensured that the procedure time measured was standardized and not confounded by the number of additional endoscopic interventions due to detection of more lesions.

Endoscopic scoring system of mucosal visibility

Before initiation of the study, all the endoscopists were instructed on the endoscopic scoring system which was based on the McNally scoring system (Figure 1)[14]. Prior to any additional flushes with diluted simethicone solution, the endoscopists evaluated and noted the McNally score for the esophagus, the gastric fundus and body, the gastric antrum and angularis, and the duodenum. The scoring per area was from the range of 1 to 4; 1 if there was no bubble at all, 2 if there were minimal bubbles which the endoscopist had to actively look out for, 3 if the bubbles were obvious but not totally obscuring the view and 4 if the bubbles were so severe that vision is obscured. The total mucosal visibility score (TMVS) was calculated by the sum of scores in all the areas and ranged from 4 to 16.

Outcomes measured

The primary end-point measured was the mean TMVS in the simethicone premedication group and the placebo group respectively. The secondary end-points measured were the mean visibility scores per area, the mean procedure time per group, the mean volume of additional flushes required per group, adverse events reported by the patient or monitoring endoscopy nurses, and the number of gastric lesions reported by the endoscopist.

Statistical analysis

We felt that a difference of 2 points in mean TMVS between the two groups would be clinically significant. An earlier study conducted in Thailand had used a similar scoring system and we adopted the standard deviation in their study results for our estimation[15]. The calculated sample size for each group was 24 patients (SD 2.4, 2 sample *t* test, $P < 0.05$, 80% power). Assuming that the drop-out rate could be around 10%, we aimed to recruit 27 patients per group.

All categorical variables were analysed with Pearson Chi-square test and all continuous variables were analysed with 2-sample *t* test, using SPSS V.19.0 software for Windows (SPSS Inc, Chicago, Illinois, United States). $P < 0.05$ was deemed statistically significant.

RESULTS

A total of 56 patients were enrolled in the study. One patient withdrew consent on the day of the gastroscopy. Another patient subsequently called to cancel the gastroscopy. The remaining 54 patients completed the study with no adverse events (Figure 2) Baseline characteristics of the patients in the 2 groups were similar in terms of mean age, gender, and premedication

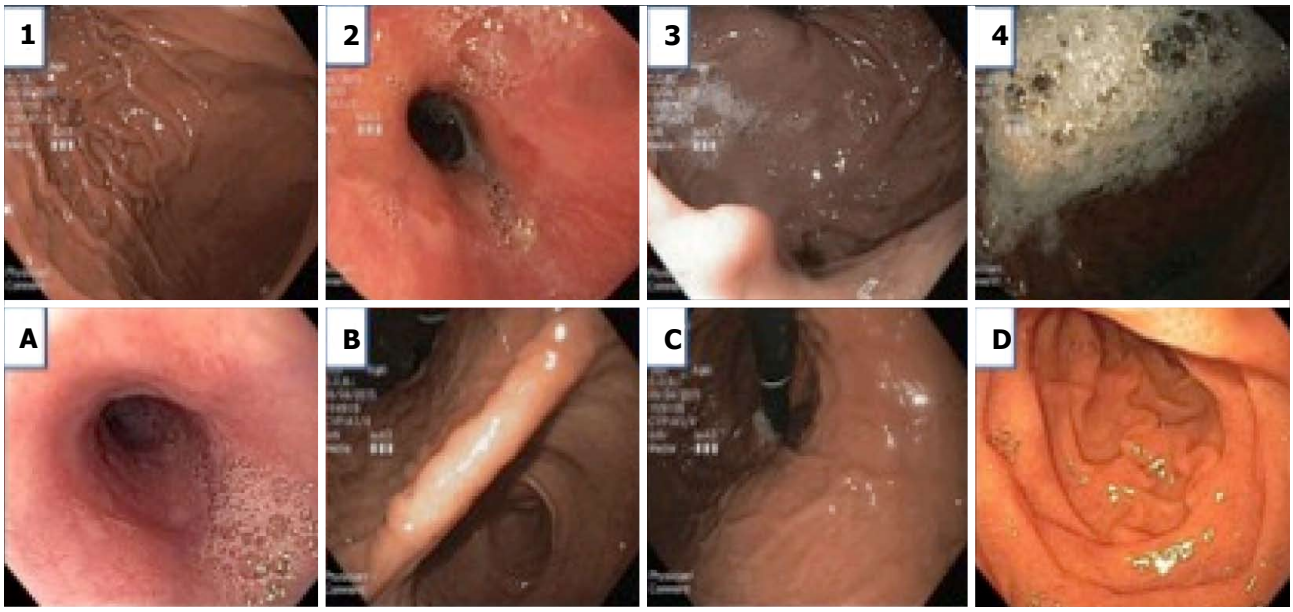

Figure 1 Endoscopic scoring system. Score of 1: No bubbles; Score of 2: Minimal bubbles which the endoscopist must actively look for; Score of 3: Foam is obviously present but not severe; Score of 4: Severe foam obscuring vision; Area A: Esophagus; Area B: The antrum and angularis of the stomach; Area C: The body and fundus of the stomach; Area D: Duodenum. TMVS is the sum of the scores of areas A, B, C and D added together. TMVS: Total mucosal visibility score.

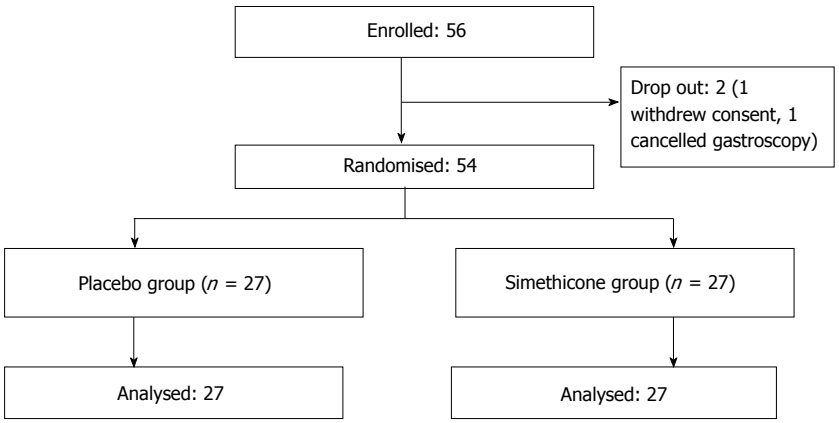

Figure 2 Workflow of patient enrollment.

time (Table 1). Six experienced endoscopists conducted the gastroscopy for all the patients, 4 of the endoscopists were involved in both groups (Figure 3). Before the study, all endoscopists separately scored the TMVS for the endoscopic images of four anonymous participants. They were blinded to the treatment allocation and patient particulars. The scores were tallied by an independent investigator to determine the interobserver agreement which was found satisfactory with a kappa statistic of 0.73.

The mean TMVS was significantly lower in the simethicone group compared to the placebo group (5.78 ± 1.65 for simethicone group, 8.89 ± 1.97 for placebo group, $P < 0.001$). This improvement in mucosal visibility score was significant for the areas of the stomach (body, fundus, antrum, and angularis) and the duodenum. However, simethicone premedication did not significantly improve mucosal visibility score of the esophagus. The simethicone group also had a shorter mean procedure time ($P = 0.049$) as well as lower mean volume of additional flushes required during gastroscopy ($P < 0.001$) (Table 2).

In the simethicone group, 51.9% of the patients had TMVS of 4 (no bubbles in all areas inspected) to 5 (one area had minimal bubbles), whereas in the placebo group only 3.7% of the patients achieved this ($P < 0.001$) (Figure 4). The number needed to treat (NNT) was 2.1 to avoid a TMVS of 6 or more.

DISCUSSION

To our knowledge, this is the first study conducted which evaluated the benefit of a very low volume of simethicone monotherapy for gastroscopy preparation, which was compared against a placebo using the McNally scoring method. Prior studies had shown that larger volumes produced better results and a 100 mL solution was generally accepted as the best. Bertoni *et al*[10] showed in their study that 90 mL solutions were superior to 30 mL solutions when ingested 5 min before the start of

Table 1 Baseline characteristics of the patients *n* (%)

	Placebo group (*n* = 27)	Simethicone group (*n* = 27)	*P* vaule
Mean age (yr ± SD)	52.9 ± 15.5	57.7 ± 12.5	0.215
Male gender (%)	9 (33.3)	15 (55.6)	0.1
Mean premedication time (min:s ± SD)	41:08 ± 9:56	44:46 ± 12:36	0.245
Indication for gastroscopy			
Dyspepsia	19 (70.4)	10 (37.0)	0.014
Reflux symptoms	3 (11.1)	6 (22.2)	0.467
Positive *H. pylori* serology	0	1 (3.7)	1
Variceal screen	1 (3.7)	2 (7.4)	1
Anemia	4 (14.8)	3 (11.1)	1
Dysphagia	0	1 (3.7)	1
Intestinal metaplasia surveillance	0	3 (11.1)	0.236
Cancer surveillance after endoscopic mucosal resection	0	1	1

H. pylori: *Helicobacter pylori*.

Table 2 Study results

	Placebo group	Simethicone group	*P* value
TMVS ± SD	8.89 ± 1.97	5.78 ± 1.65	< 0.001
Mean esophagus score ± SD	1.59 ± 0.57	1.48 ± 0.57	0.482
Mean duodenum score ± SD	2.26 ± 0.81	1.26 ± 0.53	< 0.001
Mean antrum and angularis score ± SD	2.56 ± 1.05	1.30 ± 0.54	< 0.001
Mean body and fundus score ± SD	2.44 ± 0.97	1.74 ± 0.81	0.006
Mean volume of additional water flushes required ± SD (mL)	84.81 ± 110.18	3.89 ± 11.46	< 0.001
Procedure time ± SD (s)	193.67 ± 87.04	154.85 ± 49.07	0.049

TMVS: Total mucosal visibility score.

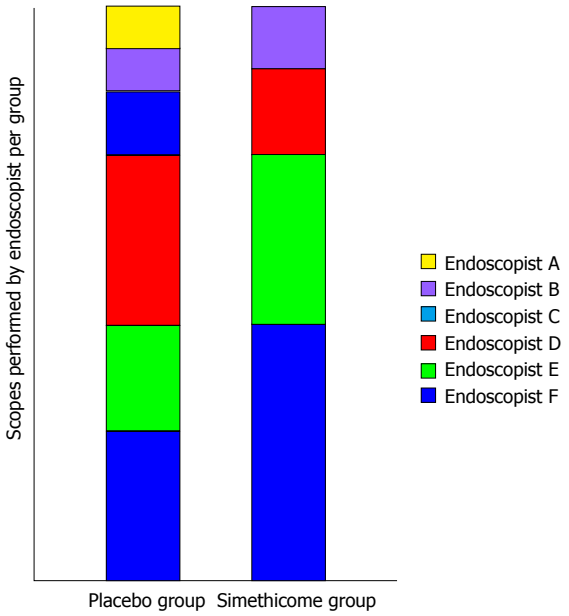

Figure 3 Endoscopist contribution per group.

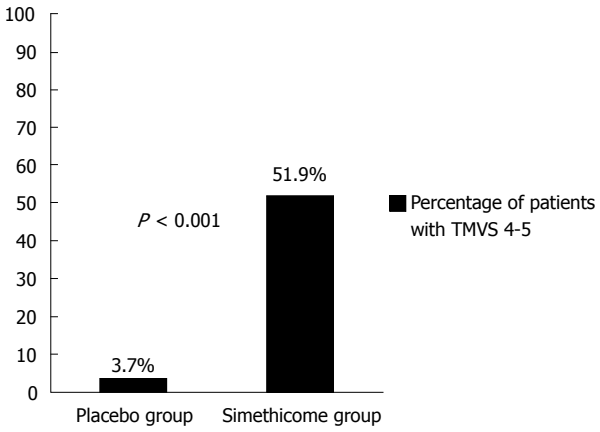

Figure 4 Percentage of patients with total mucosal visibility score of 4-5 during gastroscopy. TMVS: Total mucosal visibility score.

gastroscopy. Chang *et al*[6] showed in their recent study that when ingested within 30 min before gastroscopy, their 100 mL solution consisting of mucolytic and anti-foaming agent resulted in the best mucosal visibility scores. In this study, the premedication time was increased significantly (mean premedication times were 41:08 ± 9:56 min for placebo group and 44:46 ± 12:36 min for simethicone group), which allowed mixing of the simethicone with gastric secretion and swallowed saliva to coat the mucosal surface. This resulted in significant improvement of TMVS compared to placebo (Figures 5 and 6). Although the improvement in mucosal visibility scores was not significant for the esophageal area, the mean scores for the esophageal area were already very low to begin with (1.48 ± 0.57 in the simethicone group and 1.59 ± 0.57 in the placebo group). We postulated that this was because of the tubular structure of the esophagus as well as the peristaltic movements of the esophagus allowing mucus and secretions to flow down into the stomach. In additional, our study population is made up of healthy patients who were predominantly undergoing gastroscopy for dyspepsia; only 1 patient had dysphagia and 9 patients had reflux symptoms. This may result in the study population having a better mucosal visibility score in the esophageal area at baseline and explain why low volume simethicone solution did not make much of a difference. There was also significantly lower volume of additional flushes required during gastroscopy if simethicone was given. This, in turn, resulted in a significantly shorter procedure time for mucosal inspection (Figure 7). The ideal TMVS was 4 with absolutely no bubbles in all areas. In our study, the NNT to achieve a TMVS of 4 to 5 during gastroscopy was just 2.1.

Our study had two main limitations. Firstly, the study was not powered to investigate for the improvement of gastric lesion detection with enhanced endoscopy techniques. Our study participants were all recruited from the clinics for elective gastroscopies and the findings

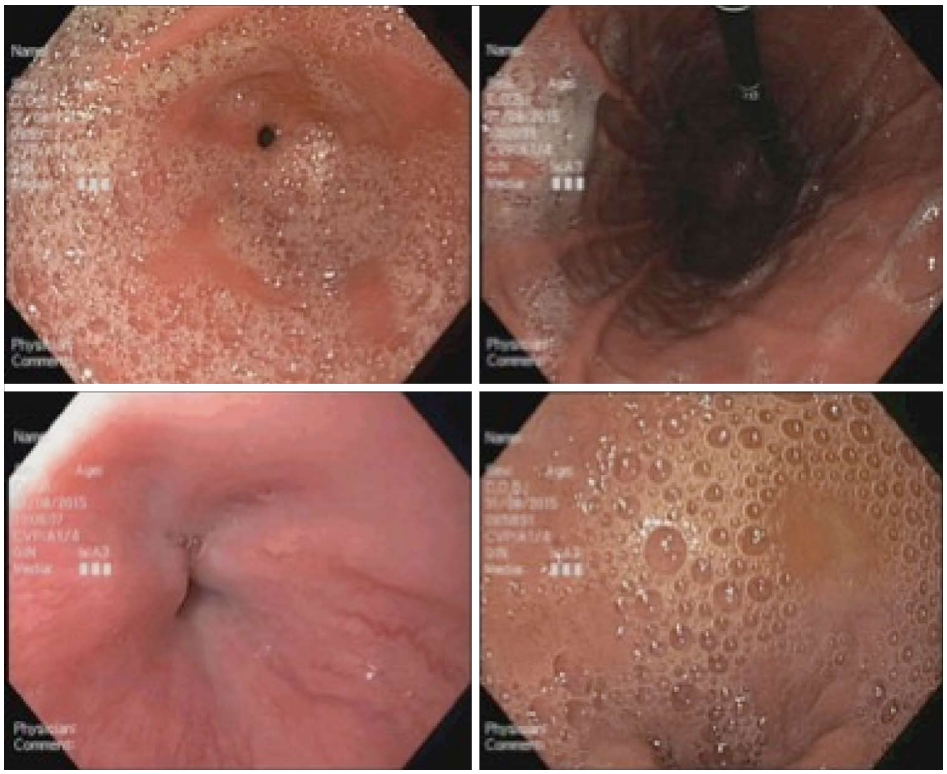

Figure 5 Endoscopic images of a patient in the placebo group (total mucosal visibility score 13).

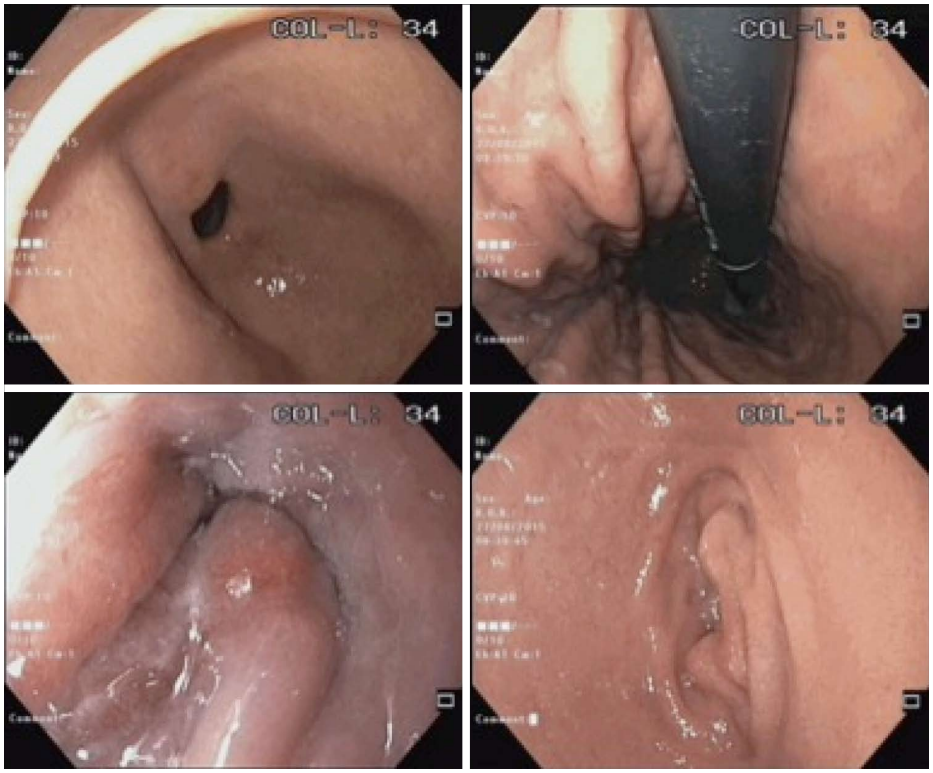

Figure 6 Endoscopic images of a patient in the simethicone group (total mucosal visibility score 4).

were predominantly benign functional dyspepsia or non-erosive gastritis which did not require further enhanced imaging as determined by the endoscopist. In order to investigate this aspect, we would have required a much larger study population which will likely require a multi-center collaboration. Secondly, we did not investigate the additional benefit of adding mucolytics to the regime which many other studies had done. This is because N-acetylcysteine is not readily available (it requires prior prescription and collection from the pharmacy by the patient) whereas simethicone is stored at all endoscopy suites and we wanted to find a convenient premedication regime that our endoscopists will be comfortable using.

None of the patients had any adverse event. As the volume of premedication required is only 5 to 6 mL in total, this is likely suitable for use in patients with

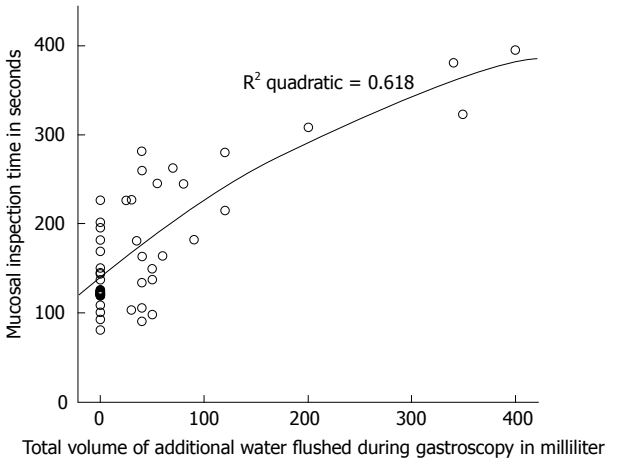

Figure 7 Correlation between volume of additional water flushes during gastroscopy (mL) and total mucosal inspection time (s).

swallowing dysfunction as such volumes are routinely used as modified water swallowing tests swallowing test[13].

ACKNOWLEDGMENTS

Ms Nway Nway Aye, department research coordinator.

COMMENTS

Background

Numerous studies have used various formulations before gastroscopy to decrease gastric foam or gastric mucus, with the overall conclusion that a larger volume with combined therapy is more effective.

Research frontiers

The potential benefit of extending premedication time has been evaluated in earlier studies with varying results.

Innovations and breakthroughs

This is the first randomized controlled study to evaluate the efficacy of a small volume simethicone solution (as compared to placebo with water) in the context of a premedication time of at least 30 min. It shows that despite earlier studies favoring combination therapy with larger volumes, a longer premedication time with a small volume monotherapy can significantly improve mucosal visibility scores. The study sample was calculated using the standard deviation from the results of a study with the same McNally scoring system. The study results offer a convenient and effective premedication that requires no additional prescription in Singapore and will likely be tolerated by patients with swallowing dysfunction.

Applications

This study's finding has resulted in the standardised use of simethicone premedication at Changi General Hospital endoscopy center prior to elective gastroscopies in the low volume formulation.

Peer-review

It is a good practical idea. They think the importance of search could be more applicable if the study done for enteroscopy not upper endoscopy. The paper is well written, well organised.

REFERENCES

1 **Song M,** Ang TL. Early detection of early gastric cancer using image-enhanced endoscopy: Current trends. *Gastrointest Inter* 2014; **3**: 1-7 [DOI: 10.1016/j.gii.2014.02.005]
2 **Singapore Cancer Registry 2015.** Cancer Survival in Singapore 1973-2012. USA: National Registry of Diseases Office, 2015
3 **Cha JM**, Won KY, Chung IK, Kim GH, Lee SY, Cho YK. Effect of pronase premedication on narrow-band imaging endoscopy in patients with precancerous conditions of stomach. *Dig Dis Sci* 2014; **59**: 2735-2741 [PMID: 24861034 DOI: 10.1007/s10620-014-3218-z]
4 **Kuo CH**, Sheu BS, Kao AW, Wu CH, Chuang CH. A defoaming agent should be used with pronase premedication to improve visibility in upper gastrointestinal endoscopy. *Endoscopy* 2002; **34**: 531-534 [PMID: 12170403 DOI: 10.1055/s-2002-33220]
5 **Lee GJ**, Park SJ, Kim SJ, Kim HH, Park MI, Moon W. Effectiveness of Premedication with Pronase for Visualization of the Mucosa during Endoscopy: A Randomized, Controlled Trial. *Clin Endosc* 2012; **45**: 161-164 [PMID: 22866258 DOI: 10.5946/ce.2012.45.2.161]
6 **Chang CC**, Chen SH, Lin CP, Hsieh CR, Lou HY, Suk FM, Pan S, Wu MS, Chen JN, Chen YF. Premedication with pronase or N-acetylcysteine improves visibility during gastroendoscopy: an endoscopist-blinded, prospective, randomized study. *World J Gastroenterol* 2007; **13**: 444-447 [PMID: 17230616 DOI: 10.3748/wjg.v13.i3.444]
7 **Neale JR**, James S, Callaghan J, Patel P. Premedication with N-acetylcysteine and simethicone improves mucosal visualization during gastroscopy: a randomized, controlled, endoscopist-blinded study. *Eur J Gastroenterol Hepatol* 2013; **25**: 778-783 [PMID: 23739279 DOI: 10.1097/MEG.0b013e32836076b2]
8 **Chen MJ**, Wang HY, Chang CW, Hu KC, Hung CY, Chen CJ, Shih SC.. The add-on N-acetylcysteine is more effective than dimethicone alone to eliminate mucus during narrow-band imaging endoscopy: a double-blind, randomized controlled trial. *Scand J Gastroenterol* 2013; **48**: 241-245 [PMID: 23268593 DOI: 10.3109/00365521.2012.749509]
9 **Asl SM**, Sivandzadeh GR. Efficacy of premedication with activated Dimethicone or N-acetylcysteine in improving visibility during upper endoscopy. *World J Gastroenterol* 2011; **17**: 4213-4217 [PMID: 22072853 DOI: 10.3748/wjg.v17.i37.4213]
10 **Bertoni G**, Gumina C, Conigliaro R, Ricci E, Staffetti J, Mortilla MG, Pacchione D. Randomized placebo-controlled trial of oral liquid simethicone prior to upper gastrointestinal endoscopy. *Endoscopy* 1992; **24**: 268-270 [PMID: 1612040 DOI: 10.1055/s-2007-1010479]
11 **Chang WK**, Yeh MK, Hsu HC, Chen HW, Hu MK. Efficacy of simethicone and N-acetylcysteine as premedication in improving visibility during upper endoscopy. *J Gastroenterol Hepatol* 2014; **29**: 769-774 [PMID: 24325147 DOI: 10.1111/jgh.12487]
12 **National Population and Talent Division**, Prime Minister's Office, Singapore Department of Statistics, Ministry of Home Affairs, Immigration and Checkpoints Authority. Population in Brief 2015. Singapore: National Population and Talent Division, Prime Minister's Office, Singapore Department of Statistics, Ministry of Home Affairs, Immigration and Checkpoints Authority, 2015
13 **Osawa A**, Maeshima S, Tanahashi N. Water-swallowing test: screening for aspiration in stroke patients. *Cerebrovasc Dis* 2013; **35**: 276-281 [PMID: 23548854 DOI: 10.1159/000348683]
14 **McNally PR**, Maydonovitch CL, Wong RK. The effectiveness of simethicone in improving visibility during colonoscopy: a double-blind randomized study. *Gastrointest Endosc* 1988; **34**: 255-258 [PMID: 3292345 DOI: 10.1016/S0016-5107(88)71324-3]
15 **Keeratichananont S**, Sobhonslidsuk A, Kitiyakara T, Achalanan N, Soonthornpun S. The role of liquid simethicone in enhancing endoscopic visibility prior to esophagogastroduodenoscopy (EGD): A prospective, randomized, double-blinded, placebo-controlled trial. *J Med Assoc Thai* 2010; **93**: 892-897 [PMID: 20718163]

P- Reviewer: De Silva AP, Shehata MMM, Yeoh SW, Zuo XL
S- Editor: Qi Y **L- Editor**: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i4.579

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 579-583
ISSN 2150-5349 (online)

CASE REPORT

Osteonecrosis of both knees in a woman with Crohn's disease

Mara Barbosa, José Cotter

Mara Barbosa, José Cotter, Department of Gastroenterology, Hospital da Senhora da Oliveira, Rua dos Cutileiros, Creixomil, 4835-044 Guimarães, Portugal

Author contributions: The authors made equal contributions to the study; Barbosa M drafted the manuscript; Cotter J critically reviewed the manuscript.

Institutional review board statement: This study was reviewed and approved by Hospital da Senhora da Oliveira Institutional Review Board.

Informed consent statement: Written informed consent was obtained from the patient described in this case report.

Conflict-of-interest statement: Mara Barbosa and José Cotter certify that they have no conflit-of-interest.

Manuscript source: Unsolicited manuscript

Correspondence to: Mara Barbosa, MD, Department of Gastroenterology, Hospital da Senhora da Oliveira, Creixomil, 4835-044 Guimarães, Portugal. maraisabelbarbosa@net.sapo.pt
Telephone: +351-933-112632
Fax: +351-253-513592

Received: April 11, 2016
Peer-review started: April 13, 2016
First decision: July 20, 2016
Revised: August 7, 2016
Accepted: September 13, 2016
Article in press: September 15, 2016
Published online: November 6, 2016

Abstract

Osteonecrosis is a very rare complication of Crohn's disease (CD). It is not clear if it is related to corticosteroid therapy or if it occurs as an extraintestinal manifestation of inflammatory bowel disease. We present the case of a patient with CD who presented with osteonecrosis of both knees. A 22 years old woman was diagnosed with CD in April 2012 (Montreal Classification A2L1 + L4B3p). She was started on prednisolone (40 mg/d), azathioprine (100 mg/d) and messalazine (3 g/d). In July 2012, due to active perianal disease, infliximab therapy was initiated. In September 2012, she had a pelvic abscess complicated by peritonitis and an ileal segmental resection and right hemicolectomy were performed. In December 2012 she was diagnosed with bilateral septic arthritis of both knees with walking impairment. She was treated with amoxicillin-clavulanic acid, started a physical rehabilitation program and progressively improved. However, then, bilateral knee pain exacerbated by movement developed. Magnetic resonance imaging showed multiple osseous medullary infarcts in the distal extremity of the femurs, proximal extremity of the tibiae and patellas and no signs of subchondral collapse, which is consistent with osteonecrosis. The patient recovered completely and maintains therapy with azathioprine and messalazine. A review of the literature is also done.

Key words: Osteonecrosis; Knee; Inflammatory bowel disease; Crohn's disease; Magnetic resonance imaging

Core tip: Although very rare, osteonecrosis is a devasting event that can occur in Crohn's disease (CD). We present the case of a 22 years old woman with CD who was diagnosed with osteonecrosis of both knees. As we demonstrate with this report, awareness of risk factors, such as corticosteroid therapy and inflammatory bowel

disease activity, is crucial to establish the diagnosis of this inflammatory bowel disease rheumatological complication. Prompt treatment is recommended. A review of the literature is also presented.

Barbosa M, Cotter J. Osteonecrosis of both knees in a woman with Crohn's disease. *World J Gastrointest Pharmacol Ther* 2016; 7(4): 579-583 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i4/579.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i4.579

INTRODUCTION

Osteonecrosis or avascular necrosis is defined as cellular death of bone components due to interruption of blood supply. Consequently, there is a collapse and destruction of articular surfaces, pain and disability[1]. Epiphysis of long bones (femoral and humeral heads and femoral condyles) are primarily involved, with the hip being the most commonly affected joint. Several clinical entities (connective tissue disorders, hemoglobinopathies, coagulation disorders, pregnancy, alcohol abuse, inflammatory bowel diseases (IBD) and corticosteroid use) have been associated with osteonecrosis, but its pathophysiology is not completely understood[2]. The true incidence of this rare manifestation in IBD is not known[1]. It has been reported to range from 0.5% to 4.3%[3]. We present the case of a 22 years old woman with Crohn's disease (CD) who was diagnosed with osteonecrosis of both knees.

CASE REPORT

A 22 years old woman was diagnosed with CD in April 2012 (Montreal Classification A2L1 + L4B3p - diagnosis at 22 years-old; ileal plus jejunal involvement; penetrant behavior and perianal disease - rectovulvar fistulae). She was initially treated with prednisolone (40 mg/d), azathioprine (100 mg corresponding to 2 mg/kg per day) and messalazine (3 g/d). In July 2012, due to fistulae non-healing, a seton was placed and infliximab therapy was started (three infusions - 0, 2 and 6 wk - 5 mg/kg). Complete closure of the rectovulvar fistulae was then confirmed. In September 2012, she had had a pelvic abscess complicated by peritonitis and she was operated. Drainage of the abscess, ileal segmental resection and right hemicolectomy was performed. From April 2012 to December 2012 a gradual weaning of corticosteroid therapy was done. In December 2002 she presented with fever, intense pain, swelling and stiffness of both knees and impaired range of motion for six weeks. Bilateral articular effusions were observed. She got bedridden. There was no history of arthritis. Laboratory studies revealed a leucocytosis with neutrophilia (17.000/mm^3 per 89%) and an elevated erythrocyte sedimentation rate (28 mm^3 per hour). Bilateral arthrocentesis was performed with diagnostic and drainage intent. Synovial fluid was purulent. Culture of the synovial fluid was positive for *S. pneumoniae.* Amoxicillin plus clavulanic acid and analgesia (acetaminophen and tramadol) was begun. Bilateral arthrotomy of knees with biopsy of the synovium was performed. The histological examination of the synovial tissue revealed synoviocyte hyperplasia, inflammatory infiltrate, mainly composed by polymorphonuclear neutrophils, and purulent exudates; these findings were consistent with the diagnosis of bilateral septic arthritis. There was no exacerbation of intestinal symptoms of CD. After an initial period of immobilization, she was started on a physical rehabilitation program and progressively improved: Inflammatory signs of knees disappeared and she started to walk with crutches. However, bilateral knee pain developed, exacerbated by movement, mainly at climbing stairs. Plain film radiographies of the knees demonstrated multiple bilateral hypotransparent areas in the distal extremity of the femurs, in the proximal extremity of the tibiae and in the patellas and also absence of signs of subchondral collapse (Figure 1). Computed tomography (CT) revealed multiple lacunar areas in the same localizations (Figure 2). Magnetic resonance imaging (MRI) showed a "geographic" pattern resulting from multiple osseous medullary infarcts in the distal 15 cm of the femurs, in the proximal 10 cm of the tibiae and in the patellas; there were also no signs of subchondral collapse (Figures 3-5). These imagiologic findings were consistent with the diagnosis of osteonecrosis. The total body radionuclide bone scan (methylene biphosphonate labeled with technetium^{-99m}) revealed an increased uptake of the agent in the distal ephiphysis of the femurs, in the proximal epiphysis of the tibiae and in the patellas; it also excluded other focus of the disease. A stage 2 of Association Research Circulation Osseous (ARCO) was established. The peripheral blood smear was normal. Lipid levels (cholesterol and triglycerides) were within normal range. Antinuclear antibody, rheumatoid factor, antismooth muscle antibody and antiphospholipid antibodies were negative. Procoagulant factors (C and S proteins, antithrombina Ⅲ and Ⅴ Leiden factor) were

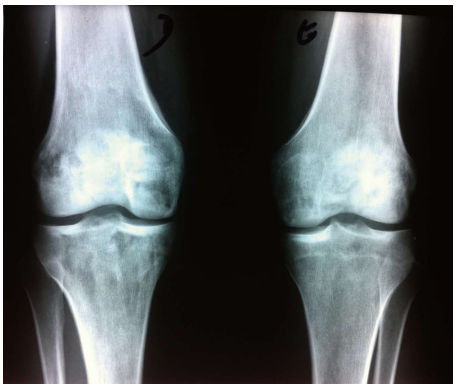

Figure 1 Plain film radiographs (bilateral knees) showing multiple bilateral hypotransparent areas.

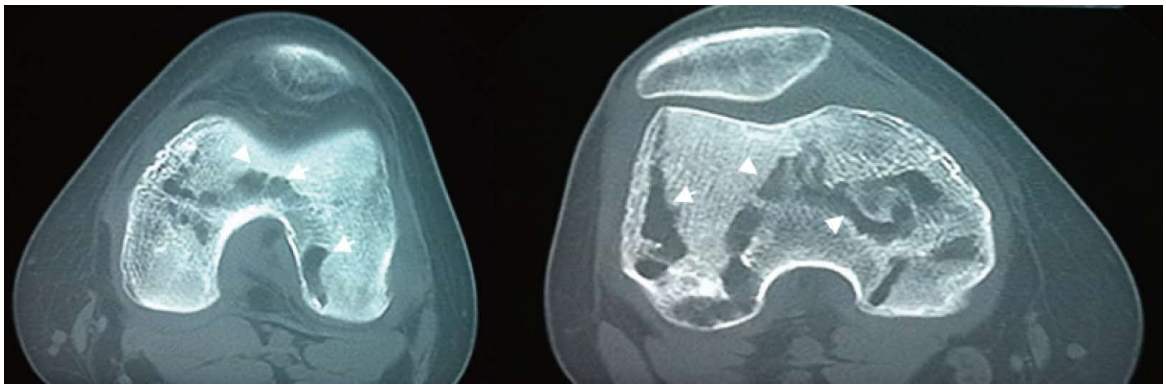

Figure 2 Computed tomography showing multiple lacunar areas in the femurs and patellas.

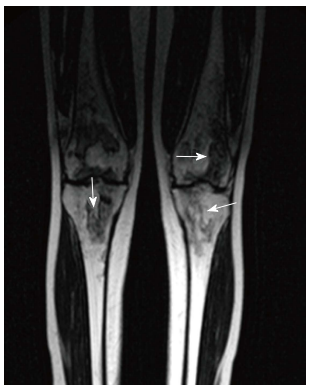

Figure 3 Magnetic resonance imaging (bilateral knees, T1-weighted images, coronal view) showing areas and serpiginous rims of low signal intensity in the femurs, tibiae and patellas, characteristic of osteonecrosis.

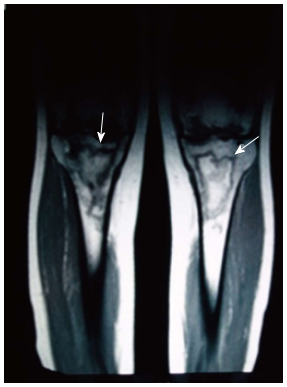

Figure 4 Magnetic resonance imaging (bilateral knees, T2-weighted images, coronal view) showing prominent medullary infarcts.

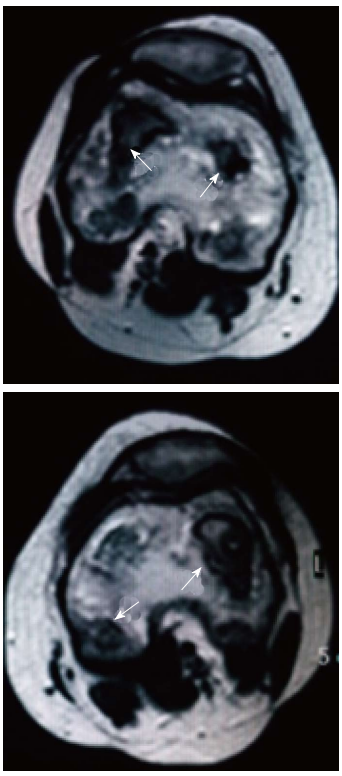

Figure 5 Magnetic resonance imaging (T2-weighted images, axial view) showing low signal serpiginous rims.

normal. The patient recovered completely and maintains therapy with azathioprine and messalazine.

DISCUSSION

The etiology and pathogenesis of osteonecrosis in IBD remain to be elucidated[3]. Some risk factors have been implicated, such as corticosteroid therapy[1,3,4] (systemic and topic) and disease activity so it can be considered an extra-intestinal manifestation of IBD[5,6]. Several studies report the occurrence of osteonecrosis in IBD patients either during or after corticosteroids use. Although initial data described a six to eight months period after initiating therapy for steroid-associated osteonecrosis to occur, this temporal relationship was not confirmed afterwards, with some other reports describing an erratic pattern of development[6]. No clear dose-response is established[1,3] but, corticosteroids doses are significantly higher in patients with osteonecrosis than in those without it[1,4,7]. Moreover, this complication may present with lower dosage of corticosteroids, comparing with other conditions[1]. All in all, a more precise association between corticosteroid use in IBD and osteonecrosis is still needed[3]. IBD patients can develop osteonecrosis unrelated to corticosteroid therapy. CD is associated with a hipercoagulable state, mainly during periods of active disease[5]; the fibrin microclots may lead to osteonecrosis

by occluding epiphyseal capillaries and limiting the blood supply to the bone. This predisposition to thrombosis supports the hypothesis that osteonecrosis might be a rheumatological condition associated with IBD, rather than a complication of its treatment. Several risk factors can be simultaneously present. Our patient needed a relatively prolonged course of corticosteroid therapy and CD was still not in complete remission before the development of osteonecrosis. In this particular case, the event of bilateral septic arthritis, almost certainly secondary to the immunossupression therapy (corticosteroids, azathioprine and infliximab), might also have contributed to its occurrence.

Pain in the affected joint, usually exacerbated by weight-bearing, is typically the presenting symptom, although patients can remain entirely asymptomatic. Despite being initially mild, pain progressively worsens over time, being present at rest, and a decrease range of motion results[1,8]. Early diagnosis is important as treatment might avert disease progression[2,9]. However, it may be challenging, as 4% to 17% of patients with CD have type 1 - pauci-articular arthropathy, in which large bearing weight joints (ankles, knees, hips, wrists, elbows and shoulders) are affected[10]. A high index of suspicion in patients with risk factors is therefore mandatory[1]. This patient had well established risk factors for osteonecrosis, the persistence of bilateral knee pain despite the disappearance of the other inflammatory signs led us to investigate the possibility of this bone complication.

Diagnosis can be made by plain film radiographs, radionuclide bone scan, CT, MRI or invasive techniques (bone marrow pressure, stress test with injection of saline, intramedullary venography, superselective angiography and bone biopsy), the later being reserved for selected cases. At present, the goldstandard for the diagnosis of osteonecrosis is MRI, as it can depict the earliest imagiologic changes of the disease, with the best sensitivity and the best accuracy (75%-100%) compared with the other methods[1,3]. MRI shows a decreased signal intensity in both T1- and T2-weighted images[1,8,11]. Plain film radiographs are usually initially unremarkable[1,8]; afterwards they can demonstrate cystic or sclerotic changes, subchondral fractures (the "crescent sign") and eventually secondary osteoarthritic changes[8]. Radionuclide bone scan using a bone-imaging agent (labeled with technetium-99m) is another helpful diagnostic imaging study in early stages of osteonecrosis[1,8], when plain films radiographs are normal or nearly normal. It is of utmost usefulness at screening, because bone scan can detect asymptomatic joint involvement[12]. However, it has the disadvantage of being non-specific, except when it shows a central area of decreased uptake surrounded by an area of increased uptake[8]. CT is a good technique at evaluating disease extension[8]. IBD patients tend to present with multifocal osteonecrosis[1,4]. Histology is the definitive method for the diagnosis of osteonecrosis, although it is usually unnecessary. Histological changes are encountered in both cortical bone and bone marrow. Necrosis of bone tissue (disappearance of the osteocytes) is followed by a regenerative process in surrounding tissues. Bone marrow lesions include edema, hemorrhage, fibrilloreticulosis, hipocellularity, necrosis of hematopoietic cells and replacement of adipocytes by eosinophilic debris[8]. The most commonly accepted classification system to stage osteonecrosis was devised by the ARCO. It encompasses 4 stages. The first one, stage 0, is defined as the presence of histological changes without any associated clinical signs or symptoms. In the last one, stage 4, there is evidence of progression to osteoarthritis (joint space narrowing and complete joint destruction)[13]. In our case, diagnosis was made by plain film radiographs, CT and MRI. A stage 2 of ARCO was established. In order to screen other localizations for osteonecrosis, a radionuclide bone scan was undertaken, which did not reveal other foci of the disease. No underlying analytical risk factor of any type (including any thrombophilic disorders) was found.

Management depends on the location and severity of joint involvement[1,8]. Conservative treatment includes restriction of weight-bearing on the affected joint or even immobilization, strengthening of the muscles surrounding the affected bone and analgesia[1,8]. No drug treatment has proven effective in averting disease progression, although bisphosphonates have shown some promise[14]. Surgical approaches include arthroplasty, core decompression, osteotomies and non-vascularized and vascularized bone grafting. In advanced cases, following subchondral collapse, total arthroplasty is the main surgical solution, although failure rates in patients with osteonecrosis are significantly higher in comparison with other conditions[1,8].

Conservative management was successful in our patient. She resumed walking without crutches and normal daily activities a few months later.

Regarding prevention, whenever possible, steroid-sparing agents should be the first option[8]. With this in mind, our patient was maintained on azathioprine and on anti-TNF therapy. If corticosteroid treatment is deemed necessary, it should be kept to the minimum effective dosage and patients may be offered a statin, as there is some evidence that it decreases the incidence of osteonecrosis in patients receiving high-dose steroids[15]. Moreover, hyperlipidemia and diabetes should be treated and alcohol ingestion avoided.

In conclusion, although very rare, osteonecrosis is a devasting event that can occur in CD. As we demonstrate with this report, awareness of risk factors, such as corticosteroid therapy and inflammatory bowel disease activity, is crucial to make the diagnosis of this rheumatological IBD complication. Prompt treatment is recommended.

COMMENTS

Case characteristics

A 22 years old woman with active Crohn's disease (CD) treated with prednisolone, messalazine, azathioprine and infliximab presented with bilateral

knee pain exacerbated by movement, after an episode of bilateral septic arthritis of both knees.

Clinical diagnosis
On clinical examination, bilateral knee pain aggravated by movement and weight-bearing was observed.

Differential diagnosis
Another causes of osteonecrosis were excluded, such as: Systemic lupus erythematosus (with or without antiphospholipid syndrome), as well as other connective-tissue diseases, hematological diseases (sickle cell disease, hemoglobinopathies), hyperlipidemia.

Laboratory diagnosis
The peripheral blood smear was normal. Lipid levels (cholesterol and triglycerides) were within normal range. Antinuclear antibody, rheumatoid factor, antismooth muscle antibody and antiphospholipid antibodies were negative. Procoagulant factors (C and S proteins, antithrombina Ⅲ and Ⅴ Leiden factor) were normal.

Imaging diagnosis
Plain film radiographies of the knees demonstrated multiple bilateral hypotransparent areas in the distal extremity of the femurs, in the proximal extremity of the tibiae and in the patellas and also absence of signs of subchondral collapse. Computed tomography revealed multiple lacunar areas in the same localizations. Magnetic resonance imaging showed a "geographic" pattern resulting from multiple osseous medullary infarcts in the distal 15 cm of the femurs, in the proximal 10 cm of the tibiae and in the patellas; there were also no signs of subchondral collapse. These imagiologic findings were consistent with the diagnosis of osteonecrosis.

Pathological diagnosis
Histological examination of both cortical bone and bone marrow was not performed because imagiological findings showed typical findings of osteonecrosis.

Treatment
A conservative strategy was adopted. After an initial period of immobilization and restriction of weight-bearing with the use of crutches, the patient was started on a rehabilitation programme. The patient recovered completely and maintains therapy with azathioprine and messalazine.

Related reports
There are few case reports in the literature of osteonecrosis in inflammatory bowel disease (IBD). The description of involvement of both knees is exceedingly rare.

Term explanation
All terms in this case report are standard and used in the field of gastroenterology.

Experiences and lessons
Although very rare, osteonecrosis is a devasting event that can occur in CD. As they demonstrate with this report, awareness of risk factors, such as corticosteroid therapy and IBD activity, is crucial to make the diagnosis of this rheumatological IBD complication. Prompt treatment is recommended.

Peer-review
This case report demonstrates very well the occurrence of osteonecrosis in the setting of inflammatory.

REFERENCES

1 **Klingenstein G**, Levy RN, Kornbluth A, Shah AK, Present DH. Inflammatory bowel disease related osteonecrosis: report of a large series with a review of the literature. *Aliment Pharmacol Ther* 2005; **21**: 243-249 [PMID: 15691298 DOI: 10.1111/j.1365-2036.2005.02231.x]

2 **Assouline-Dayan Y**, Chang C, Greenspan A, Shoenfeld Y, Gershwin ME. Pathogenesis and natural history of osteonecrosis. *Semin Arthritis Rheum* 2002; **32**: 94-124 [PMID: 12430099 DOI: 10.1053/sarh.2002.33724b]

3 **Freeman HJ**, Freeman KJ. Prevalence rates and an evaluation of reported risk factors for osteonecrosis (avascular necrosis) in Crohn's disease. *Can J Gastroenterol* 2000; **14**: 138-143 [PMID: 10694287 DOI: 10.1155/2000/958086]

4 **Hauzeur JP**, Malaise M, Gangji V. Osteonecrosis in inflammatory bowel diseases: a review of the literature. *Acta Gastroenterol Belg* 2009; **72**: 327-334 [PMID: 19902866]

5 **Khan A**, Illiffe G, Houston DS, Bernstein CN. Osteonecrosis in a patient with Crohn's disease unrelated to corticosteroid use. *Can J Gastroenterol* 2001; **15**: 765-768 [PMID: 11727007 DOI: 10.1155/2001/293059]

6 **Lanyi B**, Dienes HP, Kruis W. [Recurrent aseptic osteonecrosis in Crohn's disease - extraintestinal manifestation or steroid related complication?]. *Dtsch Med Wochenschr* 2005; **130**: 1944-1947 [PMID: 16123897 DOI: 10.1055/s-2005-872607]

7 **Kamata N**, Oshitani N, Sogawa M, Yamagami H, Watanabe K, Fujiwara Y, Arakawa T. Usefulness of magnetic resonance imaging for detection of asymptomatic osteonecrosis of the femoral head in patients with inflammatory bowel disease on long-term corticosteroid treatment. *Scand J Gastroenterol* 2008; **43**: 308-313 [PMID: 18938768 DOI: 10.1080/00365520701676773]

8 **Hochberg M**. Rheumatology. 4th edition. Londres. USA: Mosby Elsevier, 2008

9 **Madsen PV**, Andersen G. Multifocal osteonecrosis related to steroid treatment in a patient with ulcerative colitis. *Gut* 1994; **35**: 132-134 [PMID: 8307435 DOI: 10.1136/gut.35.1.132]

10 **Van Assche G**, Dignass A, Reinisch W, van der Woude CJ, Sturm A, De Vos M, Guslandi M, Oldenburg B, Dotan I, Marteau P, Ardizzone A, Baumgart DC, D'Haens G, Gionchetti P, Portela F, Vucelic B, Söderholm J, Escher J, Koletzko S, Kolho KL, Lukas M, Mottet C, Tilg H, Vermeire S, Carbonnel F, Cole A, Novacek G, Reinshagen M, Tsianos E, Herrlinger K, Oldenburg B, Bouhnik Y, Kiesslich R, Stange E, Travis S, Lindsay J. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Special situations. *J Crohns Colitis* 2010; **4**: 63-101 [PMID: 21122490 DOI: 10.1016/j.crohns.2009.09.009]

11 **Malizos KN**, Karantanas AH, Varitimidis SE, Dailiana ZH, Bargiotas K, Maris T. Osteonecrosis of the femoral head: etiology, imaging and treatment. *Eur J Radiol* 2007; **63**: 16-28 [PMID: 17555906 DOI: 10.1016/j.ejrad.2007.03.019]

12 **Sakai T**, Sugano N, Nishii T, Haraguchi K, Yoshikawa H, Ohzono K. Bone scintigraphy for osteonecrosis of the knee in patients with non-traumatic osteonecrosis of the femoral head: comparison with magnetic resonance imaging. *Ann Rheum Dis* 2001; **60**: 14-20 [PMID: 11114276 DOI: 10.1136/ard.60.1.14]

13 **Gardeniers JM**. ARCO international classification of osteonecrosis. *ARCO Newsletter* 1993; **5**: 79-82

14 **Lai KA**, Shen WJ, Yang CY, Shao CJ, Hsu JT, Lin RM. The use of alendronate to prevent early collapse of the femoral head in patients with nontraumatic osteonecrosis. A randomized clinical study. *J Bone Joint Surg Am* 2005; **87**: 2155-2159 [PMID: 16203877 DOI: 10.2106/JBJS.D.02959]

15 **Pritchett JW**. Statin therapy decreases the risk of osteonecrosis in patients receiving steroids. *Clin Orthop Relat Res* 2001; **(386)**: 173-178 [PMID: 11347831 DOI: 10.1097/00003086-200105000-00022]

P- Reviewer: Maric I **S- Editor**: Gong ZM **L- Editor**: A
E- Editor: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i4.584

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 584-586
ISSN 2150-5349 (online)

LETTERS TO THE EDITOR

Gastroenterology, hepatology and movies: A holistic insight

Guilherme Macedo, Marco Silva

Guilherme Macedo, Marco Silva, Gastroenterology Department, Centro Hospitalar São João, Faculty of Medicine of the University of Porto, 4200-319 Porto, Portugal

Author contributions: Macedo G contributed to study concept and design and drafting of the manuscript; Silva M contributed to drafting of the manuscript.

Conflict-of-interest statement: Nothing to declare.

Manuscript source: Unsolicited manuscript

Correspondence to: Guilherme Macedo, MD, PhD, WGO, FACG, AGAF, FAASLD, Former President of the Portuguese Association For the Study of the Liver, Gastroenterology Department, Centro Hospitalar São João, Faculty of Medicine of the University of Porto, Alameda Professor Hernâni Monteiro, 4200-319 Porto, Portugal. guilhermemacedo59@gmail.com
Telephone: +351-225-512100
Fax: +351-225-025766

Received: April 29, 2016
Peer-review started: April 29, 2016
First decision: July 5, 2016
Revised: September 5, 2016
Accepted: September 21, 2016
Article in press: September 23, 2016
Published online: November 6, 2016

Abstract

The Project "Movies and Health in Night talks" took place in Braga and Porto, northern Portugal, in the last 3 years. This Project demonstrated how medical knowledge may surround and integrate a cosmopolitan and holistic approach, so that we as doctors and the general public, are able to become much closer and much more prone to understand the vital cycles of our society.

Key words: Cinema; Liver; Gastroenterology; Movies; Public

Core tip: The Project "Movies and Health in Night talks", conceived and produced through the chisel of a Gastroenterologist, clearly demonstrated how medical knowledge may surround and integrate a cosmopolitan and holistic approach, so that we as doctors and the general public, are able to become much closer and much more prone to understand the vital cycles of our society. Throughout those lively nights, many brilliant remarks were brought up, unexpected comments, unengaged points of view largely discussed, almost in a libertarian atmosphere, addressing the main topics that different experts and public figures were invited to dissect, about some of the most emblematic movies from the last decades. It is our firm believe that one of Hepatologists still unexplored noble tasks is to promote an anthropologic way of addressing and solving gastrointestinal and liver diseases burden.

Macedo G, Silva M. Gastroenterology, hepatology and movies: A holistic insight. *World J Gastrointest Pharmacol Ther* 2016; 7(4): 584-586 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i4/584.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i4.584

TO THE EDITOR

A Cinema is many times defined as the art of synthesis and the art of dialectic composition of several other artistic expressions like literature, painting or music. It is

in fact the creative spring for dreams and fascination, an instrument of emotional and sometimes terror catharsis, and undoubtedly the source of social allegories. It mirrors the most extraordinary biographies, individual and collective paths, in a multitude of visions casted by the most intensive joys and sorrows. In its essence, it is capable of generating or amplify new myths and new tales, many of our civilization's poetic fancies. Essentially, it is able to enlighten new symbols, and elect new icons as models of behaviours, even promoting ritual gestures and worships; in this way, movies performers transfigure themselves into vectors of freedom or oppression, if they help to release our minds or if they condition our trails: In other words, they bear the responsibility of shaping Health and Disease in our civilization[1-3].

In fact, these are the characters that Movie Industry thrusted into our own behavioural genoma, imprinting new coordinates for individual and social references, interfering and moulding up our own values and projects.

In Politics, in Sports, in Music and Arts, among the Media, how much science and art closely live together, so that we are able to recreate our own idols, myths and symbols? What life examples do we intimately favour and how does it effect in our expectations of individual and collective Health[1,4]?

These were the inferred questions and the explicit philosophy underlying the Project "Movies and Health in Night talks" which took place in several weeks cycles, in the last 3 years in Braga and Porto, northern Portugal. This Project, conceived and produced through the chisel of a Gastroenterologist, clearly demonstrated how medical knowledge may surround and integrate a cosmopolitan and holistic approach, so that we as doctors and the general public, are able to become much closer and much more prone to understand the vital cycles of our society. Throughout those lively nights, many brilliant remarks were brought up, unexpected comments, unengaged points of view largely discussed, almost in a libertarian atmosphere, addressing the main topics that different experts and public figures were invited to dissect, about some of the most emblematic movies from the last decades.

Imagine the thrills: Before a live audience of around 100 people, in an auditorium prepared as if a movie was to be projected, but in a stage specifically arranged as if a cosy literary assembly would happen, a 2 h interview was anchored and lead by a Gastroenterologist, trying to set the pace of a most dynamic and at times provocative talk with well-known guests. Not in a medical meeting, or in a big specialty convention, but facing the lay public and even some media gurus! This was really a hard task and a brave new world to the Gastroenterologist, having to play a true pivotal task (away from his technological comfort zone…), in orienting and exploring the visions and perceptions of his 2 special guest stars. These guest stars changed every week, depending on the movie to be dissected, and consisted of widely known Portuguese artists, tv and radio public figures, sports people, and newsmen. On stage, in a TV like interview format, they expanded over the selected movie for that night. After presenting and viewing the initial trailer, the discussion was based on 4 takes of 6-8 min each; those takes were previously selected and edited by the anchor and really set the stage for a highly informal and free vivid discussion, changing points of view, also allowing handfuls of wise and bright references, sprinkled with personal experiences, funny, intimate, carefully and attentively followed by the audience. How did it work? The first part of the crosstalk, immediately after the trailer presentation, was very useful to place the topic, to put into context the subject to be addressed, focusing on the movie maker, actor's performance, interesting backgrounds and so on. Then, in a 15 min discussion after viewing the selected take, our guest stars were challenged to elaborate on their own thoughts and perspectives, and here again, the role of moderation was crucial, so that the scope has to be kept away from the initiatic and almost inexpugnable medical jargon, but at the same time health concerns should be brought under the spotlight, allowing everyone to understand and realize how extraordinarily common gastrointestinal and liver problems come along with personal decisions and behaviours or social changes.

Under the title "Cults, Vices and Fashions", which accomplished the first year's project, the selected movies were: Pollock (Ed Harris 2000), The People *vs* Harry Flynt (Milos Forman 1996), Pulp Fiction (Quentin Tarantino 1994) and 24 Hour Party People (Michael Winterbotton 2002). Those master pieces really showed how social behaviours and trends can truly influence health individually.

The second and third years projects addressed "Myths, Symbols and Idols", picking up Frida (Julie Taynor 2002), The Aviator (Martin Scorcese 2004), The Doors (Oliver Stone 2001), Ali (Michael Mann 2001), Eyes Wide Shut (Stanley Kubrick 1999), Easy Rider (Dennis Hopper 1969) and Maradona (Emir Kusturica 2008). This time the focus was on how individuals give their testimony and examples to become driving forces of our culture, again with health and disease being influenced by their own experience, in an environment loaded with alcohol, or sex, or drugs or even…sports.

Facts are that this cultural model made quite an impact in local social tissue. The auditorium was freely open to public, but a predominant fringe of university students and teachers was seen along with lay people and movie lovers (even some doubtful doctors from our Hospital and Medical School!). Many national newspapers and some radios gave echos about this Project. Again, this proved to be a new way of discovering new worlds in settled worlds, and a contribution to broaden the horizons where the skilled Gastroenterologist is generally moving, so many times being unaware of that. Just an irreverent attempts to stir up food for thought, awakening consciences and trying to shake the conventional borders of knowledge.

The truth is that, at the end of the day, we all won: Enlightened people now unexpectedly aware of how many trivial happenings might have influenced or still

may influence their own health history; media people that came to realize that doctors have much more to share than the traditional hermetic medical knowledge, and that it is written in their nature the drive for moving forward in cognitive skills about the surrounding world; and we doctors, specially we Gastroenterologists, much more conscious on that we should keep constantly in mind that diseases have larger boundaries than those anticipated in our challenging patient, and that those apparently clear cut patients have much larger landscapes than a given disease constraint. It is our firm believe that one of our still unexplored noble tasks is to promote an anthropologic way of addressing and solving gastrointestinal and liver diseases burden.

REFERENCES

1 **Díaz Membrives M**, Icart Isern MT, López Matheu MC. Literature review: Use of commercial films as a teaching resource for health sciences students. *Nurse Educ Today* 2016; **36**: 264-267 [PMID: 26490325 DOI: 10.1016/j.nedt.2015.10.002]

2 **Wahlert L**. "I Caught It at the Movies": Reflections on Medical History, Movie Theaters, and the Cinema of Contagion. *J Homosex* 2016; **63**: 323-328 [PMID: 26644058 DOI: 10.1080/00918369.2016.1124689]

3 **McKie A**. Using the arts and humanities to promote a liberal nursing education: strengths and weaknesses. *Nurse Educ Today* 2012; **32**: 803-810 [PMID: 22579881 DOI: 10.1016/j.nedt.2012.04.014]

4 **Lee A**. Health-promoting schools: evidence for a holistic approach to promoting health and improving health literacy. *Appl Health Econ Health Policy* 2009; **7**: 11-17 [PMID: 19558191 DOI: 10.1007/BF03256138]

P- Reviewer: Campo GM, Jaeschke H, Serrano-Luna J
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Lu YJ

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com