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Helicobacter pylori and pancreatic diseases

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Abstract

A possible role for *Helicobacter pylori* (*H. pylori*) infection in pancreatic diseases remains controversial. *H. pylori* infection with antral predomination leading to an increase in pancreatic bicarbonate output and inducing ductal epithelial cell proliferation could contribute to the development of pancreatic cancer *via* complex interactions with the ABO genotype, dietary and smoking habits and N-nitrosamine exposure of the host. Although the individual study data available so far is inconsistent, several meta-analyses have reported an increased risk for pancreatic cancer among *H. pylori* seropositive individuals. It has been suggested that *H. pylori* causes autoimmune pancreatitis due to molecular mimicry between *H. pylori* α-carbonic anhydrase (α-CA) and human CA type Ⅱ, and between *H. pylori* plasminogen-binding protein and human ubiquitin-protein ligase E3 component n-recognin 2, enzymes that are highly expressed in the pancreatic ductal and acinar cells, respectively. Future studies involving large numbers of cases are needed in order to examine the role of *H. pylori* in autoimmune pancreatitis more fully. Considering the worldwide pancreatic cancer burden, as well as the association between autoimmune pancreatitis and other autoimmune conditions, a complete elucidation of the role played by *H. pylori* in the genesis of such conditions could have a substantial impact on healthcare.

Key words: *Helicobacter pylori*; Pancreatic cancer; Pancreatitis; Autoimmune pancreatitis; Molecular mimicry

Core tip: *Helicobacter pylori* (*H. pylori*) infection with antral predomination could contribute to the development of pancreatic cancer through complex interactions with ABO genotypes, dietary and smoking habits and N-nitrosamine exposure of the host. It has been suggested that *H. pylori* causes autoimmune pancreatitis due to molecular mimicry between *H. pylori* α-carbonic anhydrase (α-CA) and human CA type Ⅱ, and between *H. pylori* plasminogen-binding protein and human ubiquitin-protein ligase E3 component n-recognin 2. Considering the worldwide burden of pancreatic diseases, complete elucidation of *H. pylori* role in their genesis could have substantial healthcare impact.

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INTRODUCTION

Helicobacter pylori (*H. pylori*), the ubiquitous bacterium that colonizes the human stomach, has been the subject of increased attention in the last 30 years. It has been sug-

gested that modern humans were infected with *H. pylori* before their migration from Africa over 58000 years ago and that *H. pylori* strains have been intimately associated with their human host populations ever since[1]. Over half the modern human population is infected with *H. pylori*, and its prevalence varies from 60%-90% in Japan, China, Russia and most of Central and Eastern Europe to 30%-40% in Western Europe and the United States[2]. *H. pylori* is proven to be associated with an increased risk for gastric cancer[3], peptic ulcer disease[4] and lymphoma[5]; however, a possible role for *H. pylori* infection in pancreatic disease remains controversial.

Previous studies have examined the association between *H. pylori* infection and diseases of the pancreas, including pancreatic carcinoma[6-12] and autoimmune pancreatitis[13-15], but with inconsistent results. Nevertheless, there is a solid theoretical basis for explaining the potential role for *H. pylori* in the development of these conditions. It has been proposed that *H. pylori* causes autoimmune pancreatitis due to molecular mimicry between *H. pylori* α-carbonic anhydrase (α-CA) and human CA type Ⅱ[14], and it is known that the homologous CA segments contain the binding motif of the HLA molecule DRB1*0405, which confers a risk of developing autoimmune pancreatitis. Furthermore, it has been suggested that *H. pylori* infection contributes to the development of pancreatic cancer *via* complex interactions with the ABO genotype, dietary and smoking habits and N-nitrosamine exposure of the host[16].

Pancreatic cancer is the eighth leading cause of cancer-related deaths worldwide[17] with the five year survival rate as low as 6%[18]. Autoimmune pancreatitis is a relatively novel clinical entity defined as a chronic inflammation of the pancreas due to an autoimmune mechanism[19]. Although autoimmune pancreatitis accounts for a relatively small proportion of chronic pancreatitis cases it can be associated with other autoimmune conditions, suggesting a possible involvement of the entire gastrointestinal system. With this in mind, elucidating the role of *H. pylori* in the development of pancreatic diseases could have a substantial impact on health care.

We have, therefore, conducted a comprehensive literature search in order to summarize the evidence for a role for *H. pylori* in the pathogenesis of pancreatic diseases with particular emphasis on pancreatic cancer and autoimmune pancreatitis.

H. PYLORI AND PANCREATIC CANCER

To date, no study has isolated *H. pylori* DNA in any pancreatic sample[20,21]; however, although *H. pylori* appears not to colonize the pancreas it could have an effect on pancreatic carcinogenesis through pathophysiological action. *H. pylori* shows two different colonization behaviors: one associated with pangastritis leading to hypochlorhydria, atrophic gastritis, gastric ulcer and gastric cancer, and the other associated with antral-predominant gastritis leading to hyperchlorhydria, pyloric and duodenal ulcer and, potentially, pancreatic cancer. Colonization of the antrum by *H. pylori* reduces the number of antral D-cells thus suppressing the production of somatostatin. This, in turn, leads to hyperacidity, which results in an increase in the secretion of secretin and pancreatic bicarbonate output. Secretin has been shown to have a positive effect on murine pancreatic growth as well as DNA synthesis in pancreatic ductal cells[22], and it is possible that induced ductal epithelial cell proliferation could enhance the carcinogenic effect of known carcinogens, such as N-nitrosoamines, in the pancreas, leading to the development of pancreatic cancer.

Although this assumption is hypothetical and needs to be proven there is indirect proof suggesting that *H. pylori* does play a role in pancreatic carcinogenesis. A number of serology-based studies have assessed the association between the presence of anti-*H. pylori* antibodies and pancreatic cancer[6-12]. The first of these, conducted by Raderer *et al*[6], reported a two-fold increase in the risk for pancreatic cancer among *H. pylori*-positive individuals [Odds ratio (OR) = 2.1, 95%CI: 1.09-4.05]. These findings were confirmed in the subsequent Alpha-Tocopherol, β-Carotene Cancer Prevention Study (ATBC Study), a prospective cohort study of male smokers that reported subjects positive for *H. pylori* antibodies or CagA-positive *H. pylori* strains to be at increased risk of developing pancreatic cancer (OR = 1.87, 95%CI: 1.05-3.34; OR = 2.01, 95%CI: 1.09-3.70, respectively)[7].

In contrast, two succeeding studies[8,9], each following patients for 20 years or more, reported no significant association between *H. pylori* infection and pancreatic cancer. In a nested case-control study of 104 pancreatic cancer cases and 262 matched controls, de Martel *et al*[8] selected patients from among 128992 adult subscribers to the Kaiser Permanente Medical Care Program who had been enrolled from 1964 to 1969, and found no association between *H. pylori* (OR = 0.85, 95%CI: 0.49-1.48) or its CagA protein (OR = 0.96, 95%CI: 0.48-1.92) and the subsequent development of pancreatic cancer. In the second study, Lindkvist *et al*[9] conducted a similar analysis on subjects from the Malmö Preventive Project cohort. After analysis of 87 cases and 263 matched controls the researchers reported that *H. pylori* seropositivity was not associated with pancreatic cancer (OR = 1.25, 95%CI: 0.75-2.09). Finally, a case-control study in a Polish population also reported that neither *H. pylori* (OR = 1.27, 95%CI: 0.64-2.61) nor CagA (OR = 0.90, 95%CI: 0.46-1.73) seropositivity were significant risk factors for pancreatic cancer[10].

However, Risch *et al*[11] were the first to suggest that infection with CagA-negative *H. pylori* could be a risk for pancreatic cancer. In a United States population-based case control study, conducted on 373 pancreatic cancer cases and 690 controls, the researchers reported that CagA-negative *H. pylori* seropositivity was a significant risk factor for pancreatic cancer (OR = 1.68, 95%CI: 1.07-2.66), while no significant association was reported for CagA-positive seropositivity (OR = 0.77, 95%CI: 0.52-1.16). Furthermore, the group observed the association between a pancreatic cancer risk and CagA-negative

H. pylori seropositivity only among individuals with a non-O blood type but not among those with O blood type (OR = 2.78, 95%CI: 1.49-5.20; OR = 1.28, 95%CI: 0.62-2.64, respectively), supporting a role for the ABO blood group system in mediating *H. pylori* carcinogenic potential in the pancreas. The same group conducted a similar study on the Chinese population of Shanghai and reported an increased, but not significant, risk of developing pancreatic cancer for CagA-negative *H. pylori* seropositive patients (OR = 1.28, 95%CI: 0.76-2.13)[12]. In addition, CagA-positive seropositivity was shown to protect against pancreatic cancer when compared to *H. pylori* seronegative individuals (OR = 0.68, 95%CI: 0.54-0.84).

Several meta-analyses have attempted to summarize the existing data on the role of *H. pylori* in pancreatic carcinogenesis[16,23,24] including different number of studies based on differences in inclusion criteria. All reported a significant increase in the risk of developing pancreatic cancer among *H. pylori*-positive individuals, with the summary OR ranging from 1.65 (95%CI: 1.30-2.09)[16] to 1.38 (95%CI: 1.22-1.77)[23]. However, none of the meta-analyses reported a significant association between CagA-positive seropositivity and pancreatic cancer[23,24].

Bearing all this data in mind, it could be concluded that the published scientific evidence (although somewhat inconsistent) supports a role for *H. pylori* in the development of pancreatic cancer. The exact mechanism involved in the influence of *H. pylori* on pancreatic carcinogenesis is still unclear and has yet to be explained fully. However, if *H. pylori* is found to increase the risk of developing pancreatic cancer, this could be another reason for targeting *H. pylori* for eradication, especially in individuals with a specific genetic burden, such as a family history of pancreatic cancer.

H. PYLORI AND PANCREATITIS

Although there have been some studies on animal models suggesting a possible role for *H. pylori* infection in acute pancreatitis[25], no author has so far reported a significant association between *H. pylori* infection and acute pancreatitis in humans. Khan *et al*[13] undertook a study of 50 patients with acute alcoholic pancreatitis and 50 alcoholic controls but found no association between *H. pylori* infection and the occurrence of acute pancreatitis.

However, the relationship between *H. pylori* and chronic pancreatitis, and autoimmune chronic pancreatitis in particular, has been the subject of more research. In approximately 60% of cases autoimmune pancreatitis is associated with the presence of other autoimmune diseases such as Sjögren's syndrome, sclerosing extrahepatic cholangitis, primary biliary cirrhosis, autoimmune hepatitis, retroperitoneal fibrosis, salivary gland swelling, inflammatory bowel disease, Hashimoto's thyroiditis and gastric peptic ulceration[26-28]. All of these diseases, including autoimmune pancreatitis itself, are characterized by similar pathohistological findings including fibrotic changes and/or lymphoplasmacytic inflammation. However, to date, no study has isolated *H. pylori* DNA from samples of patients affected with autoimmune pancreatitis[21].

It has been suggested previously that *H. pylori* infection exists as a possible common cause of these conditions acting *via* a mechanism involving the molecular mimicry of host structures[29]. In 2005 Guarneri *et al*[14] reported significant homology between human CA type Ⅱ and *H. pylori* α-CA, an enzyme fundamental for the survival of the bacterium in the gastric environment. As human CA type Ⅱ is expressed in the pancreatic ductal epithelium, *H. pylori* could trigger autoimmune pancreatitis by mimicking the host's CA type Ⅱ protein. Then, in 2009, Frulloni *et al*[15] identified *H. pylori* plasminogen-binding protein (PBP) antibodies in 95% of patients with autoimmune pancreatitis. However, PBP antibodies were not detected in patients with either alcohol-induced chronic pancreatitis or intraductal papillary mucinous neoplasm. *H. pylori* PBP was found to have substantial homology with ubiquitin-protein ligase E3 component n-recognin 2 (UBR2), an enzyme highly expressed in the acinar cells of the pancreas, and thus this could be another pathway through which *H. pylori* provokes molecular mimicry-induced autoimmune pancreatitis. The following year, our group (Löhr *et al*[30]) conducted a study on autoimmune pancreatitis samples using gene and protein expression profiling as well as immunoassays. Our research confirmed that acinar cells, in addition to ductal cells, are the target of immune-related inflammatory process-characterizing autoimmune pancreatitis, supporting a molecular mimicry mechanism between *H. pylori* PBP and human UBR2. All this data provides a solid theoretical basis for the hypothesis that gastric *H. pylori* infection can trigger autoimmune pancreatitis in genetically predisposed subjects. Moreover, in a series of patients with chronic pancreatitis, Dore *et al*[31] reported a reversal of elevated pancreatic enzymes after *H. pylori* eradication. However, although prevention and treatment strategies for autoimmune pancreatitis acknowledge *H. pylori* as the cause, or one of the causes, of this disease, future clinical studies that include a large number of cases will be needed in order to confirm these findings.

In conclusion, summarizing the data from available clinical studies supports a role for *H. pylori* in pancreatic carcinogenesis and autoimmune pancreatitis. Although the exact mechanisms are still unknown, molecular mimicry may play a role in the development of autoimmune pancreatitis, while pancreatic carcinoma may develop in response to *H. pylori* colonization of the antrum leading to an increase in secretin secretion and pancreatic bicarbonate output resulting in ductal epithelial cell proliferation. However, further research is needed to confirm these theoretical assumptions on the role of *H. pylori* in the development of pancreatic disease.

REFERENCES

1 **Linz B**, Balloux F, Moodley Y, Manica A, Liu H, Roumagnac P, Falush D, Stamer C, Prugnolle F, van der Merwe SW, Yamaoka Y, Graham DY, Perez-Trallero E, Wadstrom T, Suerbaum S, Achtman M. An African origin for the intimate

association between humans and Helicobacter pylori. *Nature* 2007; **445**: 915-918 [PMID: 17287725 DOI: 10.1038/nature05562]
2 **Brenner H**, Rothenbacher D, Arndt V. Epidemiology of stomach cancer. *Methods Mol Biol* 2009; **472**: 467-477 [PMID: 19107449 DOI: 10.1007/978-1-60327-492-0_23]
3 **Uemura N**, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ. Helicobacter pylori infection and the development of gastric cancer. *N Engl J Med* 2001; **345**: 784-789 [PMID: 11556297 DOI: 10.1056/NEJMoa001999]
4 **Nomura A**, Stemmermann GN, Chyou PH, Perez-Perez GI, Blaser MJ. Helicobacter pylori infection and the risk for duodenal and gastric ulceration. *Ann Intern Med* 1994; **120**: 977-981 [PMID: 7741826]
5 **Parsonnet J**, Hansen S, Rodriguez L, Gelb AB, Warnke RA, Jellum E, Orentreich N, Vogelman JH, Friedman GD. Helicobacter pylori infection and gastric lymphoma. *N Engl J Med* 1994; **330**: 1267-1271 [PMID: 8145781 DOI: 10.1056/NEJM199405053301803]
6 **Raderer M**, Wrba F, Kornek G, Maca T, Koller DY, Weinlaender G, Hejna M, Scheithauer W. Association between Helicobacter pylori infection and pancreatic cancer. *Oncology* 1998; **55**: 16-19 [PMID: 9428370 DOI: 10.1159/000011830]
7 **Stolzenberg-Solomon RZ**, Blaser MJ, Limburg PJ, Perez-Perez G, Taylor PR, Virtamo J, Albanes D. Helicobacter pylori seropositivity as a risk factor for pancreatic cancer. *J Natl Cancer Inst* 2001; **93**: 937-941 [PMID: 11416115 DOI: 10.1093/jnci/93.12.937]
8 **de Martel C**, Llosa AE, Friedman GD, Vogelman JH, Orentreich N, Stolzenberg-Solomon RZ, Parsonnet J. Helicobacter pylori infection and development of pancreatic cancer. *Cancer Epidemiol Biomarkers Prev* 2008; **17**: 1188-1194 [PMID: 18483341 DOI: 10.1158/1055-9965.EPI-08-0185]
9 **Lindkvist B**, Johansen D, Borgström A, Manjer J. A prospective study of Helicobacter pylori in relation to the risk for pancreatic cancer. *BMC Cancer* 2008; **8**: 321 [PMID: 18986545 DOI: 10.1186/1471-2407-8-321]
10 **Gawin A**, Wex T, Ławniczak M, Malfertheiner P, Starzyńska T. [Helicobacter pylori infection in pancreatic cancer]. *Pol Merkur Lekarski* 2012; **32**: 103-107 [PMID: 22590913]
11 **Risch HA**, Yu H, Lu L, Kidd MS. ABO blood group, Helicobacter pylori seropositivity, and risk of pancreatic cancer: a case-control study. *J Natl Cancer Inst* 2010; **102**: 502-505 [PMID: 20181960 DOI: 10.1093/jnci/djq007]
12 **Risch HA**, Lu L, Kidd MS, Wang J, Zhang W, Ni Q, Gao YT, Yu H. Helicobacter pylori seropositivities and risk of pancreatic carcinoma. *Cancer Epidemiol Biomarkers Prev* 2014; **23**: 172-178 [PMID: 24234587]
13 **Khan J**, Pelli H, Lappalainen-Lehto R, Järvinen S, Sand J, Nordback I. Helicobacter pylori in alcohol induced acute pancreatitis. *Scand J Surg* 2009; **98**: 221-224 [PMID: 20218418]
14 **Guarneri F**, Guarneri C, Benvenga S. Helicobacter pylori and autoimmune pancreatitis: role of carbonic anhydrase via molecular mimicry? *J Cell Mol Med* 2005; **9**: 741-744 [PMID: 16202223 DOI: 10.1111/j.1582-4934.2005.tb00506.x]
15 **Frulloni L**, Lunardi C, Simone R, Dolcino M, Scattolini C, Falconi M, Benini L, Vantini I, Corrocher R, Puccetti A. Identification of a novel antibody associated with autoimmune pancreatitis. *N Engl J Med* 2009; **361**: 2135-2142 [PMID: 19940298 DOI: 10.1056/NEJMoa0903068]
16 **Risch HA**. Pancreatic cancer: Helicobacter pylori colonization, N-nitrosamine exposures, and ABO blood group. *Mol Carcinog* 2012; **51**: 109-118 [PMID: 22162235 DOI: 10.1002/mc.20826]
17 **International agency on research of cancer**. GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012. [cited 2014 20.01.2014.]. Available from: URL: http://globocan.iarc.fr
18 **American Cancer Society**. Cancer Facts & Figures 2013. Atalanta: American Cancer Society, 2013
19 **Etemad B**, Whitcomb DC. Chronic pancreatitis: diagnosis, classification, and new genetic developments. *Gastroenterology* 2001; **120**: 682-707 [PMID: 11179244 DOI: 10.1053/gast.2001.22586]
20 **Nilsson HO**, Stenram U, Ihse I, Wadstrom T. Helicobacter species ribosomal DNA in the pancreas, stomach and duodenum of pancreatic cancer patients. *World J Gastroenterol* 2006; **12**: 3038-3043 [PMID: 16718784]
21 **Jesnowski R**, Isaksson B, Möhrcke C, Bertsch C, Bulajic M, Schneider-Brachert W, Klöppel G, Lowenfels AB, Maisonneuve P, Löhr JM. Helicobacter pylori in autoimmune pancreatitis and pancreatic carcinoma. *Pancreatology* 2010; **10**: 462-466 [PMID: 20720447 DOI: 10.1159/000264677]
22 **Haarstad H**, Petersen H. Short- and long-term effects of secretin and a cholecystokinin-like peptide on pancreatic growth and synthesis of RNA and polyamines. *Scand J Gastroenterol* 1989; **24**: 721-732 [PMID: 2479084 DOI: 10.3109/00365528909093114]
23 **Xiao M**, Wang Y, Gao Y. Association between Helicobacter pylori infection and pancreatic cancer development: a meta-analysis. *PLoS One* 2013; **8**: e75559 [PMID: 24086571 DOI: 10.1371/journal.pone.0075559]
24 **Trikudanathan G**, Philip A, Dasanu CA, Baker WL. Association between Helicobacter pylori infection and pancreatic cancer. A cumulative meta-analysis. *JOP* 2011; **12**: 26-31 [PMID: 21206097]
25 **Warzecha Z**, Dembiński A, Ceranowicz P, Dembiński M, Sendur R, Pawlik WW, Konturek SJ. Deleterious effect of Helicobacter pylori infection on the course of acute pancreatitis in rats. *Pancreatology* 2002; **2**: 386-395 [PMID: 12138227 DOI: 10.1159/000065086]
26 **Kim KP**, Kim MH, Song MH, Lee SS, Seo DW, Lee SK. Autoimmune chronic pancreatitis. *Am J Gastroenterol* 2004; **99**: 1605-1616 [PMID: 15307882 DOI: 10.1111/j.1572-0241.2004.30336.x]
27 **Shinji A**, Sano K, Hamano H, Unno H, Fukushima M, Nakamura N, Akamatsu T, Kawa S, Kiyosawa K. Autoimmune pancreatitis is closely associated with gastric ulcer presenting with abundant IgG4-bearing plasma cell infiltration. *Gastrointest Endosc* 2004; **59**: 506-511 [PMID: 15044886 DOI: 10.1016/S0016-5107(03)02874-8]
28 **Okazaki K**, Chiba T. Autoimmune related pancreatitis. *Gut* 2002; **51**: 1-4 [PMID: 12077078 DOI: 10.1136/gut.51.1.1]
29 **Kountouras J**, Zavos C, Chatzopoulos D. A concept on the role of Helicobacter pylori infection in autoimmune pancreatitis. *J Cell Mol Med* 2005; **9**: 196-207 [PMID: 15784177 DOI: 10.1111/j.1582-4934.2005.tb00349.x]
30 **Löhr JM**, Faissner R, Koczan D, Bewerunge P, Bassi C, Brors B, Eils R, Frulloni L, Funk A, Halangk W, Jesenofsky R, Kaderali L, Kleeff J, Krüger B, Lerch MM, Lösel R, Magnani M, Neumaier M, Nittka S, Sahin-Tóth M, Sänger J, Serafini S, Schnölzer M, Thierse HJ, Wandschneider S, Zamboni G, Klöppel G. Autoantibodies against the exocrine pancreas in autoimmune pancreatitis: gene and protein expression profiling and immunoassays identify pancreatic enzymes as a major target of the inflammatory process. *Am J Gastroenterol* 2010; **105**: 2060-2071 [PMID: 20407433 DOI: 10.1038/ajg.2010.141]
31 **Dore MP**, Sepulveda AR, Pedroni A, Realdi G, Delitala G. Reversal of elevated pancreatic enzymes after Helicobacter pylori eradication. *Intern Emerg Med* 2008; **3**: 269-270 [PMID: 18264669 DOI: 10.1007/s11739-008-0117-3]

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TOPIC HIGHLIGHT

WJGP 5th Anniversary Special Issues (1): *Helicobacter pylori*

Use of probiotics in the fight against *Helicobacter pylori*

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Abstract

After the discovery of *Helicobacter pylori* (*H. pylori*), and the evidence of its relationship with gastric diseases, antibiotic-based therapies were developed, which efficacy was however limited by antibiotic resistance and lack of patient compliance. A vaccine would overcome these drawbacks, but currently there is not any *H. pylori* vaccine licensed. In the frame of the studies aimed at finding alternative therapies or at increasing the efficacy of the current ones and/or reducing their side effects, the investigation on the use of probiotics plays an interesting role. *In vitro* and preclinical studies have shown the feasibility of this approach. Several clinical trials indicated that administration of probiotics can reduce the side effects of *H. pylori* eradication treatment, increasing tolerability, and often increases the overall efficacy. The results of these trials vary, likely reflecting the variety of probiotics assessed and that of the eradication treatment, as well as the differences in the geographic area that imply different *H. pylori* strains distribution, host susceptibility, and therapy efficacy. In conclusion, the use of probiotics appears promising as an adjuvant for the current *H. pylori* eradication treatment, though it still requires optimization.

Key words: *Helicobacter pylori*; Treatment; Probiotics; *Lactobacilli*; *Bifidobacteria*

Core tip: *Helicobacter pylori* (*H. pylori*) is the only bacterium that has been linked to cancer to date. The efficacy of antibiotic-based eradication treatment is hampered by antibiotic resistance and side effects that may reduce patient compliance. No vaccine is currently licensed. Thus, administration of alternative compounds that may increase the efficacy of the treatment and/or reduce side effects is of particular interest. Administration of probiotics has been proposed to increase tolerability and efficacy of the *H. pylori* eradication treatment. The results of the most recent clinical trials seem to confirm these hypotheses.

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INTRODUCTION

The gastric mucosa of more than 50% of human population is estimated to be colonized by *Helicobacter pylori* (*H. pylori*), a curved or spiral-shaped, flagellated, microaerophilic, Gram-negative bacillus. *H. pylori* was isolated and cultured from human gastric biopsies only at the beginning of 1980s[1] and classified a few years later[2], although bacteria in mammalian stomach had been already observed at the end of 19th century. Prevalence of *H. pylori* infection is much higher in developing than in developed countries[3,4], most probably as a consequence of different hygiene and living conditions.

H. pylori colonization is mostly asymptomatic, but a subset of the *H. pylori*-infected population develops chronic gastritis, peptic ulcer, or gastric mucosa-associated lymphoid tissue (MALT) lymphoma[5-7]. Moreover, *H. pylori* infection increases the risk of developing gastric cancer, thus WHO has included this pathogen among the category 1 carcinogens[8-10]. Both direct bacterial action and host

response originate chronic inflammation of the stomach and the pathological outcome in the presence of *H. pylori* infection. To make inoffensive the strong host immune response, *H. pylori* activates escaping strategies and exerts on the host immune system immunomodulatory action, through various mechanisms, including the ability of eliciting T regulatory cells and of driving T helper type 1 (Th1) and Th17 response[11-14], but establishing in the majority of the cases a relatively harmless coexistence. Nevertheless, the concomitance of certain host genetic backgrounds (such as particular polymorphisms of inflammatory cytokines[15-18]), or particular susceptibility to develop gastric autoimmunity through the activation of CD4+ Th1 cells specific for *H. pylori* peptides cross-reactive with H+, K+-ATPase[19] and factors that make *H. pylori* particularly virulent (such as CagA, the product of cytotoxin-associated gene A[20,21]), can alter this equilibrium and lead to pathological outcomes including malignant lesions.

Diagnosis of *H. pylori* infection in symptomatic subjects is generally followed by the eradication therapy. The eradication causes regression of *H. pylori*-induced peptic ulcer and MALT lymphoma[10,22], and would represent a tool for reduction of gastric cancer incidence in risk populations[23]. Current standard therapies against *H. pylori* are based on the use of one proton pump inhibitor plus two or more antibiotics for one-two weeks[24], with several variants also according to the geographic area[25-27]. The efficacy of the treatments has decreased below 80%[28], mainly due to the increase of antibiotic resistance but also to side effects (such as nausea, vomiting, diarrhea, constipation, fever, headache, *etc.*[29]), which, although generally mild, may cause poor patient compliance and discontinuing of the treatment. Thus, modifications in the combination, sequence, and duration of drug administration are continuously under investigation[24,29].

Vaccination would represent a valid alternative approach to overcome the existing problems with the antibiotic therapy. A large number of preclinical efficacy studies for vaccine candidates against *H. pylori* have been published, which however were followed by a limited number of clinical trials[30]: unfortunately, the trials that included efficacy studies failed. Presently, there is not any licensed anti-*H. pylori* vaccine.

Probiotics include several microorganisms, mostly within *Lactobacillus* or *Bifidobacterium* genus, which can be grouped under the current definition living microorganisms, which, upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition[31,32]. The beneficial effects of probiotics on gastrointestinal diseases, including antibiotic-associate diarrhea, have been widely described[33-37]. Thus, due to the gastric localization of *H. pylori* colonization and its relationships with gastric diseases, it is not surprising that several studies were carried out on the effects of probiotics on *H. pylori*. Numerous *in vitro* studies, demonstrating bacterial killing or inhibition[38], were followed by preclinical and clinical studies[38-40]. These studies indicated only partial efficacy of probiotics against *H. pylori* when administered alone, but increase of efficacy and/or reduction of side effects when probiotics were administered together with the eradication treatment[39,40].

The present review is aimed at summarizing the results of the clinical trials reported in the last two years, which assessed the efficacy of probiotics administration as an adjuvant for *H. pylori* eradication treatment. The efficacy against *H. pylori* of probiotics administered alone will be not discussed.

META-ANALYSES

Three meta-analyses on probiotics supplementation of *H. pylori* eradication therapy were published in 2013. All three meta-analyses concluded, in agreement each other, that overall probiotics exerted beneficial effects on eradication treatment, with eradication rates significantly increased. Two of these meta-analyses observed significant decrease of side effects when probiotics were added to the eradication treatment, while one of them did not observe any variation. The variety of *H. pylori* eradication treatments and of the probiotics used makes impossible a direct comparison of the results of the single studies each other; nevertheless, the overall results may provide valuable information about the possible efficacy of probiotics.

The first analysis, by Wang *et al*[41] included 10 trials (9 in adults, 1 in children), corresponding to 1469 patients overall. Of these, incidence of side effects was reported in 6 trials corresponding to 978 patients. The analysis considered only parallel controlled trials, with confirmation of eradication outcome by urea breath test or rapid urea test, and comparing at least 2 branches of treatment consisting of control group (proton pump inhibitor plus 2 antibiotics with placebo or no additional intervention) and experimental groups (the same eradication regimen plus *Lactobacillus*-containing and *Bifidobacterium*-containing probiotic compound preparation). Eradication was observed in 82.63% (535 patients eradicated/708 treated) (intention-to-treat analysis, ITT) or 87.42% (535/612) (per-protocol analysis, PP) of the subjects receiving eradication therapy supplemented by probiotics, *vs* 67.85% (517/762, ITT) or 76.43 (496/649, PP) in the control group receiving eradication therapy alone. Side effects were observed in 15.37% (71/462, ITT) of the probiotics + therapy group, *vs* 31.01% (160/516, ITT) of the control group.

The second analysis, by Zheng *et al*[42] included 9 trials (6 in adults, 3 in children), corresponding to 1163 patients. Five of these trials, corresponding to 739 patients, reported the incidence of side effects. The analysis considered only randomized controlled trials that compared the efficacy of probiotic preparations, administered together with triple or sequential therapy, with that of placebo (or blank control) in *H. pylori*-positive participants. *H. pylori* positivity was assessed by ^{13}C-urea breath test, and/or histology, and/or stool antigen test. Eradication rate increased from 68.54% (414/604, ITT) of the control group to the 78.18% (437/559, ITT) of the

probiotics+therapy group (receiving a single *Lactobacillus* species or multi-strain compounds including *Lactobacilli*). Side effects did not show variations overall, being observed in 31.21% (108/346, ITT) of probiotics + therapy group, *vs* 34.86% (137/393, ITT) of the controls. Remarkably, significant differences were found when examining separately the subgroup of five trials in which a single *Lactobacillus* species was administered; in this case, significant increase of eradication rate was accompanied by decrease of side effects as compared to control group.

The third analysis, by Li *et al*[43] included 7 pediatric randomized controlled trials, corresponding to 508 patients. Five of them, corresponding to 393 subjects, reported the incidence of side effects. The studies included in this meta-analysis compared at least two treatment groups: one receiving triple regimen (proton pump inhibitor and two antibiotics) with placebo or no extra intervention, and one receiving the same triple regimen plus probiotics. Eradication was confirmed by urea breath test or stool antigen test or histology or rapid urea test. Probiotic preparations consisted of multi-strain compounds including *Lactobacillus* and *Bifidobacterium* species, and *S. thermophilus*; one study used *S. boulardii*. Eradication was observed in 78.13% (200/256, ITT) or 82.30% (200/243, PP) of the probiotics + therapy group *vs* 66.67% (168/252, ITT) or 69.42% (168/242, PP) of the controls; the probiotics + therapy resulted efficacious in reducing side effects to 21.72% (43/198) from 42.56% (83/195) observed in control group.

RECENT CLINICAL TRIALS

The trials reported in 2012-2013, in which *H. pylori* eradication treatments with or without probiotics administration were compared, are summarized in Table 1[44-53]. All were randomized clinical trials that included at least one *H. pylori* eradication treatment group and one group that received the same eradication treatment plus probiotic compounds.

Evidence of the ability of probiotics treatment to both significantly increase the efficacy of *H. pylori* treatment and decrease the side effects was provided in 3 out of 10 studies. Efficacy only in reducing side effects was observed in 3 out of the 9 studies for which the side effects description was available; in 1 out of these 9 studies, the efficacy on *H. pylori* eradication only was observed. In 2 out of 10 studies, inefficacy of probiotics was observed, both in increasing eradication and in decreasing side effects. In summary, efficacy against *H. pylori* was reported in 5 out of 10 studies, while in 6 out of 9 studies reduction of side effects was observed; overall, efficacy against *H. pylori* and/or reduction of side effects was observed in 8 out of 10 studies.

Interestingly, in 3 out of the 5 studies in which probiotics were ineffective to increase eradication rates, the eradication rates achieved with the treatment without probiotics were already relatively high (> 80%). Conversely, in the 5 studies in which inclusion of probiotics significant increased efficacy, the treatment in the absence probiotics gave relatively low eradication rates (< 70%).

In one of these studies[45], one group received probiotics plus lactoferrin, based on a previous study[54] which hypothesized that lactoferrin could contribute to increase eradication efficacy. No differences of eradication rates were observed between the group that received and the group that did not receive lactoferrin; however, eradication rates in all groups of this study were near 90%, thus possible improvements were difficult to observe. Moreover, lactoferrin did not influence the rate of side effects[45].

POSSIBLE MECHANISMS FOR THE EFFICACY OF PROBIOTICS IN REDUCING SIDE-EFFECTS AND/OR INCREASE EFFICACY OF *H. PYLORI* ERADICATION TREATMENT

Antibiotic-associated diarrhea is a frequent phenomenon[35]. Interestingly, diarrhea is the most common side-effect of *H. pylori* eradication therapy that results to decrease upon probiotics administration (Table 1). Antibiotics are known to induce diarrhea because they alter intestinal microflora, leading to a proliferation of resistant bacterial strains, and to impairment of the fermentation processes carried out by intestinal microorganisms[35]. Some authors have already demonstrated significant reduction of antibiotic-associated diarrhea, as well as of acute diarrhea, by using probiotic compounds[34,35,37]. The action of probiotics can be ascribed to their ability to stimulate mucosal immune mechanisms (*e.g.*, activation of local macrophages to increase antigen presentation, and modulation of cytokine profiles). For instance, administration of probiotics-containing yogurt to *H. pylori*-infected children was shown able to restore the normal *Bifidobacterium* spp./*E. coli* ratio, increment serum IgA, and reduce serum interleukin 6 (*IL-6*)[55]. Probiotics action may also be exerted via non-immune mechanisms through antagonism and competition with potential pathogens; in particular, probiotics are able to produce antioxidants and antimicrobial substances, alter local pH, stimulate mucin production, enhance intestinal barrier functions, modify pathogen-derived toxins, and may affect colonization by competing with pathogens for nutrients and for the binding to the host cell surface[37,56]. Finally, microbiota, through the gut-brain connection, have been suggested to be involved in the pathophysiology of mood and anxiety disorders, and possible role of probiotics in modulating abdominal pain has been proposed, based on studies in rats[36,57].

All these general actions of probiotics have been proposed to contribute to their efficacy in increasing *H. pylori* eradication and decreasing side effects when used together with eradication therapy[58,59]. A limited number of *in vitro* or non-clinical studies have been described in

Table 1 Summary of trials using probiotics with *Helicobacer pylori* eradication treatment (2012 to date)

Treatment (oral administration)	Probiotic(s) (oral administration)	Region	Eradication rates		% side effects	Probiotic(s) efficacy	Ref.
			Intention to treat	Per protocol			
Esomeprazole 20 mg, levofloxacin 500 mg, amoxicillin 1 g, all *bid*, 7 d	10^8 CFU *Lactobacillus reuteri*, during therapy + further 7 d	Italy	80% (36/45)	80% (36/45)	66.7	Significant increase of eradication rates and reduction of side effects (nausea and diarrhea)	[44]
	Control		62.2% (28/45)	62.2% (28/45)	100.0		
Esomeprazole 20 mg and amoxicillin 1 g, both *bid*, 5 d; then esomeoprazole 20 mg, clarithromycin 500 mg, tinidazole 500 mg, all *bid*, 5 d (sequential therapy)	10^9 CFU *L. Acidophilus*, 10^9 CFU *L. bulgaricus*, 5×10^8 CFU *Bifidobacterium bifidum*, 10^9 CFU *Streptococcus thermophilus*, *bid*, during therapy	Italy	89% (65/73)	92.9% (65/70)	39.7	Eradication rates unaffected; significant decrease of side effects (metallic taste, abdominal/epigastric pain, diarrhea). Addition of lactoferrin did not influence the results achieved with probiotics	[45]
	Probiotics as above + 200 mg lactoferrin		88.5% (69/78)	93.2% (69/74)	38.5		
	Control		88.2% (67/76)	94.4% (67/71)	65.8		
Omeprazole 1 mg/kg *sid*, amoxicillin 50 mg/kg *bid*, clarithromycin 15 mg/kg *bid*, 7 d	5×10^9 CFU *L. plantarum*, 2×10^9 CFU *L. reuterii*, 2×10^9 CFU *L. casei subsp. rhamnosus*, 2×10^9 CFU *B. infantis* and *B. longum*, 10^9 CFU *L. salivarius*, 10^9 CFU *L. acidophilus*, 5×10^9 CFU *S. thermophilus*, 10^9 CFU *L. sporogenes*, *sid*., during therapy	Italy	88.2% (30/34)	88.2% (30/34)	14.5	Non-significant increase of eradication rates; significant reduction of side effects (epigastric pain, nausea, vomiting, diarrhea)	[46]
	Control		76.4% (26/34)	76.4% (26/34)	61.5		
Omeprazole 20 mg, clarithromycin 500 mg, amoxicillin 1 g, all *bid*, 7 d	*L. acidophilus* 14 d after therapy	China	79.2% (61/77)	82.4% (61/74)	89.2	Significant increase of eradication rates; no influence on side effects	[47]
	3×10^7 *L. acidophilus* 14 d before therapy		79.5% (62/78)	81.6% (62/76)	85.5		
	Control		60.8% (48/79)	61.5% (48/78)	87.2		
Omeprazole 20 mg, bismuth subcitrate 240 mg, amoxicillin 1 g, clarithromycin 500 mg, all *bid*, 14 d (quadruple therapy)	*L. casei*, *L. rhamnosus*, *L. acidophilus*, *L. bulgaricus*, *B. breve*, *B. longum*, *S. thermophilus*, total viable count 10^8 CFU, *bid*, during therapy	Iran	76.6% (69/90)	82.1% (69/84)	18.8	No significant differences in efficacy and overall side effects (decrease of diarrhea but increase of abdominal pain)	[48]
	Control		81.1% (73/90)	84.8% (73/86)	16.6		
Standard triple therapy (details not disclosed)	3×10^9 CFU *B. infantis*, *bid*, during therapy	United Arab Emirates	83% (83/100)		3.0	Significant increase of eradication rates, and reduction of incidence of antibiotic-induced side effects (diarrhea, loose bowel motion)	[49]
	3×10^9 CFU *B. infantis*, *bid*, 14 d before therapy, then during therapy		90.5% (86/95)		2.1		
	Control		68.9% (73/106)		14.2		
Sequential therapy (details not disclosed), 10 d	3×10^9 CFU *B. infantis*, *bid*, during therapy		90.8% (69/76)		1.3		
Amoxicillin 50 mg/kg, furazolidone 6 mg/kg, both *bid*, 7 d, plus omeprazole 1 mg/kg *sid* 28 d	*L. casei*, *L. rhamnosus*, *L. acidophilus*, *L. bulgaricus*, *B. infantis*, *B. breve*, *S. thermophilus*, total viable count 10^9 CFU, *sid*, during therapy	Iran	90.1% (30/33)		21.2	Significant increase of eradication rates, and reduction of side effects (nausea, vomiting, diarrhea)	[50]
	Control		69.7% (23/33)		63.6		
Furazolidone 200 mg, tetracycline 500 mg, lansoprazole 30 mg, *bid*, 7 d	*L. acidophilus*, *L. rhamnosus*, *B. bifidum*, *S. faecium*, 1.25×10^9 CFU each, *sid*,during therapy and further 23 d	Brazil	81.8% (45/55)	89.8% (44/49)	59.3/44.9	Non-significant increase of eradication rates and non-significant reduction of side effects (at 7 and 30 d)	[51]
	control		76.9% (40/52)	85.1% (41/48)	71.2/60.4		
Standard triple therapy	*L. acidophilus*, *B. bifidum* during and after therapy	China	83.7% (36/43)			Increase of eradication rates	[52]
	Control		64.4% (29/45)				
Omeprazole 20 mg, amoxicillin 1 g, clarithromycin 500 mg, all *bid*, 14 d	2×10^8 CFU *L. reuteri*, *sid*, during therapy and further 14 d	Egypt	74.3% (26/35)	74.3% (26/35)	28.6%	Non-significant increase of eradication rates; significant decrease of side effects (taste disorders, diarrhea)	[53]
	Control		65.7% (23/35)	65.7% (23/35)	68.6%		

CFU: Colony forming unit; Control: Group that received the eradication treatment without probiotics or with placebo; the term "significant" was used when $P < 0.05$ was reported in the corresponding paper.

the literature that can help to understand possible direct and specific activity of probiotics against *H. pylori*. The most recent studies are described below.

H. pylori urease catalyzes the conversion of urea to carbon dioxide and ammonia; ammonia in turn forms ammonium hydroxide, which neutralizes the local acidity in favor of *H. pylori* survival. Some studies reported the ability of *Lactobacillus casei* (*L. casei*) to inhibit *H. pylori* ure-

ase[60,61]; the specific effect on urease was suggested by the fact that such inhibition was observed under experimental conditions that did not influence the bacterial growth. This activity may be due to the activity of lactic acid[60]. More in general, anti *H. pylori* activity exerted by lactic acid bacteria has been proposed to be due to organic acids produced by these bacteria[56,60,62].

Recently, some *Lactobacillus* strains (*L. gasseri* Chen and *L. plantarum*) have been reported to be able to inhibit *H. pylori* adherence to gastric epithelial cells[63]. Similar results were described for some *Lactobacillus* strains (including *L. acidophilus*, *L. johnsonii*, and *L. salivarius* subsp. *salicinius*) that were able to reduce *H. pylori* adhesion to the human gastric adenocarcinoma cell line AGS, and also its intracellular growth; generally, this activity was more evident using culture supernatants of *Lactobacilli* rather than using bacterial cells[64]. *L. salivarius* was also able to counteract the increase of IL-8 production induced by *H. pylori* in AGS cells[64]. Administration of *L. johnsonii* or *L. salivarius* to rats infected by *H. pylori* revealed a reduction of bacterial load, of local IL-8 production, and of gastric inflammation[64]. Moreover, *L. johnsonii* La1 culture supernatant was found able to reduce *H. pylori* motility and its adherence to the human gastric epithelial cell line MKN74, providing a possible explanation of the ability of *L. johnsonii* La1 to reduce gastric colonization in *H. pylori*-infected Mongolian gerbils[65]. In the same animal model, long-term administration of yogurt supplemented with probiotics (*L. acidophilus, L. bulgaricus, B. lactis, S. thermophilus)* was found to reduce *H. pylori* colonization, TNF-α expression, gastric inflammation and intestinal metaplasia as compared with infected controls not receiving probiotics[66].

Probiotics may also interfere with the activation of specific host pathways by *H. pylori*. *L. acidophilus* produces conjugated linoleic acids (CLA) that have been shown by some studies able to interfere with inflammatory outcomes of *H. pylori* infection[67]. This interference targets nuclear factor-κB pathway[67,68], which is known to be induced by *H. pylori*. Consistently with these observations, CLA from *L. acidophilus* or *L. plantarum* was also shown to suppress the *H. pylori*-induced IL-8 and TNF-α expression by the AGS cell line[69].

Further studies showed that *L. acidophilus* can also interfere with the Smad7 activation, also in this case resulting in reduced inflammatory events[68]. Conditioned media from *L. salivarius*, *L. rhamnosus*, and *L. plantarum* were found to suppress the *H. pylori*-induced Il-8 expression and NFκB activation in AGS cells, without inhibiting *H. pylori* growth; *L. plantarum* was also able to suppress the activation of *c-jun* (which is one of the proto-oncogenes activated by *H. pylori* CagA[70]) *in vitro*, and to attenuate gastric inflammation in a rat model of *H. pylori* infection[71].

CONCLUSION

Probiotics are generally considered safe to administer to humans, and several strains have already received indication for use in specific disorders[37]. Probiotics treatment as an adjuvant of eradication treatment showed in the recent trials efficacy against *H. pylori* and/or decreased side effects of the treatment in most of the studies - but not in all. This confirms the previously reported results. It must be remarked that the efficacy of probiotics treatment in increasing eradication can be evaluated only when eradication rates in the controls that did not receive probiotics are low enough; on the other hand, the efficacy in reducing side effects can be observed when side effects are present, *i.e.,* in almost all studies. To date, it does not appear clear whether probiotics may be more effective in particular subgroups, and if predictive factors for treatment success can be identified. The meta-analysis by Zheng *et al*[42] suggested that using single *Lactobacillus* species could achieve better results than administering multi-strain compounds; however, this was not highlighted by other meta-analyses, and remains a point to be further clarified. Possible influence of age, lifestyle (dietary habit in particular), grade of infection, type of gastroduodenal symptoms, and other similar factors could be analyzed in wider meta-analyses, as this information is provided at least in part in the reports of the clinical trials. Conversely, the possible influence on probiotics efficacy of essential factors such as for instance the *H. pylori* infecting strain, the host genetic background, and the host microbiome, could be assessed only by studies specifically designed to investigate the relevance of these factors.

It is known that *H. pylori* isolates are different according to the geographic areas, and that the susceptibility to *H. pylori* infection and the outcome of the infection vary according to both *H. pylori* and/or host genetic background, that may result in combinations much more harmful than others[17,18,21], and may also influence the eradication rates achievable. Thus, it is not unexpected that some studies, in disagreement with others, did not find beneficial effects of probiotics adjunctive treatment: having more information of *H. pylori* isolates and on genetic background of the hosts would strongly help to understand the reasons of success or of failure of probiotics.

Possible specific activity of probiotics against defined *H. pylori* factors is still largely to be understood. Indeed, the decrease of inflammatory cytokines, restoration of IL-10, suppression of NFκB activation, *etc.*, in the majority of the cases may be indirect effects, *i.e.,* related to the ability of probiotics to reduce *H. pylori* adhesion (*in vitro* studies) or colonization (*in vivo* studies). To date, the only proposed possible specific *H. pylori* target for probiotics has been urease[60,61]. It would be interesting to experimentally assess the possible interference of probiotics or probiotics factors with the *in vitro* and/or *in vivo* activities of other well-characterized *H. pylori* factors such as for instance CagA and VacA, besides urease. However, it must be said that probiotics may have low chance of entering in direct and massive contact with *H. pylori* as the latter resides under the mucus layer of the gastric mucosa, in large part adherent to the epithelial cells, where probiotics are unlikely to arrive in significant amount. In fact, the optimal conditions for probiotics colonization are present

in the large intestine, where the highest concentration of probiotics is found, while scarce concentration of probiotics is usually found in the stomach[72]. Thus, at least for therapeutic use, it seems more likely that probiotics exert indirect and non-specific rather than direct and specific anti-*H. pylori* activity.

In conclusion, administration of safe probiotics as an adjuvant for the current *H. pylori* eradication treatment appears promising, though it still requires optimization; even in the cases in which the treatments achieve high eradication rates, probiotics may reduce side effects. Further investigation on the mechanisms behind the direct and indirect effects of probiotics on *H. pylori* could help not only to better refine the type of treatment, but also may contribute to better understand some aspects of *H. pylori* pathogenesis.

REFERENCES

1 **Marshall BJ**, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1984; **1**: 1311-1315 [PMID: 6145023 DOI: 10.3410/f.1092121.545496]

2 **Goodwin CS**, Armstrong JA, Chilvers T, Peters M, Collins MD, Sly L, McConnel W, Harper WES. Transfer of Campylobacter pylori and Campylobacter mustelae to Helicobacter gen. nov. as Helicobacter pylori comb. nov. and Helicobacter mustelae comb. nov., respectively. *Int J Syst Bacteriol* 1989; **39**: 397-405 [DOI: 10.1099/00207713-39-4-397]

3 **Rothenbacher D**, Brenner H. Burden of Helicobacter pylori and H. pylori-related diseases in developed countries: recent developments and future implications. *Microbes Infect* 2003; **5**: 693-703 [PMID: 12814770 DOI: 10.1016/S1286-4579(03)00111-4]

4 **Frenck RW**, Clemens J. Helicobacter in the developing world. *Microbes Infect* 2003; **5**: 705-713 [PMID: 12814771 DOI: 10.1016/S1286-4579(03)00112-6]

5 **Goodwin CS**, Armstrong JA, Marshall BJ. Campylobacter pyloridis, gastritis, and peptic ulceration. *J Clin Pathol* 1986; **39**: 353-365 [PMID: 3517070 DOI: 10.1136/jcp.39.4.353]

6 **Sontag SJ**. Guilty as charged: bugs and drugs in gastric ulcer. *Am J Gastroenterol* 1997; **92**: 1255-1261 [PMID: 9260785]

7 **Du MQ**, Isaccson PG. Gastric MALT lymphoma: from aetiology to treatment. *Lancet Oncol* 2002; **3**: 97-104 [PMID: 11902529 DOI: 10.1016/S1470-2045(02)00651-4]

8 Schistosomes, liver flukes and Helicobacter pylori. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7-14 June 1994. *IARC Monogr Eval Carcinog Risks Hum* 1994; **61**: 1-241 [PMID: 7715068]

9 **Uemura N**, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ. Helicobacter pylori infection and the development of gastric cancer. *N Engl J Med* 2001; **345**: 784-789 [PMID: 11556297 DOI: 10.1056/NEJMoa001999]

10 **IARC Working Group on the Evaluation of Carcinogenic Risks to Humans**. Biological agents. Volume 100 B. A review of human carcinogens. *IARC Monogr Eval Carcinog Risks Hum* 2012; **100**: 1-441 [PMID: 23189750]

11 **Sundquist M**, Quiding-Järbrink M. Helicobacter pylori and its effect on innate and adaptive immunity: new insights and vaccination strategies. *Expert Rev Gastroenterol Hepatol* 2010; **4**: 733-744 [PMID: 21108593 DOI: 10.1586/egh.10.71]

12 **Raghavan S**, Quiding-Järbrink M. Immune modulation by regulatory T cells in Helicobacter pylori-associated diseases. *Endocr Metab Immune Disord Drug Targets* 2012; **12**: 71-85 [PMID: 22214337 DOI: 10.2174/187153012799278974]

13 **D'Elios MM**, Andersen LP. Helicobacter pylori inflammation, immunity, and vaccines. *Helicobacter* 2007; **12** Suppl 1: 15-19 [PMID: 17727455 DOI: 10.1111/j.1523-5378.2007.00530.x]

14 **Amedei A**, Munari F, Bella CD, Niccolai E, Benagiano M, Bencini L, Cianchi F, Farsi M, Emmi G, Zanotti G, de Bernard M, Kundu M, D'Elios MM. Helicobacter pylori secreted peptidyl prolyl cis, trans-isomerase drives Th17 inflammation in gastric adenocarcinoma. *Intern Emerg Med* 2014; **9**: 303-309 [PMID: 23054412 DOI: 10.1007/s11739-012-0867-9]

15 **Zhang Y**, Liu C, Peng H, Zhang J, Feng Q. IL1 receptor antagonist gene IL1-RN variable number of tandem repeats polymorphism and cancer risk: a literature review and meta-analysis. *PLoS One* 2012; **7**: e46017 [PMID: 23049925 DOI: 10.1371/journal.pone.0046017]

16 **Santos JC**, Ladeira MS, Pedrazzoli J, Ribeiro ML. Relationship of IL-1 and TNF-α polymorphisms with Helicobacter pylori in gastric diseases in a Brazilian population. *Braz J Med Biol Res* 2012; **45**: 811-817 [PMID: 22714811 DOI: 10.1590/S0100-879X2012007500099]

17 **Persson C**, Canedo P, Machado JC, El-Omar EM, Forman D. Polymorphisms in inflammatory response genes and their association with gastric cancer: A HuGE systematic review and meta-analyses. *Am J Epidemiol* 2011; **173**: 259-270 [PMID: 21178102 DOI: 10.1093/aje/kwq370]

18 **Ruggiero P**. Helicobacter pylori and inflammation. *Curr Pharm Des* 2010; **16**: 4225-4236 [PMID: 21184659 DOI: 10.3389/fimmu.2013.00328]

19 **D'Elios MM**, Bergman MP, Amedei A, Appelmelk BJ, Del Prete G. Helicobacter pylori and gastric autoimmunity. *Microbes Infect* 2004; **6**: 1395-1401 [PMID: 15596126 DOI: 10.1016/j.micinf.2004.10.001]

20 **Stein M**, Ruggiero P, Rappuoli R, Bagnoli F. Helicobacter pylori CagA: From Pathogenic Mechanisms to Its Use as an Anti-Cancer Vaccine. *Front Immunol* 2013; **4**: 328 [PMID: 24133496]

21 **Pacchiani N**, Censini S, Buti L, Covacci A. Echoes of a distant past: The cag pathogenicity island of Helicobacter pylori. *Cold Spring Harb Perspect Med* 2013; **3**: a010355 [PMID: 24097901 DOI: 10.1101/cshperspect.a010355]

22 **Ong SP**, Duggan A. Eradication of Helicobacter pylori in clinical situations. *Clin Exp Med* 2004; **4**: 30-38 [PMID: 15598083 DOI: 10.1007/s10238-004-0035-2]

23 **Fock KM**, Talley N, Moayyedi P, Hunt R, Azuma T, Sugano K, Xiao SD, Lam SK, Goh KL, Chiba T, Uemura N, Kim JG, Kim N, Ang TL, Mahachai V, Mitchell H, Rani AA, Liou JM, Vilaichone RK, Sollano J. Asia-Pacific consensus guidelines on gastric cancer prevention. *J Gastroenterol Hepatol* 2008; **23**: 351-365 [PMID: 18318820 DOI: 10.1111/j.1440-1746.2008.05314.x]

24 **Selgrad M**, Kandulski A, Malfertheiner P. Helicobacter pylori: diagnosis and treatment. *Curr Opin Gastroenterol* 2009; **25**: 549-556 [PMID: 19696666 DOI: 10.1097/MOG.0b013e32833159f2]

25 **O'Connor A**, Molina-Infante J, Gisbert JP, O'Morain C. Treatment of Helicobacter pylori infection 2013. *Helicobacter* 2013; **18** Suppl 1: 58-65 [PMID: 24011247 DOI: 10.1111/hel.12075]

26 **Couturier MR**, Marshall BJ, Goodman KJ, Mégraud F. Helicobacter pylori diagnostics and treatment: could a lack of universal consensus be the best consensus? *Clin Chem* 2014; **60**: 589-594 [PMID: 23908455 DOI: 10.1373/clinchem.2012.201475]

27 **Ierardi E**, Giorgio F, Losurdo G, Di Leo A, Principi M. How antibiotic resistances could change Helicobacter pylori treatment: A matter of geography? *World J Gastroenterol* 2013; **19**: 8168-8180 [PMID: 24363506 DOI: 10.3748/wjg.v19.i45.8168]

28 **Graham DY**, Lu H, Yamaoka Y. A report card to grade Helicobacter pylori therapy. *Helicobacter* 2007; **12**: 275-278 [PMID: 17669098 DOI: 10.1111/j.1523-5378.2007.00518.x]

29 **Yuan Y**, Ford AC, Khan KJ, Gisbert JP, Forman D, Leontiadis GI, Tse F, Calvet X, Fallone C, Fischbach L, Oderda G, Bazzoli F, Moayyedi P. Optimum duration of regimens for Helicobacter pylori eradication. *Cochrane Database Syst Rev*

2013; **12**: CD008337 [PMID: 24338763 DOI: 10.1002/14651858.CD008337.pub2]
30 **Del Giudice G**, Malfertheiner P, Rappuoli R. Development of vaccines against Helicobacter pylori. *Expert Rev Vaccines* 2009; **8**: 1037-1049 [PMID: 19627186 DOI: 10.1586/erv.09.62]
31 **Guarner F**, Schaafsma GJ. Probiotics. *Int J Food Microbiol* 1998; **39**: 237-238 [PMID: 9553803 DOI: 10.1016/S0168-1605(97)00136-0]
32 **FAO/WHO Expert Consultation.** Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria. Córdoba, Argentina 1-4 Oct. 2001. Available from: URL: http://www.who.int/foodsafety/publications/fs_management/en/probiotics.pdf
33 **Behnsen J**, Deriu E, Sassone-Corsi M, Raffatellu M. Probiotics: properties, examples, and specific applications. *Cold Spring Harb Perspect Med* 2013; **3**: a010074 [PMID: 23457295 DOI: 10.1101/cshperspect.a010074]
34 **Petschow B**, Doré J, Hibberd P, Dinan T, Reid G, Blaser M, Cani PD, Degnan FH, Foster J, Gibson G, Hutton J, Klaenhammer TR, Ley R, Nieuwdorp M, Pot B, Relman D, Serazin A, Sanders ME. Probiotics, prebiotics, and the host microbiome: the science of translation. *Ann N Y Acad Sci* 2013; **1306**: 1-17 [PMID: 24266656 DOI: 10.1111/nyas.12303]
35 **Sarowska J**, Choroszy-Król I, Regulska-Ilow B, Frej-Mądrzak M, Jama-Kmiecik A. The therapeutic effect of probiotic bacteria on gastrointestinal diseases. *Adv Clin Exp Med* 2013; **22**: 759-766 [PMID: 24285463]
36 **Foligné B**, Daniel C, Pot B. Probiotics from research to market: the possibilities, risks and challenges. *Curr Opin Microbiol* 2013; **16**: 284-292 [PMID: 23866974 DOI: 10.1016/j.mib.2013.06.008]
37 **Guarner F**, Khan AG, Garisch J, Eliakim R, Gangl A, Thomson A, Krabshuis J, Lemair T, Kaufmann P, de Paula JA, Fedorak R, Shanahan F, Sanders ME, Szajewska H, Ramakrishna BS, Karakan T, Kim N. World Gastroenterology Organisation Global Guidelines: probiotics and prebiotics October 2011. *J Clin Gastroenterol* 2012; **46**: 468-481 [PMID: 22688142 DOI: 10.1097/MCG.0b013e3182549092]
38 **Hamilton-Miller JM**. The role of probiotics in the treatment and prevention of Helicobacter pylori infection. *Int J Antimicrob Agents* 2003; **22**: 360-366 [PMID: 14522098 DOI: 10.1016/S0924-8579(03)00153-5]
39 **Wilhelm SM**, Johnson JL, Kale-Pradhan PB. Treating bugs with bugs: the role of probiotics as adjunctive therapy for Helicobacter pylori. *Ann Pharmacother* 2011; **45**: 960-966 [PMID: 21693698 DOI: 10.1345/aph.1Q104]
40 **Patel A**, Shah N, Prajapati JB. Clinical application of probiotics in the treatment of Helicobacter pylori infection--a brief review. *J Microbiol Immunol Infect* 2014; **47**: 429-437 [PMID: 23757373 DOI: 10.1016/j.jmii.2013.03.010]
41 **Wang ZH**, Gao QY, Fang JY. Meta-analysis of the efficacy and safety of Lactobacillus-containing and Bifidobacterium-containing probiotic compound preparation in Helicobacter pylori eradication therapy. *J Clin Gastroenterol* 2013; **47**: 25-32 [PMID: 23090045 DOI: 10.1097/MCG.0b013e318266f6cf]
42 **Zheng X**, Lyu L, Mei Z. Lactobacillus-containing probiotic supplementation increases Helicobacter pylori eradication rate: evidence from a meta-analysis. *Rev Esp Enferm Dig* 2013; **105**: 445-453 [PMID: 24274441 DOI: 10.4321/S1130-01082013000800002]
43 **Li S**, Huang XL, Sui JZ, Chen SY, Xie YT, Deng Y, Wang J, Xie L, Li TJ, He Y, Peng QL, Qin X, Zeng ZY. Meta-analysis of randomized controlled trials on the efficacy of probiotics in Helicobacter pylori eradication therapy in children. *Eur J Pediatr* 2014; **173**: 153-161 [PMID: 24323343 DOI: 10.1007/s00431-013-2220-3]
44 **Ojetti V**, Bruno G, Ainora ME, Gigante G, Rizzo G, Roccarina D, Gasbarrini A. Impact of Lactobacillus reuteri Supplementation on Anti-Helicobacter pylori Levofloxacin-Based Second-Line Therapy. *Gastroenterol Res Pract* 2012; **2012**: 740381 [PMID: 22690211 DOI: 10.1155/2012/740381]
45 **Manfredi M**, Bizzarri B, Sacchero RI, Maccari S, Calabrese L, Fabbian F, De'Angelis GL. Helicobacter pylori infection in clinical practice: probiotics and a combination of probiotics + lactoferrin improve compliance, but not eradication, in sequential therapy. *Helicobacter* 2012; **17**: 254-263 [PMID: 22759324 DOI: 10.1111/j.1523-5378.2012.00944.x]
46 **Tolone S**, Pellino V, Vitaliti G, Lanzafame A, Tolone C. Evaluation of Helicobacter Pylori eradication in pediatric patients by triple therapy plus lactoferrin and probiotics compared to triple therapy alone. *Ital J Pediatr* 2012; **38**: 63 [PMID: 23114016 DOI: 10.1186/1824-7288-38-63]
47 **Du YQ**, Su T, Fan JG, Lu YX, Zheng P, Li XH, Guo CY, Xu P, Gong YF, Li ZS. Adjuvant probiotics improve the eradication effect of triple therapy for Helicobacter pylori infection. *World J Gastroenterol* 2012; **18**: 6302-6307 [PMID: 23180952 DOI: 10.3748/wjg.v18.i43.6302]
48 **Shavakhi A**, Tabesh E, Yaghoutkar A, Hashemi H, Tabesh F, Khodadoostan M, Minakari M, Shavakhi S, Gholamrezaei A. The effects of multistrain probiotic compound on bismuth-containing quadruple therapy for Helicobacter pylori infection: a randomized placebo-controlled triple-blind study. *Helicobacter* 2013; **18**: 280-284 [PMID: 23433200 DOI: 10.1111/hel.12047]
49 **Dajani AI**, Abu Hammour AM, Yang DH, Chung PC, Nounou MA, Yuan KY, Zakaria MA, Schi HS. Do probiotics improve eradication response to Helicobacter pylori on standard triple or sequential therapy? *Saudi J Gastroenterol* 2013; **19**: 113-120 [PMID: 23680708 DOI: 10.4103/1319-3767.111953]
50 **Ahmad K**, Fatemeh F, Mehri N, Maryam S. Probiotics for the treatment of pediatric helicobacter pylori infection: a randomized double blind clinical trial. *Iran J Pediatr* 2013; **23**: 79-84 [PMID: 23446685]
51 **Navarro-Rodriguez T**, Silva FM, Barbuti RC, Mattar R, Moraes-Filho JP, de Oliveira MN, Bogsan CS, Chinzon D, Eisig JN. Association of a probiotic to a Helicobacter pylori eradication regimen does not increase efficacy or decreases the adverse effects of the treatment: a prospective, randomized, double-blind, placebo-controlled study. *BMC Gastroenterol* 2013; **13**: 56 [PMID: 23530767 DOI: 10.1186/1471-230X-13-56]
52 **Wang YH**, Huang Y. Effect of Lactobacillus acidophilus and Bifidobacterium bifidum supplementation to standard triple therapy on Helicobacter pylori eradication and dynamic changes in intestinal flora. *World J Microbiol Biotechnol* 2014; **30**: 847-853 [PMID: 24233772 DOI: 10.1007/s11274-013-1490-2]
53 **Emara MH**, Mohamed SY, Abdel-Aziz HR. Lactobacillus reuteri in management of Helicobacter pylori infection in dyspeptic patients: a double-blind placebo-controlled randomized clinical trial. *Therap Adv Gastroenterol* 2014; **7**: 4-13 [PMID: 24381643 DOI: 10.1177/1756283X13503514]
54 **de Bortoli N**, Leonardi G, Ciancia E, Merlo A, Bellini M, Costa F, Mumolo MG, Ricchiuti A, Cristiani F, Santi S, Rossi M, Marchi S. Helicobacter pylori eradication: a randomized prospective study of triple therapy versus triple therapy plus lactoferrin and probiotics. *Am J Gastroenterol* 2007; **102**: 951-956 [PMID: 17313499 DOI: 10.1111/j.1572-0241.2007.01085.x]
55 **Yang YJ**, Sheu BS. Probiotics-containing yogurts suppress Helicobacter pylori load and modify immune response and intestinal microbiota in the Helicobacter pylori-infected children. *Helicobacter* 2012; **17**: 297-304 [PMID: 22759330 DOI: 10.1111/j.1523-5378.2012.00941.x]
56 **Ljungh A**, Wadström T. Lactic acid bacteria as probiotics. *Curr Issues Intest Microbiol* 2006; **7**: 73-89 [PMID: 16875422]
57 **Rousseaux C**, Thuru X, Gelot A, Barnich N, Neut C, Dubuquoy L, Dubuquoy C, Merour E, Geboes K, Chamaillard M, Ouwehand A, Leyer G, Carcano D, Colombel JF, Ardid D, Desreumaux P. Lactobacillus acidophilus modulates intestinal pain and induces opioid and cannabinoid receptors. *Nat Med* 2007; **13**: 35-37 [PMID: 17159985 DOI: 10.1038/nm1521]

58 **Marteau P**, Rambaud JC. Potential of using lactic acid bacteria for therapy and immunomodulation in man. *FEMS Microbiol Rev* 1993; **12**: 207-220 [PMID: 8398215 DOI: 10.1111/j.1574-6976.1993.tb00019.x]

59 **Vítor JM**, Vale FF. Alternative therapies for Helicobacter pylori: probiotics and phytomedicine. *FEMS Immunol Med Microbiol* 2011; **63**: 153-164 [PMID: 22077218 DOI: 10.1111/j.1574-695X.2011.00865.x]

60 **Aiba Y**, Suzuki N, Kabir AM, Takagi A, Koga Y. Lactic acid-mediated suppression of Helicobacter pylori by the oral administration of Lactobacillus salivarius as a probiotic in a gnotobiotic murine model. *Am J Gastroenterol* 1998; **93**: 2097-2101 [PMID: 9820379 DOI: 10.1111/j.1572-0241.1998.00600.x]

61 **Sgouras D**, Maragkoudakis P, Petraki K, Martinez-Gonzalez B, Eriotou E, Michopoulos S, Kalantzopoulos G, Tsakalidou E, Mentis A. In vitro and in vivo inhibition of Helicobacter pylori by Lactobacillus casei strain Shirota. *Appl Environ Microbiol* 2004; **70**: 518-526 [PMID: 14711683 DOI: 10.1128/AEM.70.1.518-526.2004]

62 **Lin WH**, Wu CR, Fang TJ, Guo JT, Huang SY, Lee MS, Yang HL. Anti-Helicobacter pylori activity of fermented milk with lactic acid bacteria. *J Sci Food Agric* 2011; **91**: 1424-1431 [PMID: 21445876 DOI: 10.1002/jsfa.4327]

63 **Chen X**, Liu XM, Tian F, Zhang Q, Zhang HP, Zhang H, Chen W. Antagonistic activities of lactobacilli against Helicobacter pylori growth and infection in human gastric epithelial cells. *J Food Sci* 2012; **77**: M9-14 [PMID: 22181017 DOI: 10.1111/j.1750-3841.2011.02498.x]

64 **Hsieh PS**, Tsai YC, Chen YC, Teh SF, Ou CM, King VA. Eradication of Helicobacter pylori infection by the probiotic strains Lactobacillus johnsonii MH-68 and L. salivarius ssp. salicinius AP-32. *Helicobacter* 2012; **17**: 466-477 [PMID: 23067294 DOI: 10.1111/j.1523-5378.2012.00992.x]

65 **Isobe H**, Nishiyama A, Takano T, Higuchi W, Nakagawa S, Taneike I, Fukushima Y, Yamamoto T. Reduction of overall Helicobacter pylori colonization levels in the stomach of Mongolian gerbil by Lactobacillus johnsonii La1 (LC1) and its in vitro activities against H. pylori motility and adherence. *Biosci Biotechnol Biochem* 2012; **76**: 850-852 [PMID: 22484956 DOI: 10.1271/bbb.110921]

66 **Kuo CH**, Wang SS, Lu CY, Hu HM, Kuo FC, Weng BC, Wu CC, Liu CJ, Tsai PY, Lee TC, Chen LW, Cheng KH, Chang LL, Wu DC. Long-Term Use of Probiotic-Containing Yogurts Is a Safe Way to Prevent Helicobacter pylori: Based on a Mongolian Gerbil's Model. *Biochem Res Int* 2013; **2013**: 594561 [PMID: 24349780 DOI: 10.1155/2013/594561]

67 **Kim JM**, Kim JS, Kim YJ, Oh YK, Kim IY, Chee YJ, Han JS, Jung HC. Conjugated linoleic acids produced by Lactobacillus dissociates IKK-gamma and Hsp90 complex in Helicobacter pylori-infected gastric epithelial cells. *Lab Invest* 2008; **88**: 541-552 [PMID: 18347582 DOI: 10.1038/labinvest.2008.16]

68 **Yang YJ**, Chuang CC, Yang HB, Lu CC, Sheu BS. Lactobacillus acidophilus ameliorates H. pylori-induced gastric inflammation by inactivating the Smad7 and NFκB pathways. *BMC Microbiol* 2012; **12**: 38 [PMID: 22429929 DOI: 10.1186/1471-2180-12-38]

69 **Hwang SW**, Kim N, Kim JM, Huh CS, Ahn YT, Park SH, Shin CM, Park JH, Lee MK, Nam RH, Lee HS, Kim JS, Jung HC, Song IS. Probiotic suppression of the H. pylori-induced responses by conjugated linoleic acids in a gastric epithelial cell line. *Prostaglandins Leukot Essent Fatty Acids* 2012; **86**: 225-231 [PMID: 22521089 DOI: 10.1016/j.plefa.2012.04.002]

70 **Meyer-ter-Vehn T**, Covacci A, Kist M, Pahl HL. Helicobacter pylori activates mitogen-activated protein kinase cascades and induces expression of the proto-oncogenes c-fos and c-jun. *J Biol Chem* 2000; **275**: 16064-16072 [PMID: 10747974 DOI: 10.1074/jbc.M000959200]

71 **Thiraworawong T**, Spinler JK, Werawatganon D, Klaikeaw N, Venable SF, Versalovic J, Tumwasorn S. Anti-inflammatory properties of gastric-derived Lactobacillus plantarum XB7 in the context of Helicobacter pylori infection. *Helicobacter* 2014; **19**: 144-155 [PMID: 24387083 DOI: 10.1111/hel.12105]

72 **DiBaise JK**, Zhang H, Crowell MD, Krajmalnik-Brown R, Decker GA, Rittmann BE. Gut microbiota and its possible relationship with obesity. *Mayo Clin Proc* 2008; **83**: 460-469 [PMID: 18380992 DOI: 10.4065/83.4.460]

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TOPIC HIGHLIGHT

WJGP 5th Anniversary Special Issues (1): *Helicobacter pylori*

Treatment of *Helicobacter pylori* infection: Past, present and future

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Abstract

Helicobacter pylori (*H. pylori*) is a major human pathogen associated with significant morbidity and mortality. However, after decades of efforts, treatment of *H. pylori* remains a challenge for physicians, as there is no universally effective regimen. Due to the rising prevalence of antimicrobial resistance, mainly to clarithromycin, efficacy of standard triple therapies has declined to unacceptably low levels in most parts of the world. Novel regimens, specifically experimented to improve the therapeutic outcome against antibiotic-resistant *H. pylori* strains, are now recommended as first-line empirical treatment options providing high efficacy (reportedly > 90% in intention to treat analysis) even in high clarithromycin resistance settings. These include the bismuth quadruple, concomitant, sequential and hybrid therapies. Due to the rapid development of quinolone resistance, levofloxacin-based regimens should be reserved as second-line/rescue options. Adjunct use of probiotics has been proposed in order to boost eradication rates and decrease occurrence of treatment-related side effects. Molecular testing methods are currently available for the characterization of *H. pylori* therapeutic susceptibility, including genotypic detection of macrolide resistance and evaluation of the cytochrome P450 2C19 status known to affect the metabolism of proton pump inhibitors. In the future, use of these techniques may allow for culture-free, non-invasive tailoring of therapy for *H. pylori* infection.

Key words: *Helicobacter pylori*; Antibiotic resistance; Bismuth-quadruple; Concomitant; Sequential; Probiotics

Core tip: Worldwide increase in prevalence of macrolide resistance has accounted for the failure of standard therapies for the treatment of *Helicobacter pylori* (*H. pylori*) infection. Bismuth quadruple, concomitant, sequential and hybrid therapies are now recommended as first-line empirical treatments providing improved efficacy in high clarithromycin resistance settings. As quinolone resistance is rapidly increasing, levofloxacin should be preferentially used in second-line/rescue therapies. There is increasing evidence that adjunct probiotic supplementation improves the therapeutic outcome and tolerability. Genotypic characterization of *H. pylori* susceptibility to therapy may allow for a tailored therapeutic approach in the future.

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INTRODUCTION

Treatment of *Helicobacter pylori* (*H. pylori*) infection is paramount for the management of prevalent gastrointestinal

Table 1 Factors reported to negatively affect the outcome of therapies for *Helicobacter pylori* infection

Pathogen-related	Host-related
Development of resistance to antibiotics	Non-compliance to treatment
High bacterial load in the stomach	Non-ulcer dyspepsia
Protective effect of the gastric mucus layer	Smoking
Intracellular location of many bacteria	CYP2C19 status (rapid metabolizer)
CagA negative	
Presence of dormant coccoid forms (not susceptible to antibiotics)	
Heteroresistant status (co-existence of strains susceptible and resistant to the same antibiotic)	

diseases, including peptic ulcer disease, gastric cancer and functional dyspepsia[1-3]. Moreover, extra-digestive disorders are now included as indications for eradication of *H. pylori*: idiopathic thrombocytopenic purpura, vitamin B12 deficiency and unexplained iron deficiency anemia[4]. Contrarily to other bacterial infections, for which susceptibility testing is commonly performed to guide treatment, culture of *H. pylori* is not widely available and requires performing endoscopy which is not well-tolerated by all patients and has a series of limitations, including the fact that *in vitro* susceptibility does not always guarantee *in vivo* eradication[5]. Hence, regimens for *H. pylori* have been routinely prescribed empirically, provided they have been previously tested and sufficiently tailored with regard to various parameters (*i.e.*; treatment dose, duration, dosing intervals *etc.*) to optimize cure rates and minimize side effects. However, the optimal treatment to eradicate *H. pylori* remains to be established, as no regimen is effective universally. Worldwide increase in resistance to key antibiotics, mainly clarithromycin (CAM), but also metronidazole (MNZ) and levofloxacin, is the main determinant of failure in the treatment of *H. pylori* infection[6,7]. In a recent systematic review, the global incidence of CAM resistance has been reported to be 17.2% ranging from 11.1% in Europe to 29.3% in America, whereas, in the same analysis, continental rates of resistance to MNZ were 17% and 44.1% respectively[8]. Antibiotic consumption for infections other than *H. pylori* is accounting for the wide increase in *H. pylori* antibiotic resistance rates[9,10]. Indeed, different national policies for antibiotic use are largely reflecting geographical distribution of *H. pylori* resistance: CAM resistance has been reported to be significantly higher in Southern European countries (reaching 49% in some areas of Spain) as compared to Northern Europe (*e.g.,* only 1% in the Netherlands) where policies for antibiotic use are more stringent[9]. Additionally to the development of antibiotic resistance, a series of both host and pathogen related factors may negatively impact on the performance of regimens to eradicate *H. pylori* (Table 1)[11,12].

Despite decades of efforts, treatment of *H. pylori* infection remains a challenging issue for both researchers and practicing physicians. In the present article we aim to provide a comprehensive overview of perspectives on the past, present and future of *H. pylori* eradication.

CLARITHROMYCIN-BASED TRIPLE THERAPIES: A DECLINING CLINICAL STANDARD

Historically, the first truly effective therapy for *H. pylori* infection, comprising of bismuth, tetracycline and MNZ, was proposed in 1990[13]. A few years later, use of CAM in a triple therapy, proposed by Bazzoli *et al*[14], was the start of CAM-based triple regimens, thereafter representing the gold standard in the treatment of *H. pylori*. In studies conducted during the 90's, standard triple therapies (STT) comprising of a proton pump inhibitor (PPI) *bid*, CAM 500 mg *bid* and amoxicillin 1000 mg *bid* or MNZ 500 mg (or 400 mg in England), all given for 7-14 d, provided consistently good results yielding > 80% eradication success and even > 90% was feasible[15,16]. Due to this high efficacy and relative simplicity, optimal safety profile, and large pharmaceutical company commitment, these regimens have been widely accepted in national expert panels and consensus recommendations worldwide as standard of care treatments for first-line eradication of *H. pylori*[17-20]. However, rising prevalence of CAM resistance has accounted for a significant decline in the efficacy of standard regimens. This decreasing efficacy was already evident in the meta-analyses published by the early 2000's, prompting significant changes in the paradigm of treating the infection. These included the introduction of the concept of cumulative treatment efficacy (requiring the patient to comply with more treatment courses; thus, more side effects and spreading of secondary antibiotic resistance), and later the introduction of a local threshold (15%-20%) of CAM resistance at which CAM should not be used empirically[17,18]. The decreased efficacy of standard treatments against CAM-resistant strains has been well-documented on a meta-analytic basis: In a meta-analysis by Fischbach and Evans, the success of triple therapy was decreased by 66.2% (95%CI: 58.2%-74.2%) when strains of *H. pylori* were resistant *vs* susceptible to CAM[7]. Congruently, a more recent analysis by Venerito *et al*[21], revealed similar results: including antimicrobial susceptibility data from 4 randomized clinical trials (RCTs), standard triple therapies successfully eradicated 88% of CAM-sensitive but only 14% of CAM-resistant *H. pylori* strains (risk difference = 0.75, 95%CI: 0.63-0.87). If MNZ is used, presence of MNZ resistance may also affect the therapeutic outcome[22], although it is generally considered less important clinically. This is due to the fact that MNZ resistance may be largely overcome by increasing dose and prolonging treatment duration[23]. Lastly, *H. pylori* resistance to amoxicillin is exceptional and generally is not relevant clinically. In the light of increasing data confirming suboptimal performance (< 70%) in most

Table 2 Current regimens to treat *Helicobacter pylori* infection

Treatment	Regimen
Bismuth-containing quadruple therapy	A PPI (standard dose, *bid*), bismuth (standard dose, *qid*) tetracycline (500 mg, *qid*) and metronidazole (500 mg, *qid*) for 10-14 d
Sequential therapy	A 5-d dual therapy with a PPI (standard dose, *bid*) and amoxicillin (1 g, *bid*) followed by a 5-d triple therapy with a PPI (standard dose, *bid*), clarithromycin (500 mg, *bid*) and metronidazole (500 mg, *bid*)
Concomitant therapy	A PPI (standard dose, *bid*), clarithromycin (500 mg, *bid*), amoxicillin (1 g, *bid*) and metronidazole (500 mg, *bid*) for 7-10 d
Hybrid therapy	A 7-d dual therapy with a PPI (standard dose, *bid*) and amoxicillin (1 g, *bid*) followed by a 7-d quadruple therapy with a PPI (standard dose, *bid*), amoxicillin (1 g, *bid*), clarithromycin (500 mg, *bid*) and metronidazole (500 mg, *bid*)
Levofloxacin-based triple therapy	A PPI (standard dose, *bid*), levofloxacin (500 mg, *bid*) and amoxicillin (1 g, *bid*) for 10 d

PPI: Proton pump inhibitor.

European countries, the recent Maastricht IV/ Florence consensus report has definitively displaced standard regimens as the empirical gold standard to eradicate *H. pylori*[4]. Instead, use of legacy triple regimens should take into account the local resistance pattern (thus, used only in areas in which CAM resistance is < 20%) or rely on susceptibility testing provided that pre-treatment culture is available (*i.e.*, used as tailored treatments).

CURRENT THERAPIES FOR *H. PYLORI* INFECTION

Novel regimens, specifically experimented to improve the therapeutic outcome against antibiotic-resistant *H. pylori* strains, are now recommended as first-line empirical treatment options providing improved efficacy (reportedly > 90% in intention to treat analysis) in high CAM resistance settings. These regimens are summarized in Table 2.

BISMUTH QUADRUPLE THERAPY

Bismuth quadruple therapy (BQT) currently represents a preferred first-line treatments option for areas with a high (≥ 20%) incidence of CAM resistance but also a valuable second-line treatment option when a CAM-based regimen has previously failed. It works independently to CAM achieving > 90% eradication in the presence of CAM resistance, whereas implementation of a high MNZ dose (1500-1600 mg/d) and prolonged (10-14 d) treatment duration allow for minimizing the impact on MNZ resistance, providing eradication rates > 85% even in regions with a high resistance to this drug[24]. A patient-friendly monocapsule (containing bismuth, MNZ and tetracycline) is available (Pylera®, Aptalis, Mont St Hilaire, QC, Canada) providing intention-to-treat eradication rates of 86% and 80% in two large RCTs conducted in North America and Europe respectively[25-27]. Contrarily, the ITT eradication rate with BQT was only 77.8% in a recent meta-analysis (*vs* 77% for STT), questioning both the efficacy as well as the superiority of the BQT over STT[28]. However, a substantial grade of study heterogeneity, especially with respect to MNZ dosing, should be acknowledged. The second-line efficacy of BQT has been also confirmed on a meta-analytic basis (30 studies) showing an average 77% second-line efficacy (ITT) after failure of STT[29]. Third-line efficacy of BQT after two previous eradication failures with CAM- and levofloxacin-containing triple therapies was 65% (ITT) in a multi-center study from Spain[30]. Non-availability of bismuth salts or tetracycline in some countries as well as the potential toxicity of bismuth are the main limitations. However, including 4763 patients no differences with respect to tolerability were shown between non-bismuth and bismuth-containing groups except from dark stools being more common in the later[31].

SEQUENTIAL THERAPY

Sequential therapy uses the same antibiotics contained in STT but administered sequentially. It has been postulated that the initial course of amoxicillin disrupts the bacterial cell wall preventing the development of efflux channels transferring CAM out of the bacteria[32]. Although in the initial RCTs[33] (most of them conducted in Italy) and earlier meta-analyses sequential therapy was clearly superior to STT [ITT eradication 91.7% (95%CI: 90%-93%) *vs* 76.7% (95%CI: 75%-79%) for STT][34], more recent data from South America, Iran and South Korea revealed lower eradication rates (< 80%)[35-37]. Despite this sequential therapy seems to be fairly effective against CAM mono-resistant strains, being able to eradicate 72.8% of them, its efficacy against dual resistant (CAM and MNZ) strains was only 37% (range: 16.2% to 60.7%) when 8 studies with antibiotic susceptibility data were evaluated[38]. Critically, sequential therapy was not superior to either a 14-d triple therapy (RR = 1, 95%CI: 0.94-1.06) or a bismuth-based therapy (RR = 0.99, 95%CI: 0.94-1.05) in an extensive evaluation of 46 RCTs[38].

NON-BISMUTH QUADRUPLE (CONCOMITANT) THERAPY

A non-bismuth quadruple "concomitant" therapy is another valid first-line treatment option for areas with a high incidence of CAM resistance[39,40]. In 19 studies (2070 patients) the overall eradication rate with concomitant therapy was 88% (95%CI: 85%-91%) and 91% when 3 outlying studies with inherently short treatment duration (3-5 d) were excluded[41]. Indeed, treatment duration of

at least 7 d has been shown to be necessary for the success of concomitant therapy[42], whereas extra-prolonging treatment to 14 d combined with a high PPI dose (omeprazole 40 mg × 2) may further boost cure rates to > 95%, as revealed by a non-inferiority multi-center trial[43]. An increased efficacy against dual resistant *H. pylori* strains has been proposed as the main strength of the concomitant over the sequential therapy[44], though the two regimens have performed equally when compared using 338 patients in a high antibiotic resistance country (Spain)[45]. Indeed, by evaluating 106 patients with pre-treatment susceptibility testing, the concomitant therapy eradicated only 55% of dual-resistant strains *vs* 100% and 91% with CAM and MNZ resistance respectively[46]. Thus, both regimens seem to be prone to the deleterious impact of dual resistance, performing comparably (with about 81% of efficacy each) by pooling data of 6 comparative RCTs[38].

HYBRID THERAPY

A two-step dual-concomitant (hybrid) regimen, proposed by Hsu *et al*[47], is another valuable treatment option competing with both the sequential and concomitant treatments. By evaluating data from 2 RCTs, hybrid therapy performed marginally, though not significantly, better as compared to sequential therapy (86.6% *vs* 81%)[38], and comparably to concomitant therapy in a comparative study in which, interestingly, fewer adverse events occurred in the group treated with the hybrid regimen[43]. Further data is warranted to allow for definitive conclusions on the efficacy and tolerability of hybrid therapy.

LEVOFLOXACIN-BASED THERAPIES

To overcome increasing CAM resistance, levofloxacin, a broad spectrum quinolone, has been used as a substitute of CAM in either triple or sequential regimens achieving > 90% cure rates, and even > 95% is feasible provided that the local resistance to levofloxacin is low (< 10%)[48,49]. However, levofloxacin also encounters clinically significant problems of antibiotic resistance, as resistance to quinolones currently exceeds 40% in America, 20% in Europe and 10% in Asia[8]. Due to the rapid development of secondary quinolone resistance, first-line use of levofloxacin is generally discouraged, and the drug is reserved for use in second-line/rescue regimens after failure of a CAM- and/or a MNZ-based regimen[50]. The good (cure rates 81%-87%) second-line efficacy of a levofloxacin triple therapy (LTT) has been confirmed in two meta-analyses published in 2006, both showing better results with LTT in comparison with second-line BQT[51,52]. Congruently, second-line efficacy of LTT was 88.7% in a more recent meta-analysis including RCTs up to October 2010[53]. Critically, use of LTT after failure of either a sequential or concomitant regimen has been reported to provide up to 97.8% of cumulative therapeutic efficacy[54]. Use of other quinolone agents, such as Moxifloxacin and Sitafloxacin, has shown promising results[55,56], though there is no evidence to support any therapeutic advantage over levofloxacin.

FUTURE PERSPECTIVES

Adjunct probiotics

Albeit different attempts have been made to restore the efficacy of standard treatments, such as increasing the PPI dose or prolonging treatment duration, none has been proved at a level to overcome today's antimicrobial resistance. An approach which has attracted growing interest is using probiotics in conjunction with regimens to eradicate *H. pylori*[57]. The expected benefit is twofold: boosting eradication and improving tolerability by preventing occurrence of treatment-related side effects. The pathogenic basis of a possible beneficial effect of probiotics on *H. pylori* eradication remains to be clarified, though some hypothesis have been put forward including strength of the mucosal barrier, competition for adhesion and immunomodulatory mechanisms[58]. Different trials used probiotics adjunctively to either standard or novel regimens in recent years providing contradictory results[59-62]. Although different single- or multi-strain compounds have been evaluated, there is currently evidence to support use of *Saccharomyces boulardi* (OR = 1.13; 95%CI: 1.05-1.21) or *Lactobacillus spp.* (OR = 1.78; 95%CI: 1.2-2.6) supplementation adjunctively to standard triple therapy[63,64]. In the most recent analysis assessing the effect of Lactobacillus-containing and Bifidobacterium-containing supplementation, the pooled odds ratio (ITT) with probiotic supplementation was 2.066 (95%CI: 1.398-3.055) for eradication and 0.305 (95%CI: 0.117-0.793) for the incidence of total side effects[65]. Interestingly, with respect to the prevention of side-effects, use of probiotics may be relevant only in a subset of patients, in particularly those with recurrent infection or history of gastrointestinal antibiotic-related side effects[57]. Further data is awaited to clarify the role, standardize regimens and assess the cost-effectiveness of probiotics in the treatment of *H. pylori* infection.

Culture-free, non-invasive determination of H. pylori antibiotic susceptibility

Critically, even the novel treatments discussed above are to some (although to a lesser as compared to legacy therapies) degree prone to the impact of antibiotic resistance; eradication rates > 95% are infrequent, and even > 90% are disputed in some studies[35,66,67]. Furthermore, it is possible that the success of empirical treatments will further decline in the future as resistance to key antibiotics is constantly growing worldwide. In order to maintain high therapeutic efficacy, tailored treatment of *H. pylori* infection based on pre-treatment susceptibility testing appears as the ideal approach. This will prevent exposing the patient to repeated empirical treatments which increase the risk for side effects and promote development of secondary resistance. However, as

mentioned, current means of performing endoscopy and *H. pylori* culture are invasive, do not 100% reflect in vivo susceptibility, and are time-consuming as culture requires 3-10 d and susceptibility testing (*e.g.*, by Etest, AB bioMerieux, Solna, Sweden) will require additional 3-4 d. These limitations preclude systematical performance of *H. pylori* culture, which is currently recommended only for cases with at least two empirical treatment failures. A class-wide resistance to macrolides is the result of point mutations in three adjacent nucleotide positions (*A2143G, A2142G and A2142C*) in the peptidyl transferase loop of the *23SrRNA* gene[68,69]. These three point mutations account for 90% of cases of primary CAM resistance in Western countries. In recent years, molecular testing methods have been developed for these mutations including a standard polymerase chain reaction (PCR) and other PCR-based methods including PCR-restriction fragment length polymorphism, PCR-DNA enzyme immunoassay, PCR oligonucleotide ligation assay and PCR-line probe assay, as well as Real-time PCR assay which represents a powerful advancement of the basic PCR[70-72]. These methods may offer rapid and highly accurate results in the genotypic detection of CAM resistance, including detection of the heteroresistant status (*i.e.*, co-existence of strains susceptible and resistant to the same antibiotic) known to account for a significant number of treatment failures[73,74]. These techniques can be directly applied on gastric biopsy specimens or used in association with minimally-invasive techniques (*e.g.,* oro-gastric brushing or gastric wash) or non-invasively using stool specimens[75-77]. Importantly, genotypic detection of CAM resistance is also possible with Fluorescence In-Situ Hybridization, which can be also applied on paraffin-embedded specimens[78,79]. Detection of levofloxacin resistance based on the detection of *gyrA* mutations is also available[80]. Two Asian studies have provided data on the potential utility of a tailored therapeutic approach based on the molecular detection of *H. pylori* resistance to CAM. Tailored treatment using a simple PPI/MNZ regimen successfully eradicated the pathogen in 94.3% *vs* 71.4% using empirical standard treatment[77]. In a larger study (218 patients), CAM was replaced by MNZ in the triple regimen if a CAM-resistant strain was detected. Eradication rates were 91.2% in the tailored group *vs* 79.1% and 75.9% by using empirical MNZ- and CAM-based triple therapies (n = 308 in each control group) respectively ($P < 0.001$)[81].

Pharmacogenomics

Genetic variability in the activity of the cytochrome P450 (CYP) 2C19 (CYP2C19) is known to influence the plasma levels of PPIs, and thus treatment of *H. pylori* infection[82,83]. Three distinct genotypes are recognized: rapid, intermediate and poor metabolizers. Preliminary data on the potential use of pharmacogenomics has been provided by a RCT with 300 *H. pylori*-positive patients randomized to either a 1 wk standard regimen or to personalized therapy based on both CYP2C19 and CAM susceptibility status assessed by genetic testing[84]. The ITT eradication rates were significantly higher in the tailored group (96% *vs* 70%) without an increase of the final per-patient cost for successful eradication. In the future, both practical and logistic issues should be addressed before a molecular-based approach can be widely adopted as a genuine basis for the individualization of *H. pylori* eradication therapies.

CONCLUSION

For more than a decade, triple regimens have been the standard of care therapies for *H. pylori* infection. However, in more recent years, rising prevalence of macrolide resistance has accounted for a significant decline in the performance of these regimens, resulting in the necessity of more treatment courses in order to eradicate the pathogen. In order to maintain high therapeutic efficacy, regimens with an improved performance against antibiotic-resistant *H. pylori* strains are now recommended as preferred first-line treatments. The concomitant and sequential regimens are currently the best validated first-line therapeutic options. Hybrid therapy is another effective CAM-based alternative and a relevant competitor to both these treatments. BQT is also a valid treatment for high CAM resistance settings, but also an effective second-line regimen when a CAM-based regimen fails. Due to the rapid development of quinolone resistance, levofloxacin-based regimens should be currently reserved as second-or-more-line treatment options. While efforts to improve empirical treatments continue, the fields of genotypic detection of *H. pylori* antimicrobial susceptibility and pharmacogenomics offer a fascinating new perspective. This is to guarantee 100% therapeutic efficacy: fast, culture-free and non-invasive.

REFERENCES

1 **Moayyedi P**, Hunt RH. Helicobacter pylori public health implications. *Helicobacter* 2004; **9** Suppl 1: 67-72 [PMID: 15347308 DOI: 10.1111/j.1083-4389.2004.00250.x]

2 **Malfertheiner P**, Chan FK, McColl KE. Peptic ulcer disease. *Lancet* 2009; **374**: 1449-1461 [PMID: 19683340 DOI: 10.1016/S0140-6736(09)60938-7]

3 **Kuipers EJ**. Helicobacter pylori and the risk and management of associated diseases: gastritis, ulcer disease, atrophic gastritis and gastric cancer. *Aliment Pharmacol Ther* 1997; **11** Suppl 1: 71-88 [PMID: 9146793]

4 **Malfertheiner P**, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, Gensini GF, Gisbert JP, Graham DY, Rokkas T, El-Omar EM, Kuipers EJ. Management of Helicobacter pylori infection--the Maastricht IV/ Florence Consensus Report. *Gut* 2012; **61**: 646-664 [PMID: 22491499 DOI: 10.1136/gutjnl-2012-302084]

5 **Gisbert JP**. Is culture necessary before first-line treatment for Helicobacter pylori infection? *Intern Med* 2011; **50**: 2717; author reply 2719-2720 [PMID: 22041399]

6 **Mégraud F**. H pylori antibiotic resistance: prevalence, importance, and advances in testing. *Gut* 2004; **53**: 1374-1384 [PMID: 15306603 DOI: 10.1136/gut.2003.022111]

7 **Fischbach L**, Evans EL. Meta-analysis: the effect of antibiotic resistance status on the efficacy of triple and quadruple first-line therapies for Helicobacter pylori. *Aliment Pharmacol Ther* 2007; **26**: 343-357 [PMID: 17635369 DOI: 10.1111/

j.1365-2036.2007.03386.x]
8 **De Francesco V**, Giorgio F, Hassan C, Manes G, Vannella L, Panella C, Ierardi E, Zullo A. Worldwide H. pylori antibiotic resistance: a systematic review. *J Gastrointestin Liver Dis* 2010; **19**: 409-414 [PMID: 21188333]
9 **Megraud F**, Coenen S, Versporten A, Kist M, Lopez-Brea M, Hirschl AM, Andersen LP, Goossens H, Glupczynski Y. Helicobacter pylori resistance to antibiotics in Europe and its relationship to antibiotic consumption. *Gut* 2013; **62**: 34-42 [PMID: 22580412 DOI: 10.1136/gutjnl-2012-302254]
10 **Boyanova L**, Ilieva J, Gergova G, Davidkov L, Spassova Z, Kamburov V, Katsarov N, Mitov I. Numerous risk factors for Helicobacter pylori antibiotic resistance revealed by extended anamnesis: a Bulgarian study. *J Med Microbiol* 2012; **61**: 85-93 [PMID: 21873378 DOI: 10.1099/jmm.0.035568-0]
11 **Georgopoulos SD**, Ladas SD, Karatapanis S, Mentis A, Spiliadi C, Artikis V, Raptis SA. Factors that may affect treatment outcome of triple Helicobacter pylori eradication therapy with omeprazole, amoxicillin, and clarithromycin. *Dig Dis Sci* 2000; **45**: 63-67 [PMID: 10695615]
12 **Yang JC**, Lin CJ. CYP2C19 genotypes in the pharmacokinetics/pharmacodynamics of proton pump inhibitor-based therapy of Helicobacter pylori infection. *Expert Opin Drug Metab Toxicol* 2010; **6**: 29-41 [PMID: 19968574 DOI: 10.1517/17425250903386251]
13 **George LL**, Borody TJ, Andrews P, Devine M, Moore-Jones D, Walton M, Brandl S. Cure of duodenal ulcer after eradication of Helicobacter pylori. *Med J Aust* 1990; **153**: 145-149 [PMID: 1974027]
14 **Bazzoli F**, Pozzato P. Therapy of H. pylori infection. *J Physiol Pharmacol* 1997; **48** Suppl 4: 39-46 [PMID: 9440054]
15 Current European concepts in the management of Helicobacter pylori infection. The Maastricht Consensus Report. European Helicobacter Pylori Study Group. *Gut* 1997; **41**: 8-13 [PMID: 9274464]
16 **Lind T**, Veldhuyzen van Zanten S, Unge P, Spiller R, Bayerdörffer E, O'Morain C, Bardhan KD, Bradette M, Chiba N, Wrangstadh M, Cederberg C, Idström JP. Eradication of Helicobacter pylori using one-week triple therapies combining omeprazole with two antimicrobials: the MACH I Study. *Helicobacter* 1996; **1**: 138-144 [PMID: 9398894]
17 **Malfertheiner P**, Mégraud F, O'Morain C, Hungin AP, Jones R, Axon A, Graham DY, Tytgat G. Current concepts in the management of Helicobacter pylori infection--the Maastricht 2-2000 Consensus Report. *Aliment Pharmacol Ther* 2002; **16**: 167-180 [PMID: 11860399]
18 **Malfertheiner P**, Megraud F, O'Morain C, Bazzoli F, El-Omar E, Graham D, Hunt R, Rokkas T, Vakil N, Kuipers EJ. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. *Gut* 2007; **56**: 772-781 [PMID: 17170018 DOI: 10.1136/gut.2006.101634]
19 **Chey WD**, Wong BC. American College of Gastroenterology guideline on the management of Helicobacter pylori infection. *Am J Gastroenterol* 2007; **102**: 1808-1825 [PMID: 17608775 DOI: 10.1111/j.1572-0241.2007.01393.x]
20 **Fock KM**, Katelaris P, Sugano K, Ang TL, Hunt R, Talley NJ, Lam SK, Xiao SD, Tan HJ, Wu CY, Jung HC, Hoang BH, Kachintorn U, Goh KL, Chiba T, Rani AA. Second Asia-Pacific Consensus Guidelines for Helicobacter pylori infection. *J Gastroenterol Hepatol* 2009; **24**: 1587-1600 [PMID: 19788600 DOI: 10.1111/j.1440-1746.2009.05982.x]
21 **Venerito M**, Krieger T, Ecker T, Leandro G, Malfertheiner P. Meta-analysis of bismuth quadruple therapy versus clarithromycin triple therapy for empiric primary treatment of Helicobacter pylori infection. *Digestion* 2013; **88**: 33-45 [PMID: 23880479 DOI: 10.1159/000350719]
22 **Houben MH**, van de Beek D, Hensen EF, de Craen AJ, Rauws EA, Tytgat GN. A systematic review of Helicobacter pylori eradication therapy--the impact of antimicrobial resistance on eradication rates. *Aliment Pharmacol Ther* 1999; **13**: 1047-1055 [PMID: 10468680]
23 **Graham DY**, Qureshi WA. Antibiotic-resistant H. pylori infection and its treatment. *Curr Pharm Des* 2000; **6**: 1537-1544 [PMID: 10974150]
24 **Fischbach LA**, van Zanten S, Dickason J. Meta-analysis: the efficacy, adverse events, and adherence related to first-line anti-Helicobacter pylori quadruple therapies. *Aliment Pharmacol Ther* 2004; **20**: 1071-1082 [PMID: 15569109 DOI: 10.1111/j.1365-2036.2004.02248.x]
25 **de Boer WA**, van Etten RJ, Schneeberger PM, Tytgat GN. A single drug for Helicobacter pylori infection: first results with a new bismuth triple monocapsule. *Am J Gastroenterol* 2000; **95**: 641-645 [PMID: 10710051 DOI: 10.1111/j.1572-0241.2000.01971.x]
26 **Laine L**, Hunt R, El-Zimaity H, Nguyen B, Osato M, Spénard J. Bismuth-based quadruple therapy using a single capsule of bismuth biskalcitrate, metronidazole, and tetracycline given with omeprazole versus omeprazole, amoxicillin, and clarithromycin for eradication of Helicobacter pylori in duodenal ulcer patients: a prospective, randomized, multicenter, North American trial. *Am J Gastroenterol* 2003; **98**: 562-567 [PMID: 12650788]
27 **Malfertheiner P**, Bazzoli F, Delchier JC, Celiński K, Giguère M, Rivière M, Mégraud F. Helicobacter pylori eradication with a capsule containing bismuth subcitrate potassium, metronidazole, and tetracycline given with omeprazole versus clarithromycin-based triple therapy: a randomised, open-label, non-inferiority, phase 3 trial. *Lancet* 2011; **377**: 905-913 [PMID: 21345487]
28 **Luther J**, Higgins PD, Schoenfeld PS, Moayyedi P, Vakil N, Chey WD. Empiric quadruple vs. triple therapy for primary treatment of Helicobacter pylori infection: Systematic review and meta-analysis of efficacy and tolerability. *Am J Gastroenterol* 2010; **105**: 65-73 [PMID: 19755966 DOI: 10.1038/ajg.2009.508]
29 **Gisbert JP**. Rescue Therapy for Helicobacter pylori Infection 2012. *Gastroenterol Res Pract* 2012; **2012**: 974594 [PMID: 22536225 DOI: 10.1155/2012/974594]
30 **Gisbert JP**, Perez-Aisa A, Rodrigo L, Molina-Infante J, Modolell I, Bermejo F, Castro-Fernández M, Antón R, Sacristán B, Cosme A, Barrio J, Harb Y, Gonzalez-Barcenas M, Fernandez-Bermejo M, Algaba A, Marín AC, McNicholl AG. Third-line rescue therapy with bismuth-containing quadruple regimen after failure of two treatments (with clarithromycin and levofloxacin) for H. pylori infection. *Dig Dis Sci* 2014; **59**: 383-389 [PMID: 24126798 DOI: 10.1007/s10620-013-2900-x]
31 **Ford AC**, Malfertheiner P, Giguere M, Santana J, Khan M, Moayyedi P. Adverse events with bismuth salts for Helicobacter pylori eradication: systematic review and meta-analysis. *World J Gastroenterol* 2008; **14**: 7361-7370 [PMID: 19109870]
32 **Webber MA**, Piddock LJ. The importance of efflux pumps in bacterial antibiotic resistance. *J Antimicrob Chemother* 2003; **51**: 9-11 [PMID: 12493781]
33 **Zullo A**, Vaira D, Vakil N, Hassan C, Gatta L, Ricci C, De Francesco V, Menegatti M, Tampieri A, Perna F, Rinaldi V, Perri F, Papadìa C, Fornari F, Pilati S, Mete LS, Merla A, Potì R, Marinone G, Savioli A, Campo SM, Faleo D, Ierardi E, Miglioli M, Morini S. High eradication rates of Helicobacter pylori with a new sequential treatment. *Aliment Pharmacol Ther* 2003; **17**: 719-726 [PMID: 12641522]
34 **Gisbert JP**, Calvet X, O'Connor A, Mégraud F, O'Morain CA. Sequential therapy for Helicobacter pylori eradication: a critical review. *J Clin Gastroenterol* 2010; **44**: 313-325 [PMID: 20054285 DOI: 10.1097/MCG.0b013e3181c8a1a3]
35 **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]
36 **Lim JH**, Lee DH, Choi C, Lee ST, Kim N, Jeong SH, Kim JW, Hwang JH, Park YS, Lee SH, Shin CM, Jo HJ, Jang ES, Song Is, Jung HC. Clinical outcomes of two-week sequential and concomitant therapies for Helicobacter pylori eradication: a randomized pilot study. *Helicobacter* 2013; **18**: 180-186 [PMID: 23305083 DOI: 10.1111/hel.12034]
37 **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]
38 **Gatta L**, Vakil N, Vaira D, Scarpignato C. Global eradication rates for Helicobacter pylori infection: systematic review and meta-analysis of sequential therapy. *BMJ* 2013; **347**: f4587 [PMID: 23926315 DOI: 10.1136/bmj.f4587]
39 **Georgopoulos S**, Papastergiou V, Xirouchakis E, Laoudi F, Lisgos P, Spiliadi C, Papantoniou N, Karatapanis S. Non-bismuth quadruple "concomitant" therapy versus standard triple therapy, both of the duration of 10 days, for first-line H. pylori eradication: a randomized trial. *J Clin Gastroenterol* 2013; **47**: 228-232 [PMID: 22858517 DOI: 10.1097/MCG.0b013e31826015b0]
40 **Georgopoulos S**, Papastergiou V, Xirouchakis E, Laudi F, Papantoniou N, Lisgos P, Spiliadi C, Fragou P, Skorda L, Karatapanis S. Evaluation of a four-drug, three-antibiotic, nonbismuth-containing "concomitant" therapy as first-line Helicobacter pylori eradication regimen in Greece. *Helicobacter* 2012; **17**: 49-53 [PMID: 22221616 DOI: 10.1111/j.1523-5378.2011.00911.x]
41 **Gisbert JP**, Calvet X. Update on non-bismuth quadruple (concomitant) therapy for eradication of Helicobacter pylori. *Clin Exp Gastroenterol* 2012; **5**: 23-34 [PMID: 22457599 DOI: 10.2147/CEG.S25419]
42 **Essa AS**, Kramer JR, Graham DY, Treiber G. Meta-analysis: four-drug, three-antibiotic, non-bismuth-containing "concomitant therapy" versus triple therapy for Helicobacter pylori eradication. *Helicobacter* 2009; **14**: 109-118 [PMID: 19298338 DOI: 10.1111/j.1523-5378.2009.00671.x]
43 **Molina-Infante J**, Romano M, Fernandez-Bermejo M, Federico A, Gravina AG, Pozzati L, Garcia-Abadia E, Vinagre-Rodriguez G, Martinez-Alcala C, Hernandez-Alonso M, Miranda A, Iovene MR, Pazos-Pacheco C, Gisbert JP. Optimized nonbismuth quadruple therapies cure most patients with Helicobacter pylori infection in populations with high rates of antibiotic resistance. *Gastroenterology* 2013; **145**: 121-128.e1 [PMID: 23562754 DOI: 10.1053/j.gastro.2013.03.050]
44 **Wu DC**, Hsu PI, Wu JY, Opekun AR, Kuo CH, Wu IC, Wang SS, Chen A, Hung WC, Graham DY. Sequential and concomitant therapy with four drugs is equally effective for eradication of H pylori infection. *Clin Gastroenterol Hepatol* 2010; **8**: 36-41.e1 [PMID: 19804842 DOI: 10.1016/j.cgh.2009.09.030]
45 **McNicholl AG**, Marin AC, Molina-Infante J, Castro M, Barrio J, Ducons J, Calvet X, de la Coba C, Montoro M, Bory F, Perez-Aisa A, Forné M, Gisbert JP. Randomised clinical trial comparing sequential and concomitant therapies for Helicobacter pylori eradication in routine clinical practice. *Gut* 2014; **63**: 244-249 [PMID: 23665990 DOI: 10.1136/gutjnl-2013-304820]
46 **Georgopoulos SD**, Xirouchakis E, Martinez-Gonzalez B, Sgouras DN, Spiliadi C, Mentis AF, Laoudi F. Clinical evaluation of a ten-day regimen with esomeprazole, metronidazole, amoxicillin, and clarithromycin for the eradication of Helicobacter pylori in a high clarithromycin resistance area. *Helicobacter* 2013; **18**: 459-467 [PMID: 23714140 DOI: 10.1111/hel.12062]
47 **Hsu PI**, Wu DC, Wu JY, Graham DY. Modified sequential Helicobacter pylori therapy: proton pump inhibitor and amoxicillin for 14 days with clarithromycin and metronidazole added as a quadruple (hybrid) therapy for the final 7 days. *Helicobacter* 2011; **16**: 139-145 [PMID: 21435092 DOI: 10.1111/j.1523-5378.2011.00828.x]
48 **Romano M**, Cuomo A, Gravina AG, Miranda A, Iovene MR, Tiso A, Sica M, Rocco A, Salerno R, Marmo R, Federico A, Nardone G. Empirical levofloxacin-containing versus clarithromycin-containing sequential therapy for Helicobacter pylori eradication: a randomised trial. *Gut* 2010; **59**: 1465-1470 [PMID: 20947881 DOI: 10.1136/gut.2010.215350]
49 **Berning M**, Krasz S, Miehlke S. Should quinolones come first in Helicobacter pylori therapy? *Therap Adv Gastroenterol* 2011; **4**: 103-114 [PMID: 21694812 DOI: 10.1177/1756283X10384171]
50 **Carothers JJ**, Bruce MG, Hennessy TW, Bensler M, Morris JM, Reasonover AL, Hurlburt DA, Parkinson AJ, Coleman JM, McMahon BJ. The relationship between previous fluoroquinolone use and levofloxacin resistance in Helicobacter pylori infection. *Clin Infect Dis* 2007; **44**: e5-e8 [PMID: 17173210 DOI: 10.1086/510074]
51 **Gisbert JP**, Morena F. Systematic review and meta-analysis: levofloxacin-based rescue regimens after Helicobacter pylori treatment failure. *Aliment Pharmacol Ther* 2006; **23**: 35-44 [PMID: 16393278 DOI: 10.1111/j.1365-2036.2006.02737.x]
52 **Saad RJ**, Schoenfeld P, Kim HM, Chey WD. Levofloxacin-based triple therapy versus bismuth-based quadruple therapy for persistent Helicobacter pylori infection: a meta-analysis. *Am J Gastroenterol* 2006; **101**: 488-496 [PMID: 16542284 DOI: 10.1111/j.1572-0241.1998.455_t.x]
53 **Di Caro S**, Fini L, Daoud Y, Grizzi F, Gasbarrini A, De Lorenzo A, Di Renzo L, McCartney S, Bloom S. Levofloxacin/amoxicillin-based schemes vs quadruple therapy for Helicobacter pylori eradication in second-line. *World J Gastroenterol* 2012; **18**: 5669-5678 [PMID: 23155306 DOI: 10.3748/wjg.v18.i40.5669]
54 **Gisbert JP**, Molina-Infante J, Marin AC, Vinagre G, Barrio J, McNicholl AG. Second-line rescue triple therapy with levofloxacin after failure of non-bismuth quadruple "sequential" or "concomitant" treatment to eradicate H. pylori infection. *Scand J Gastroenterol* 2013; **48**: 652-656 [PMID: 23556551 DOI: 10.3109/00365521.2013.786132]
55 **Murakami K**, Okimoto T, Kodama M, Tanahashi J, Fujioka T, Ikeda F, Muraoka H, Takigawa M, Saika T, Hasegawa M, Kobayashi I. Sitafloxacin activity against Helicobacter pylori isolates, including those with gyrA mutations. *Antimicrob Agents Chemother* 2009; **53**: 3097-3099 [PMID: 19380599 DOI: 10.1128/AAC.01552-08]
56 **Wenzhen Y**, Kehu Y, Bin M, Yumin L, Quanlin G, Donghai W, Lijuan Y. Moxifloxacin-based triple therapy versus clarithromycin-based triple therapy for first-line treatment of Helicobacter pylori infection: a meta-analysis of randomized controlled trials. *Intern Med* 2009; **48**: 2069-2076 [PMID: 20009394]
57 **Wilhelm SM**, Johnson JL, Kale-Pradhan PB. Treating bugs with bugs: the role of probiotics as adjunctive therapy for Helicobacter pylori. *Ann Pharmacother* 2011; **45**: 960-966 [PMID: 21693698 DOI: 10.1345/aph.1Q104]
58 **Lesbros-Pantoflickova D**, Corthésy-Theulaz I, Blum AL. Helicobacter pylori and probiotics. *J Nutr* 2007; **137**: 812S-818S [PMID: 17311980]
59 **Du YQ**, Su T, Fan JG, Lu YX, Zheng P, Li XH, Guo CY, Xu P, Gong YF, Li ZS. Adjuvant probiotics improve the eradication effect of triple therapy for Helicobacter pylori infection. *World J Gastroenterol* 2012; **18**: 6302-6307 [PMID: 23180952 DOI: 10.3748/wjg.v18.i43.6302]
60 **Efrati C**, Nicolini G, Cannaviello C, O'Sed NP, Valabrega S. Helicobacter pylori eradication: sequential therapy and Lactobacillus reuteri supplementation. *World J Gastroenterol* 2012; **18**: 6250-6254 [PMID: 23180945 DOI: 10.3748/wjg.v18.i43.6250]
61 **Manfredi M**, Bizzarri B, Sacchero RI, Maccari S, Calabrese L, Fabbian F, De'Angelis GL. Helicobacter pylori infection in clinical practice: probiotics and a combination of probiot-

ics + lactoferrin improve compliance, but not eradication, in sequential therapy. *Helicobacter* 2012; **17**: 254-263 [PMID: 22759324 DOI: 10.1111/j.1523-5378.2012.00944.x]
62 **Shavakhi A**, Tabesh E, Yaghoutkar A, Hashemi H, Tabesh F, Khodadoostan M, Minakari M, Shavakhi S, Gholamrezaei A. The effects of multistrain probiotic compound on bismuth-containing quadruple therapy for Helicobacter pylori infection: a randomized placebo-controlled triple-blind study. *Helicobacter* 2013; **18**: 280-284 [PMID: 23433200 DOI: 10.1111/hel.12047]
63 **Szajewska H**, Horvath A, Piwowarczyk A. Meta-analysis: the effects of Saccharomyces boulardii supplementation on Helicobacter pylori eradication rates and side effects during treatment. *Aliment Pharmacol Ther* 2010; **32**: 1069-1079 [PMID: 21039671 DOI: 10.1111/j.1365-2036.2010.04457.x]
64 **Zou J**, Dong J, Yu X. Meta-analysis: Lactobacillus containing quadruple therapy versus standard triple first-line therapy for Helicobacter pylori eradication. *Helicobacter* 2009; **14**: 97-107 [PMID: 19751434 DOI: 10.1111/j.1523-5378.2009.00716.x]
65 **Wang ZH**, Gao QY, Fang JY. Meta-analysis of the efficacy and safety of Lactobacillus-containing and Bifidobacterium-containing probiotic compound preparation in Helicobacter pylori eradication therapy. *J Clin Gastroenterol* 2013; **47**: 25-32 [PMID: 23090045 DOI: 10.1097/MCG.0b013e318266f6cf]
66 **Liou JM**, Chen CC, Chen MJ, Chen CC, Chang CY, Fang YJ, Lee JY, Hsu SJ, Luo JC, Chang WH, Hsu YC, Tseng CH, Tseng PH, Wang HP, Yang UC, Shun CT, Lin JT, Lee YC, Wu MS. Sequential versus triple therapy for the first-line treatment of Helicobacter pylori: a multicentre, open-label, randomised trial. *Lancet* 2013; **381**: 205-213 [PMID: 23158886 DOI: 10.1016/S0140-6736(12)61579-7]
67 **Vakil N**, Vaira D. Treatment for H. pylori infection: new challenges with antimicrobial resistance. *J Clin Gastroenterol* 2013; **47**: 383-388 [PMID: 23388847 DOI: 10.1097/MCG.0b013e318277577b]
68 **Taylor DE**, Ge Z, Purych D, Lo T, Hiratsuka K. Cloning and sequence analysis of two copies of a 23S rRNA gene from Helicobacter pylori and association of clarithromycin resistance with 23S rRNA mutations. *Antimicrob Agents Chemother* 1997; **41**: 2621-2628 [PMID: 9420030]
69 **Versalovic J**, Shortridge D, Kibler K, Griffy MV, Beyer J, Flamm RK, Tanaka SK, Graham DY, Go MF. Mutations in 23S rRNA are associated with clarithromycin resistance in Helicobacter pylori. *Antimicrob Agents Chemother* 1996; **40**: 477-480 [PMID: 8834903]
70 **Lehours P**, Siffré E, Mégraud F. DPO multiplex PCR as an alternative to culture and susceptibility testing to detect Helicobacter pylori and its resistance to clarithromycin. *BMC Gastroenterol* 2011; **11**: 112 [PMID: 22004003 DOI: 10.1186/1471-230X-11-112]
71 **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]
72 **Schabereiter-Gurtner C**, Hirschl AM, Dragosics B, Hufnagl P, Puz S, Kovách Z, Rotter M, Makristathis A. Novel real-time PCR assay for detection of Helicobacter pylori infection and simultaneous clarithromycin susceptibility testing of stool and biopsy specimens. *J Clin Microbiol* 2004; **42**: 4512-4518 [PMID: 15472302 DOI: 10.1128/JCM.42.10.4512-4518.2004]
73 **De Francesco V**, Zullo A, Ierardi E, Giorgio F, Perna F, Hassan C, Morini S, Panella C, Vaira D. Phenotypic and genotypic Helicobacter pylori clarithromycin resistance and therapeutic outcome: benefits and limits. *J Antimicrob Chemother* 2010; **65**: 327-332 [PMID: 20008044 DOI: 10.1093/jac/dkp445]
74 **Rimbara E**, Fischbach LA, Graham DY. Optimal therapy for Helicobacter pylori infections. *Nat Rev Gastroenterol Hepatol* 2011; **8**: 79-88 [PMID: 21293508 DOI: 10.1038/nrgastro.2010.210]
75 **Baba S**, Oishi Y, Watanabe Y, Oikawa R, Morita R, Yoshida Y, Hiraishi T, Maehata T, Nagase Y, Fukuda Y, Nakazawa M, Ishigouoka S, Hattori N, Suzuki H, Toyota M, Niwa H, Suzuki M, Itoh F. Gastric wash-based molecular testing for antibiotic resistance in Helicobacter pylori. *Digestion* 2011; **84**: 299-305 [PMID: 22057261 DOI: 10.1159/000332570]
76 **Graham DY**, Kudo M, Reddy R, Opekun AR. Practical rapid, minimally invasive, reliable nonendoscopic method to obtain Helicobacter pylori for culture. *Helicobacter* 2005; **10**: 1-3 [PMID: 15691309 DOI: 10.1111/j.1523-5378.2005.00285.x]
77 **Kawai T**, Yamagishi T, Yagi K, Kataoka M, Kawakami K, Sofuni A, Itoi T, Sakai Y, Moriyasu F, Osaka Y, Takagi Y, Aoki T, Rimbara E, Noguchi N, Sasatsu M. Tailored eradication therapy based on fecal Helicobacter pylori clarithromycin sensitivities. *J Gastroenterol Hepatol* 2008; **23** Suppl 2: S171-S174 [PMID: 19120893 DOI: 10.1111/j.1440-1746.2008.05408.x]
78 **Morris JM**, Reasonover AL, Bruce MG, Bruden DL, McMahon BJ, Sacco FD, Berg DE, Parkinson AJ. Evaluation of seaFAST, a rapid fluorescent in situ hybridization test, for detection of Helicobacter pylori and resistance to clarithromycin in paraffin-embedded biopsy sections. *J Clin Microbiol* 2005; **43**: 3494-3496 [PMID: 16000488 DOI: 10.1128/JCM.43.7.3494-3496.2005]
79 **Yilmaz O**, Demiray E, Tümer S, Altungöz O, Yörükoğlu K, Soytürk M, Simşek I. Detection of Helicobacter pylori and determination of clarithromycin susceptibility using formalin-fixed, paraffin-embedded gastric biopsy specimens by fluorescence in situ hybridization. *Helicobacter* 2007; **12**: 136-141 [PMID: 17309750 DOI: 10.1111/j.1523-5378.2007.00483.x]
80 **Cambau E**, Allerheiligen V, Coulon C, Corbel C, Lascols C, Deforges L, Soussy CJ, Delchier JC, Megraud F. Evaluation of a new test, genotype HelicoDR, for molecular detection of antibiotic resistance in Helicobacter pylori. *J Clin Microbiol* 2009; **47**: 3600-3607 [PMID: 19759218 DOI: 10.1128/JCM.00744-09]
81 **Lee HJ**, Kim JI, Cheung DY, Kim TH, Jun EJ, Oh JH, Chung WC, Kim BW, Kim SS, Park SH, Kim JK. Eradication of Helicobacter pylori according to 23S ribosomal RNA point mutations associated with clarithromycin resistance. *J Infect Dis* 2013; **208**: 1123-1130 [PMID: 23801607 DOI: 10.1093/infdis/jit287]
82 **Furuta T**, Shirai N, Takashima M, Xiao F, Hanai H, Sugimura H, Ohashi K, Ishizaki T, Kaneko E. Effect of genotypic differences in CYP2C19 on cure rates for Helicobacter pylori infection by triple therapy with a proton pump inhibitor, amoxicillin, and clarithromycin. *Clin Pharmacol Ther* 2001; **69**: 158-168 [PMID: 11240980 DOI: 10.1067/mcp.2001.113959]
83 **Sim SC**, Risinger C, Dahl ML, Aklillu E, Christensen M, Bertilsson L, Ingelman-Sundberg M. A common novel CYP2C19 gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants. *Clin Pharmacol Ther* 2006; **79**: 103-113 [PMID: 16413245 DOI: 10.1016/j.clpt.2005.10.002]
84 **Furuta T**, Shirai N, Kodaira M, Sugimoto M, Nogaki A, Kuriyama S, Iwaizumi M, Yamade M, Terakawa I, Ohashi K, Ishizaki T, Hishida A. Pharmacogenomics-based tailored versus standard therapeutic regimen for eradication of H. pylori. *Clin Pharmacol Ther* 2007; **81**: 521-528 [PMID: 17215846 DOI: 10.1038/sj.clpt.6100043]

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TOPIC HIGHLIGHT

WJGP 5th Anniversary Special Issues (1): *Helicobacter pylori*

Helicobacter pylori and neurological diseases: Married by the laws of inflammation

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Abstract

The purpose of this paper is to review current information about the role of inflammation caused by *Helicobacter pylori* (*H. pylori*) infection in neurological diseases such as Parkinson's disease, Alzheimer's disease, Guillain-Barré syndrome, multiple sclerosis, and other inflammatory diseases including ischemic stroke. Infection with *H. pylori* usually persists throughout life, resulting in a chronic inflammatory response with local secretion of numerous inflammatory mediators including chemokines [interleukin (IL)-8, macrophage chemotactic protein, growth-regulated oncogene (GRO)-α, chemokine (C-X-C motif) ligand 1] and cytokines [IL-1β, tumor necrosis factor-α, IL-6, IL-12, interferon-γ], which can pass into the circulation and have a systemic effect. The persistence of detectable systemic and local concentrations of inflammatory mediators is likely to alter the outcome of neurological diseases. These proinflammatory factors can induce brain inflammation and the death of neurons and could eventually be associated to Parkinson's disease and also may be involved in the development of Alzheimer's disease. However, most neurological diseases are the result of a combination of multiple factors, but the systemic inflammatory response is a common component and determinant in the onset, evolution, and outcome of diseases. However, more studies are needed to allow understanding of the effects and mechanisms by which the inflammatory response generated by *H. pylori* infection affects neurological diseases.

Key words: Gastrointestinal diseases; *Helicobacter pylori*; Immune disease; Mediators of inflammation; Neurodegenerative diseases

Core tip: Neurological diseases such as Parkinson, Alzheimer, Guillain-Barré syndrome, multiple sclerosis, and ischemic stroke are the result of a combination of multiple factors, but the chronic and systemic inflammatory response to *Helicobacter pylori* could be a common component and determinant in the onset, evolution, and outcome of these diseases.

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INTRODUCTION

Helicobacter pylori (*H. pylori*) is a Gram-negative bacterium that chronically infects more than 50% of the human population[1]. It is well known that infection with the bacterium increases the risk of gastric diseases including peptic ulcers and gastric cancer[2,3]. *H. pylori* is able to infect and live persistently in the human stomach and elicits

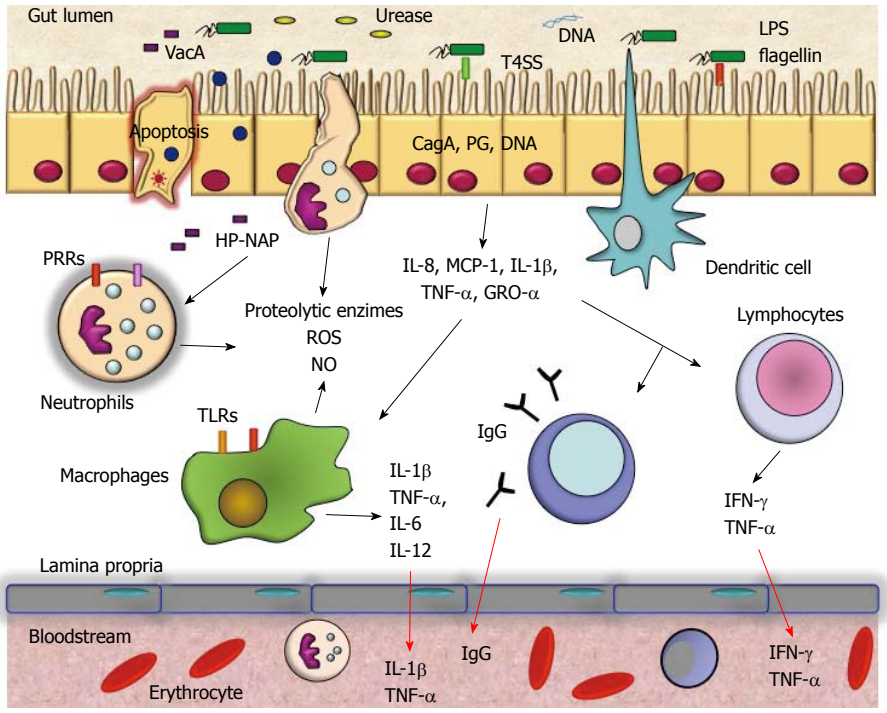

Figure 1 The inflammatory response in *Helicobacter pylori* infection. Immune cells are recruited to the lamina propria of the gastric epithelium by chemokines and cytokines (IL-8, MCP-1, GRO-α, IL-1β, TNF-α) produced by epithelial cells or directly by bacterial products including *H. pylori* neutrophil-activating protein, VacA, and urease. At the site of infection, the immune cells are activated and exert their effector functions, including the production of cytokines (IL-1β, TNF-α, IL-6, IL-12, IFN-γ, chemokines (IL-8, MCP-1), proteolytic enzymes, oxide nitric (NO) and reactive oxygen species (ROS). PG: Peptidoglycan; T4SS: Type IV secretion system; IL: Interleukin; TNF: Tumor necrosis factor; MCP: Macrophage chemotactic protein; GRO: Growth-regulated oncogene.

severe acute and chronic inflammatory responses, which may be of variable magnitude depending on the host' s genetic makeup and lifestyle[4]. Differing inflammatory responses among hosts may help to explain the different outcomes of infection with *H. pylori*[5]. *H. pylori* induces infiltration to the gastric mucosa of neutrophils, macrophages, dendritic cells, T and B cells, and stimulates secretion of interleukin (IL)-8, tumor necrosis factor (TNF)-α, IL-6, IL-1β, IL-12, IL-10, and interferon (IFN)-γ[5]. This persistent response causes significant changes in the physiology of the stomach by direct damage to the cells or by regulating cell proliferation and apoptosis (Figure 1). Neutrophils and macrophages release reactive oxygen and nitrogen species, which may induce irreversible changes in the gene expression of cells of the gastric mucosa. The levels of these chemical species decrease when *H. pylori* infection is eradicated[6]. The secretion of inflammatory mediators is likely to have serious biological implications at the local and systemic level. For example, IL-8 is a chemokine induced early by *H. pylori* infection. It is a chemoattractant of neutrophils and mediates responses to bacterial infection and to autoimmune disease[7]. IL-6 activates target genes involved in cellular differentiation, survival, apoptosis, and proliferation[8]. IL-6 may perpetuate inflammation by inducing antiapoptotic signals mediated by signal transducer and activator of transcription 3. IL-1β signaling is absolutely necessary for the efficient control of *H. pylori* infection. IL-1R(-/-) mice failed to developed protective immunity against Helicobacter-associated gastritis and gastric preneoplasia as a result of their inability to generate Helicobacter-specific T helper (Th)1 and Th17 responses[9].

IL-10 is an anti-inflammatory cytokine that inhibits the production of proinflammatory cytokines by inhibition of Th1 lymphocytes and stimulation of B cells and Th2 lymphocytes, and consequently it downregulates the inflammatory response[10]. This cytokine is very important for the maintenance of a balanced response in gastric inflammation. In contrast, IFN-γ mediates responses to bacterial infection and autoimmune disease. It is upregulated in the gastric mucosa by chronic Helicobacter infection[11]. This cytokine is important in the production of gastric acid and its levels are correlated with the damage found in gastritis.

H. pylori infection has been associated with the development and progression of neurological diseases, principally by inducing systemic inflammation, molecular mimicry, and interference with the absorption of drugs. In this review, we summarize the most important research on this issue.

PARKINSON'S DISEASE

Parkinson's disease is the second most common neurodegenerative disease worldwide. It is characterized by the accumulation of cytoplasmic proteins, including α-synuclein, which leads to the progressive loss of dopaminergic neurons. The loss of dopaminergic neurons causes the resting tremors, rigidity and bradykinesia that are characteristic symptoms of the disease[12]. *H. pylori* infection may

increase the risk of Parkinson's disease[13]. The administration of L-3,4-dihydroxyphenylalanine (L-dopa), a precursor of dopamine, is used as a treatment for Parkinson's disease. *H. pylori* infection may affect the bioavailability of L-dopa by disrupting the duodenal mucosa, which is the site of primary absorption of L-dopa[14,15]. Recent studies suggested that *H. pylori* eradication in patients with Parkinson's disease might improve the bioavailability of L-dopa and reduce motor fluctuations[15-18].

Parkinsonism is a neurological syndrome that shares symptoms found in Parkinson's disease. It has been suggested that a peripheral immune response characterized by the presence of proinflammatory cytokines such as IL-8, IL-1β, and TNF-α in the bloodstream induces a disruption of the blood-brain barrier and promotes microglia-mediated inflammation and neurotoxicity[19-21]. Several studies have established that that proinflammatory factors associated with chronic gastrointestinal disease can induce brain inflammation and the death of dopaminergic neurons and could eventually be responsible for parkinsonism[22-24]. Dobbs *et al*[25] proposed that *H. pylori* infection predisposes to autoimmunity that results in neuronal damage leading to eventual parkinsonism. This was based on the observation of an age-associated increase in the levels of antibodies against *H. pylori* in Parkinson's patients, but this association is not clear, and other investigations are required to clarify it.

ALZHEIMER'S DISEASE

Alzheimer's disease is a progressive neurodegenerative disease characterized by both synaptic loss and neuronal death as a result of extracellular and intracellular accumulation of β-amyloid deposits and neurofibrillary tangles in brain regions important for memory and cognitive processes[26]. The inflammatory response plays an important role in the pathophysiology of Alzheimer's disease. Higher levels of *H. pylori*-specific IgG antibody, IL-8 and TNF-α have been found in cerebrospinal fluid (CSF) of cognitively impaired Alzheimer's disease patients infected with *H. pylori*[27,28], and it has been proposed that the inflammatory response induced by *H. pylori* may be involved in the development of Alzheimer's disease. This is consistent with some studies that observed an improvement in parameters of cognitive and functional status and the survival rate of Alzheimer's disease patients after eradication of *H. pylori*[29,30]. However, Alzheimer's disease was independent of *H. pylori* status in a Japanese population[31].

H. pylori-induced chronic atrophic gastritis causes a decrease in serum vitamin B concentration, thereby increasing the concentration of homocysteine[26]. It has been shown that the serum homocysteine concentration correlates with the severity of dementia. Homocysteine-induced oxidative damage has been described in the brain of subjects with mild cognitive impairment, suggesting that oxidative damage may be one of the earliest events in the onset and progression of Alzheimer's disease[30].

GUILLAIN-BARRÉ SYNDROME

Guillain-Barré syndrome is an acute inflammatory autoimmune neuropathy, presenting as a progressive motor weakness usually beginning in the legs, and can be triggered by a preceding bacterial or viral infection. Molecular mimicry of host structures by antigens present in the gastrointestinal pathogens *Campylobacter jejuni* and *H. pylori* are thought to be connected with the development of the autoimmune sequelae observed in Guillain-Barré syndrome[32,33]. In a case-control study, it was found that serum anti-*H. pylori* IgG of patients was significantly higher than that of controls, that CSF anti-*H. pylori* IgG was positive in 80% of patients and in 20% of controls and that the CSF IgG titer was also significantly higher in patients than controls[34]. Furthermore, specific IgG antibodies to vacuolating cytotoxin A (VacA) of *H. pylori* have been detected in the CSF of patients with Guillain-Barré syndrome. The sequence homology found between VacA and human ATPase A subunit suggests that antibodies to VacA bind to ion channels in Schwann cells, resulting in demyelination of motor neurons in these patients[35]. Moreover, high levels of serum anti-*H. pylori* IgG antibodies closely correlate with a more advanced clinical status, and elevation of anti-*H. pylori*-specific IgG antibodies is associated with involvement of the proximal parts of peripheral nerves in patients with acute inflammatory demyelinating polyradiculoneuropathy, the most commonly observed subtype of Guillain-Barré syndrome[32]. Most studies have included only a small sample, so more research is needed to confirm the association between *H. pylori* infection and Guillain-Barré syndrome.

MULTIPLE SCLEROSIS

Multiple sclerosis is the most common inflammatory demyelinating disease of the central nervous system. The association between *H. pylori* infection and multiple sclerosis is a controversial issue and there are few studies that address the problem. *H. pylori* infection is significantly less frequent in patients with conventional multiple sclerosis than in healthy controls or patients with opticospinal multiple sclerosis[36]. However, *H. pylori* infection seems to be one of the risk factors for the development of anti-aquaporin 4 (AQP4) antibody-positive multiple sclerosis, and the eradication of *H. pylori* may be a possible adjunct therapy[37]. Neuromyelitis optica is an inflammatory disease selectively affecting the optic nerves and spinal cord. Chronic persistent infection may contribute to the development of neuromyelitis optica through molecular mimicry between human AQP4 and bacterial AQP. In addition, *H. pylori* neutrophil-activating protein (HP-NAP) contributes to the pathology by inducing migration and activation of neutrophils[37].

ISCHEMIC STROKE

The pathophysiologic mechanism for the majority of

ischemic strokes is occlusion of carotid or cerebral vessels. Infection with *H. pylori* as a risk factor for stroke is still an unresolved issue because of conflicting results. However, a recent meta-analysis showed that chronic infection with *H. pylori* and the presence of CagA-positive strains are statistically significant risk factors for ischemic stroke, especially for noncardioembolic ischemic stroke[38,39]. Similarly, CagA-positive strains of *H. pylori* are significantly associated with atherosclerotic stroke in patients with an active infection[34].

The mechanisms of the high risk for ischemic stroke conferred by chronic *H. pylori* infection are still not understood. It has been hypothesized that *H. pylori* activates platelets and affects coagulation, and it has been shown that six months after eradication of *H. pylori* infection, the plasma levels of total cholesterol, low-density lipoprotein-cholesterol, fibrinogen, and IL-8 were significantly lower than those in *H. pylori*-positive stroke patients and controls[40].

CONCLUSION

Most neurological diseases are the result of a combination of multiple factors, but the systemic inflammatory response and the production of autoantibodies are common components and determinants in the onset, evolution, and outcome of these diseases. Future studies need to focus on determining the molecular mechanisms by which inflammatory mediators induced by *H. pylori* act on the brain, tipping the balance toward a pathological condition.

REFERENCES

1 **Montecucco C**, Rappuoli R. Living dangerously: how Helicobacter pylori survives in the human stomach. *Nat Rev Mol Cell Biol* 2001; **2**: 457-466 [PMID: 11389469 DOI: 10.1038/35073084]
2 **Labenz J**, Börsch G. Evidence for the essential role of Helicobacter pylori in gastric ulcer disease. *Gut* 1994; **35**: 19-22 [PMID: 8307443]
3 Infection with Helicobacter pylori. *IARC Monogr Eval Carcinog Risks Hum* 1994; **61**: 177-240 [PMID: 7715070]
4 **Hamajima N**, Goto Y, Nishio K, Tanaka D, Kawai S, Sakakibara H, Kondo T. Helicobacter pylori eradication as a preventive tool against gastric cancer. *Asian Pac J Cancer Prev* 2004; **5**: 246-252 [PMID: 15373702]
5 **Peek RM**, Fiske C, Wilson KT. Role of innate immunity in Helicobacter pylori-induced gastric malignancy. *Physiol Rev* 2010; **90**: 831-858 [PMID: 20664074 DOI: 10.1152/physrev.00039.2009]
6 **Ando T**, Goto Y, Maeda O, Watanabe O, Ishiguro K, Goto H. Causal role of Helicobacter pylori infection in gastric cancer. *World J Gastroenterol* 2006; **12**: 181-186 [PMID: 16482615]
7 **Matsuo Y**, Ochi N, Sawai H, Yasuda A, Takahashi H, Funahashi H, Takeyama H, Tong Z, Guha S. CXCL8/IL-8 and CXCL12/SDF-1alpha co-operatively promote invasiveness and angiogenesis in pancreatic cancer. *Int J Cancer* 2009; **124**: 853-861 [PMID: 19035451 DOI: 10.1002/ijc.24040]
8 **Lin MT**, Lin BR, Chang CC, Chu CY, Su HJ, Chen ST, Jeng YM, Kuo ML. IL-6 induces AGS gastric cancer cell invasion via activation of the c-Src/RhoA/ROCK signaling pathway. *Int J Cancer* 2007; **120**: 2600-2608 [PMID: 17304514 DOI: 10.1002/ijc.22599]
9 **Hitzler I**, Sayi A, Kohler E, Engler DB, Koch KN, Hardt WD, Müller A. Caspase-1 has both proinflammatory and regulatory properties in Helicobacter infections, which are differentially mediated by its substrates IL-1β and IL-18. *J Immunol* 2012; **188**: 3594-3602 [PMID: 22403439 DOI: 10.4049/jimmunol.1103212]
10 **Xue H**, Lin B, An J, Zhu Y, Huang G. Interleukin-10-819 promoter polymorphism in association with gastric cancer risk. *BMC Cancer* 2012; **12**: 102 [PMID: 22436502 DOI: 10.1186/1471-2407-12-102]
11 **Lindgren Å**, Yun CH, Sjöling Å, Berggren C, Sun JB, Jonsson E, Holmgren J, Svennerholm AM, Lundin SB. Impaired IFN-γ production after stimulation with bacterial components by natural killer cells from gastric cancer patients. *Exp Cell Res* 2011; **317**: 849-858 [PMID: 21255568 DOI: 10.1016/j.yexcr.2011.01.006]
12 **Gelb DJ**, Oliver E, Gilman S. Diagnostic criteria for Parkinson disease. *Arch Neurol* 1999; **56**: 33-39 [PMID: 9923759]
13 **Nielsen HH**, Qiu J, Friis S, Wermuth L, Ritz B. Treatment for Helicobacter pylori infection and risk of Parkinson's disease in Denmark. *Eur J Neurol* 2012; **19**: 864-869 [PMID: 22248366 DOI: 10.1111/j.1468-1331.2011.03643.x]
14 **Pierantozzi M**, Pietroiusti A, Sancesario G, Lunardi G, Fedele E, Giacomini P, Frasca S, Galante A, Marciani MG, Stanzione P. Reduced L-dopa absorption and increased clinical fluctuations in Helicobacter pylori-infected Parkinson's disease patients. *Neurol Sci* 2001; **22**: 89-91 [PMID: 11487216 DOI: 10.1007/s100720170061]
15 **Fasano A**, Bove F, Gabrielli M, Petracca M, Zocco MA, Ragazzoni E, Barbaro F, Piano C, Fortuna S, Tortora A, Di Giacopo R, Campanale M, Gigante G, Lauritano EC, Navarra P, Marconi S, Gasbarrini A, Bentivoglio AR. The role of small intestinal bacterial overgrowth in Parkinson's disease. *Mov Disord* 2013; **28**: 1241-1249 [PMID: 23712625 DOI: 10.1002/mds.25522]
16 **Lee WY**, Yoon WT, Shin HY, Jeon SH, Rhee PL. Helicobacter pylori infection and motor fluctuations in patients with Parkinson's disease. *Mov Disord* 2008; **23**: 1696-1700 [PMID: 18649391 DOI: 10.1002/mds.22190]
17 **Lyte M**. Microbial endocrinology as a basis for improved L-DOPA bioavailability in Parkinson's patients treated for Helicobacter pylori. *Med Hypotheses* 2010; **74**: 895-897 [PMID: 19962247 DOI: 10.1016/j.mehy.2009.11.001]
18 **Rahne KE**, Tagesson C, Nyholm D. Motor fluctuations and Helicobacter pylori in Parkinson's disease. *J Neurol* 2013; **260**: 2974-2980 [PMID: 24002418 DOI: 10.1007/s00415-013-7089-6]
19 **Candelario-Jalil E**, Taheri S, Yang Y, Sood R, Grossetete M, Estrada EY, Fiebich BL, Rosenberg GA. Cyclooxygenase inhibition limits blood-brain barrier disruption following intracerebral injection of tumor necrosis factor-alpha in the rat. *J Pharmacol Exp Ther* 2007; **323**: 488-498 [PMID: 17704356 DOI: 10.1124/jpet.107.127035]
20 **Block ML**, Zecca L, Hong JS. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat Rev Neurosci* 2007; **8**: 57-69 [PMID: 17180163 DOI: 10.1038/nrn2038]
21 **Lo YC**, Shih YT, Wu DC, Lee YC. In vitro effects of Helicobacter pylori-induced infection in gastric epithelial AGS cells on microglia-mediated toxicity in neuroblastoma SH-SY5Y cells. *Inflamm Res* 2009; **58**: 329-335 [PMID: 19247579 DOI: 10.1007/s00011-009-8075-4]
22 **Dobbs RJ**, Charlett A, Purkiss AG, Dobbs SM, Weller C, Peterson DW. Association of circulating TNF-alpha and IL-6 with ageing and parkinsonism. *Acta Neurol Scand* 1999; **100**: 34-41 [PMID: 10416510]
23 **Bardou I**, Kaercher RM, Brothers HM, Hopp SC, Royer S, Wenk GL. Age and duration of inflammatory environment differentially affect the neuroimmune response and catecholaminergic neurons in the midbrain and brainstem. *Neurobiol Aging* 2014; **35**: 1065-1073 [PMID: 24315728 DOI: 10.1016/j.neurobiolaging.2013.11.006]

24 **Villarán RF**, Espinosa-Oliva AM, Sarmiento M, De Pablos RM, Argüelles S, Delgado-Cortés MJ, Sobrino V, Van Rooijen N, Venero JL, Herrera AJ, Cano J, Machado A. Ulcerative colitis exacerbates lipopolysaccharide-induced damage to the nigral dopaminergic system: potential risk factor in Parkinson`s disease. *J Neurochem* 2010; **114**: 1687-1700 [PMID: 20584104 DOI: 10.1111/j.1471-4159.2010.06879.x]

25 **Dobbs SM**, Dobbs RJ, Weller C, Charlett A. Link between Helicobacter pylori infection and idiopathic parkinsonism. *Med Hypotheses* 2000; **55**: 93-98 [PMID: 10904422 DOI: 10.1054/mehy.2000.1110]

26 **Selkoe DJ**. Deciphering the genesis and fate of amyloid beta-protein yields novel therapies for Alzheimer disease. *J Clin Invest* 2002; **110**: 1375-1381 [PMID: 12438432 DOI: 10.1172/JCI16783]

27 **Roubaud-Baudron C**, Krolak-Salmon P, Quadrio I, Mégraud F, Salles N. Impact of chronic Helicobacter pylori infection on Alzheimer's disease: preliminary results. *Neurobiol Aging* 2012; **33**: 1009.e11-1009.e19 [PMID: 22133280 DOI: 10.1016/j.neurobiolaging.2011.10.021]

28 **Kountouras J**, Boziki M, Gavalas E, Zavos C, Deretzi G, Grigoriadis N, Tsolaki M, Chatzopoulos D, Katsinelos P, Tzilves D, Zabouri A, Michailidou I. Increased cerebrospinal fluid Helicobacter pylori antibody in Alzheimer's disease. *Int J Neurosci* 2009; **119**: 765-777 [PMID: 19326283 DOI: 10.1080/00207450902782083]

29 **Kountouras J**, Boziki M, Gavalas E, Zavos C, Grigoriadis N, Deretzi G, Tzilves D, Katsinelos P, Tsolaki M, Chatzopoulos D, Venizelos I. Eradication of Helicobacter pylori may be beneficial in the management of Alzheimer's disease. *J Neurol* 2009; **256**: 758-767 [PMID: 19240960 DOI: 10.1007/s00415-009-5011-z]

30 **Kountouras J**, Boziki M, Gavalas E, Zavos C, Deretzi G, Chatzigeorgiou S, Katsinelos P, Grigoriadis N, Giartza-Taxidou E, Venizelos I. Five-year survival after Helicobacter pylori eradication in Alzheimer disease patients. *Cogn Behav Neurol* 2010; **23**: 199-204 [PMID: 20829670 DOI: 10.1097/WNN.0b013e3181df3034]

31 **Shiota S**, Murakami K, Yoshiiwa A, Yamamoto K, Ohno S, Kuroda A, Mizukami K, Hanada K, Okimoto T, Kodama M, Abe K, Yamaoka Y, Fujioka T. The relationship between Helicobacter pylori infection and Alzheimer's disease in Japan. *J Neurol* 2011; **258**: 1460-1463 [PMID: 21336779 DOI: 10.1007/s00415-011-5957-5]

32 **Kountouras J**, Deretzi G, Zavos C, Karatzoglou P, Touloumis L, Nicolaides T, Chatzopoulos D, Venizelos I. Association between Helicobacter pylori infection and acute inflammatory demyelinating polyradiculoneuropathy. *Eur J Neurol* 2005; **12**: 139-143 [PMID: 15679702 DOI: 10.1111/j.1468-1331.2004.00977.x]

33 **Moran AP**, Prendergast MM. Molecular mimicry in Campylobacter jejuni and Helicobacter pylori lipopolysaccharides: contribution of gastrointestinal infections to autoimmunity. *J Autoimmun* 2001; **16**: 241-256 [PMID: 11334489 DOI: 10.1006/jaut.2000.0490]

34 **De Bastiani R**, Gabrielli M, Ubaldi E, Benedetto E, Sanna G, Cottone C, Candelli M, Zocco MA, Saulnier N, Santoliquido A, Papaleo P, Gasbarrini G, Gasbarrini A. High prevalence of Cag-A positive H. pylori strains in ischemic stroke: a primary care multicenter study. *Helicobacter* 2008; **13**: 274-277 [PMID: 18665936 DOI: 10.1111/j.1523-5378.2008.00610.x]

35 **Chiba S**, Sugiyama T, Yonekura K, Tanaka S, Matsumoto H, Fujii N, Ebisu S, Sekiguchi K. An antibody to VacA of Helicobacter pylori in cerebrospinal fluid from patients with Guillain-Barre syndrome. *J Neurol Neurosurg Psychiatry* 2002; **73**: 76-78 [PMID: 12082053]

36 **Li W**, Minohara M, Su JJ, Matsuoka T, Osoegawa M, Ishizu T, Kira J. Helicobacter pylori infection is a potential protective factor against conventional multiple sclerosis in the Japanese population. *J Neuroimmunol* 2007; **184**: 227-231 [PMID: 17296235 DOI: 10.1016/j.jneuroim.2006.12.010]

37 **Li W**, Minohara M, Piao H, Matsushita T, Masaki K, Matsuoka T, Isobe N, Su JJ, Ohyagi Y, Kira J. Association of anti-Helicobacter pylori neutrophil-activating protein antibody response with anti-aquaporin-4 autoimmunity in Japanese patients with multiple sclerosis and neuromyelitis optica. *Mult Scler* 2009; **15**: 1411-1421 [PMID: 19965522 DOI: 10.1177/1352458509348961]

38 **Wang ZW**, Li Y, Huang LY, Guan QK, Xu da W, Zhou WK, Zhang XZ. Helicobacter pylori infection contributes to high risk of ischemic stroke: evidence from a meta-analysis. *J Neurol* 2012; **259**: 2527-2537 [PMID: 22688569 DOI: 10.1007/s00415-012-6558-7]

39 **Zhang S**, Guo Y, Ma Y, Teng Y. Relationship between the cytotoxin-associated gene-A status of H pylori strains and cerebral infarction in European Caucasians and Chinese Han: a meta-analysis. *World J Gastroenterol* 2008; **14**: 1286-1292 [PMID: 18300359]

40 **Majka J**, Róg T, Konturek PC, Konturek SJ, Bielański W, Kowalsky M, Szczudlik A. Influence of chronic Helicobacter pylori infection on ischemic cerebral stroke risk factors. *Med Sci Monit* 2002; **8**: CR675-CR684 [PMID: 12388919]

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TOPIC HIGHLIGHT

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Contemporary review of drug-induced pancreatitis: A different perspective

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Abstract

Although gallstone and alcohol use have been considered the most common causes of acute pancreatitis, hundreds of frequently prescribed medications are associated with this disease state. The true incidence is unknown since there are few population based studies available. The knowledge of drug induced acute pancreatitis is limited by the availability and the quality of the evidence as the majority of data is extrapolated from case reports. Establishing a definitive causal relationship between a drug and acute pancreatitis poses a challenge to clinicians. Several causative agent classification systems are often used to identify the suspected agents. They require regular updates since new drug induced acute pancreatitis cases are reported continuously. In addition, infrequently prescribed medications and herbal medications are often omitted. Furthermore, identification of drug induced acute pancreatitis with new medications often requires accumulation of post market case reports. The unrealistic expectation for a comprehensive list of medications and the multifactorial nature of acute pancreatitis call for a different approach. In this article, we review the potential mechanisms of drug induced acute pancreatitis and provide the perspective of deductive reasoning in order to allow clinicians to identify potential drug induced acute pancreatitis with limited data.

Key words: Drug-induced pancreatitis; Mechanism

Core tip: The knowledge of drug-induced acute pancreatitis (DIAP) is limited by the availability and the quality of the evidence. Potential publication bias may also impact our knowledge of DIAP. Several causative agent classification systems have been proposed, but they require regular updates. In addition, Infrequent prescribed medications and herbal medications are often omitted from those summarized lists. We review the potential mechanisms of DIAP and provide the perspective of deductive reasoning in order to allow clinicians to identify potential DIAP with limited data.

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INTRODUCTION

Acute pancreatitis (AP) is an acute inflammatory condition of the pancreas that may extend to local and distant extra-pancreatic tissues. The annual incidence of AP in the United States is approximately 17 cases per 100000. Acute pancreatitis results in 100000 hospitalizations per year, based on previous reports[1]. An average of 2000 patients per year die from complications related to AP. Although gallstones and alcohol are responsible for more than 90% of all cases in adults, medications have

been recognized as a potential cause of AP[2]. Since the first reported case with chlorthalidone and cortisone in the 1950s, hundreds of commonly prescribed medications from different classes have been reported to induce pancreatic damage. It is expected that the list of drug induced acute pancreatitis (DIAP) will continue to expand with newly approved medications, new cases identified for older agents, and the alternative medicines which have less clinical research support in general. While medications are considered as a common cause of AP, reports of DIAP range from 0.1%-2% of overall cases[2,3].

It is not clear if the true incidence of DIAP has been established due to a lack of mandatory adverse drug report (ADR) system to clinicians, potential publication bias, and the challenge to associate AP with medications. Data from clinical trials of new drugs usually are not informative due to the idiosyncratic character of DIAP. In general, idiosyncratic adverse drug reactions occur with a frequency lower than 1:10000[4]. It is extremely difficult to identify adverse reactions in phase Ⅰ to phase Ⅲ clinical investigational trials. Incretin mimetics have been recently introduced in the treatment of diabetes and are widely used in many countries. Incretin mimetics, including exenatide and sitagliptin, were reported to induce AP shortly after those were approved which resulted in warranted an FDA drug safety communication regarding those agents. However, a meta-analysis of randomized clinical trials of most incretin mimetics including sitagliptin did not show any relevant effect on the incidence of pancreatitis. Overall, the incidence was only 0.1% (22 pancreatitis cases found in a pool of 20312 patients). This is an example of the limitation of clinical trials in finding adverse event with low incidence such as DIAP[5].

The knowledge of DIAP is also limited by the availability and the quality of the evidence. It can be difficult to rule out other causes of DIAP, especially in patients who have multiple comorbidities, medications, and underlying risk factors. Since all reports depend on the judgment of the clinicians to exclude other possible causes, reporting more severe ADR has also lead to publication bias. Due to its rarity, most of the evidence comes from case reports of individual drugs and few from case control studies. With a lack of standard ADR reporting format, inadequate data collection in several domains, such as the drug dose, onset of DIAP relative to the use of the medication, and exclusions of other causes, makes it difficult to establish a true causality. In addition, a causal relationship between the agents and DIAP may be difficult to establish due to ethical and practical considerations of re-challenge with the suspected agents. Therefore, the definite relationship between DIAP and medications has only been established in no more than 6% of the agents that have been shown to cause DIAP[6]. Since the identification of DIAP has relied mostly on individual case reports, specific drugs instead of the entire class are usually noted, which makes it even more challenging to identify possible cases in a timely manner.

Potential publication bias may also impact our understanding of DIAP and influence how DIAP is being managed. New drugs or medications with known severe side effects are usually more closely monitored than those that have been in existence for a long time, infrequently prescribed, or considered harmless (*i.e.*, over the counter medications or herbal supplements). Despite the low incidence of drug-induced AP, it is associated with higher morbidity, extended hospital stays, and increased healthcare cost[7]. Approximately 25% of the cases may require intensive care treatment[8]. Developing a systemic approach of identifying potential DIAP is warranted. The aim of this review is to offer a different perspective of approaching DIAP by examining the potential mechanisms of DIAP in order to allow clinicians to identity possible cases with limited data.

ETIOLOGY OF ACUTE PANCREATITIS

Acute pancreatitis is an inflammatory process of the pancreas with varying involvement of other regional tissues or remote organ systems. Gallstone and alcohol use have been considered the most common causes of acute pancreatitis. Gallstone-associated AP is mainly identified by imaging. Previous association with tobacco use is directly linked to alcohol abuse. More evidence associates tobacco use as another toxin that can be directly linked to both acute and recurrent pancreatitis. Other potential mechanical etiologies include periampullary pathologies including intraductal tumor or parasites that are possible in developing countries. In addition to the most common causes, other etiological risk factors for acute pancreatitis are associated with mechanical factors including pancreas divisum, endoscopic retrograde cholangiopancreatography and manometry, as well as trauma or surgical procedures near the pancreas.

Metabolic or systemic process such as hyperlipidemia, infection, and chronic hypercalcemia are well known causes of pancreatitis as well[9]. Infections and toxins, including viral etiologies: mumps, coxsackievirus, hepatitis B, cytomegalovirus, varicella-zoster, herpes simplex virus, human immunodeficiency virus. Bacteria such as *Mycoplasma*, *Legionella*, *Leptospira*, *Salmonella*, *Aspergillus*, *Toxoplasma*, *Cryptosporidium* and *Ascaris* are potential causes of AP. Last, but not least, vascular diseases and pregnancy are also described as causes for pancreatitis.

AP can occur if there is damage to the acinar cells and/or injury to the pancreatic duct that leads to inappropriate accumulation and activation of proenzymes within the pancreas. The activated pancreatic enzymes digest the cell membranes of the pancreas and activate an inflammatory response, which increases the vascular permeability of the pancreas. Hemorrhage, edema, ischemia, and necrosis can result[1,9]. Data from animal studies show that reduced exocytosis and premature fusion of zymogen granules to lysosomes in pancreatic exocrine cells may activate pancreatic proenzymes and lead to cellular autodigestion.

Table 1 Classification system of drug-induced acute pancreatitis according to Badalov *et al*[11]

	Definition	Example
Class Ⅰ drug	Ia: at least one case report, evidence of a positive re-challenge, and exclusion of other causes of AP	Codeine, cytarabine, dapsone, enalapril, furosemide, isoniazid, mesalamine, metronidazole, pentamidine, pravastatin, procainamide, simvastatin, sulfamethoxazole, sulindac, tetracycline, valproic acid
	Ib: similar to class Ia, except that other causes of AP could not be ruled out	Amiodarone, azathioprine, dexamethasone, ifosfaide, lamivudine, losartan, 6-MP, premarin, TMP-SMZ
Class Ⅱ drugs	Include at least four case reports with a consistent latency period for at least 75% of the cases	Acetaminophen, Clozapine, DDI, erythromycin, estrogen, l-asparaginase, propofol, tamoxifen
Class Ⅲ drug	At least two case reports but do not have re-challenge data or a consistent latency period	Alendronate, carbamazepine, ceftriaxone, clarithromycin, cyclosporin, hydrochlorothiazide, interferone/ribavirin, metformin, minocycline, naproxen, paclitaxel, prednisone, prednisolone
Class Ⅳ drug	One case report without re-challenge data	Ampicillin, cisplatin, colchicine, cyclophosphamide, diclofenac, doxorubicin, interleukin-2, octreotide, propoxyphene, rifampin, risperidone, sertaline, tacrolimus, vincristine

AP: Acute pancreatitis; 6-MP: 6-mercaptopurine; TMP-SMZ: Trimethoprim and sulfamethoxazole.

CLASSIFICATION OF CAUSATIVE AGENTS

It is difficult to determine if the effects are intrinsic for all members of a drug class despite reports of DIAP incidence within the class. Several classification systems have been proposed. A substantial number of medications are known to cause AP, however, the underlying mechanism is still not well understood. The classification systems rely on summarized lists of medications from previously published reviews to help make the diagnosis of DIAP. Mallory and Kern in 1980s classified drugs that may cause pancreatitis into three groups: definite, probable, or possible association with pancreatitis[5,9]. In order to improve the quality of evidence, different classification systems have also been proposed that categorized DIAP in classes based on the number of reports and re-challenge results[5,10].

Badalov *et al*[11] in 2007 expanded the classification system to five categories: Ⅰa, Ⅰb, Ⅱ, Ⅲ, and Ⅳ (Table 1). Classifications are based on the published reports from 1955 to 2005. Class Ⅰa includes drugs with at least one case report, evidence of a positive re-challenge, and exclusion of other causes of AP. Class Ⅰb is similar to class Ⅰa, except that other causes of AP could not be ruled out. Criteria for class Ⅱ drugs include at least four case reports with a consistent latency period for at least 75% of the cases. Class Ⅲ drugs have at least two case reports but do not have re-challenge data or a consistent latency period. Finally, class Ⅳ drugs have one case report without re-challenge data. This classification provides a quick reference of potential causative agents based on the available data at the time of the review. However, regular updates of existing classification are needed since new cases of DIAP are reported continuously. Furthermore, infrequently prescribed medications and alternative medications are often omitted from these summarized lists.

Mechanism of DIAP

The majority of the reported DIAP cases seem to have an idiosyncratic character. Idiosyncratic reactions to drugs are adverse effects that are not directly related to pharmacodynamic mechanisms of the drugs. These adverse events can occur unpredictably via abnormal interactions between the drugs and the organism, which is usually mediated by immunologic or cytotoxic effects triggered by the drug or its metabolites in a specific organ, in this case, the pancreas[11]. Although the exact mechanism of DIAP is not always known, the pathogenesis should not differ from other causes of AP. It is believed that the pathogenesis of AP differs only in the injury mechanism. It consists of three steps: (1) premature activation of trypsin in acinar cells; (2) intrapancreatic inflammation; and (3) extrapancreatic inflammation[5]. Several mechanisms have been hypothesized including immune-mediated, direct pancreatic toxicity, pancreatic-duct constriction, influence of medication on the bile flow, thrombosis, metabolic effects, and hypersensitivity[12,13]. Mechanisms of DIAP is showed in Figure 1.

Researchers have also used latency to classify the potential mechanisms of DIAP[5]. It is hypothesized that direct immunological effects are usually observed within the first month of drug exposure, whereas toxic effects are noted after a few months of treatment. Potential mechanisms of DIAP include hypersensitivity (onset after four to eight weeks of use), accumulation of a toxic metabolite (onset after several months of use), hypertriglyceridemia (onset after several months of use), and intrinsic toxicity, which is sometimes related to overdose (onset may be almost immediate)[14]. However, there are exceptions that exist. Clinicians should not ignore long lasting medications while DIAP is a concern.

The following reviews summarize five major types of mechanisms of DIAP (Table 2), namely structural, toxins, metabolic, vascular, and other.

Structural

The structural damage such as compression, obstruction, or inflammation of the pancreatic duct may lead to AP. The most common cause for obstruction is choledochlithiasis, or gallstones. Obstruction can also be caused by

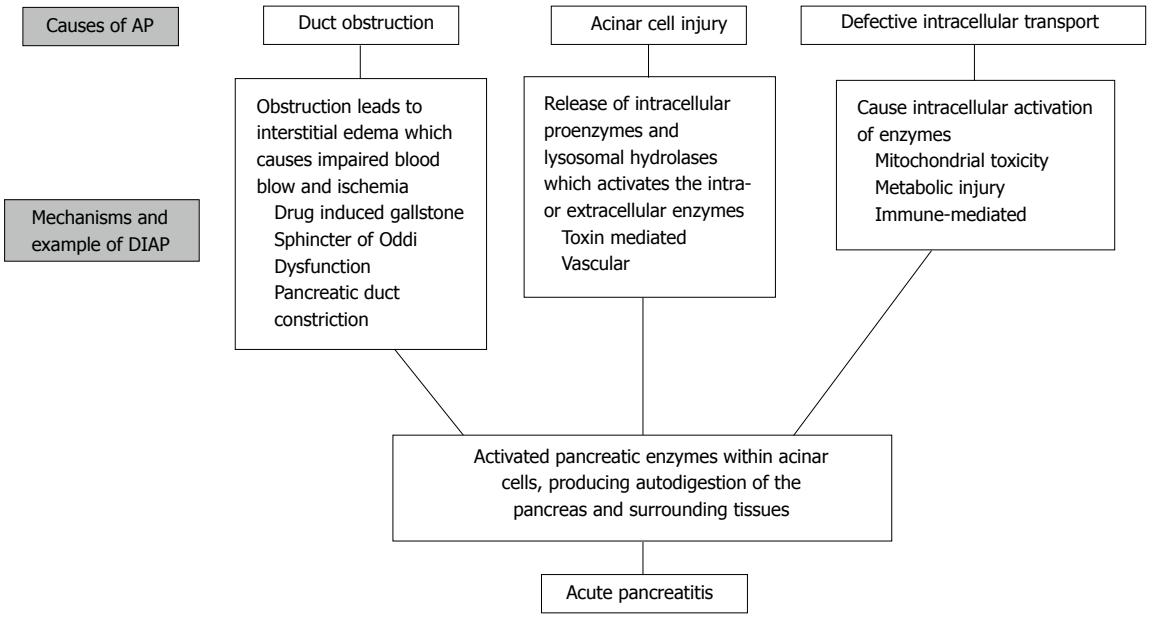

Figure 1 Mechanism of drug-induced pancreatitis. AP: Acute pancreatitis; DIAP: Drug-induced acute pancreatitis.

Table 2 Mechanism of drug induced pancreatitis with drugs associated with acute pancreatitis

Mechanism of DIAP	Drugs with a definite relationship or with class I / II to AP	Probable	Similar structure/class/mechanism with reported cases
Structural	Cholestatic liver injury		Rofecoxib
	Azathioprine		
	Cytarabine		
	Spasm of the sphincter of Oddi	Octreotide	Opium
	Opioids		Marcolides
	Codeine		
	Erythromycin		
	Obstruction		ACE-inhibitors
	Enalapril-angioedema		
	Duct constriction		NSAIDs
	Sulindac		
	Stone		Ceftriaxone
			Dipyridamole
Toxins	Acetaminophen	Metformin	Minocycline
	Didanosine		Tigecycline
	Isoniazid		Doxycycline
	Metronidazole		NRTI
	Valproic acid		HMG-CoA reductase inhibitors
	Mesalamine		
	Pentamidine		
	Asparaginase		
	Sitaliptin		
	Exenatide		
	Tetracycline		
	Pravastatin		
Metabolic	Hypertriglyceridemia	Hydrochlorothiazide	Isotretinoin
	Estrogens	Interferon alfa	Retinoid derivaties
	Corticosteroids	Propofol	Protease inhibitors
	Furosemide	Tamoxifen	Saw palmetto
	β-blocker		Ethacrynic acid
	Clomiphene		Anti-psychotics (aripiprazole, clozapine, olanzapine, quetiapine, risperidone)
	Hypercalcemia		Ⅳ calcium
			Vitamin D
Vascular			Contrast media - iopamidol Procainamide
Immune-mediated	Azathioprine/mercaptopurine sulfasalazine		

AP: Acute pancreatitis; NRTI: Nucleoside reverse transcriptase inhibitor; NSAIDs: Nonsteroidal anti-inflammatory drugs.

duodenal inflammation in Crohn's disease[1].

Medications with risk of gallstones: Ceftriaxone, a third-generation cephalosporin that is excreted from bile duct, has been associated with the development of sludge or stones in the gallbladders for some patients treated with this medication. Secondary pancreatitis has been considered in association with ceftriaxone-induced pseudolithiasis[15]. Unlike ceftriaxone, the kidney pathway is the major means of elimination for most of cephalosporins. It could potentially explain why DIAP has not been reported as class wide induced disease.

Based on an increased amount of cholesterol secreted in bile, causing an increased risk of gallstones which may explain the mechanism of 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitor-induced AP[10]. Long-term administration of dipyridamole and octreotide can form insoluble substances that precipitate in the gallbladder bile to promote gallstone formation as they are highly excreted from the bile[16,17].

Medications that Cause Sphincter of Oddi Dysfunction: As another example of structural disturbance, the Sphincter of Oddi (SO) is situated at the junction of the bile and pancreatic ducts where they enter the duodenum and serves to regulate the flow of bile and pancreatic juices as well as preventing reflux of duodenal contents into the pancreatobiliary system. SO dysfunction refers to two possible conditions-papillary stenosis (edema or hypertrophy) and dyskinesia (tachyoddia, induced spasm) that lead to partial or complete obstruction of the pancreatic duct resulting in pancreatitis[18]. SO dysfunction is implicated as a cause of various forms of AP including gallstone pancreatitis, pancreatitis secondary to alcohol, scorpion envenomation, and organophosphate poisoning. Medications such as octreotide, opioids, opium, and codeine reportedly induce AP in association with SO dysfunction[19,20]. Erythromycin can cause DIAP due to its prokinetic effect on the smooth muscle of the gastrointestinal track and the gallbladder subsequently increasing the pressure of the SO[21]. Since all macrolide antibiotics have prokinetic effect of different degrees, it is reasonable to consider that AP could potentially be drug related when patients are treated with these agents. As an example, clarithromycin and azithromycin have been reported to be associated with AP[6].

The mechanism of action of the class of drug is also an important factor when evaluating the relatedness of the adverse event to the drug. The probable mechanism of aspirin- or nonsteroidal anti-inflammatory drug (NSAID)- induced pancreatitis is due to inhibition of prostaglandins that otherwise may cause pancreatic duct constriction[12]. Aspirin is shown to increase pancreatic duct permeability in animal models. It increases calcium secretion from the pancreas, which is considered a marker of pancreatic damage. Experimental studies suggested that prostaglandins may have a protective effect on pancreatic cells[22]. Membrane stabilization of pancreatic cells may be the mechanism behind the cytoprotection conferred by prostaglandins. In NSAID-associated AP, sulindac seems to stand out as the individual drug from the class with the highest number of published cases[23-26].

Toxins

Cumulative dose-dependent effect of toxic metabolites is also hypothesized in drugs showing a consistent long latency (more than 30 d) at the onset of the first episode of DIAP such as valproic acid[5]. Below we discussed a few classic examples of toxin-mediated DIAP.

Nucleoside reverse transcriptase inhibitor: The leading hypothesis of nucleoside reverse transcriptase inhibitor (NRTI)-associated pancreatitis involves mitochondrial toxicity caused by the inhibition of human mitochondrial DNA polymerase-gamma[27]. This inhibition leads to impaired oxidative phosphorylation and failure to synthesize ATP, which is vital for energy-requiring reactions within the cell. Tissues with the highest energy demand appear to be most susceptible. Mitochondrial toxicity is shared among nucleoside analogues and AP attributed to these agents has been described[28]. The degree of mitochondrial impairment and the resultant tissue-specific clinical manifestations vary depending on the NRTI. Among non-nucleoside reverse transcriptase inhibitors, nevirapine is associated with pancreas-related toxicities, whereas efavirenz is not[29,30].

Metronidazole: One speculative mechanism of metronidazole-induced pancreatitis is that under aerobic conditions, it may undergo redox cycling and yield hydrogen peroxide, superoxide, and other free radicals, which can be toxic to pancreatic beta cells and induce pancreatitis[31].

Pentamidine: Pentamidine has a cytotoxic effect on pancreatic β-cells isle and can cause hypoglycemia or hyperglycemia[32]. Same effect can be expected on acinar pancreatic cells.

L-asparaginase: Animal models suggested that L-asparaginase -induced pancreatic injury can involve disruption of the plasma amino acid balance. Disruption of protein synthesis in acinar cells can cause inhibition of exocytosis following the histologic morphologic changes[33].

Tetracycline: Medications in the tetracycline class, including tetracycline, minocycline, and oxytetracycline, are also associated with AP[34-37]. Tetracycline-induced fatty metamorphosis of the liver usually accompanies evidence of pancreatitis, but pancreatitis without evidence of liver disease has also been observed after administration of tetracycline. Steinberg hypothesized that accumulation of an unidentified toxic metabolite may be the cause of tetracycline-induced pancreatitis[38]. Others suggest that high biliary concentration of tetracycline may be associated with tetracycline-induced pancreatitis[39]. Bile concentrations of minocycline after a 200 mg loading dose followed

by one single 100 mg dose were observed to be more than 10 times higher than concurrent serum concentrations (mean serum concentration 0.65 mcg/mL, range 0.07-1.85 mcg/mL)[40]. Tigecycline, the first available member of the glycylcycline group, is a derivate of minocycline and can share similar side effects. Concentrations of tigecycline in bile (median 75.2 mg/L, range 15.9-1150 mg/L) were also found to be several logs greater than concurrent serum concentration (median 0.112 mg/L, range 0.042-0.25 mg/L) after a single 100 mg dose. The mean and median bile-to-serum 24-h area under the concentration-time curve (AUC0-24) ratios were 537 and 368 respectively[41]. It is reasonable to suspect DIAP as a possible complication in tigecycline treated patients. Several cases have been reported previously[42].

Metabolic

Hypertriglyceridemia: It is generally accepted that levels of triglycerides (TG) greater than 1000 mg/dL may increase the risk of precipitating an episode of pancreatitis[43]. The breakdown products of TG are probably responsible for inducing pancreatitis. When lipase in the pancreatic capillary bed acts on the high levels of TG in serum, toxic free fatty acids are generated. The endothelial lining of small pancreatic blood vessels is the first site of injury. Damages of small blood vessels lead to recruitment of inflammatory cells and thrombosis. Hyperlipidemic pancreatitis may be associated with normal serum amylase but with elevated serum lipase levels[44]. With excessive TG, local ischemia and acidemia may occur due to capillary obstruction[45]. This damage exposes TG to pancreatic lipases, which impact degradation of TG[46]. Hydrolysis of TG by pancreatic lipase, excessive formation of free fatty acids with inflammatory changes, capillary injury, and hyperviscosity are postulated to account for the development of hypertriglyceridemia-induced pancreatitis.

Drugs including estrogens, isotretinoin, propofol, retinoid derivatives, HIV protease inhibitors, β-blockers, thiazides, and furosemide are thought to induce AP owing to hypertriglyceridemia. Estrogen is the most well studied drug in this manner. Exogenous estrogens increase serum TG and fatty acids primarily by reducing levels of lipoprotein and hepatic lipases, which subsequently decrease clearance and aggravate insulin resistance[47]. Typically, estrogen-related pancreatitis occurs within the first months following estrogen initiation. Obese patients with underlying glucose intolerance or fasting hypertriglyceridemia are at greater risk[44]. However, reports have also shown that estrogen-associated DIAP can happen without elevated serum lipid concentrations[48,49]. It is thought that arteriolar thrombosis may be another potential mechanism of action[48,50]. Tamoxifen and clomiphene are synthetic estrogen analogues with mixed agonist-antagonist actions. Cases of tamoxifen- or clomiphene-associated AP have been reported with mechanisms similar to that of estrogen.

Dibenzodiazepine-derived atypical antipsychotics (*i.e.*, clozapine, olanzapine, and quetiapine) may also be a potential cause of DIAP. Both risperidone and ziprasidone are non-dibenzodiazepine atypical antipsychotics and appear to have minimal effect on serum lipids[51]. This is another example where clinicians can apply the general knowledge of each medication when evaluating the likelihood of DIAP for the newer medications.

The previous section discussed that tetracyclines-associated DIAP due to its toxic metabolite and high biliary concentrations. Elmore and Rogge[36] also proposed a tetracycline-induced hypertriglyceridemia mechanism with subsequent pancreatitis. Tetracycline inhibits protein synthesis by binding to the 30S ribosomal subunit in the messenger ribonucleic acid (mRNA) translation complex. Blockage of protein synthesis could result in accumulation of defective proteins within hepatocytes. This inhibits the release of TG from the liver, which may lead to pancreatitis.

Hypercalcemia: Calcium is identified as the most important intracellular element in acinar cell stimulus-secretion coupling[52]. Disruption in the secretory process could be the mechanism by which hypercalcemia induces pancreatitis. Based on experimental studies, increase in extracellular calcium leads to a functional secretory block with dose-dependent characteristics[53]. Acinar cell stimulation induces spikes in cytosolic calcium concentration by repetitively releasing calcium from intracellular stores, which activates the normal secretory process of digestive enzymes from intracellular zymogen stores. Excessive extracellular calcium concentration leads to sustained increases in cytosolic calcium. It results in vacuole formation and trypsinogen activation and eventually leads to edematous or necrotizing pancreatitis[54]. Research indicates that hypercalcemia is associated with an increase in serum enzymes[44]. Intravenous calcium administration has been associated with pancreatitis in at least two published reports. Additionally, pancreatitis has been correlated to cases of vitamin D poisoning and to patients receiving total parenteral nutrition[55]. It is suspected that all drugs which can cause hypercalcemia carry risk of inducing AP.

Thiazides, a class with hypertriglyceridemia potential, could also induce hypercalcemia and hypophosphatemia. Thiazide-induced reductions in blood pressure may lead to pancreatic ischemia. They may act directly on the pancreas or indirectly by altering calcium metabolism. Therefore, there are multiple mechanisms exhibited by thiazides that could potentially lead to AP.

Vascular

Ischemia is an uncommon cause of AP. Pancreatic infarcts may occur in patients with underlying atherosclerotic vascular disease, but they are unusual because the pancreas is richly perfused from several different arterial sources. Cholesterol emboli may cause pancreatitis, cholecystitis, or bowel ulceration or infarction, and should be suspected when AP occurs after vascular interventions such as cardiac catheterization. Patients may have associated evidence

of renal, gut, or peripheral cholesterol emboli. Ischemic pancreatic and hepatic injury may be associated with malignant hypertension, low flow states due to severe heart failure, or administration of potent vasoconstrictors. Vasculitis may cause pancreatitis associated with systemic autoimmune diseases. Acute pancreatitis secondary to drug-induced lupus syndrome has also been described[56].

Contrast-induced pancreatitis may be related to decreased oxygenation and impaired circulation of the pancreas. Iopamidol has a viscosity of 9.4 cP at 37 degrees centigrade versus human plasma of 1.72 cP at hematocrit of 43%. A similar pathophysiologic process has been proposed in contrast-induced kidney injury. Cholesterol crystal embolization may be another mechanism that results in occlusion of small arteries[57].

Immune-mediated reaction

Direct immunological effects are usually observed within the first month of drug exposure, whereas toxic effects are noted after a few months of treatment[45]. Researchers have also considered AP an immune-mediated reaction if relapse occurs rapidly after re-challenge as seen with sulfonamides and aminosalicylates (*e.g.*, sulfasalazine and mesalazine)[58-60]. The latency between initiation of the drug and the onset of DIAP is usually one week to a month, but reexposure can lead to a new episode in one to three days[5]. Cases of azathioprine or the thiopurine bases mercaptopurine-induced pancreatitis are well documented. Studies have shown patients with decreased levels of thiopurine metabolizing enzyme inosine triphosphate pyrophosphatase may be at an increased risk of developing thiopurine-induced AP. However, 6-tioguanine-induced pancreatitis is less common than conventional thiopurine. Only 1% of inflammatory bowel disease (IBD) patients previously intolerant to the conventional thiopurines are reported to have 6-tioguanine-induced AP after treatment[61]. A strong correlation with immune disorders, mainly Crohn's disease and HIV infections, implies an immune-mediated reaction as a chief causative factor of the disease.

Angiotensin-converting-enzyme inhibitors: Captopril, enalapril, lisinopril, perindopril, benazepril, and quinapril have all been associated with AP[25,52]. Pancreatic duct obstruction by local angioedema may be the mechanism by which angiotensin-converting-enzyme-inhibitors cause pancreatitis. Others propose a direct toxic effect on pancreatic cells. Since captopril is structurally dissimilar to enalapril and lisinopril, an allergic reaction seems less likely. Angiotensin receptor blockers may share a similar mechanism for pancreatitis, at this point definitive cases are not described in literature[10].

Alternative medicines including herbal medication

There are very limited data of DIAP associated with herbal or over the counter medications when compared to prescription medications. Although a mechanism for saw palmetto-induced AP has not been thoroughly established, cases of saw palmetto-induced cholestatic hepatitis associated with AP have been reported. Currently, there are only two reported cases of saw palmetto induced AP. Another theory suggests that it occurs through its estrogenic effects by stimulating estrogen receptors and then induces a hypercoagulable state that leads to pancreatic necrosis[2]. This information should prompt clinicians to consider saw palmetto a potential cause of AP.

A WORK UP FOR ACUTE PANCREATITIS

Since no specific test for establishing the diagnosis of DIAP is available, the diagnosis is usually based on excluding all other common causes. Pancreatitis is suspected when a patient presents with clinical features including acute onset of persistent and severe epigastric abdominal pain, and is then confirmed by laboratory and imaging studies that exclude other serious intra-abdominal conditions. Most patients will have elevations in serum levels of amylase or lipase within a few hours of the onset of symptoms. Lipase tends to remain elevated longer than amylase. Amylase and lipase levels above three times the upper limit of normal are mostly associated with pancreatitis. Once these levels are elevated, serial measurements are of no clinical significance for prognosis or outcomes. They should not be obtained routinely after the initial measurements are obtained.

Imaging studies are used to establish the diagnosis, but also to determine etiology and prognosis. Both abdominal ultrasound and abdominal computed tomography (CT) can be used interchangeably; however, the latter is preferred as it can provide alternative diagnoses.

As part of the investigation for potential causes of AP, a history of alcohol/tobacco use, previous biliary colic, medication history, family history, and recent trauma should be elicited. Gallstone-associated pancreatitis should be suspected if stones are seen on imaging studies or if liver chemistries are abnormal and then improve over a few days. A three-fold elevation of ALT has a high predictive value for gallstone-associated AP. Hypertriglyceridemia, especially with levels above 1000 mg/dL, and hypercalcemia can be evaluated based on laboratory data. Infections, including viral etiologies, are potential causes of AP as well.

A suspected drug etiology should be considered after the exclusion of more common causes of illness. As mentioned above, it is challenging to establish the causality between medications and associated AP. The use of classification systems may be useful as the first screening tool. Since the mean interval between initial drug administration and start of the symptoms is approximately 5 wk, with a range of 2 to 36 wk[46], clinicians can target those medications when reviewing the medication profiles. If the mediations are not listed on these summarized lists, clinicians should identify if similar structured medications have been associated with DIAP and evaluate the possibility of sharing a similar mechanism of inducing pancreatitis. Once the target agent is identified, the offending agent

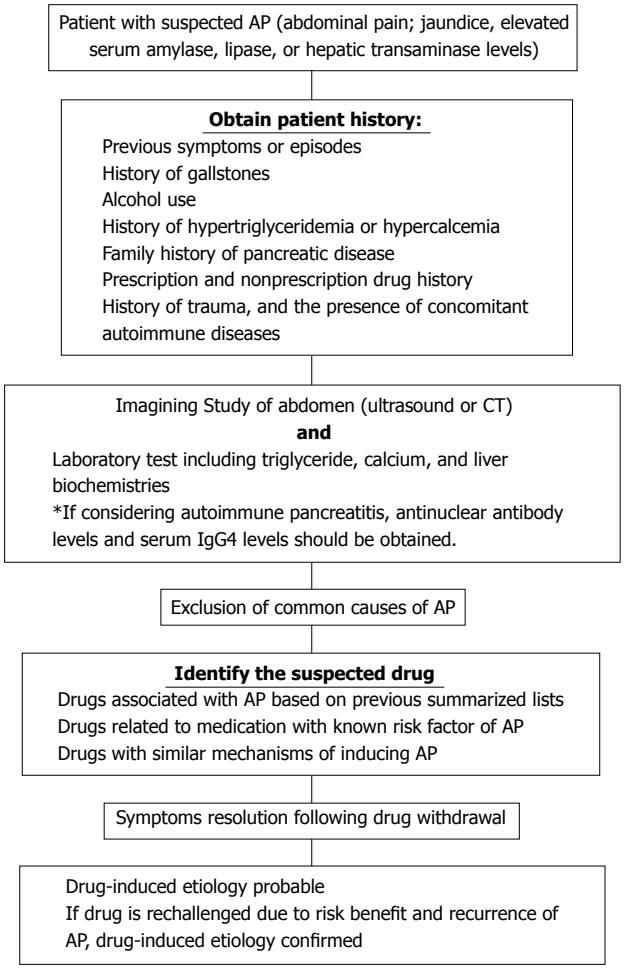

Figure 2 Algorithm of Identifying A potential case of drug induced acute pancreatitis. AP: Acute pancreatitis.

should be discontinued, preferably one at the time to avoid confounders. Most reactions are reversible and resolve on their own within 3-7 d after the offending agent has been discontinued. Due to the nature of the disease state and ethical consideration, re-challenge of the suspected drug is usually not possible. Often times, the medication can be just a possible/probable cause of AP. If re-challenge of the suspect drug is considered necessary, the patient's written informed consent should be obtained. An algorithm of identifying a potential case of drug induced AP is presented in Figure 2.

DISCUSSION

Hundreds of medications have been suggested to be the potential cause of AP, although the true incidence of DIAP is unknown. Evidence associating drugs with AP is largely based on individual cases. It is unrealistic to expect a comprehensive list that includes all agents associated with AP due to continuously reported new cases. Although relapse of pancreatitis after controlled re-challenge confirms a causal relationship, such proof is uncommon. Furthermore, re-challenge is only ethical when the same treatment is absolutely necessary for the patient. It leads to only few causal relationships for the reported agents.

Few causative agent classifications have been proposed. These classifications have helped clinicians understand the quality of evidence behind each potential causative agent. However, with the exception of a few agents with a definite relationship confirmed by re-challenge, it depends on each individual report to exclude all other possible causes, especially drug effects that may be difficult to separate from the underlying conditions. Clinically, certain subpopulations such as children, women, the elderly, patients with IBD and patients with HIV appear to be at a higher risk[2]. Mesalazine, azathioprine, and corticosteroids, for instance, are used in the treatment of IBD which itself increases the risk of AP. Anti-retroviral agents is another example as HIV is an independent risk factor of AP. A study that compared patients with and without HIV infection found a drug-related etiology in 41% and 5% of the patients with AP respectively[62]. The use of NRTIs such as didanosine, stavudine, and lamivudine and co-administration of other medications such as pentamidine, cotrimoxazole, antimycobacterial therapy, or cytotoxic chemotherapy for at least 6 mo were found to be a significant risk factor for at least a three-fold increase in serum pancreatic enzymes ($P < 0.05$). Certain medications, such as proton pump inhibitors and histamine2-receptor antagonists as well as NSAIDs, may be initiated in response to early symptoms of unrecognized pancreatitis. This may have led to erroneously attributing the pancreatitis to these medications[63,64]. Repeated cases of DIAP are more likely to be published or even diagnosed than those without prior reports. Due to underreporting incidence rates from spontaneous reports and potential publication bias with only reporting severe cases, it has further complicated the assessment of the causal relationship between drugs and AP based on current proposed classification.

Efforts have been devoted to improve drug safety surveillance strategies. Vilar *et al*[65] have shown promising results of detecting adverse drug events related to pancreatitis by developing molecular fingerprint-based models. The models were based on the premise that similar molecules can have comparable biological properties. For example, tigecycline is structurally related to minocycline and shares similar pharmacokinetic properties and side effects with tetracyclines. Not surprisingly, cases of tigecycline-induced AP were reported soon after its introduction to the market[42]. Nevertheless, DIAP is generally not considered as a drug class effect, so specific drugs are usually noted instead of the entire class[14]. It is suggested that clinicians take the potential mechanism of DIAP into account. For example, ceftriaxone has different pharmacokinetic properties than other cephalosporins and may lead to secondary pancreatitis caused by only ceftriaxone induced pseudolithiasis.

Limited data exist regarding the mechanisms of DIAP. The pathogenesis is not completely understood. Nevertheless, DIAP should not have unique features that

distinguish it from AP due to other causes. Drugs may lead to pancreatitis by inducing known risk factors of AP such as structural (*e.g.*, cholestatic liver injury, spasm of the SO, duct obstruction/constriction, and stones), metabolic (*e.g.*, hypertriglyceridemia and hypercalcemia), and vascular effects. Some drugs or drug metabolites may theoretically have a direct toxic effect on the pancreas. Other than known mechanisms of toxicity such as mitochondrial toxicity and protein synthesis inhibition, the high level of gastrointestinal drug concentration may be needed to cause cytotoxic damage. Drugs with a definite causal relationship to AP including isoniazid, metronidazole, valproic acid, mesalamine, and tetracycline share similar pharmacokinetic properties by extensive hepatic metabolism. If other potential causes of DIAP have been ruled out, drugs that are highly concentrated in the gastrointestinal tract could be potential suspects of DIAP. For other drugs, an immunoallergic idiosyncratic reaction is more likely. Re-challenge with these drugs usually leads to prompt recurrence of symptoms in a dose-independent manner. In an animal study, the results suggest that DIAP is multifactorial and may explain why the incidence of DIAP is low[18].

Establishing a definitive causal relationship between a drug and AP poses a challenge to clinicians. Depending on the agents, the time from the initiation of therapy to the onset of pancreatitis symptoms varies. Pancreatitis can occur within a short time after administration of the first dose to years after therapy begins for most of the drugs. The unrealistic expectation of the comprehensive list and the multifactorial natures of the causes of AP call for a different approach. This article reviews the potential mechanisms of DIAP and provides the perspective of deductive reasoning in order to identify potential DIAP.

REFERENCES

1 **Tenner S**, Baillie J, DeWitt J, Vege SS. American College of Gastroenterology guideline: management of acute pancreatitis. *Am J Gastroenterol* 2013; **108**: 1400-1415; 1416 [PMID: 23896955 DOI: 10.1038/ajg.2013.218]

2 **Balani AR**, Grendell JH. Drug-induced pancreatitis: incidence, management and prevention. *Drug Saf* 2008; **31**: 823-837 [PMID: 18759507 DOI: 10.2165/00002018-200831100-00002]

3 **Lankisch PG**, Dröge M, Gottesleben F. Drug induced acute pancreatitis: incidence and severity. *Gut* 1995; **37**: 565-567 [PMID: 7489946 DOI: 10.1136/gut.37.4.565]

4 **Urbanek K**, Vinklerova I, Krystynik O, Prochazka V. Acute pancreatitis induced by drugs. *Acute pancreatitis* 2011; **(2)**: 17-34 [DOI: 10.5772/25876]

5 **Monami M**, Dicembrini I, Martelli D, Mannucci E. Safety of dipeptidyl peptidase-4 inhibitors: a meta-analysis of randomized clinical trials. *Curr Med Res Opin* 2011; **27** Suppl 3: 57-64 [PMID: 22106978 DOI: 10.1185/03007995.2011.602964]

6 **Drug-Induced Pancreatitis**. Pharmacist's Letter/Prescriber's Letter. April 2013. Available from: URL: http://prescribersletter.therapeuticresearch.com/home.aspx?cs=&s=PRL&AspxAutoDetectCookieSupport=1

7 **Neoptolemos JP**, Raraty M, Finch M, Sutton R. Acute pancreatitis: the substantial human and financial costs. *Gut* 1998; **42**: 886-891 [PMID: 9691932 DOI: 10.1136/gut.42.6.886]

8 **Sekimoto M**, Takada T, Kawarada Y, Hirata K, Mayumi T, Yoshida M, Hirota M, Kimura Y, Takeda K, Isaji S, Koizumi M, Otsuki M, Matsuno S. JPN Guidelines for the management of acute pancreatitis: epidemiology, etiology, natural history, and outcome predictors in acute pancreatitis. *J Hepatobiliary Pancreat Surg* 2006; **13**: 10-24 [PMID: 16463207 DOI: 10.1007/s00534-005-1047-3]

9 **Soran A**, Chelluri L, Lee KK, Tisherman SA. Outcome and quality of life of patients with acute pancreatitis requiring intensive care. *J Surg Res* 2000; **91**: 89-94 [PMID: 10816356 DOI: 10.1006/jsre.2000.5925]

10 **Fagenholz PJ**, Castillo CF, Harris NS, Pelletier AJ, Camargo CA. Increasing United States hospital admissions for acute pancreatitis, 1988-2003. *Ann Epidemiol* 2007; **17**: 491-497 [PMID: 17448682 DOI: 10.1016/j.annepidem.2007.02.002]

11 **Badalov N**, Baradarian R, Iswara K, Li J, Steinberg W, Tenner S. Drug-induced acute pancreatitis: an evidence-based review. *Clin Gastroenterol Hepatol* 2007; **5**: 648-651; quiz 644 [PMID: 17395548 DOI: 10.1016/j.cgh.2006.11.023]

12 **Zaccara G**, Franciotta D, Perucca E. Idiosyncratic adverse reactions to antiepileptic drugs. *Epilepsia* 2007; **48**: 1223-1244 [PMID: 17386054 DOI: 10.1111/j.1528-1167.2007.01041.x]

13 **Underwood TW**, Frye CB. Drug-induced pancreatitis. *Clin Pharm* 1993; **12**: 440-448 [PMID: 8403815]

14 **Tenner S**, Steinberg WM. Acute Pancreatitis. In: Feldman M, Friedman LS, Brandt LJ, editors. 9th ed. St. Louis, MO: Saunders: Sleisenger & Fordtran's Gastrointestinal and Liver Disease, 2010

15 **Zinberg J**, Chernaik R, Coman E, Rosenblatt R, Brandt LJ. Reversible symptomatic biliary obstruction associated with ceftriaxone pseudolithiasis. *Am J Gastroenterol* 1991; **86**: 1251-1254 [PMID: 1882806]

16 **Trendle MC**, Moertel CG, Kvols LK. Incidence and morbidity of cholelithiasis in patients receiving chronic octreotide for metastatic carcinoid and malignant islet cell tumors. *Cancer* 1997; **79**: 830-834 [PMID: 9024721]

17 **Tauber JP**, Poncet MF, Harris AG, Barthel HR, Simonetta-Chateauneuf C, Buscail L, Bayard F. The impact of continuous subcutaneous infusion of octreotide on gallstone formation in acromegalic patients. *J Clin Endocrinol Metab* 1995; **80**: 3262-3266 [PMID: 7593435 DOI: 10.1210/jcem.80.11.7593435]

18 **Chen JW**, Thomas A, Woods CM, Schloithe AC, Toouli J, Saccone GT. Sphincter of Oddi dysfunction produces acute pancreatitis in the possum. *Gut* 2000; **47**: 539-545 [PMID: 10986215 DOI: 10.1136/gut.47.4.539]

19 **Vidal J**, Sacanella E, Muñoz E, Miro JM, Navarro S. Acute pancreatitis related to octreotide in a patient with acquired immunodeficiency syndrome. *Pancreas* 1994; **9**: 395-397 [PMID: 8022766]

20 **Soule S**, Conway G, Hatfield A, Jacobs H. Effectiveness and tolerability of slow release lanreotide treatment in active acromegaly: six-month report on an Italian multicentre study. *J Clin Endocrinol Metab* 1996; **81**: 4502-4503 [PMID: 8954072 DOI: 10.1210/jc.81.12.4502]

21 **Berger TM**, Cook WJ, O'Marcaigh AS, Zimmerman D. Acute pancreatitis in a 12-year-old girl after an erythromycin overdose. *Pediatrics* 1992; **90**: 624-626 [PMID: 1408521]

22 **Chen HM**, Chen JC, Ng CJ, Chiu DF, Chen MF. Melatonin reduces pancreatic prostaglandins production and protects against caerulein-induced pancreatitis in rats. *J Pineal Res* 2006; **40**: 34-39 [PMID: 16313496]

23 **Goldstein J**, Laskin DA, Ginsberg GH. Sulindac associated with pancreatitis. *Ann Intern Med* 1980; **93**: 151 [PMID: 7396304 DOI: 10.7326/0003-4819-93-1-151_1]

24 **Siefkin AD**. Sulindac and pancreatitis. *Ann Intern Med* 1980; **93**: 932-933 [PMID: 7447197 DOI: 10.7326/0003-4819-93-6-932_2]

25 **Memon AN**. Pancreatitis and sulindac. *Ann Intern Med* 1982; **97**: 139 [PMID: 7091990 DOI: 10.7326/0003-4819-97-1-139_1]

26 **Lerche A**, Vyberg M, Kirkegaard E. Acute cholangitis and pancreatitis associated with sulindac (clinoril). *Histopa-*

thology 1987; **11**: 647-653 [PMID: 3623432 DOI: 10.1111/j.1365-2559.1987.tb02675.x]
27 **Brinkman K**, ter Hofstede HJ, Burger DM, Smeitink JA, Koopmans PP. Adverse effects of reverse transcriptase inhibitors: mitochondrial toxicity as common pathway. *AIDS* 1998; **12**: 1735-1744 [PMID: 9792373 DOI: 10.1097/00002030-199814000-00004]
28 **Dalton SD**, Rahimi AR. Emerging role of riboflavin in the treatment of nucleoside analogue-induced type B lactic acidosis. *AIDS Patient Care STDS* 2001; **15**: 611-614 [PMID: 11788075]
29 **Wester CW**, Thomas AM, Bussmann H, Moyo S, Makhema JM, Gaolathe T, Novitsky V, Essex M, deGruttola V, Marlink RG. Non-nucleoside reverse transcriptase inhibitor outcomes among combination antiretroviral therapy-treated adults in Botswana. *AIDS* 2010; **24** Suppl 1: S27-S36 [PMID: 20023437 DOI: 10.1097/01.aids.0000366080.91192.55]
30 **Kesselring AM**, Wit FW, Sabin CA, Lundgren JD, Gill MJ, Gatell JM, Rauch A, Montaner JS, de Wolf F, Reiss P, Mocroft A. Risk factors for treatment-limiting toxicities in patients starting nevirapine-containing antiretroviral therapy. *AIDS* 2009; **23**: 1689-1699 [PMID: 19487907 DOI: 10.1097/QAD.0b013e32832d3b54]
31 **Sura ME**, Heinrich KA, Suseno M. Metronidazole-associated pancreatitis. *Ann Pharmacother* 2000; **34**: 1152-1155 [PMID: 11054984 DOI: 10.1345/aph.10021]
32 **Assan R**, Perronne C, Assan D, Chotard L, Mayaud C, Matheron S, Zucman D. Pentamidine-induced derangements of glucose homeostasis. Determinant roles of renal failure and drug accumulation. A study of 128 patients. *Diabetes Care* 1995; **18**: 47-55 [PMID: 7698047 DOI: 10.2337/diacare.18.1.47]
33 **Suzuki M**, Shimizu T, Kudo T, Shoji H, Ohtsuka Y, Yamashiro Y. Octreotide prevents L-asparaginase-induced pancreatic injury in rats. *Exp Hematol* 2008; **36**: 172-180 [PMID: 18023522 DOI: 10.1016/j.exphem.2007.09.005]
34 **Nicolau DP**, Mengedoht DE, Kline JJ. Tetracycline-induced pancreatitis. *Am J Gastroenterol* 1991; **86**: 1669-1671 [PMID: 1951248]
35 **Elmore MF**, Rogge JD. Tetracycline-induced pancreatitis. *Gastroenterology* 1981; **81**: 1134-1136 [PMID: 6456964]
36 **Kunelis CT**, Peters JL, Edmondson HA. Fatty liver of pregnancy and its relationship to tetracycline therapy. *Am J Med* 1965; **38**: 359-377 [PMID: 14266828 DOI: 10.1016/0002-9343(65)90145-2]
37 **Steinberg WM**. Acute drug and toxin induced pancreatitis. *Hosp Pract* (Off Ed) 1985; **20**: 95-102 [PMID: 3932442]
38 **Gilson M**, Moachon L, Jeanne L, Dumaine V, Eyrolle L, Morand P, Ben m'Rad M, Salmon D. Acute pancreatitis related to tigecycline: case report and review of the literature. *Scand J Infect Dis* 2008; **40**: 681-683 [PMID: 18979610 DOI: 10.1080/00365540801938949]
39 **Macdonald H**, Kelly RG, Allen ES, Noble JF, Kanegis LA. Pharmacokinetic studies on minocycline in man. *Clin Pharmacol Ther* 1973; **14**: 852-861 [PMID: 4199710]
40 **Wyeth Pharmaceutics**. Tygacil® (package insert). Philadelphia, PA: Wyeth Pharmaceutics, 2009
41 **Hung WY**, Kogelman L, Volpe G, Iafrati M, Davidson L. Tigecycline-induced acute pancreatitis: case report and literature review. *Int J Antimicrob Agents* 2009; **34**: 486-489 [PMID: 19540093 DOI: 10.1016/j.ijantimicag.2009.05.004]
42 **Athyros VG**, Giouleme OI, Nikolaidis NL, Vasiliadis TV, Bouloukos VI, Kontopoulos AG, Eugenidis NP. Long-term follow-up of patients with acute hypertriglyceridemia-induced pancreatitis. *J Clin Gastroenterol* 2002; **34**: 472-475 [PMID: 11907366 DOI: 10.1097/00004836-200204000-00020]
43 **Frick TW**, Spycher MA, Heitz PU, Largiadèr F, Goodale RL. Hypercalcaemia and pancreatic ultrastructure in cats. *Eur J Surg* 1992; **158**: 289-294 [PMID: 1354495]
44 **Kimura W**, Mössner J. Role of hypertriglyceridemia in the pathogenesis of experimental acute pancreatitis in rats. *Int J Pancreatol* 1996; **20**: 177-184 [PMID: 9013278 DOI: 10.1007/BF02803766]
45 **Wilde PJ**, Chu BS. Interfacial & amp; colloidal aspects of lipid digestion. *Adv Colloid Interface Sci* 2011; **165**: 14-22 [PMID: 21377138 DOI: 10.1016/j.cis.2011.02.004]
46 **Perseghin G**, Scifo P, Pagliato E, Battezzati A, Benedini S, Soldini L, Testolin G, Del Maschio A, Luzi L. Gender factors affect fatty acids-induced insulin resistance in nonobese humans: effects of oral steroidal contraception. *J Clin Endocrinol Metab* 2001; **86**: 3188-3196 [PMID: 11443187 DOI: 10.1210/jc.86.7.3188]
47 **Foster ME**, Powell DE. Pancreatitis, multiple infarcts and oral contraception. *Postgrad Med J* 1975; **51**: 667-669 [PMID: 1197171 DOI: 10.1136/pgmj.51.599.667]
48 **Mungall IP**, Hague RV. Pancreatitis and the pill. *Postgrad Med J* 1975; **51**: 855-857 [PMID: 1208296 DOI: 10.1136/pgmj.51.602.855]
49 **Murley RS**. Pancreatitis from oral contraceptives. *BMJ* 1974; **1**: 161 [DOI: 10.1136/bmj.1.5899.161-f]
50 **Meyer JM**. A retrospective comparison of weight, lipid, and glucose changes between risperidone- and olanzapine-treated inpatients: metabolic outcomes after 1 year. *J Clin Psychiatry* 2002; **63**: 425-433 [PMID: 12019668 DOI: 10.4088/JCP.v63n0509]
51 **Petersen OH**. Stimulus-excitation coupling in plasma membranes of pancreatic acinar cells. *Biochim Biophys Acta* 1982; **694**: 163-184 [PMID: 6128029 DOI: 10.1016/0304-4157(82)90023-5]
52 **Frick TW**, Mithöfer K, Fernández-del Castillo C, Rattner DW, Warshaw AL. Hypercalcemia causes acute pancreatitis by pancreatic secretory block, intracellular zymogen accumulation, and acinar cell injury. *Am J Surg* 1995; **169**: 167-172 [PMID: 7817987 DOI: 10.1016/S0002-9610(99)80127-5]
53 **Frick TW**. The role of calcium in acute pancreatitis. *Surgery* 2012; **152**: S157-S163 [PMID: 22906890 DOI: 10.1016/j.surg.2012.05.013]
54 **Khan AS**, Latif SU, Eloubeidi MA. Controversies in the etiologies of acute pancreatitis. *JOP* 2010; **11**: 545-552 [PMID: 21068485]
55 **Gorges R**, Ghalayini W, Zughaib M. A case of contrast-induced pancreatitis following cardiac catheterization. *J Invasive Cardiol* 2013; **25**: E203-E204 [PMID: 24088437]
56 **Falko JM**, Thomas FB. Letter: Acute pancreatitis due to procainamide-induced lupus erythematosus. *Ann Intern Med* 1975; **83**: 832-833 [PMID: 1200531 DOI: 10.7326/0003-4819-83-6-832]
57 **Widdison AL**, Karanjia ND. Pancreatic infection complicating acute pancreatitis. *Br J Surg* 1993; **80**: 148-154 [PMID: 8443638 DOI: 10.1002/bjs.1800800208]
58 **Schaad UB**, Wedgwood-Krucko J, Tschaeppeler H. Reversible ceftriaxone-associated biliary pseudolithiasis in children. *Lancet* 1988; **2**: 1411-1413 [PMID: 2904533 DOI: 10.1016/S0140-6736(88)90596-X]
59 **Niemelä S**, Lehtola J, Karttunen T, Lähde S. Pancreatitis in patients with chronic inflammatory bowel disease. *Hepatogastroenterology* 1989; **36**: 175-177 [PMID: 2753464]
60 **de Boer NK**, Derijks LJ, Gilissen LP, Hommes DW, Engels LG, de-Boer SY, den Hartog G, Hooymans PM, Mäkelburg AB, Westerveld BD, Naber AH, Mulder CJ, de Jong DJ. On tolerability and safety of a maintenance treatment with 6-thioguanine in azathioprine or 6-mercaptopurine intolerant IBD patients. *World J Gastroenterol* 2005; **11**: 5540-5544 [PMID: 16222751]
61 **Bruminhent J**, Carrera P, Li Z, Amankona R, Roberts IM. Acute pancreatitis with saw palmetto use: a case report. *J Med Case Rep* 2011; **5**: 414 [PMID: 21867545 DOI: 10.1186/1752-1947-5-414]
62 **Cappell MS**, Marks M. Acute pancreatitis in HIV-seropositive patients: a case control study of 44 patients. *Am J Med* 1995; **98**: 243-248 [PMID: 7872340 DOI: 10.1016/

S0002-9343(99)80370-2]
63 **Eland IA**, Alvarez CH, Stricker BH, Rodríguez LA. The risk of acute pancreatitis associated with acid-suppressing drugs. *Br J Clin Pharmacol* 2000; **49**: 473-478 [PMID: 10792205 DOI: 10.1046/j.1365-2125.2000.00196.x]
64 **Evans JM**, McMahon AD, Steinke DT, McAlpine RR, MacDonald TM. Do H2-receptor antagonists cause acute pancreatitis? *Pharmacoepidemiol Drug Saf* 1998; **7**: 383-388 [PMID: 15073967]
65 **Vilar S**, Harpaz R, Santana L, Uriarte E, Friedman C. Enhancing adverse drug event detection in electronic health records using molecular structure similarity: application to pancreatitis. *PLoS One* 2012; **7**: e41471 [PMID: 22911794 DOI: 10.1371/journal.pone.0041471]

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TOPIC HIGHLIGHT

WJGP 5th Anniversary Special Issues (3): Pancreatitis

Acute pancreatitis in children and adolescents

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Abstract

In this Topic Highlight, the causes, diagnosis, and treatment of acute pancreatitis in children are discussed. Acute pancreatitis should be considered during the differential diagnosis of abdominal pain in children and requires prompt treatment because it may become life-threatening. The etiology, clinical manifestations, and course of acute pancreatitis in children are often different than in adults. Therefore, the specific features of acute pancreatitis in children must be considered. The etiology of acute pancreatitis in children is often drugs, infections, trauma, or anatomic abnormalities. Diagnosis is based on clinical symptoms (such as abdominal pain and vomiting), serum pancreatic enzyme levels, and imaging studies. Several scoring systems have been proposed for the assessment of severity, which is useful for selecting treatments and predicting prognosis. The basic pathogenesis of acute pancreatitis does not greatly differ between adults and children, and the treatments for adults and children are similar. In large part, our understanding of the pathology, optimal treatment, assessment of severity, and outcome of acute pancreatitis in children is taken from the adult literature. However, we often find that the common management of adult pancreatitis is difficult to apply to children. With advances in diagnostic techniques and treatment methods, severe acute pancreatitis in children is becoming better understood and more controllable.

Key words: Acute pancreatitis; Children; Pathophysiology; Etiology; Diagnosis; Treatment

Core tip: The etiology, manifestations, and course of acute pancreatitis in children are often different than in adults, and these differences should be highlighted. The etiology of acute pancreatitis in children is drugs, infections, trauma, or anatomic abnormalities. The diagnosis of acute pancreatitis is based on clinical symptoms, serum pancreatic enzyme levels, and imaging studies. Treatments in adults and children are similar. With advances in diagnostic techniques and treatments, severe acute pancreatitis in children is becoming better understood and more controllable.

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INTRODUCTION

Acute pancreatitis is not necessarily a rare disease, even in children and adolescents (hereinafter referred to as "children"), and may be life-threatening if it is severe[1,2]. Therefore, acute pancreatitis should always be considered during the differential diagnosis of abdominal pain in children, and appropriate treatment should be started promptly when necessary. However, many treatment regimens are based on consensus conferences and evidence in adults, so a search for the cause and appropriate treatment in children is often difficult[3,4]. This paper discusses the causes, diagnosis, and treatment of acute pancreatitis in children, including a review based on our own experiences.

Table 1 Etiology of childhood acute pancreatitis

Congenital anomalies, periampullary obstruction
Choledochal cyst, abnormal union of the pancreaticobiliary junction, gallstone, cholecystitis, pancreatic divisum, tumor, ascaris aberrant
Infectious
Mumps, measles, coxsackie, echo, lota, influenza, epstein-barr virus, Mycoplasma, salmonella, gram-negative bacteria
Drugs
L-asparaginase, steroid, valproic acid, azathioprine, Mercaptopurine, mesalazine, Cytarabine, Salicylic acid, indomethacin, tetracycline, chlorothiazide, isoniazid, anticoagulant drug, borate, alcohol
Trauma
Blunt injury, child abuse, ERCR, After surgery
Systemic disease
Reye syndrom, systemic lupus erythematosus, polyarteritis nodosa, Juvenile rheumatoid arthritis, sepsis, multiple organ failure, Organ transplantation, hemolytic-uremic syndrome, henoch-schoenlein purpura, kawasaki disease, inflammatory bowel disease, chronic intestinal pseudo-obstruction, gastric ulcer, anorexia nervosa, food allergy, cystic fibrosis
Metabolic
Hyperlipoproteinemia (I, IV, V), hypercalcemia, diabetes, α1 antitrypsin deficiency
Nutrition
Malnutrition, high-calorie infusion, vitamin A and D deficiency
Others
Familial, idiopathic

ERCP: Endoscopic retrograde cholangiopancreatography.

Table 2 Cause of acute pancreatitis in children and adolescents

Ref.	Location	Cases	Etiology (%) Systemic	Biliary[1]	Anatomic[2]	Trauma	Familial	Metabolic[3]	Drugs	Others[4]	Idiopathic
Lopez[50]	United States	274	48	10	NA	19	NA	0.7	5	0.4	17
DeBanto *et al*[1]	United States	301	3.5	10.5	1.5	13.5	5.5	4	11	16.5	34
Werlin *et al*[8]	United States	180	14	12	7.5	14	3	5.5	12	24	8
Nydegger *et al*[4]	Australia	279	22.2	5.4	NA	36.3	NA	5.8	3.2	2.2	25.1
Suzuki *et al*[19]	Japan	135	8.9	30.4	25.9	9.6	NA	NA	11.1	3.7	10.4
Lantz *et al*[2]	United States	211	3.3	11.8	5.2	7.6	0.9	6.2	19.9	13.8	31.3

All studies contained more than 100 cases. NA: Not available. [1]Gallstone, biliary sludge, choledochal cyst; [2]Abnormal union of the pancreaticobiliary junction, pancreatic divisum; [3]Diabetic acidosis, hyperlipidemia, organic acidemias, hypercalcemia; [4]Associated viral infection, postendoscopic retrograde cholangiopancreatography, alcohol, autoimmune, cystic fibrosis, post-surgery.

ETIOLOGY

Alcohol and gallstones are the etiology of acute pancreatitis in many adults, and although some differences exist based on sex and ethnicity, these two etiologies account for more than 60% of cases of acute pancreatitis in adults[5,6]. However, the etiology in children is often drugs, infections, trauma, and anatomic anomalies such as choledochal cysts and abnormal union of the pancreatobiliary junction (Table 1)[1,4,7,8]. Table 2 shows the incidence of acute pancreatitis by etiology. There is a considerable difference in the etiology of acute pancreatitis in Western and Asian children[9].

Drugs

Among drugs used in childhood and adolescence, L-asparaginase (ASNase), steroids, and valproic acid often cause pancreatitis as an adverse reaction. In particular, ASNase, a key drug used in treatment of childhood leukemia, is associated with a higher incidence of pancreatitis as compared to other drugs, ranging from 2%-16% when mild cases are included[10-12]. A characteristic of pancreatitis associated with ASNase, in addition to clinical symptoms of abdominal pain and tenderness, is the early absence of elevated serum amylase levels in about half of patients[13,14]. This phenomenon is attributed to inhibition of protein synthesis by ASNase[14]. Therefore, when acute pancreatitis is suspected based on clinical findings, even in the absence of serum amylase elevation, acute pancreatitis must always be considered in the differential diagnosis, and it is important not to miss the opportunity for early treatment. Azathioprine and mesalazine can also cause pancreatic toxicity, so if serum pancreatic enzyme levels increase during the treatment of inflammatory bowel disease, drug-related pancreatitis must also be considered[15].

Infectious disease

Mumps is often encountered in daily clinical practice, but few patients develop pancreatitis that requires additional treatment. Pancreatitis as a complication is reported in 0.3%-15% of patients when mild cases are included[16]. Abdominal symptoms such as pain and tenderness may occur before the clinical onset of mumps (4-8 d after viral infection) and often spontaneously resolve in about 1 wk. In addition, pancreatitis may occur without parotid

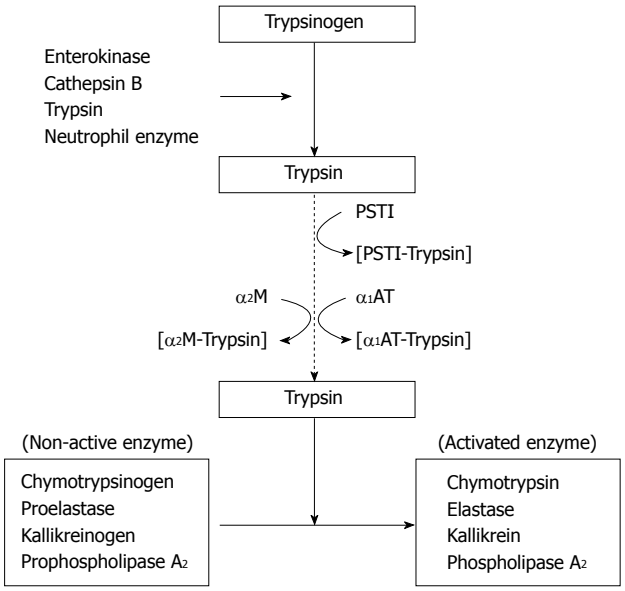

Figure 1 Suppression mechanisms for pancreatic enzyme activation. PSTI: Pancreatic secretory trypsin inhibitor; α2M: α2-macroglobulin; α1AT: α1-antitrypsin.

gland swelling in a few patients. When pancreatitis of unknown etiology occurs, testing for the mumps virus is recommended. Two deaths have been reported to date, so although rare, possible serious infection must be kept in mind[17].

Pancreatitis associated with mycoplasma infection is broadly classified into two types: early onset type during early infection (days 1-3) and late-onset type after respiratory tract symptoms have occurred (days 7-14). The mechanism in the former is thought to be direct invasion of mycoplasma into the pancreas, and in the latter, pancreatic injury caused by autoantibodies to acinar cells[18]. The prognosis in pancreatitis due to mycoplasma is generally good.

Congenital anomalies

Among anomalies of the pancreatobiliary system, choledochal cyst is the most common cause of acute pancreatitis[1,2,4,19]. In fact, many choledochal cysts are discovered because of symptoms of acute pancreatitis. In children with acute pancreatitis in whom the etiology is unclear, ultrasonography, endoscopic retrograde cholangiopancreatography (ERCP), or magnetic resonance cholangiopancreatography (MRCP) should be performed[20,21]. Most choledochal cysts, with the exception of Todani classification type Ⅱ (bile duct diverticulum) and type Ⅲ (choledochocele), are associated with abnormal union[22]. The sphincter of Oddi is usually most thickened in the duodenal muscularis mucosa; however, in abnormal union, because this sphincter surrounds a common channel after union of the main pancreatic duct and common bile duct, there is communication between the ducts during sphincter contraction[23]. Therefore, reflux of bile into the pancreatic duct, a protein plug in the common channel, or gallstone impaction is probably involved in the onset of pancreatitis.

PANCREATITIS CAUSED BY GENETIC MUTATIONS

Hereditary pancreatitis is due to autosomal dominant inheritance with about 80% penetrance. A relationship between a mutation in the cationic trypsinogen gene (protease serine 1, *PRSS1*) and hereditary pancreatitis was identified in 1996[24]. In 2000, a mutation in the serine protease inhibitor gene (*Kazal* type 1: *SPINK1*) was reported to be related to chronic idiopathic pancreatitis of unknown cause[25]. Patients with hereditary pancreatitis due to a *PRSS1* gene mutation or relapsing pancreatitis due to a *SPINK1* gene mutation can develop pancreatic exocrine insufficiency and diabetes in the future, and they are a high-risk group for pancreatic cancer[26-28]. The cause of these complications like cancer, as in chronic pancreatitis due to other etiologies, involves hyperplasia and metaplasia of the pancreatic duct epithelium due to recurrent or chronic inflammation. *K-ras* gene mutations also play a role[29]. Diabetes or pancreatic cancer developing in childhood cases has not been reported.

Recently, variants in *CPA1,* which encodes carboxypeptidase A1, were implicated in early onset pancreatitis in children up to 10 years old. The mechanism by which *CPA1* variants confer increased pancreatitis risk may involve misfolding-induced endoplasmic reticulum stress rather than elevated trypsin activity[30].

Other causes

In malignant lymphoma, lymphoma invasion near the head of the pancreas may compress the pancreatic duct and lead to acute pancreatitis[31]. In addition, in solid pseudopapillary neoplasms, intratumoral hemorrhage due to trauma can cause transient tumor enlargement, leading to pancreatic duct obstruction and acute pancreatitis[32].

PATHOPHYSIOLOGY

To understand the pathophysiology of acute pancreatitis, knowledge about the inhibitory mechanisms of activation of pancreatic enzymes under physiological conditions is necessary. In normal pancreatic acinar cells, lysosomes containing cathepsin B, which are involved in intracellular and extracellular digestion, and zymogen granules containing digestive proenzymes, such as trypsinogen, are released; and these inactive proenzymes remain inactivated[33,34]. In addition, even if trypsin is aberrantly activated in the pancreas for some reason, its activity is blocked by pancreatic secretory trypsin inhibitor (PSTI). Moreover, if trypsin leaks into the blood, the endogenous trypsin inhibitors α1-antitrypsin (α1AT) and α2-macroglobulin (α2M) bind to trypsin and suppress its activity (Figure 1)[35]. Anatomically, the sphincter of Oddi located in the duodenal ampulla of Vater prevents reflux of duodenal fluid into the pancreatic duct. Pancreatic duct pressure is also usually higher than bile duct pressure, so there is no bile reflux into the pancreatic duct[23].

Excessive stimulation of pancreatic exocrine secre-

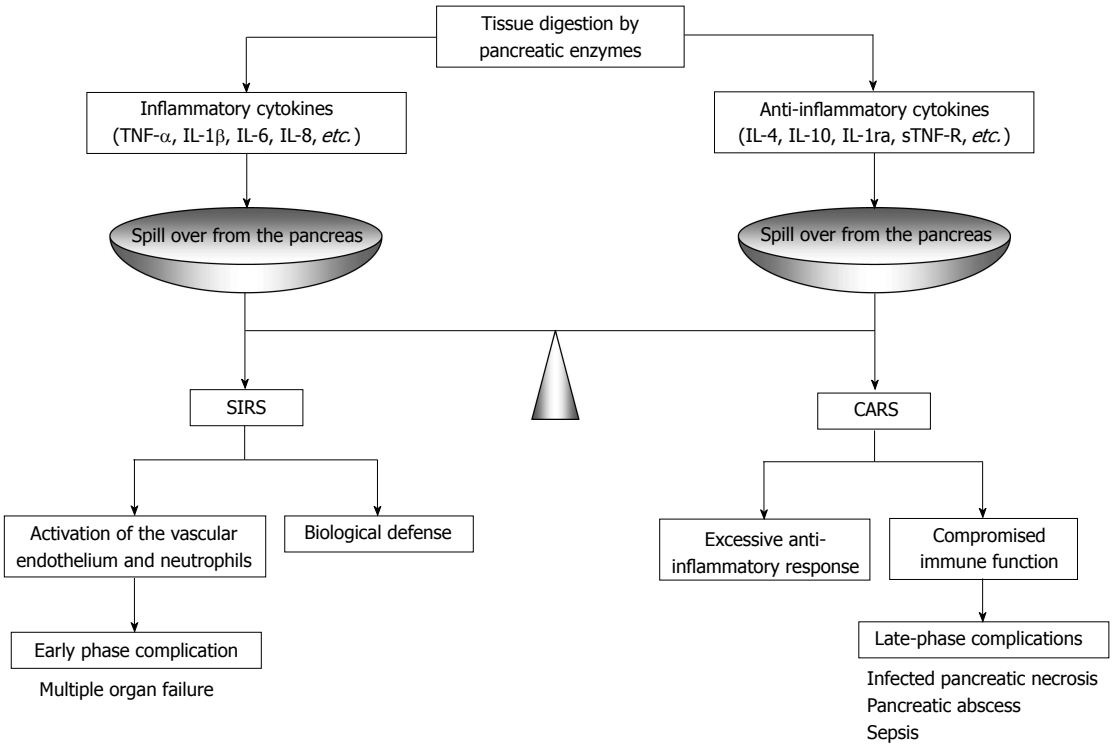

Figure 2 Compensatory anti-inflammatory response syndrome and systemic inflammatory response syndrome during acute pancreatitis. TNF: Tumor necrosis factor; IL: Interleukin; sTNF-R: Soluble tumor necrosis factor receptor; CARS: Compensatory anti-inflammatory response syndrome; SIRS: Systemic inflammatory response syndrome.

tions can cause reflux of pancreatic juices and enterokinase, pancreatic duct obstruction, and inflammation. These conditions can disrupt the above-mentioned defense mechanisms, activate trypsin beyond the ability for trypsin inactivation, and increase attacking factors, thus leading to acute pancreatitis[36]. Enterokinase is the most efficient activator, but trypsin itself, lysosomal enzymes (cathepsin B) in pancreatic acinar cells, and neutrophilic enzymes are also activators[34,36]. In experimental models of early acute pancreatitis, blockage of secretion has been suggested as the initiating event, leading to the accumulation of zymogen granules within acinar cells. This event is followed by a co-localization of digestive enzymes and lysosomal enzymes within vacuoles and, finally, an activation of enzymes that cause acute intracellular injury[37]. The activation of zymogen protease in pancreatic acinar cells is thought to play an important role in the development of acute pancreatitis[36,38].

Mild pancreatitis mainly involves the pancreas and local surrounding lesions. It is generally reversible, and about 6 mo after clinical remission, the pancreas recovers its normal morphology and function. In severe pancreatitis, vasoactive substances such as histamine and bradykinin are produced in large amounts with trypsin activation. As this vasoactive process increases, third spacing of fluids and shock due to hypovolemia may occur. In addition, leakage of activated enzymes from the pancreas causes secondary cytokine production. These cytokines trigger the systemic inflammatory response syndrome (SIRS)[39,40]. SIRS results in hyperactivation of macrophages and neutrophils throughout the body and the release of tissue injury mediators; multiorgan failure, including shock, circulatory failure, and acute respiratory distress syndrome (ARDS), may occur[41-43].

Meanwhile, as a biological defense response, anti-inflammatory cytokines and cytokine antagonists are induced to prevent prolongation of SIRS. This predominance of cytokine antagonists is called compensatory anti-inflammatory response syndrome (CARS)[44]. Because CARS inhibits new cytokine production, susceptibility to infection is increased, and infection of vital organs can occur. As a result of infection, endotoxins in the blood stimulate neutrophil aggregation in distal organs, tissue injury mediators are released, and distal organ failure occurs (Figure 2).

CLINICAL DIAGNOSIS AND ASSESSMENT OF SEVERITY

The diagnosis of acute pancreatitis is in principle based on clinical findings, biochemical tests, and imaging studies. Both a differential diagnosis and assessment of severity are necessary. The etiology of acute pancreatitis in children often differs from that in adults, and differences in the clinical manifestations and course may occur. Therefore, the diagnosis should be made keeping in mind specific features of the disease in children and after obtaining a past medical and family medical history (Figure 3).

Clinical manifestations

More than 90% of adults with acute pancreatitis report

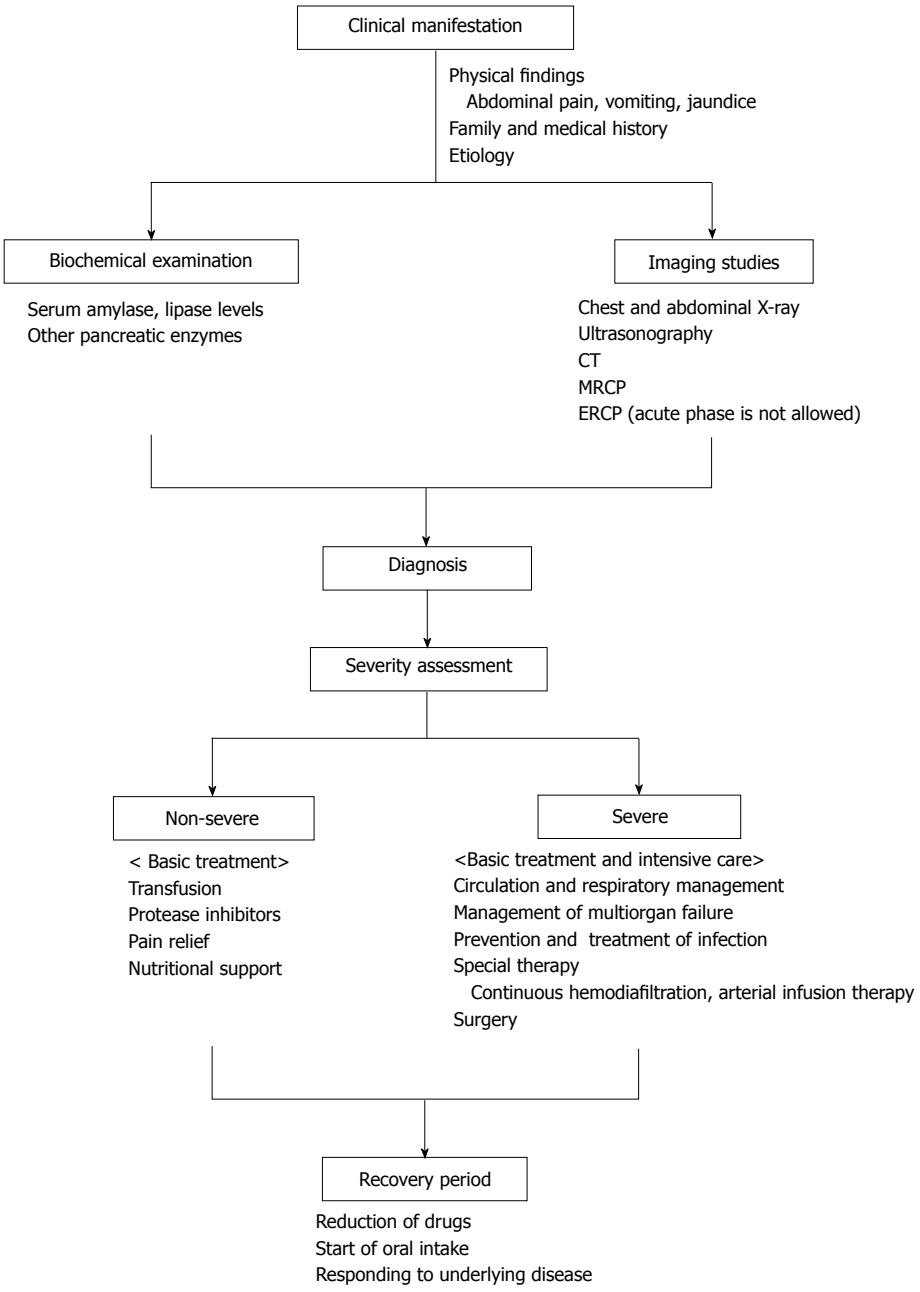

Figure 3 Clinical diagnosis of acute pancreatitis. CT: Computed tomography; ERCP: Endoscopic retrograde cholangiopancreatography; MRCP: Magnetic resonance cholangiopancreatography.

abdominal pain[45,46]. Abdominal pain is also an important early symptom in children. Weizman *et al*[47] reported that all 61 of their pediatric patients with acute pancreatitis initially had abdominal pain. Ziegler *et al*[48] also reported abdominal pain in 40 of 49 patients (82%). Table 3 shows the initial symptoms by age in our series of 135 children with acute pancreatitis[19]. In older children, the frequency of abdominal pain as a first symptom was similar to that in adults, whereas in younger children, vomiting was an important clinical symptom[49]. However, very young children and those with mild pancreatitis sometimes have non-specific abdominal pain. The location, characteristics, and triggers of abdominal pain, as well as physical examination of the abdomen, are important clues in the diagnosis of acute pancreatitis.

Other symptoms may include jaundice, fever, diarrhea, back pain, irritability, and lethargy. Jaundice and clay-colored stools suggest an abnormality of the biliary system such as a choledochal cyst, and there may be a palpable abdominal mass[8]. Infants and toddlers cannot verbalize abdominal pain, but vomiting, irritability, and lethargy are common[48]. In severe acute pancreatitis, children may initially present with shock, followed by symptoms of multiorgan failure, including dyspnea, oliguria, hemorrhage, and mental status changes[1].

Laboratory investigations

The prompt measurement of serum amylase is useful for

Table 3 First symptoms and chief complaints by age *n* (%)

	Age, yr			
	1-5 (*n* = 53)	6-10 (*n* = 47)	11-17 (*n* = 35)	Total (*n* = 135)
Abdominal pain	46 (86.8)	39 (83.0)	32 (91.4)	116 (85.9)
Fever	21 (39.6)	21 (44.7)	10 (28.6)	52 (38.5)
Vomiting	29 (54.7)	16 (34)	6 (17.1)	51 (37.8)
Jaundice	9 (17)	2 (4.3)	0	11 (8.1)
Back pain	0	1 (2.1)	5 (14.3)	6 (4.4)
Pale stool	3 (5.7)	1 (2.1)	0	4 (3)
Diarrhea	0	1 (2.1)	2 (5.7)	3 (2.2)
Loss of consciousness	1 (1.9)	1 (2.1)	1 (2.0)	3 (2.2)
Others	5 (9.5)	2 (4.2)	2 (5.8)	9 (6.6)

a diagnosis of acute pancreatitis[50]. However, elevated levels are also seen in gastrointestinal diseases such as pancreatobiliary tract obstruction and perforative peritonitis, as well as in salivary gland disease and renal failure. Therefore, low disease specificity is a problem. Serum lipase has a sensitivity of 86.5%-100% and specificity of 84.7%-99.0% for diagnosing acute pancreatitis[51]. Thus, its sensitivity is higher compared to serum amylase. In severe pancreatitis, serum lipase levels 7 times higher than normal have been reported within 24 h after onset of pancreatitis[52]. The degree of elevation and serial changes, however, generally do not correlate with disease severity[53]. In acute pancreatitis due to ASNase or valproic acid, which is fairly common in children, serum amylase may not be elevated[13]. Therefore, other serum pancreatic enzymes should also be measured.

Imaging

When acute pancreatitis is suspected, plain chest and abdominal X-rays are essential. A plain chest X-ray may show a pleural effusion, ARDS, or pneumonia. Although these findings are not specific for acute pancreatitis, they are important for the assessment of disease severity. A plain abdominal X-ray may show an ileus, colon cut-off sign, sentinel loop sign, calcified gallstones, pancreatic stones, or retroperitoneal gas. This information is important in assessing the clinical course of acute pancreatitis and is necessary for a differential diagnosis to rule out other diseases such as gastrointestinal perforation[54,55].

Ultrasonography is a convenient and non-invasive test. It is the test of first choice for screening to diagnose acute pancreatitis in children and for following the clinical course. The ultrasound diagnosis of acute pancreatitis is based on pancreatic morphology, appearance of the pancreatic parenchyma and pancreatic duct, and extrapancreatic findings[56,57].

CT scanning together with ultrasonography is essential for diagnosing acute pancreatitis. CT is useful to evaluate any extrapancreatic lesions, monitor the clinical course, and assess severity. In particular, CT is superior for early assessment of acute pancreatitis when ultrasound findings are nonspecific because of abdominal gas[56,58].

Pancreatitis in children is often caused by pancreatobiliary tract anomalies such as a choledochal cyst or abnormal union of the pancreatobiliary junction. Therefore, ERCP should be performed in pancreatitis of unknown cause. MRCP imaging has also improved and is useful in searching for a cause of acute pancreatitis in children[59]. In particular, MRCP should be performed before ERCP to detect any pancreatobiliary tract disease in children with initial onset of acute pancreatitis of unknown cause. However, in younger children, abnormal union of the pancreatobiliary junction is often difficult to delineate[21].

Severity assessment

Rapid and accurate assessment of severity is useful for selecting appropriate initial treatment and predicting the prognosis. In 2002, DeBanto *et al*[1] were the first to suggest a scoring system for predicting the severity of acute pancreatitis in children. This system is modified from the Ranson and Glasgow systems and consists of the following eight parameters: age (< 7 years old), weight (< 23 kg), white blood cell count at admission (> 18500 cells/μL), lactic dehydrogenase at admission (> 2000 U/L), 48-h trough Ca^{2+} (< 8.3 mg/dL), 48-h trough albumin (< 2.6 g/dL), 48-h fluid sequestration (> 75 mL/kg per 48 h), and 48-h rise in blood urea nitrogen (> 5 mg/dL). They set the cutoff for predicting a severe outcome at three criteria. However, this scoring system is not exact for Asian children[18]. Lautz *et al*[2] also reported that DeBanto pediatric scores have limited ability to predict acute pancreatitis severity in children and adolescents in the United States. Recently, we reported the usefulness of a new severity assessment that modified the acute pancreatitis severity scoring system of the Ministry of Health, Labour and Welfare of Japan (JPN score) for use in children[60,61]. The parameters of the pediatric JPN score were as follows: (1) base excess ≤ -3 mEq or shock (systolic blood pressure cutoffs according to age group); (2) PaO_2 ≤ 60 mmHg (room air) or respiratory failure; (3) blood urea nitrogen ≥ 40 mg/dL [or creatinine (Cr) ≥ 2.0 mg/dL] or oliguria (< 0.5 mL/kg per h); (4) lactate dehydrogenase ≥ 2 × the value of the upper limits; (5) platelet count ≤ $1 \times 10^5/mm^3$; (6) calcium ≤ 7.5 mg/dL; (7) C-reactive protein ≥ 15 mg/dL; (8) number of positive measures in pediatric SIRS score ≥ 3; and (9) age < 7 years old or/and weight < 23 kg. The cutoff for predicting a severe outcome was set at three criteria.

The CT severity index has proven to be very useful in adults[62]. Recently, Lautz *et al*[58] also reported that the CT severity index was superior to a clinical scoring system for identifying children with acute pancreatitis at heightened risk for developing serious complications.

TREATMENT

The initial treatment for acute pancreatitis is to withhold oral intake of food or fluid to allow the pancreas to rest (*i.e.*, prevent stimulation of pancreatic exocrine secretions). Fluid and electrolyte supplementation, enzyme inhibition therapy, and treatment to relieve pain and

prevent infection are provided. It is important to gradually permit liquid and food intake at a suitable time while continuing treatment. This treatment strategy is based on a consensus conference and evidence accumulated in adult patients. The basic pathogenesis of acute pancreatitis does not greatly differ between adults and children, and the treatment selected for children should be similar to that in adults.

Infusion of extracellular fluid

Because fluid leaks into the surrounding tissue due to inflammation associated with acute pancreatitis, adequate infusion to supplement extracellular fluid is needed during initial treatment. In severe cases, increased vascular permeability and decreased colloid osmotic pressure causes extravasation of extracellular fluids into the surrounding tissue and retroperitoneum and then into the peritoneal cavity and pleural cavity, thus leading to large losses in circulating plasma volume[63]. This acute circulatory impairment causes a rapidly deteriorating condition in early acute pancreatitis.

DRUG THERAPY

Analgesics

Pain in acute pancreatitis is often intense and persistent, and pain control is required. Appropriate use of analgesics can effectively reduce pain, but this should not interfere with making a diagnosis or providing other treatments[64-66]. The analgesics used include pentazocine, metamizole, and morphine.

Antibiotics

In mild cases of acute pancreatitis, the incidence of infectious complications and mortality rates are low, and prophylactic antibiotics are usually not necessary. However, even in mild cases, antibiotics should be considered if severity increases or complications like cholangitis develop. In severe cases, antibiotics can reduce infectious pancreatitis complications and improve the prognosis[67]. Drugs should be selected with good tissue distribution to the pancreas.

Pancreatic protease inhibitors and octreotide

The Santorini Consensus Conference in 1997 concluded that gabexate mesilate did not contribute to reduced mortality rates in acute pancreatitis[68]. However, in severe acute pancreatitis, continuous infusion of large doses of gabexate mesilate may decrease complications and mortality rates[69]. Similar efficacy in children has been reported, but no clear evidence exists[70]. Protease inhibitors may be a part of combined modality therapy (especially to improve hemodynamic status), but judicious administration is advised in severe cases.

Octreotide was introduced in the early 1980s and offers several advantages over somatostatin, such as a much longer half-life and the option for either subcutaneous or intravenous administration[71]. Octreotide is a powerful inhibitor of exocrine pancreatic secretion and cholecystokinin production[72]. Several studies have evaluated the effect of octreotide on the incidence of clinical pancreatitis after ERCP and postoperative complications such as pancreatic duct fistula following pancreaticoduodenectomy and pancreatic transplantation[73,74]. Effectiveness in reducing complications in acute pancreatitis has not been demonstrated[75]. However, at the case report level, octreotide has been effective in treating pancreatic pseudocysts as a complication in acute pancreatitis and in preventing and treating drug-related pancreatitis due to ASNase, a key drug used to treat lymphocytic leukemia in children[76-78]. As a somatostatin derivative, the most common adverse effect of octreotide is abdominal distention, but adverse effects such as failure to thrive are unlikely if octreotide is given for only 2-6 wk.

NUTRITIONAL SUPPORT

In severe pancreatitis, the early initiation of enteral nutrition reduces the incidence of infections and leads to shorter hospital stays[79]. An enteral feeding tube is placed in the duodenum or in the jejunum past the ligament of Treitz[80]. This type of nutrition is recommended to reduce stimulation of exocrine pancreatic secretion.

Control of abdominal pain and serum pancreatic enzyme levels should be considered in deciding when to resume oral intake. If serum pancreatic enzymes are decreasing, overall status is good, and abdominal pain has subsided, liquid intake can be started. If serum amylase and lipase levels are approximately less than two times the upper normal limits, a fat-restricted diet should be started[81]. Energy and fat intake can gradually be increased with careful monitoring.

Specific treatment for severe pancreatitis

In patients with infected pancreatic necrosis, surgical drainage and pancreatectomy may be indicated. Specific treatments such as continuous hemodiafiltration to remove humoral mediators and continuous regional arterial infusion of a protease inhibitor and antibiotics have been effective in adults[82,83]. These specific treatments have also been effective and lifesaving in children[84,85]. Although there is no universally acceptable scoring system for predicting the severity of childhood acute pancreatitis, consideration should be given to early transfer of severe patients to a medical center where intensive treatment is available.

Endoscopic treatment and surgery

Anatomic anomalies such as abnormal union of the pancreatobiliary junction are an indication for surgery. In patients with outflow tract obstruction of pancreatic juices caused by ampulla of Vater anomalies or pancreatic divisum, endoscopic sphincterotomy is effective.

Infectious complications should be clinically suspected if fever or signs of inflammation recur during the course of acute pancreatitis. Symptoms often become

prominent 2 wk or more after the onset of pancreatitis. The definitive diagnosis of infected pancreatic necrosis can be made by CT- or ultrasound-guided local fine-needle aspiration and bacteriologic cultures[86,87]. However, this procedure may be difficult in children. Therefore, worsening blood test results, positive blood cultures, positive blood endotoxins, elevated serum procalcitonin levels, and CT findings of the pancreas may serve as clues to a diagnosis of infected pancreatic necrosis[88].

Patients whose general condition is stable can be conservatively treated with antibiotics and observed, but if their condition does not improve, a necrosectomy is required. Necrosectomy early in pancreatitis is associated with a high mortality rate, so it should ideally be performed after the patient's hemodynamic status and general condition have stabilized[89]. Percutaneous necrosectomy, endoscopic transgastric necrosectomy and laparoscopic pancreatic necrosectomy have recently been reported as less invasive treatments in adults and a few children[90-92]. Pancreatic abscesses generally require percutaneous, endoscopic, or surgical drainage.

Pancreatic pseudocysts are cysts that develop due to injury of the pancreatic duct and extravasation of fluid. These occur 4 wk or later after the onset of pancreatitis. Treatment is indicated for pseudocysts if their size does not decrease, if they are accompanied by abdominal pain, or if there are complications of infection or hemorrhage. Endoscopic ultrasound-guided transgastric puncture and drainage can safely be performed in these cases[93,94].

CONCLUSION

Currently, our approach to acute pancreatitis in children mainly depends on physician experience and knowledge gained from acute pancreatitis in adults. Acute pancreatitis in children tends to be considered a difficult disease, even by pediatric gastroenterologists. However, with recent advances in diagnostic techniques and treatment methods, unfamiliar and difficult diseases are becoming controllable diseases once they are better understood. In order to improve treatment outcomes in patients with childhood acute pancreatitis, future studies focusing on developing a scoring system for predicting the severity of acute pancreatitis and identifying the potential effective treatment modalities for children should be conducted.

REFERENCES

1 **DeBanto JR**, Goday PS, Pedroso MR, Iftikhar R, Fazel A, Nayyar S, Conwell DL, Demeo MT, Burton FR, Whitcomb DC, Ulrich CD, Gates LK. Acute pancreatitis in children. *Am J Gastroenterol* 2002; **97**: 1726-1731 [PMID: 12135026]

2 **Lautz TB**, Chin AC, Radhakrishnan J. Acute pancreatitis in children: spectrum of disease and predictors of severity. *J Pediatr Surg* 2011; **46**: 1144-1149 [PMID: 21683213 DOI: 10.1016/j.jpedsurg.2011.03.044]

3 **Banks PA**, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG, Tsiotos GG, Vege SS. Classification of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus. *Gut* 2013; **62**: 102-111 [PMID: 23100216 DOI: 10.1136/gutjnl-2012-302779]

4 **Nydegger A**, Couper RT, Oliver MR. Childhood pancreatitis. *J Gastroenterol Hepatol* 2006; **21**: 499-509 [PMID: 16638090 DOI: 10.1111/j.1440-1746.2006.04246.x]

5 **Banks PA**. Epidemiology, natural history, and predictors of disease outcome in acute and chronic pancreatitis. *Gastrointest Endosc* 2002; **56**: S226-S230 [PMID: 12447272 DOI: 10.1067/mge.2002.129022]

6 **Yadav D**, Lowenfels AB. Trends in the epidemiology of the first attack of acute pancreatitis: a systematic review. *Pancreas* 2006; **33**: 323-330 [PMID: 17079934 DOI: 10.1097/01.mpa.0000236733.31617.52]

7 **Benifla M**, Weizman Z. Acute pancreatitis in childhood: analysis of literature data. *J Clin Gastroenterol* 2003; **37**: 169-172 [PMID: 12869890]

8 **Werlin SL**, Kugathasan S, Frautschy BC. Pancreatitis in children. *J Pediatr Gastroenterol Nutr* 2003; **37**: 591-595 [PMID: 14581803]

9 **Tomomasa T**, Tabata M, Miyashita M, Itoh K, Kuroume T. Acute pancreatitis in Japanese and Western children: etiologic comparisons. *J Pediatr Gastroenterol Nutr* 1994; **19**: 109-110 [PMID: 7965459]

10 **Müller HJ**, Boos J. Use of L-asparaginase in childhood ALL. *Crit Rev Oncol Hematol* 1998; **28**: 97-113 [PMID: 9768345 DOI: 10.1002/cncr.23716]

11 **Flores-Calderón J**, Exiga-Gonzaléz E, Morán-Villota S, Martín-Trejo J, Yamamoto-Nagano A. Acute pancreatitis in children with acute lymphoblastic leukemia treated with L-asparaginase. *J Pediatr Hematol Oncol* 2009; **31**: 790-793 [PMID: 19770681 DOI: 10.1097/MPH.0b013e3181b794e8]

12 **Raja RA**, Schmiegelow K, Frandsen TL. Asparaginase-associated pancreatitis in children. *Br J Haematol* 2012; **159**: 18-27 [PMID: 22909259 DOI: 10.1111/bjh.12016]

13 **Shimizu T**, Yamashiro Y, Igarashi J, Fujita H, Ishimoto K. Increased serum trypsin and elastase-1 levels in patients undergoing L-asparaginase therapy. *Eur J Pediatr* 1998; **157**: 561-563 [PMID: 9686816]

14 **Minowa K**, Suzuki M, Fujimura J, Saito M, Koh K, Kikuchi A, Hanada R, Shimizu T. L-asparaginase-induced pancreatic injury is associated with an imbalance in plasma amino acid levels. *Drugs R D* 2012; **12**: 49-55 [PMID: 22594522 DOI: 10.2165/11632990-000000000-00000]

15 **Trivedi CD**, Pitchumoni CS. Drug-induced pancreatitis: an update. *J Clin Gastroenterol* 2005; **39**: 709-716 [PMID: 16082282]

16 **Hviid A**, Rubin S, Mühlemann K. Mumps. *Lancet* 2008; **371**: 932-944 [PMID: 18342688 DOI: 10.1016/S0140-6736(08)60419-5]

17 **Parenti DM**, Steinberg W, Kang P. Infectious causes of acute pancreatitis. *Pancreas* 1996; **13**: 356-371 [PMID: 8899796]

18 **Mårdh PA**, Ursing B. The occurrence of acute pancreatitis in Mycoplasma pneumoniae infection. *Scand J Infect Dis* 1974; **6**: 167-171 [PMID: 4605307]

19 **Suzuki M**, Fujii T, Takahiro K, Ohtsuka Y, Nagata S, Shimizu T. Scoring system for the severity of acute pancreatitis in children. *Pancreas* 2008; **37**: 222-223 [PMID: 18665087 DOI: 10.1097/MPA.0b013e31816618e1]

20 **Suzuki R**, Shimizu T, Suzuki M, Yamashiro Y. Detection of abnormal union of pancreaticobiliary junction by magnetic resonance cholangiopancreatography in a girl with acute pancreatitis. *Pediatr Int* 2002; **44**: 183-185 [PMID: 11896881]

21 **Suzuki M**, Shimizu T, Kudo T, Suzuki R, Ohtsuka Y, Yamashiro Y, Shimotakahara A, Yamataka A. Usefulness of nonbreath-hold 1-shot magnetic resonance cholangiopancreatography for the evaluation of choledochal cyst in children. *J Pediatr Gastroenterol Nutr* 2006; **42**: 539-544 [PMID: 16707978 DOI: 10.1097/01.mpg.0000221894.44124.8e]

22 **Todani T**, Watanabe Y, Narusue M, Tabuchi K, Okajima K. Congenital bile duct cysts: Classification, operative procedures, and review of thirty-seven cases including cancer arising from choledochal cyst. *Am J Surg* 1977; **134**: 263-269 [PMID: 889044]

23 **Matsumoto Y**, Fujii H, Itakura J, Matsuda M, Nobukawa B,

Suda K. Recent advances in pancreaticobiliary maljunction. *J Hepatobiliary Pancreat Surg* 2002; **9**: 45-54 [PMID: 12021897 DOI: 10.1007/s005340200004]
24 **Whitcomb DC**, Gorry MC, Preston RA, Furey W, Sossenheimer MJ, Ulrich CD, Martin SP, Gates LK, Amann ST, Toskes PP, Liddle R, McGrath K, Uomo G, Post JC, Ehrlich GD. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. *Nat Genet* 1996; **14**: 141-145 [PMID: 8841182 DOI: 10.1038/ng1096-141]
25 **Witt H**, Luck W, Hennies HC, Classen M, Kage A, Lass U, Landt O, Becker M. Mutations in the gene encoding the serine protease inhibitor, Kazal type 1 are associated with chronic pancreatitis. *Nat Genet* 2000; **25**: 213-216 [PMID: 10835640]
26 **Masamune A**, Mizutamari H, Kume K, Asakura T, Satoh K, Shimosegawa T. Hereditary pancreatitis as the premalignant disease: a Japanese case of pancreatic cancer involving the SPINK1 gene mutation N34S. *Pancreas* 2004; **28**: 305-310 [PMID: 15084977]
27 **Teich N**, Schulz HU, Witt H, Böhmig M, Keim V. N34S, a pancreatitis associated SPINK1 mutation, is not associated with sporadic pancreatic cancer. *Pancreatology* 2003; **3**: 67-68 [PMID: 12649567 DOI: 10.1159/000069145]
28 **Whitcomb DC**, Applebaum S, Martin SP. Hereditary pancreatitis and pancreatic carcinoma. *Ann N Y Acad Sci* 1999; **880**: 201-209 [PMID: 10415865]
29 **Apple SK**, Hecht JR, Lewin DN, Jahromi SA, Grody WW, Nieberg RK. Immunohistochemical evaluation of K-ras, p53, and HER-2/neu expression in hyperplastic, dysplastic, and carcinomatous lesions of the pancreas: evidence for multistep carcinogenesis. *Hum Pathol* 1999; **30**: 123-129 [PMID: 10029438]
30 **Witt H**, Beer S, Rosendahl J, Chen JM, Chandak GR, Masamune A, Bence M, Szmola R, Oracz G, Macek M, Bhatia E, Steigenberger S, Lasher D, Bühler F, Delaporte C, Tebbing J, Ludwig M, Pilsak C, Saum K, Bugert P, Masson E, Paliwal S, Bhaskar S, Sobczynska-Tomaszewska A, Bak D, Balascak I, Choudhuri G, Nageshwar Reddy D, Rao GV, Thomas V, Kume K, Nakano E, Kakuta Y, Shimosegawa T, Durko L, Szabó A, Schnúr A, Hegyi P, Rakonczay Z, Pfützer R, Schneider A, Groneberg DA, Braun M, Schmidt H, Witt U, Friess H, Algül H, Landt O, Schuelke M, Krüger R, Wiedenmann B, Schmidt F, Zimmer KP, Kovacs P, Stumvoll M, Blüher M, Müller T, Janecke A, Teich N, Grützmann R, Schulz HU, Mössner J, Keim V, Löhr M, Férec C, Sahin-Tóth M. Variants in CPA1 are strongly associated with early onset chronic pancreatitis. *Nat Genet* 2013; **45**: 1216-1220 [PMID: 23955596 DOI: 10.1038/ng.2730]
31 **Amodio J**, Brodsky JE. Pediatric Burkitt lymphoma presenting as acute pancreatitis: MRI characteristics. *Pediatr Radiol* 2010; **40**: 770-772 [PMID: 20135116 DOI: 10.1007/s00247-009-1475-3]
32 **Suzuki M**, Shimizu T, Minowa K, Ikuse T, Baba Y, Ohtsuka Y. Spontaneous shrinkage of a solid pseudopapillary tumor of the pancreas: CT findings. *Pediatr Int* 2010; **52**: 335-336 [PMID: 20500490 DOI: 10.1111/j.1442-200X.2010.03039.x]
33 **Halangk W**, Lerch MM, Brandt-Nedelev B, Roth W, Ruthenbuerger M, Reinheckel T, Domschke W, Lippert H, Peters C, Deussing J. Role of cathepsin B in intracellular trypsinogen activation and the onset of acute pancreatitis. *J Clin Invest* 2000; **106**: 773-781 [PMID: 10995788 DOI: 10.1172/JCI9411]
34 **Steer ML**, Meldolesi J. The cell biology of experimental pancreatitis. *N Engl J Med* 1987; **316**: 144-150 [PMID: 3540666 DOI: 10.1056/NEJM198701153160306]
35 **Hedström J**, Kemppainen E, Andersén J, Jokela H, Puolakkainen P, Stenman UH. A comparison of serum trypsinogen-2 and trypsin-2-alpha1-antitrypsin complex with lipase and amylase in the diagnosis and assessment of severity in the early phase of acute pancreatitis. *Am J Gastroenterol* 2001; **96**: 424-430 [PMID: 11232685 DOI: 10.1111/j.1572-0241.2001.03457.x]
36 **Rinderknecht H**. Activation of pancreatic zymogens. Normal activation, premature intrapancreatic activation, protective mechanisms against inappropriate activation. *Dig Dis Sci* 1986; **31**: 314-321 [PMID: 2936587]
37 **Suzuki M**, Shimizu T, Kudo T, Shoji H, Ohtsuka Y, Yamashiro Y. Octreotide prevents L-asparaginase-induced pancreatic injury in rats. *Exp Hematol* 2008; **36**: 172-180 [PMID: 18023522 DOI: 10.1016/j.exphem.2007.09.005]
38 **Thrower EC**, Diaz de Villalvilla AP, Kolodecik TR, Gorelick FS. Zymogen activation in a reconstituted pancreatic acinar cell system. *Am J Physiol Gastrointest Liver Physiol* 2006; **290**: G894-G902 [PMID: 16339296 DOI: 10.1152/ajpgi.00373.2005]
39 **Ogawa M**. Acute pancreatitis and cytokines: "second attack" by septic complication leads to organ failure. *Pancreas* 1998; **16**: 312-315 [PMID: 9548672]
40 **Buter A**, Imrie CW, Carter CR, Evans S, McKay CJ. Dynamic nature of early organ dysfunction determines outcome in acute pancreatitis. *Br J Surg* 2002; **89**: 298-302 [PMID: 11872053 DOI: 10.1046/j.0007-1323.2001.02025.x]
41 **Beger HG**, Bittner R, Büchler M, Hess W, Schmitz JE. Hemodynamic data pattern in patients with acute pancreatitis. *Gastroenterology* 1986; **90**: 74-79 [PMID: 3940259]
42 **Bradley EL**, Hall JR, Lutz J, Hamner L, Lattouf O. Hemodynamic consequences of severe pancreatitis. *Ann Surg* 1983; **198**: 130-133 [PMID: 6870367]
43 **Mofidi R**, Duff MD, Wigmore SJ, Madhavan KK, Garden OJ, Parks RW. Association between early systemic inflammatory response, severity of multiorgan dysfunction and death in acute pancreatitis. *Br J Surg* 2006; **93**: 738-744 [PMID: 16671062 DOI: 10.1002/bjs.5290]
44 **Gunjaca I**, Zunic J, Gunjaca M, Kovac Z. Circulating cytokine levels in acute pancreatitis-model of SIRS/CARS can help in the clinical assessment of disease severity. *Inflammation* 2012; **35**: 758-763 [PMID: 21826480 DOI: 10.1007/s10753-011-9371-z]
45 **Malfertheiner P**, Kemmer TP. Clinical picture and diagnosis of acute pancreatitis. *Hepatogastroenterology* 1991; **38**: 97-100 [PMID: 1855780]
46 **Koizumi M**, Takada T, Kawarada Y, Hirata K, Mayumi T, Yoshida M, Sekimoto M, Hirota M, Kimura Y, Takeda K, Isaji S, Otsuki M, Matsuno S. JPN Guidelines for the management of acute pancreatitis: diagnostic criteria for acute pancreatitis. *J Hepatobiliary Pancreat Surg* 2006; **13**: 25-32 [PMID: 16463208 DOI: 10.1007/s00534-005-1048-2]
47 **Weizman Z**, Durie PR. Acute pancreatitis in childhood. *J Pediatr* 1988; **113**: 24-29 [PMID: 2455030]
48 **Ziegler DW**, Long JA, Philippart AI, Klein MD. Pancreatitis in childhood. Experience with 49 patients. *Ann Surg* 1988; **207**: 257-261 [PMID: 3345113]
49 **Lerner A**, Branski D, Lebenthal E. Pancreatic diseases in children. *Pediatr Clin North Am* 1996; **43**: 125-156 [PMID: 8596678]
50 **Lopez MJ**. The changing incidence of acute pancreatitis in children: a single-institution perspective. *J Pediatr* 2002; **140**: 622-624 [PMID: 12032533 DOI: 10.1067/mpd.2002.123880]
51 **Agarwal N**, Pitchumoni CS, Sivaprasad AV. Evaluating tests for acute pancreatitis. *Am J Gastroenterol* 1990; **85**: 356-366 [PMID: 2183590]
52 **Coffey MJ**, Nightingale S, Ooi CY. Serum lipase as an early predictor of severity in pediatric acute pancreatitis. *J Pediatr Gastroenterol Nutr* 2013; **56**: 602-608 [PMID: 23403441 DOI: 10.1097/MPG.0b013e31828b36d8]
53 **Banks PA**, Freeman ML. Practice guidelines in acute pancreatitis. *Am J Gastroenterol* 2006; **101**: 2379-2400 [PMID: 17032204 DOI: 10.1111/j.1572-0241.2006.00856.x]
54 **Stein GN**, Kalser MH, Sarian NN, Finkelstein A. An evaluation of the roentgen changes in acute pancreatitis: correlation with clinical findings. *Gastroenterology* 1959; **36**: 354-361 [PMID: 13640153]

55 **Pickhardt PJ**. The colon cutoff sign. *Radiology* 2000; **215**: 387-389 [PMID: 10796912 DOI: 10.1148/radiology.215.2.r00ma18387]

56 **Silverstein W**, Isikoff MB, Hill MC, Barkin J. Diagnostic imaging of acute pancreatitis: prospective study using CT and sonography. *AJR Am J Roentgenol* 1981; **137**: 497-502 [PMID: 7025598 DOI: 10.2214/ajr.137.3.497]

57 **Jeffrey RB**, Laing FC, Wing VW. Extrapancreatic spread of acute pancreatitis: new observations with real-time US. *Radiology* 1986; **159**: 707-711 [PMID: 3517954 DOI: 10.1148/radiology.159.3.3517954]

58 **Lautz TB**, Turkel G, Radhakrishnan J, Wyers M, Chin AC. Utility of the computed tomography severity index (Balthazar score) in children with acute pancreatitis. *J Pediatr Surg* 2012; **47**: 1185-1191 [PMID: 22703791 DOI: 10.1016/j.jpedsurg.2012.03.023]

59 **Shimizu T**, Suzuki R, Yamashiro Y, Segawa O, Yamataka A, Kuwatsuru R. Magnetic resonance cholangiopancreatography in assessing the cause of acute pancreatitis in children. *Pancreas* 2001; **22**: 196-199 [PMID: 11249076]

60 **Shimizu T**. Pancreatic disease in Children. *J Jpn Pediatr Soc* 2009; **113**: 1-11 (in Japanese)

61 **Takeda K**, Yokoe M, Takada T, Kataoka K, Yoshida M, Gabata T, Hirota M, Mayumi T, Kadoya M, Yamanouchi E, Hattori T, Sekimoto M, Amano H, Wada K, Kimura Y, Kiriyama S, Arata S, Takeyama Y, Hirota M, Hirata K, Shimosegawa T. Assessment of severity of acute pancreatitis according to new prognostic factors and CT grading. *J Hepatobiliary Pancreat Sci* 2010; **17**: 37-44 [PMID: 20012329 DOI: 10.1007/s00534-009-0213-4]

62 **Balthazar EJ**. Acute pancreatitis: assessment of severity with clinical and CT evaluation. *Radiology* 2002; **223**: 603-613 [PMID: 12034923 DOI: 10.1148/radiol.2233010680]

63 **Mao EQ**, Tang YQ, Fei J, Qin S, Wu J, Li L, Min D, Zhang SD. Fluid therapy for severe acute pancreatitis in acute response stage. *Chin Med J* (Engl) 2009; **122**: 169-173 [PMID: 19187641]

64 **Kahl S**, Zimmermann S, Pross M, Schulz HU, Schmidt U, Malfertheiner P. Procaine hydrochloride fails to relieve pain in patients with acute pancreatitis. *Digestion* 2004; **69**: 5-9 [PMID: 14755147 DOI: 10.1159/000076541]

65 **Peiró AM**, Martínez J, Martínez E, de Madaria E, Llorens P, Horga JF, Pérez-Mateo M. Efficacy and tolerance of metamizole versus morphine for acute pancreatitis pain. *Pancreatology* 2008; **8**: 25-29 [PMID: 18235213 DOI: 10.1159/000114852]

66 **Layer P**, Bronisch HJ, Henniges UM, Koop I, Kahl M, Dignass A, Ell C, Freitag M, Keller J. Effects of systemic administration of a local anesthetic on pain in acute pancreatitis: a randomized clinical trial. *Pancreas* 2011; **40**: 673-679 [PMID: 21562445 DOI: 10.1097/MPA.0b013e318215ad38]

67 **Manes G**, Uomo I, Menchise A, Rabitti PG, Ferrara EC, Uomo G. Timing of antibiotic prophylaxis in acute pancreatitis: a controlled randomized study with meropenem. *Am J Gastroenterol* 2006; **101**: 1348-1353 [PMID: 16771960]

68 **Dervenis C**, Johnson CD, Bassi C, Bradley E, Imrie CW, McMahon MJ, Modlin I. Diagnosis, objective assessment of severity, and management of acute pancreatitis. Santorini consensus conference. *Int J Pancreatol* 1999; **25**: 195-210 [PMID: 10453421]

69 **Yasuda T**, Ueda T, Takeyama Y, Shinzeki M, Sawa H, Nakajima T, Matsumoto I, Fujita T, Sakai T, Ajiki T, Fujino Y, Kuroda Y. Treatment strategy against infection: clinical outcome of continuous regional arterial infusion, enteral nutrition, and surgery in severe acute pancreatitis. *J Gastroenterol* 2007; **42**: 681-689 [PMID: 17701132 DOI: 10.1007/s00535-007-2081-5]

70 **Kim SC**, Yang HR. Clinical efficacy of gabexate mesilate for acute pancreatitis in children. *Eur J Pediatr* 2013; **172**: 1483-1490 [PMID: 23812506 DOI: 10.1007/s00431-013-2068-6]

71 **Pless J**, Bauer W, Briner U, Doepfner W, Marbach P, Maurer R, Petcher TJ, Reubi JC, Vonderscher J. Chemistry and pharmacology of SMS 201-995, a long-acting octapeptide analogue of somatostatin. *Scand J Gastroenterol Suppl* 1986; **119**: 54-64 [PMID: 2876507]

72 **Greenberg R**, Haddad R, Kashtan H, Kaplan O. The effects of somatostatin and octreotide on experimental and human acute pancreatitis. *J Lab Clin Med* 2000; **135**: 112-121 [PMID: 10695655 DOI: 10.1067/mlc.2000.104457]

73 **Li-Ling J**, Irving M. Somatostatin and octreotide in the prevention of postoperative pancreatic complications and the treatment of enterocutaneous pancreatic fistulas: a systematic review of randomized controlled trials. *Br J Surg* 2001; **88**: 190-199 [PMID: 11167865 DOI: 10.1046/j.1365-2168.2001.01659.x]

74 **Bonatti H**, Tabarelli W, Berger N, Wykypiel H, Jaschke W, Margreiter R, Mark W. Successful management of a proximal pancreatic duct fistula following pancreatic transplantation. *Dig Dis Sci* 2006; **51**: 2026-2030 [PMID: 17053956 DOI: 10.1007/s10620-006-9373-0]

75 **Xu W**, Zhou YF, Xia SH. Octreotide for primary moderate to severe acute pancreatitis: a meta-analysis. *Hepatogastroenterology* 2013; **60**: 1504-1508 [PMID: 24298575]

76 **Gullo L**, Barbara L. Treatment of pancreatic pseudocysts with octreotide. *Lancet* 1991; **338**: 540-541 [PMID: 1678802]

77 **Wu SF**, Chen AC, Peng CT, Wu KH. Octreotide therapy in asparaginase-associated pancreatitis in childhood acute lymphoblastic leukemia. *Pediatr Blood Cancer* 2008; **51**: 824-825 [PMID: 18726919 DOI: 10.1002/pbc.21721]

78 **Suzuki M**, Takata O, Sakaguchi S, Fujimura J, Saito M, Shimizu T. Retherapy using L-asparaginase with octreotide in a patient recovering from L-asparaginase-induced pancreatitis. *Exp Hematol* 2008; **36**: 253-254 [PMID: 18279714 DOI: 10.1016/j.exphem.2007.11.010]

79 **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]

80 **Gupta R**, Patel K, Calder PC, Yaqoob P, Primrose JN, Johnson CD. A randomised clinical trial to assess the effect of total enteral and total parenteral nutritional support on metabolic, inflammatory and oxidative markers in patients with predicted severe acute pancreatitis (APACHE II & gt; or =6). *Pancreatology* 2003; **3**: 406-413 [PMID: 14526151]

81 **Petrov MS**, van Santvoort HC, Besselink MG, Cirkel GA, Brink MA, Gooszen HG. Oral refeeding after onset of acute pancreatitis: a review of literature. *Am J Gastroenterol* 2007; **102**: 2079-2084; quiz 2085 [PMID: 17573797 DOI: 10.1111/j.1572-0241.2007.01357.x]

82 **Wada K**, Takada T, Hirata K, Mayumi T, Yoshida M, Yokoe M, Kiriyama S, Hirota M, Kimura Y, Takeda K, Arata S, Hirota M, Sekimoto M, Isaji S, Takeyama Y, Gabata T, Kitamura N, Amano H. Treatment strategy for acute pancreatitis. *J Hepatobiliary Pancreat Sci* 2010; **17**: 79-86 [PMID: 20012325 DOI: 10.1007/s00534-009-0218-z]

83 **Piaścik M**, Rydzewska G, Milewski J, Olszewski S, Furmanek M, Walecki J, Gabryelewicz A. The results of severe acute pancreatitis treatment with continuous regional arterial infusion of protease inhibitor and antibiotic: a randomized controlled study. *Pancreas* 2010; **39**: 863-867 [PMID: 20431422 DOI: 10.1097/MPA.0b013e3181d37239]

84 **Morimoto A**, Imamura T, Ishii R, Nakabayashi Y, Nakatani T, Sakagami J, Yamagami T. Successful management of severe L-asparaginase-associated pancreatitis by continuous regional arterial infusion of protease inhibitor and antibiotic. *Cancer* 2008; **113**: 1362-1369 [PMID: 18661511]

85 **Fukushima H**, Fukushima T, Suzuki R, Enokizono T, Matsunaga M, Nakao T, Koike K, Mori K, Matsueda K, Sumazaki R. Continuous regional arterial infusion effective for children with acute necrotizing pancreatitis even under neutropenia. *Pediatr Int* 2013; **55**: e11-e13 [PMID: 23679174 DOI: 10.1111/j.1442-200X.2012.03702.x]

86 **Banks PA**, Gerzof SG, Langevin RE, Silverman SG, Sica GT, Hughes MD. CT-guided aspiration of suspected pancreatic infection: bacteriology and clinical outcome. *Int J Pancreatol* 1995; **18**: 265-270 [PMID: 8708399 DOI: 10.1007/BF02784951]

87 **Rau B**, Pralle U, Mayer JM, Beger HG. Role of ultrasonographically guided fine-needle aspiration cytology in the diagnosis of infected pancreatic necrosis. *Br J Surg* 1998; **85**: 179-184 [PMID: 9501810 DOI: 10.1046/j.1365-2168.1998.00707.x]

88 **Raizner A**, Phatak UP, Baker K, Patel MG, Husain SZ, Pashankar DS. Acute necrotizing pancreatitis in children. *J Pediatr* 2013; **162**: 788-792 [PMID: 23102790 DOI: 10.1016/j.jpeds.2012.09.037]

89 **Wittau M**, Scheele J, Gölz I, Henne-Bruns D, Isenmann R. Changing role of surgery in necrotizing pancreatitis: a single-center experience. *Hepatogastroenterology* 2010; **57**: 1300-1304 [PMID: 21410076]

90 **Pattillo JC**, Funke R. Laparoscopic pancreatic necrosectomy in a child with severe acute pancreatitis. *J Laparoendosc Adv Surg Tech A* 2012; **22**: 123-126 [PMID: 22044514]

91 **Gómez Beltrán O**, Roldán Molleja L, Garrido Pérez JI, Medina Martínez M, Granero Cendón R, González de Caldas Marchal R, Rodríguez Salas M, Gilbert Pérez J, Paredes Esteban RM. [Acute pancreatitis in children]. *Cir Pediatr* 2013; **26**: 21-24 [PMID: 23833923]

92 **Gardner TB**, Coelho-Prabhu N, Gordon SR, Gelrud A, Maple JT, Papachristou GI, Freeman ML, Topazian MD, Attam R, Mackenzie TA, Baron TH. Direct endoscopic necrosectomy for the treatment of walled-off pancreatic necrosis: results from a multicenter U.S. series. *Gastrointest Endosc* 2011; **73**: 718-726 [PMID: 21237454 DOI: 10.1016/j.gie.2010.10.053]

93 **Jazrawi SF**, Barth BA, Sreenarasimhaiah J. Efficacy of endoscopic ultrasound-guided drainage of pancreatic pseudocysts in a pediatric population. *Dig Dis Sci* 2011; **56**: 902-908 [PMID: 20676768 DOI: 10.1007/s10620-010-1350-y]

94 **Patty I**, Kalaoui M, Al-Shamali M, Al-Hassan F, Al-Naqeeb B. Endoscopic drainage for pancreatic pseudocyst in children. *J Pediatr Surg* 2001; **36**: 503-505 [PMID: 11227007 DOI: 10.1053/jpsu.2001.21620]

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TOPIC HIGHLIGHT

WJGP 5th Anniversary Special Issues (3): Pancreatitis

Genetics of acute and chronic pancreatitis: An update

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Abstract

Progress made in identifying the genetic susceptibility underlying acute and chronic pancreatitis has benefitted the clinicians in understanding the pathogenesis of the disease in a better way. The identification of mutations in cationic trypsinogen gene (*PRSS1* gene; functional gain mutations) and serine protease inhibitor kazal type 1 (*SPINK1* gene; functional loss mutations) and other potential susceptibility factors in genes that play an important role in the pancreatic secretory functions or response to inflammation during pancreatic injury has changed the current concepts and understanding of a complex multifactorial disease like pancreatitis. An individual's susceptibility to the disease is governed by genetic factors in combination with environmental factors. Candidate gene and genetic linkage studies have identified polymorphisms in cationic trypsinogen (*PRSS1*), *SPINK1*, cystic fibrosis trans-membrane conductance regulator (*CFTR*), Chymotrypsinogen C (*CTRC*), Cathepsin B (*CTSB*) and calcium sensing receptor (*CASR*). Individuals with polymorphisms in the mentioned genes and other as yet identified genes are at an enhanced risk for the disease. Recently, polymorphisms in genes other than those involved in "intra-pancreatic trypsin regulatory mechanism" namely Claudin-2 (*CLDN2*) and Carboxypeptidase A1 (*CPA1*) gene have also been identified for their association with pancreatitis. With ever growing number of studies trying to identify the genetic susceptibility in the form of single nucleotide polymorphisms, this review is an attempt to compile the available information on the topic.

Key words: Chronic pancreatitis; Acute pancreatitis; Genetic susceptibility; Single nucleotide polymorphisms; Inflammation

Core tip: Pancreatitis is a progressive inflammatory disease. Though the pancreas has adequate protection against environmental and metabolic stress, if the magnitude of this stress exceeds the threshold which the organ can handle, it leads to pathologic effects. Although genetic variables have been identified that affect the function of pancreas, namely polymorphisms in serine protease inhibitor kazal type 1 (*SPINK1*), polymorphisms in cationic trypsinogen (*PRSS1*) and Chymotrypsinogen C (*CTRC*) genes in the acinar cells and cystic fibrosis trans-membrane conductance regulator (*CFTR*), calcium sensing receptor (*CASR* genes in the ductal cells leading to pancreatitis, off late many genetic factors outside of the "intra-pancreatic trypsin regulatory mechanism" have been identified for their role in pancreatitis. This review is an update on the genetic aspects of acute and chronic pancreatitis.

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INTRODUCTION

Chronic pancreatitis (CP) is a disease associated with

inflammation where the secretory parenchyma of the pancreas is progressively destroyed. There is involvement of several known risk factors and processes such as inflammation, necrosis, apoptosis or duct obstruction despite the heterogeneity in pathogenesis. The process of fibrosis usually leads to progressive worsening in lobular morphology, structure of pancreas, changes in arrangement and composition of the islets and deformation of the large ducts[1]. These conditions lead to diabetes that is due to irreversible morphological and structural changes and exocrine and endocrine dysfunction[2]. The major types of pancreatitis are acute pancreatitis (AP), recurrent acute pancreatitis (RAP) and CP.

In spite of an individual carrying a genetic risk and being subjected to oxidative or metabolic stress, the pancreas is histologically normal in appearance in the pre-acute phase. "First hit" in terms of injury due to excess alcohol consumption, metabolic factors, hyperlipidemia, gallstones and genetic factors leads to AP-which is a sentinel AP event (SAPE)[3]. During this proinflammatory phase, inflammatory related damage occurs due to the infiltration of the pancreas with inflammatory cells. This phase may end through an anti-inflammatory response that is mediated partly by tissue macrophages and is associated with the activation of stellate cells and subsequent proliferation causing fibrosis. However clinical recovery is attained in most of the cases.

If this phase is followed by RAP due to genetic risks namely polymorphisms in serine protease inhibitor kazal type 1 *(SPINK1),* polymorphisms in cationic trypsinogen (*PRSS1*), cystic fibrosis trans-membrane conductance regulator (*CFTR*) genes and other as yet unknown genes) or chronic cell stressors develop like alcohol, smoking, oxidative stress, *etc.*, after the SAPE (second hit), it leads to CP which is due to chronic inflammation and progressive fibrosis. CP may also manifest as a direct result of extensive pancreatic necrosis, duct obstruction in the proximal region directly resulting from severe AP which is independent and without the second hit[4].

Many risk factors that contribute varyingly to pancreatitis have been identified. These include alcohol, metabolic factors, toxins, insecticides, certain medications, viral and bacterial infections, trauma caused by surgery[5]. Growing evidence suggests a substantial contribution of genetic predisposition to pancreatitis. As early as 1950's, genetic studies on pancreatitis suggested that it may be an inherited disease[6]. After this initial description, a mutation inherited in autosomal dominant mode was identified in the cationic trypsinogen gene that is located on 7th chromosome in individuals with hereditary pancreatitis[7,8]. Further to this, a number of other mutations/polymorphisms in genes that have a role in inhibition, regulation or modulation of the pancreatic trypsin activity, secretory function and inflammatory injury respectively were identified. Mutations in the *PRSS1*, *SPINK1*, *CFTR* and polymorphisms in other genes namely the ones regulating the response to inflammation [tumor necrosis factor (TNF), interleukin-1 (IL-1) and IL-10][9] are the major genetic contributors to the development of AP and CP.

A model (two hit model) for the pathogenesis of pancreatitis has been proposed[10], suggesting that "there is a loss of balance between events associated with activation and degradation of active trypsin enzyme leading to the presence of persistent "super-trypsin" with in the acinar cell that is due to mutations or polymorphisms in genes namely *SPINK1,* Cathepsin B (*CTSB*), Chymotrypsinogen C (*CTRC*) and other yet to be identified susceptibility genes. This loss of balance leads to inflammation and these events are the first hits that contribute to the pathogenesis of pancreatitis". The presence of additional genetic and/or environmental risks leading to one or more phenotypes namely fibrosis, stone formation and/or diabetes and these events are the second hit.

AP: DEFINITION, SYMPTOMS AND RISK FACTORS

AP is a syndrome of acute and sudden inflammation of the pancreas. Clinically, it is detected by upper abdominal pain with sudden onset, digestive enzymes namely pancreatic amylase and lipase that are elevated in the serum and/or typical findings like edema, peripancreatic fat stranding, fluid collection on the abdominal imaging studies. The process in AP is initiated by an injury that is acute followed by an inflammatory response (also acute) which is mostly out of proportion and to the extent of tissue injury. The above response is due to premature activation of digestive enzymes in the pancreas that digest the tissue, consequently activating the inflammatory cascade. The immune system may also be cross-activated by the activated pancreatic digestive enzymes. Many risk factors for AP have been identified. The most important of them being duct obstruction by gall stones, parasites, tumors, anatomical abnormalities and endoscopic retrograde cholangio-pancreatography; metabolic factors like hyperlipidemia, hypercalcemia and acidosis; toxins like ethyl alcohol, insecticides, scorpion toxins, medications (azathioprine, NSAIDs, tetracycline, *etc.*); Bacterial and viral infections, trauma caused by blunt or penetrating or surgery apart from genetic susceptibility namely mutations in *PRSS1*, *SPINK1* and *CFTR*[5].

CP: DEFINITION, SYMPTOMS AND RISK FACTORS

CP is a disease associated with inflammation that is progressive and is characterized by three main features. Abdominal pain that is recurrent or persisting at the clinical level, damage of the parenchyma in pancreas with irregular sclerosis and inflammation, accompanied by ductal dilation, strictures or stones at the morphological level and finally a progressive loss of exocrine and endocrine functions at the functional level[11-13]. Based on the etiologies and risk factors, a working classification for CP

Table 1 General genetic information of the genes which confer susceptibility to pancreatitis

Name of the gene	Upstream gene variants	Downstream gene variants	Non-coding exon variants	Synonymous variants	Missense variants	Stop gained	Intron variants
CTRC	490	430	102	28	57	5	789
CASR	580	732	129	433	1459	57	4707
PRSS1	1031	1634	431	126	280	6	637
CTSB	5763	11413	621	682	1261	10	18675
SPINK1	366	252	38	8	37	0	236
CFTR	1193	2377	87	447	2533	558	13723
CLDN2	205	171	0	36	78	0	560

CTRC: Chymotrypsin C; *CASR*: Calcium sensing Receptor; *PRSS1*: Trypsinogen Gene; *CTSB*: Cathepsin B; *SPINK1*: Serine protease inhibitor kazal type 1; *CFTR*: Cystic fibrosis transmembrane conductance regulator; *CLDN2*: Claudin 2.

Table 2 Summary[1] of the polymorphisms in genes related to pancreatitis

Name of the gene	Chromosome	No. of splice variants	Length (bp) of exon region	No. of exons
CTRC	1	4	898	8
CASR	3	4	5009	7
PRSS1	7	6	800	5
CTSB	8	35	3875	10
SPINK1	5	3	542	4
CFTR	7	11	6128	27
CLDN2	X	3	3150	2

[1]Extracted from ENSEMBL. Upstream Gene variants: A sequence variant located 5′ of a gene. Downstream gene variants: A sequence variant located 3′ of a gene. Non-coding exon variants: A sequence variant that changes non-coding exon sequence. Synonymous variants: There is no change in the resulting aminoacid. Missense variants: Variant that changes one or more bases, resulting in a different aminoacid but where the length is preserved. Stop gained: Sequence variant whereby at least one base of a codon is changed, resulting in premature stop codon, leading to a shortened transcript. Intron variants: a variant occurring within an intron. *CTRC*: Chymotrypsin C; *CASR*: Calcium sensing Receptor; *PRSS1*: Trypsinogen Gene; *CTSB*: Cathepsin B; *SPINK1*: Serine protease inhibitor kazal type 1; *CFTR*: Cystic fibrosis transmembrane conductance regulator; *CLDN2*: Claudin 2.

has been elaborated by the American Gastroenterological Association according to its prevalence and mechanism named TIGAR-O classification system (toxic-metabolic, idiopathic, genetic, autoimmune, recurrent and severe AP, obstruction)[14]. The toxic metabolic include alcohol, smoking (tobacco), hyperlipidemia, hypercalcemia, chronic renal failure and certain medications; idiopathic includes early onset, late onset and tropical; mutations in cationic *PRSS1* gene, *CFTR* gene, *SPINK1*, α-1 antitrypsin deficiency and other unidentified genes comprise genetic risk; autoimmune includes isolated autoimmune chronic pancreatitis, autoimmune syndromic CP including Sjogren's syndrome-associated CP, primary biliary cirrhosis-associated CP and inflammatory bowel disease-associated CP. Recurrent and severe AP-associated CP includes post necrotic (severe AP), vascular disease/ischemic and post-irradiation. Obstructible risk factors include sphincter of Oddi disorders, pancreas divisum, duct obstruction (tumor), preampullary duodenal wall cysts and post-traumatic pancreatic duct scars.

GENETIC RISK FACTORS FOR ACUTE AND CP

It has long been suggested that inappropriate activation of trypsinogen in the pancreas is the first and most important step in the development of pancreatitis[15] and all the known genetic susceptibility factors for pancreatitis identified till date can be categorized as members of the intra-pancreatic trypsin regulatory mechanism and were identified employing a candidate-gene approach based on the above mechanism and they include polymorphisms/mutations in genes namely *CTRC*, *CASR*, Trypsinogen gene (*PRSS1, 2* and *3*), Cathepsin B (*CTSB*), *SPINK1/PST1*, *CFTR* gene. General information about the genes is presented in Table 1. A recent study[16] identified an underlying genetic susceptibility in approximately half of idiopathic CP patients, when they screened for mutations in *PRSS1, SPINK1, CTRC* and *CFTR* genes, emphasizing the important role of genetics in CP. A detailed list of different types of polymorphisms identified in these genes till date has been extracted from ENSEMBL and presented in Table 2 and the list of polymorphisms in these genes are also listed in the web site www.pacreasgenetics.org, however only the important polymorphisms/mutations have been discussed in detail in this review.

Trypsinogen (PRSS1, 2 and 3) genes

PRSS1, anionic trypsinogen (*PRSS2*) and mesotrypsinogen (*PRSS3*) are the three types of trypsinogen that are expressed by the pancreas to an extent of two-thirds to one-third to less than 5% respectively[17,18]. Eight trypsinogen genes are shown to be located in the beta T-cell receptor locus at 7q35[19]. The *PRSS1* gene that is mapped to the long arm of chromosome 7 encodes the trypsin-1 (TRY-1) protein[8,20]. Important mutations (gain of function namely A16V, N29I, R122H) have been identified in the *PRSS1* gene that are associated with hereditary pancreatitis in Caucasians[21,22], French[23], D162D variant in Chinese[24] however a study from India reported that *PRSS1* gene mutations are not associated with CP[25]. A study from Korea reported that 5.4% of subjects with idiopathic CP and 40% with pancreatitis that is hereditary carried R122H mutation in the *PRSS1* gene and other variants were not reported apart from R122H. None

of the 50 controls had the mutation[26]. One important study[27] screened for *PRSS1* mutations in a Belgian patient with sporadic CP and observed a migration pattern that is altered different from the transition (g.133283G > A) in exon 3 of the gene. Subsequent analysis by DNA sequencing revealed a DNA variant that was novel (g.133283-133284GC > AT) also resulting in R122H, however they concluded that in contrast to the change in codon CGC to CAC, codon CGC > CAT strongly suggested an alternative mutational mechanism of gene conversion.

Apart from the polymorphisms and their associations with pancreatitis, studies have also looked in to the copy number variations (CNVs) for their role in pancreatitis. A study[28] identified a duplication and triplication of 605kb segment on chromosome 7q35 in French ICP patients, which increased the copy number of *PRSS1* and 2 genes that code for anionic trypsinogen. The same study identified a trypsinogen gene that was hybrid with exon 1, 2 from *PRSS2* and exons 3 to 5 from *PRSS1*, which had two gain of function effects namely increase in trypsinogen gene copy number with N29I mutation in it. The 605kb segment duplication was also assessed further in French and Indian patients with idiopathic CP (ICP) and concluded that it was associated with French ICP but not in Indian patients with CP[29], however the CNVs in *PRSS3* were not associated[30].

Serine protease inhibitor Kazal type 1/pancreatic secretory trypsin inhibitor gene

SPINK 1/pancreatic secretory trypsin inhibitor (*PSTI*) is a specific trypsin inhibitor and an acute phase protein which is secreted by the acinar cells[31]. The gene encoding *SPINK1* has 4 exons and 3 introns that is located at 5q32 and is approximately 7.5kb long[32]. SPINK1 protein plays a role in the prevention of premature activation of zymogen that is catalyzed by trypsin within the pancreatic duct system or the acinar tissue. A reactive site in the protein serves as a specific target substrate for trypsin[33] and it can inhibit up to 20% of the activity of pancreatic trypsin. It is the first line of defense against auto digestion, thereby protecting the pancreas[9], however inhibition of trypsin by *SPINK1* is temporary as trypsin may target the trypsin-SPINK1 complex and subsequently degrade the inhibitory molecule and restore trypsin activity[34]. *SPINK1* mutations cause a loss of function mutations as against *PRSS1* which generate gain of function mutations. There are several mutations/polymorphisms that are identified till date in the *SPINK1* gene (Table 2), however N34S is the most common missense mutation, that is a substitution of asparagine by serine at codon 34. N34S polymorphism was found in individuals especially without a family history and many studies have confirmed its association in different ethnic groups[25,35-37]. A substantial number of patients (15%-40%) with ICP carry N34S mutation in either heterozygous or homozygous state based on the above studies. The *SPINK1* polymorphisms (N34S) are in complete linkage disequilibrium with other variants that are located in the introns[38]. Other mutations/polymorphisms have also been identified namely a promoter mutation (-215-A and -215 G > T), a mutation in the start codon that destroys the only translational initiation codon of *SPINK1* (2 T-C, Met to Thr; MIT)[39], -53C > T; -41G > A, -2C > A; L14P; D50E; IVS3 + 125C > A; IVS3 + 184T > A; R65Q; R67C which were reported predominantly in single patients or families[35,38,40].

Polymorphisms in *SPINK1* gene are generally associated with loss of function. Although the *SPINK1* N34S polymorphism is associated with pancreatitis, the association is weak with very few individuals with the mutation developing pancreatitis some time during their life time[35,41]. Furthermore there is no difference in the severity of the disease with respect to the heterozygous and homozygous genotypes of SPINK1; there are complex interactions and the effect of the mutation depends on the reduction in the enzyme. Pancreatitis may be initiated in the homozygous N34S state, however the heterozygous genotype may only cause a lowering of the enzyme level and it requires other additional factors (genetic and environmental) to initiate the disease[42]. Therefore in general *SPINK1* polymorphism is hypothesized to be a susceptibility factor for a polygenic complex trait or a disease modifier[3] with polymorphisms in other genes being involved.

Apart from the above polymorphisms, two copy number mutations (deletions) in the SPINK1 gene that were associated with loss of function and encoding pancreatic secretory trypsin inhibitor (*PSTI*) were identified by a study[38]. In a particular family these deletions were co-inherited with a missense mutation (p.L997F) in the *CFTR* gene, suggesting complex interactions between the CNVs and single nucleotide substitutions contributing to the disease phenotype. SPINK1 polymorphisms are common in the general population (approximately 2%) but are shown to be significantly associated with pancreatitis.

Chymotrypsin C gene

CTRC encodes Chymotrypsin C, a digestive enzyme. It is produced by the acinar cells in the pancreas. It is packaged with zymogen granules and is secreted along with other digestive enzymes from the pancreas. Prematurely activated trypsin is destroyed by CTRC by acting on the molecule within the calcium-binding loop in the absence of calcium and therefore is a crucial candidate gene in the pathogenesis of pancreatitis[43]. Many polymorphisms have been identified in this gene till date (Table 2). A study[44] had sequenced all the 8 exons (8.2 kb) of the *CTRC* gene in a total of 621 individuals with idiopathic or hereditary CP and 614 control subjects of German origin and identified that the large majority of the variants were in 2nd, 3rd and 7th exons. Only exons 2, 3 and 7 were sequenced in an additional 280 CP patients and 2075 controls for exons 2 and 3 and 2190 controls for exons 7. Although a number of missense and deletion variants were found they concluded that the two most frequent variants

which were significantly overrepresented in the pancreatitis group as compared to the controls were c.760C > T (p.R254W) and c.738_761del24 (p.K247_R254del) (30/901 (3.3%) affected individuals but only in 21/2804 (0.7%) controls), both of which were located in exon 7. Furthermore, this group also studied 71 and 84 individuals of Indian origin with tropical pancreatitis and controls respectively, and suggested a higher frequency of *CTRC* alterations in this cohort [10/71 (14.1%) in Tropical pancreatitis Vs 1/84 (1.2%) controls] as compared to the German cohort and two relatively frequent variants were found in the Indian cohort namely c.217G > A (p.A73T) missense alteration and the c.190_193del ATTG (p.I64LfsX69) frame shift deletion[44]. Another study from India[45] identified 14 variants in 584 CP patients and 598 normal subjects [71/584 CP patients (12.2%) and 22/598 controls (3.7%)], when all the eight exons and flanking regions of the *CTRC* gene were sequenced. It was p.V235I variant which was common in the Indian CP patients as against the p.K247_R254del variant in the Caucasians. Apart from this variant the study also identified other pathogenic variants namely p.A73T and c.180C > T as significantly associated with Indian CP.

Cathepsin B gene

The human *CTSB* is 25.6kb. It has 12 exons. Several transcript species are known to be produced by alternative splicing[46]. It is hypothesized that chronic pancreatitis is a result of mutations in the *CTSB* gene and they may be involved in premature activation of trypsinogen or inappropriate localization[47]. A study on the CTSB gene polymorphisms and tropic calcific pancreatitis identified significant association of Val26Val polymorphism (allele frequency of 0.48 in patients *vs* 0.30 in controls) with Odds of 2.15 apart from differences in the mutant allele frequencies that are significant at Ser53Gly (allele frequency of 0.10 *vs* 0.04 in patients and controls respectively) and C595T SNPs (allele frequency of 0.12 *vs* 0.20 in patients and controls respectively. Further L26V polymorphism was equally as common in N34S positive and wild type patients suggesting that CTSB is involved independently with the disease. This study suggested that *CTSB* polymorphisms may be associated with pancreatitis more so in the absence of mutations in *PRSS1* gene and N34S *SPINK1* polymorphism proposed to play a disease modifier role[47], however another study failed to associate polymorphisms in this gene with pancreatitis in European cohort (allele frequency of 0.398 in patients and 0.48 in cotrols)[48].

Calcium-sensing receptor gene

Auto-activation and autolysis are processes in which trypsinogen molecule is activated to trypsin and is also degraded by other trypsin molecules. For the mentioned purpose, two specific cleavage sites exist for potential attack by other trypsin molecules. Lysine 23 (L23) is the first site and arginine 1122 (R122) the second. The cleavage of L23 causes trypsinogen activation to trypsin with 8-amino acid trypsinogen activation peptide being released while R122 cleavage causes inactivation of trypsin. The susceptibility of the two sites for an attack is regulated by calcium concentration and concentration dependent occupation of the calcium binding sites[49]. In normal acinar cells low calcium concentrations are prevalent and these low concentrations limit the activation of trypsinogen, thereby promoting inactivation of trypsin by exposing the second site (R122), however calcium hyper stimulation or dysregulation in the acinar cells favors activation of trypsinogen and prevention of trypsin inactivation[50]. Thus regulation of calcium levels (intra-acinar) is critical for preventing trypsinogen activation and pancreatic injury. CASR plays a major and important role in maintaining the calcium homeostasis through its effect on renal tubules and parathyroid gland. A variety of hypercalcemia-associated syndromes are associated with genetic variants in the *CASR* gene[51]. The first of the reports associating *CASR* mutations with CP came from a family study of 5 individuals who were all heterozygous for the N34S *SPINK1* polymorphism. Only two of the 5 heterozygous individuals developed CP and both these individuals presented with a T > C mutation at position 518 in the *CASR* gene, that is a leucine to proline amino acid change in the extracellular domain of the CASR protein[52], suggesting that *CASR* mutations may be a predisposing genetic factor that may increase the susceptibility for CP. Another study[53] that screened for mutations in *SPINK1* and *CASR* gene on a small Indian cohort of 35 patients with Tropical chronic pancreatitis (TCP) and an equal number of controls reported that a combination of mutations in both the genes was seen in 6% of the patients, while 22% had mutation in single gene, suggesting that *CASR* mutations may be a risk for TCP and that risk may be further increased with associated *SPINK1* mutation. A study by Muddana *et al*[54] initially included 115 subjects with pancreatitis and 66 controls. Of the study group, 57 patients and 21 controls were predetermined to carry the N34S *SPINK1* polymorphism. Based on the initial results, the study included an additional 223 patients and 239 controls to analyze the three common non-synonymous SNPs in exon 7 that were found to be significant from the initial study. The *CASR* exon 7 polymorphism (R990G) was significantly (Odds, 2.01 and P = 0.01) associated with CP and the association of this SNP was stronger in subjects with moderate to heavy alcohol consumption. This study however did not find any significant associations between the various *CASR* genotypes and *SPINK1* N34S in CP. None of the earlier reported polymorphisms from Germany and India were also detected in this US-based study. All the association studies suggest that recurrent trypsin activation/dysregulated calcium and failed inhibition increase the risk of pancreatitis *via* the intracellular calcium dysregulation.

CFTR gene

The impact of *CFTR* gene continues to be debated, although variants in this gene are strongly associated

with pancreatitis. *CFTR* gene in humans has 27 exons, is located at 7q31 and is 250 kb in length[55]. For the proper functioning of the duct cells in the pancreas and other anion secreting epithelial cells, CFTR anion channel is a critical molecule. CFTR apart from regulating the functions of other channels also conducts both chloride and bicarbonate channels, the opening and closing of which controls the bulk of fluid secretion from the pancreas[50]. The association between idiopathic CP and *CFTR* mutations was demonstrated in 1998[56,57]. More than 1200 mutations have been identified and based on the mechanism by which they disrupt the function; they are classified in to five different groups with group V mutations subsequently being included in group I (as they cause functional alterations in the levels of mRNA)[58]. Class I mutations affects biosynthesis, class Ⅱ mutations affect protein maturation, class Ⅲ affect chloride channel regulation/gating while class Ⅳ mutations affect chloride conductance[59]. An additional class of mutations was proposed by Haardt *et al*[60] as class Ⅵ which included protein stability mutations.

A higher frequency of mutations in the *CFTR* gene was seen in a significant number of patients (30%) with ICP. There was six and two times higher frequency of CFTR mutations and 5T allele respectively in patients[56,57,61]. With few of these mutations there was a reduction in the amount of functional CFTR. The others might be a combination of a severe and a mild mutation or either type of mutations with 5T allele in intron 8 of the gene[9]. There is an increased risk (up to 40 fold) for pancreatitis when individuals are compound heterozygotes[62]. Complete coding sequences of the *CFTR,* P*RSS1* and *SPINK1* genes were analyzed for mutations and it was seen that 25%-30% of the patients with CP carried at least a single mutation in the *CFTR* gene and majority were compound heterozygotes for a *CFTR* mutation or were trans-heterozygotes for *CFTR, PRSS1* and *SPINK1* mutations[62,63]. Furthermore, a combination of two *CFTR* mutations and N34S in *SPINK1* gene increases the risk of pancreatitis by 900 fold[9]. It is clear from these studies that *CFTR* variants are associated with CP, however the mechanisms of the complex interactions of various susceptibility loci has to be understood in a better way.

Proinflammatory cytokine genes

It is already established that the cytokine profile with in the pancreas is different in CP as compared to normal pancreas[64]. A potential factor that could affect the production of proinflammatory cytokines are polymorphisms in these genes. Association studies involving polymorphisms in various cytokine genes have shown varying results in various populations. Various genes namely *TNF-a* (tumor necrosis factor-α), *Monocyte chemoattractant protein-1*, and *IL-8*[65-67] have been studied for their association with pancreatitis.

It is known that *TNF-a* along with *IL-1* is a major early cytokine to mediate the systemic inflammatory response syndrome (SIRS)[68-70]. A study[71] reported the association between *TNF-a* -238 AG but not -308 SNP genotype with organ failure (shock and/or respiratory failure) and in the IL-6 gene the CC genotype at position 174 was associated with biliary etiology of AP. The study included 84 patients with AP (no controls were included) and known polymorphisms in *TNF-a*, interleukin 1 (*IL-1*), IL-1 receptor antagonist (*IL1RN, IL-6* and *IL-10)* were genotyped for etiology associated susceptibility and severity, however other polymorphisms like *TNF-a*-1031, -863 and -857 SNPs were not included in the study. Another study[72] reported a negative association between *TNF-a*-308 and severity of pancreatitis (397 patients and 300 controls with major allele frequency in *TNF* gene being 0.87 for patients with AP and 0.86 for controls) from Finland, however they did not study the *TNF-a*-238 SNP. These results were similar to studies reported from United Kingdom, by[73], who studied 190 and 102 AP patients and controls respectively and Sargen *et al*[74], who studied 135 AP and and 107 controls repesctively (78.3% and 84.4% for *TNF-a*-308 and 21.7% and 15.6% for *TNF-a*-238 in controls and AP repectively). However, *TNF-a*-308 allele was reported to be associated with severe AP in Hungarian patients[75]. The study included 77 patients (mixed etiology and grouped according to the severity of the disease on the basis of Ranson scores) and 71 controls. Another study[76] associated *TNF-a*-308 allele with shock in patients with severe AP, however suggested that the polymorphism played no part in disease severity or susceptibility. The study included 208 AP cases and 116 ethnicity matched controls. A recent meta-analysis[77] integrated the previous findings on *TNF-a*-308 G > A and -238 G > A alleles and explored whether the polymorphisms were associated with susceptibility and severity to pancreatitis. The study included 1569 pancreatitis cases and 1330 controls from 12 published case-control studies and concluded that polymorphisms in these two sites did not alter the risk of pancreatitis.

Monocyte chemoattractant protein 1 (MCP-1) is a member of the C-C chemokine family. It exerts a strong chemo attractant activity in macrophages, lymphocytes and monocytes[78]. A common polymorphism-2518 A > G alters the expression of the gene with G allele being associated with higher levels of MCP-1 protein which is associated with higher risk of pancreatitis. A study from United States[65] included 77 consecutive patients and 116 controls for the mentioned genotype and concluded that the -2518 genotype is a risk factor for severe AP (12 of 14; 86% with AP *vs* 50 of 116; 43% control subjects) and also suggested that MCP-1 serum levels appear to be an accurate predictor of severity of AP and death when measured early in the course of the disease. Another study from Italy[79] studied 118 AP, 64 ARP, and 142 CP patients and 88 controls and concluded that all patients with pancreatic inflammatory disease had significantly higher serum MCP-1 levels. A study[80] studied the relationship between a polymorphism in the *MCP-1* gene (-2518A/G) and AP in the Han population of Suzhou, China and suggested an increased risk of AP associated

with G allele [72.4% (113/156) and 76.1% (35/46) in severe AP; 47.1% (113/240)]. However, the 2518A/G polymorphism in the *MCP-1* gene did not significantly alter the susceptibility to CP[81].

Interleukins are proinflammatory cytokines and polymorphisms in these genes have been shown to affect the immune response[82]. A meta-analysis[83] on the inteleukin gene polymorphisms which included a total of 10 studies, covering a total of 1220 AP cases and 1351 controls showed evidence for significant association between *IL-8* -251 T/A (rs4073) polymorphism and AP risk, suggesting that *IL-8* -251 A allele was associated with an increased risk of AP. However, there were no significant associations between *IL-1β* [IL-1β +3954 C/T (rs1143634) and IL-1β -511 C/T (rs16944)], IL-6 [IL-6 -174 G/C (rs1800795) and *IL-6* -634 C/G (rs1800796)] and *IL-10* [IL-10 -1082 A/G (rs1800896), *IL-10* -819 C/T (rs1800871) and *IL-10* -592 C/A (rs1800872)] gene polymorphisms and AP risk. In summary, the study concluded that the IL-8 -251 T/A polymorphism was associated with an increased risk of AP. In addition, there were no significant associations between IL-1β, IL-6 and IL-10 gene polymorphisms and AP risk.

Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine. It is released by macrophages and lymphocytes[84]. It plays an important pathogenic role in AP and a study[85] investigated the role of -173 G > C polymorphism and the (CATT) n repeat microsatellite at position -794 in 164 patients with AP and 197 controls C allele 58/160 [18.1% in AP *vs* 47/097 (11.9%) in controls]. There was no significant difference in the repeat length of the microsatellite marker between patients and controls, however the C allele of the -173 G > C genotype was significantly higher in patients.

Claudin-2 and Carboxypeptidase A1 gene

New susceptibility loci for CP have been identified. The first SNP in the Claudin-2 (*CLDN2*) locus is the outcome of the first and only reported Genome wide association study for pancreatitis till date, which included 1676 cases and 4507 controls in stage Ⅰ and 910 cases and 4170 controls in stage Ⅱ. The study identified two SNPs namely one SNP in *PRSS1-PRSS2* locus (allele frequency of 0.576 in controls *vs* 0.634 in pancreatitis) and the other in the Claudin-2 locus (*CLDN2*) (allele frequency of 0.261 in controls *vs* 0.322 in pancreatitis). The SNP in the *PRSS1* locus affects susceptibility by altering the expression of trypsinogen and the SNP in the *CLDN2* is associated with atypical localization of claudin-2 in pancreatic acinar cells. Homozygous or hemizyogus genotype (in females and males) confers the greatest risk and the alleles also interact with alcohol consumption to increase the risk of pancreatitis[86]. Another study[87] analyzed variants in Carboxypeptidase A1 (*CPA1*) encoding carboxypeptidase A1, primarily in Germany discover set and in thee replication sets from Europe, India and Japan. *CPA1* variants were associated with non-alcoholic CP with varying levels of significance in the discovery [29/944 (3.1%) of German cases and 5/3,938 (0.1%) controls] as well as all the three replication sets 8/600 (1.3%) of European cases and 9/2,432 (0.4%) controls, 5/230 (2.2%) of Indian cases and 0/264 controls and 5/247 (2.0%) of Japanese cases and 0/341 controls. The study concluded that variants may confer an increased risk of CP and the mechanism may involve endoplasmic reticulum stress that may be induced by misfolding rather than trypsin activity that is elevated.

GENETIC TESTING FOR *PRSS1, SPINK1* AND *CFTR* GENES - WHEN TO ORDER THE TEST?

A valuable diagnostic genetic test to investigate acute and CP has been added ever since a point mutation in the *PRSS1* gene has been identified. Consensus guidelines for ethical molecular genetic testing for hereditary pancreatitis has been proposed[88] which recommends it under the following conditions: (1) Unexplained two or more (recurrent) episodes of documented pain that are separate with hyoeramylasemia attack; (2) Idiopathic CP; (3) Family history of pancreatitis [in a parent, sib, child (first degree) and in aunt, uncle or grand parent (second degree)]; (4) A need to exclude significant concern of hereditary pancreatitis in a child with an unexplained episode of documented pancreatitis that required a hospitalization; (5) As part of research protocol that is approved. Genetic testing (*PRSS1* mutations) in children below 16 years is indicated after; (6) Hospitalization that was required in an individual because of an episode of documented pancreatitis of unknown etiology that is severe enough; (7) Pancreatitis of unknown etiology in an individual with two or more documented episodes; (8) A child with an episode of documented pancreatitis, who has a relative with hereditary pancreatitis mutation that is known; (9) Recurrent abdominal pain (unknown etiology) in a child, where there is a distinct clinical possibility of hereditary pancreatitis; and (10) Diagnosis of hereditary pancreatitis as a distinct clinical possibility in an individual with CP of unknown etiology[88].

Currently genetic testing for mutations in SPINK1 or CFTR genes is considered as premature as the identification of mutations in these genes neither convincingly explains the disease in an individual who has been diagnosed with pancreatitis or has the ability to predict the possibility of developing the disease[88-90].

The significance of a positive test result for *PRSS1* genetic testing should be explained clearly to the subjects. Variable clinical course, mode of inheritance and incomplete penetrance are the important aspects apart from others, where counseling needs to be imparted to the patients. Strategies should be discussed to prevent future episodes of AP namely avoiding concomitant risk factors like alcohol, metabolic disturbances and drugs.

Important risk factors namely choledocolithiasis and other obstructive factors that contribute to AP have to be

identified and treated. Therefore patients have to be advised to undergo radiological and endoscopic evaluation to identify the above risks[91]. Furthermore, as these mutations (R122H or N29I) also significantly increase the risk for pancreatic cancer, the patients should be counseled for abstinence from tobacco and smoking[92] and counseling may be imparted and genetic testing ordered for at risk relatives if warranted[3].

CONCLUSION

As emphasized earlier many of the susceptibility loci identified till date have taken the candidate-gene approach and to the best of our knowledge there are no GWAS (Genome wide association studies) which are available apart from the only study which identified *PRSS1* and *CLDN2* polymorphisms recently[86]. Furthermore, a better understanding of the interactions of the etiological factors with susceptibility SNPs will aid in diagnosing and treating the disease at an early stage. There is an urgent need to utilize the advances in genomics namely GWAS and/or exome sequencing on NGS platform to unravel as yet unidentified susceptibility loci for pancreatitis, which is a multifactorial and a complex disease for a better understanding at the molecular level.

REFERENCES

1 **Brock C**, Nielsen LM, Lelic D, Drewes AM. Pathophysiology of chronic pancreatitis. *World J Gastroenterol* 2013; **19**: 7231-7240 [PMID: 24259953 DOI: 10.3748/wjg.v19.i42.7231]

2 **Klöppel G**, Detlefsen S, Feyerabend B. Fibrosis of the pancreas: the initial tissue damage and the resulting pattern. *Virchows Arch* 2004; **445**: 1-8 [PMID: 15138818 DOI: 10.1007/s00428-004-1021-5]

3 **Whitcomb DC**. Value of genetic testing in the management of pancreatitis. *Gut* 2004; **53**: 1710-1717 [PMID: 15479696 DOI: 10.1136/gut.2003.015511]

4 **Yadav D**, Whitcomb DC. The role of alcohol and smoking in pancreatitis. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 131-145 [PMID: 20125091]

5 **Whitcomb DC**. Genetic aspects of pancreatitis. *Annu Rev Med* 2010; **61**: 413-424 [PMID: 20059346 DOI: 10.1146/annurev.med.041608.121416]

6 **Comfort MW**, STEINBERG AG. Pedigree of a family with hereditary chronic relapsing pancreatitis. *Gastroenterology* 1952; **21**: 54-63 [PMID: 14926813]

7 **Le Bodic L**, Bignon JD, Raguénès O, Mercier B, Georgelin T, Schnee M, Soulard F, Gagne K, Bonneville F, Muller JY, Bachner L, Férec C. The hereditary pancreatitis gene maps to long arm of chromosome 7. *Hum Mol Genet* 1996; **5**: 549-554 [PMID: 8845851 DOI: 10.1093/hmg/5.4.549]

8 **Whitcomb DC**, Preston RA, Aston CE, Sossenheimer MJ, Barua PS, Zhang Y, Wong-Chong A, White GJ, Wood PG, Gates LK, Ulrich C, Martin SP, Post JC, Ehrlich GD. A gene for hereditary pancreatitis maps to chromosome 7q35. *Gastroenterology* 1996; **110**: 1975-1980 [PMID: 8964426]

9 **Grendell JH**. Genetic factors in pancreatitis. *Curr Gastroenterol Rep* 2003; **5**: 105-109 [PMID: 12631449]

10 **Mahurkar S**, Reddy DN, Rao GV, Chandak GR. Genetic mechanisms underlying the pathogenesis of tropical calcific pancreatitis. *World J Gastroenterol* 2009; **15**: 264-269 [PMID: 19140225 DOI: 10.3748/wjg.15.264]

11 **Sarner M**, Cotton PB. Classification of pancreatitis. *Gut* 1984; **25**: 756-759 [PMID: 6735257]

12 **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]

13 **Sarles H**, Adler G, Dani R, Frey C, Gullo L, Harada H, Martin E, Norohna M, Scuro LA. The pancreatitis classification of Marseilles-Rome 1988. *Scand J Gastroenterol* 1989; **24**: 641-642 [PMID: 2814334]

14 **Etemad B**, Whitcomb DC. Chronic pancreatitis: diagnosis, classification, and new genetic developments. *Gastroenterology* 2001; **120**: 682-707 [PMID: 11179244]

15 **Steer ML**, Meldolesi J. The cell biology of experimental pancreatitis. *N Engl J Med* 1987; **316**: 144-150 [PMID: 3540666 DOI: 10.1056/NEJM198701153160306]

16 **Masson E**, Chen JM, Audrézet MP, Cooper DN, Férec C. A conservative assessment of the major genetic causes of idiopathic chronic pancreatitis: data from a comprehensive analysis of PRSS1, SPINK1, CTRC and CFTR genes in 253 young French patients. *PLoS One* 2013; **8**: e73522 [PMID: 23951356 DOI: 10.1371/journal.pone.0073522]

17 **Scheele G**, Bartelt D, Bieger W. Characterization of human exocrine pancreatic proteins by two-dimensional isoelectric focusing/sodium dodecyl sulfate gel electrophoresis. *Gastroenterology* 1981; **80**: 461-473 [PMID: 6969677]

18 **Rinderknecht H**, Renner IG, Abramson SB, Carmack C. Mesotrypsin: a new inhibitor-resistant protease from a zymogen in human pancreatic tissue and fluid. *Gastroenterology* 1984; **86**: 681-692 [PMID: 6698368]

19 **Rowen L**, Koop BF, Hood L. The complete 685-kilobase DNA sequence of the human beta T cell receptor locus. *Science* 1996; **272**: 1755-1762 [PMID: 8650574 DOI: 10.1126/science.272.5269.1755]

20 **Pandya A**, Blanton SH, Landa B, Javaheri R, Melvin E, Nance WE, Markello T. Linkage studies in a large kindred with hereditary pancreatitis confirms mapping of the gene to a 16-cM region on 7q. *Genomics* 1996; **38**: 227-230 [PMID: 8954806]

21 **Whitcomb DC**, Gorry MC, Preston RA, Furey W, Sossenheimer MJ, Ulrich CD, Martin SP, Gates LK, Amann ST, Toskes PP, Liddle R, McGrath K, Uomo G, Post JC, Ehrlich GD. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. *Nat Genet* 1996; **14**: 141-145 [PMID: 8841182 DOI: 10.1038/ng1096-141]

22 **Witt H**, Luck W, Becker M. A signal peptide cleavage site mutation in the cationic trypsinogen gene is strongly associated with chronic pancreatitis. *Gastroenterology* 1999; **117**: 7-10 [PMID: 10381903]

23 **Rebours V**, Boutron-Ruault MC, Schnee M, Férec C, Le Maréchal C, Hentic O, Maire F, Hammel P, Ruszniewski P, Lévy P. The natural history of hereditary pancreatitis: a national series. *Gut* 2009; **58**: 97-103 [PMID: 18755888 DOI: 10.1136/gut.2008.149179]

24 **Liu QC**, Gao F, Ou QS, Zhuang ZH, Lin SR, Yang B, Cheng ZJ. Novel mutation and polymorphism of PRSS1 gene in the Chinese patients with hereditary pancreatitis and chronic pancreatitis. *Chin Med J* (Engl) 2008; **121**: 108-111 [PMID: 18272034]

25 **Chandak GR**, Idris MM, Reddy DN, Mani KR, Bhaskar S, Rao GV, Singh L. Absence of PRSS1 mutations and association of SPINK1 trypsin inhibitor mutations in hereditary and non-hereditary chronic pancreatitis. *Gut* 2004; **53**: 723-728 [PMID: 15082592 DOI: 10.1136/gut.2003.026526]

26 **Oh HC**, Kim MH, Choi KS, Moon SH, Park do H, Lee SS, Seo DW, Lee SK, Yoo HW, Kim GH. Analysis of PRSS1 and SPINK1 mutations in Korean patients with idiopathic and familial pancreatitis. *Pancreas* 2009; **38**: 180-183 [PMID: 18852684 DOI: 10.1097/MPA.0b013e31818d1b90]

27 **Chen JM**, Raguenes O, Ferec C, Deprez PH, Verellen-Dumoulin C. A CGC& gt; CAT gene conversion-like event resulting in the R122H mutation in the cationic trypsinogen gene and its implication in the genotyping of pancreatitis.

J Med Genet 2000; **37**: E36 [PMID: 11073545 DOI: 10.1136/jmg.37.11.e36]
28 **Chen JM**, Masson E, Le Maréchal C, Férec C. Copy number variations in chronic pancreatitis. *Cytogenet Genome Res* 2008; **123**: 102-107 [PMID: 19287144 DOI: 10.1159/000184697]
29 **Masson E**, Le Maréchal C, Chandak GR, Lamoril J, Bezieau S, Mahurkar S, Bhaskar S, Reddy DN, Chen JM, Férec C. Trypsinogen copy number mutations in patients with idiopathic chronic pancreatitis. *Clin Gastroenterol Hepatol* 2008; **6**: 82-88 [PMID: 18063422]
30 **Masson E**, Le Maréchal C, Chen JM, Férec C. Absence of mesotrypsinogen gene (PRSS3) copy number variations in patients with chronic pancreatitis. *Pancreas* 2008; **37**: 227-228 [PMID: 18665091 DOI: 10.1097/MPA.0b013e3181654b6a]
31 **KAZAL LA**, SPICER DS, BRAHINSKY RA. Isolation of a crystalline trypsin inhibitor-anticoagulant protein from pancreas. *J Am Chem Soc* 1948; **70**: 3034-3040 [PMID: 18882536 DOI: 10.1021/ja01189a060]
32 **Horii A**, Kobayashi T, Tomita N, Yamamoto T, Fukushige S, Murotsu T, Ogawa M, Mori T, Matsubara K. Primary structure of human pancreatic secretory trypsin inhibitor (PSTI) gene. *Biochem Biophys Res Commun* 1987; **149**: 635-641 [PMID: 3501289]
33 **Bartelt DC**, Shapanka R, Greene LJ. The primary structure of the human pancreatic secretory trypsin inhibitor. Amino acid sequence of the reduced S-aminoethylated protein. *Arch Biochem Biophys* 1977; **179**: 189-199 [PMID: 843082]
34 **Laskowski M**, Wu FC. Temporary inhibition of trypsin. *J Biol Chem* 1953; **204**: 797-805 [PMID: 13117856]
35 **Pfützer RH**, Barmada MM, Brunskill AP, Finch R, Hart PS, Neoptolemos J, Furey WF, Whitcomb DC. SPINK1/PSTI polymorphisms act as disease modifiers in familial and idiopathic chronic pancreatitis. *Gastroenterology* 2000; **119**: 615-623 [PMID: 10982753]
36 **Schneider A**, Suman A, Rossi L, Barmada MM, Beglinger C, Parvin S, Sattar S, Ali L, Khan AK, Gyr N, Whitcomb DC. SPINK1/PSTI mutations are associated with tropical pancreatitis and type II diabetes mellitus in Bangladesh. *Gastroenterology* 2002; **123**: 1026-1030 [PMID: 12360464 DOI: 10.1053/gast.2002.36059]
37 **Gomez-Lira M**, Bonamini D, Castellani C, Unis L, Cavallini G, Assael BM, Pignatti PF. Mutations in the SPINK1 gene in idiopathic pancreatitis Italian patients. *Eur J Hum Genet* 2003; **11**: 543-546 [PMID: 12825076 DOI: 10.1038/sj.ejhg.5200989]
38 **Chen JM**, Mercier B, Audrezet MP, Ferec C. Mutational analysis of the human pancreatic secretory trypsin inhibitor (PSTI) gene in hereditary and sporadic chronic pancreatitis. *J Med Genet* 2000; **37**: 67-69 [PMID: 10691414 DOI: 10.1136/jmg.37.1.67]
39 **Witt H**, Luck W, Hennies HC, Classen M, Kage A, Lass U, Landt O, Becker M. Mutations in the gene encoding the serine protease inhibitor, Kazal type 1 are associated with chronic pancreatitis. *Nat Genet* 2000; **25**: 213-216 [PMID: 10835640 DOI: 10.1038/76088]
40 **Kuwata K**, Hirota M, Sugita H, Kai M, Hayashi N, Nakamura M, Matsuura T, Adachi N, Nishimori I, Ogawa M. Genetic mutations in exons 3 and 4 of the pancreatic secretory trypsin inhibitor in patients with pancreatitis. *J Gastroenterol* 2001; **36**: 612-618 [PMID: 11578065 DOI: 10.1007/s005350170045]
41 **Whitcomb DC**. How to think about SPINK and pancreatitis. *Am J Gastroenterol* 2002; **97**: 1085-1088 [PMID: 12014708 DOI: 10.1111/j.1572-0241.2002.05697.x]
42 **Witt H**. Chronic pancreatitis and cystic fibrosis. *Gut* 2003; **52** Suppl 2: ii31-ii41 [PMID: 12651880 DOI: 10.1136/gut.52.suppl_2.ii31]
43 **Szmola R**, Sahin-Tóth M. Chymotrypsin C (caldecrin) promotes degradation of human cationic trypsin: identity with Rinderknecht's enzyme Y. *Proc Natl Acad Sci USA* 2007; **104**: 11227-11232 [PMID: 17592142 DOI: 10.1073/pnas.0703714104]
44 **Rosendahl J**, Witt H, Szmola R, Bhatia E, Ozsvári B, Landt O, Schulz HU, Gress TM, Pfützer R, Löhr M, Kovacs P, Blüher M, Stumvoll M, Choudhuri G, Hegyi P, te Morsche RH, Drenth JP, Truninger K, Macek M, Puhl G, Witt U, Schmidt H, Büning C, Ockenga J, Kage A, Groneberg DA, Nickel R, Berg T, Wiedenmann B, Bödeker H, Keim V, Mössner J, Teich N, Sahin-Tóth M. Chymotrypsin C (CTRC) variants that diminish activity or secretion are associated with chronic pancreatitis. *Nat Genet* 2008; **40**: 78-82 [PMID: 18059268 DOI: 10.1038/ng.2007.44]
45 **Paliwal S**, Bhaskar S, Mani KR, Reddy DN, Rao GV, Singh SP, Thomas V, Chandak GR. Comprehensive screening of chymotrypsin C (CTRC) gene in tropical calcific pancreatitis identifies novel variants. *Gut* 2013; **62**: 1602-1606 [PMID: 22580415 DOI: 10.1136/gutjnl-2012-302448]
46 **Berquin IM**, Cao L, Fong D, Sloane BF. Identification of two new exons and multiple transcription start points in the 5'-untranslated region of the human cathepsin-B-encoding gene. *Gene* 1995; **159**: 143-149 [PMID: 7622042]
47 **Mahurkar S**, Idris MM, Reddy DN, Bhaskar S, Rao GV, Thomas V, Singh L, Chandak GR. Association of cathepsin B gene polymorphisms with tropical calcific pancreatitis. *Gut* 2006; **55**: 1270-1275 [PMID: 16492714 DOI: 10.1136/gut.2005.084772]
48 **Weiss FU**, Behn CO, Simon P, Ruthenbürger M, Halangk W, Lerch MM. Cathepsin B gene polymorphism Val26 is not associated with idiopathic chronic pancreatitis in European patients. *Gut* 2007; **56**: 1322-1323 [PMID: 17698872 DOI: 10.1136/gut.2007.122507]
49 **Whitcomb DC**, Lowe ME. Human pancreatic digestive enzymes. *Dig Dis Sci* 2007; **52**: 1-17 [PMID: 17205399 DOI: 10.1007/s10620-006-9589-z]
50 **Whitcomb DC**. Mechanisms of disease: Advances in understanding the mechanisms leading to chronic pancreatitis. *Nat Clin Pract Gastroenterol Hepatol* 2004; **1**: 46-52 [PMID: 16265044 DOI: 10.1038/ncpgasthep0025]
51 **Pidasheva S**, D'Souza-Li L, Canaff L, Cole DE, Hendy GN. CASRdb: calcium-sensing receptor locus-specific database for mutations causing familial (benign) hypocalciuric hypercalcemia, neonatal severe hyperparathyroidism, and autosomal dominant hypocalcemia. *Hum Mutat* 2004; **24**: 107-111 [PMID: 15241791 DOI: 10.1002/humu.20067]
52 **Felderbauer P**, Hoffmann P, Einwächter H, Bulut K, Ansorge N, Schmitz F, Schmidt WE. A novel mutation of the calcium sensing receptor gene is associated with chronic pancreatitis in a family with heterozygous SPINK1 mutations. *BMC Gastroenterol* 2003; **3**: 34 [PMID: 14641934 DOI: 10.1186/1471-230X-3-34]
53 **Murugaian EE**, Premkumar RM, Radhakrishnan L, Vallath B. Novel mutations in the calcium sensing receptor gene in tropical chronic pancreatitis in India. *Scand J Gastroenterol* 2008; **43**: 117-121 [PMID: 18938753]
54 **Muddana V**, Lamb J, Greer JB, Elinoff B, Hawes RH, Cotton PB, Anderson MA, Brand RE, Slivka A, Whitcomb DC. Association between calcium sensing receptor gene polymorphisms and chronic pancreatitis in a US population: role of serine protease inhibitor Kazal 1type and alcohol. *World J Gastroenterol* 2008; **14**: 4486-4491 [PMID: 18680227 DOI: 10.3748/wjg.14.4486]
55 **Zielenski J**, Rozmahel R, Bozon D, Kerem B, Grzelczak Z, Riordan JR, Rommens J, Tsui LC. Genomic DNA sequence of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. *Genomics* 1991; **10**: 214-228 [PMID: 1710598]
56 **Sharer N**, Schwarz M, Malone G, Howarth A, Painter J, Super M, Braganza J. Mutations of the cystic fibrosis gene in patients with chronic pancreatitis. *N Engl J Med* 1998; **339**: 645-652 [PMID: 9725921 DOI: 10.1056/NEJM199809033391001]
57 **Cohn JA**, Friedman KJ, Noone PG, Knowles MR, Silverman LM, Jowell PS. Relation between mutations of the cystic fibro-

sis gene and idiopathic pancreatitis. *N Engl J Med* 1998; **339**: 653-658 [PMID: 9725922 DOI: 10.1056/NEJM199809033391002]
58 **Welsh M**, Ramsey BW, Accurso E and Cutting GR. Cystic Fibrosis: In the molecular and metabolic basis of inherited Disease. (ed A.B.CL Scriver, WS Sly & D. Valle). New York: Mc-Graw Hill, 2001: 5121-5188
59 **Rowntree RK**, Harris A. The phenotypic consequences of CFTR mutations. *Ann Hum Genet* 2003; **67**: 471-485 [PMID: 12940920 DOI: 10.1046/j.1469-1809.2003.00028.x]
60 **Haardt M**, Benharouga M, Lechardeur D, Kartner N, Lukacs GL. C-terminal truncations destabilize the cystic fibrosis transmembrane conductance regulator without impairing its biogenesis. A novel class of mutation. *J Biol Chem* 1999; **274**: 21873-21877 [PMID: 10419506 DOI: 10.1074/jbc.274.31.21873]
61 **Ockenga J**, Stuhrmann M, Ballmann M, Teich N, Keim V, Dörk T, Manns MP. Mutations of the cystic fibrosis gene, but not cationic trypsinogen gene, are associated with recurrent or chronic idiopathic pancreatitis. *Am J Gastroenterol* 2000; **95**: 2061-2067 [PMID: 10950058 DOI: 10.1111/j.1572-0241.2000.02228.x]
62 **Noone PG**, Zhou Z, Silverman LM, Jowell PS, Knowles MR, Cohn JA. Cystic fibrosis gene mutations and pancreatitis risk: relation to epithelial ion transport and trypsin inhibitor gene mutations. *Gastroenterology* 2001; **121**: 1310-1319 [PMID: 11729110]
63 **Audrézet MP**, Chen JM, Le Maréchal C, Ruszniewski P, Robaszkiewicz M, Raguénès O, Quéré I, Scotet V, Férec C. Determination of the relative contribution of three genes-the cystic fibrosis transmembrane conductance regulator gene, the cationic trypsinogen gene, and the pancreatic secretory trypsin inhibitor gene-to the etiology of idiopathic chronic pancreatitis. *Eur J Hum Genet* 2002; **10**: 100-106 [PMID: 11938439 DOI: 10.1038/sj/ejhg/5200786]
64 **Saurer L**, Reber P, Schaffner T, Büchler MW, Buri C, Kappeler A, Walz A, Friess H, Mueller C. Differential expression of chemokines in normal pancreas and in chronic pancreatitis. *Gastroenterology* 2000; **118**: 356-367 [PMID: 10648464]
65 **Papachristou GI**, Sass DA, Avula H, Lamb J, Lokshin A, Barmada MM, Slivka A, Whitcomb DC. Is the monocyte chemotactic protein-1 -2518 G allele a risk factor for severe acute pancreatitis? *Clin Gastroenterol Hepatol* 2005; **3**: 475-481 [PMID: 15880317]
66 **Chen WC**, Nie JS. Genetic polymorphism of MCP-1-2518, IL-8-251 and susceptibility to acute pancreatitis: a pilot study in population of Suzhou, China. *World J Gastroenterol* 2008; **14**: 5744-5748 [PMID: 18837094 DOI: 10.3748/wjg.14.5744]
67 **Hofner P**, Balog A, Gyulai Z, Farkas G, Rakonczay Z, Takács T, Mándi Y. Polymorphism in the IL-8 gene, but not in the TLR4 gene, increases the severity of acute pancreatitis. *Pancreatology* 2006; **6**: 542-548 [PMID: 17124436 DOI: 10.1159/000097363]
68 **Dinarello CA**, Gelfand JA, Wolff SM. Anticytokine strategies in the treatment of the systemic inflammatory response syndrome. *JAMA* 1993; **269**: 1829-1835 [PMID: 8459516 DOI: 10.1001/jama.1993.03500140081040]
69 **Cavaillon JM**, Adrie C, Fitting C, Adib-Conquy M. Reprogramming of circulatory cells in sepsis and SIRS. *J Endotoxin Res* 2005; **11**: 311-320 [PMID: 16263005 DOI: 10.1177/09680519050110050901]
70 **Matsuda N**, Hattori Y. Systemic inflammatory response syndrome (SIRS): molecular pathophysiology and gene therapy. *J Pharmacol Sci* 2006; **101**: 189-198 [PMID: 16823257]
71 **de-Madaria E**, Martínez J, Sempere L, Lozano B, Sánchez-Payá J, Uceda F, Pérez-Mateo M. Cytokine genotypes in acute pancreatitis: association with etiology, severity, and cytokine levels in blood. *Pancreas* 2008; **37**: 295-301 [PMID: 18815552 DOI: 10.1097/MPA.0b013e31816726d5]
72 **Tukiainen E**, Kylänpää ML, Puolakkainen P, Kemppainen E, Halonen K, Orpana A, Methuen T, Salaspuro M, Haapiainen R, Repo H. Polymorphisms of the TNF, CD14, and HSPA1B genes in patients with acute alcohol-induced pancreatitis. *Pancreas* 2008; **37**: 56-61 [PMID: 18580445 DOI: 10.1097/MPA.0b013e31815d9bad]
73 **Powell JJ**, Fearon KC, Siriwardena AK, Ross JA. Evidence against a role for polymorphisms at tumor necrosis factor, interleukin-1 and interleukin-1 receptor antagonist gene loci in the regulation of disease severity in acute pancreatitis. *Surgery* 2001; **129**: 633-640 [PMID: 11331456 DOI: 10.1067/msy.2001.113375]
74 **Sargen K**, Demaine AG, Kingsnorth AN. Cytokine gene polymorphisms in acute pancreatitis. *JOP* 2000; **1**: 24-35 [PMID: 11852287]
75 **Balog A**, Gyulai Z, Boros LG, Farkas G, Takács T, Lonovics J, Mándi Y. Polymorphism of the TNF-alpha, HSP70-2, and CD14 genes increases susceptibility to severe acute pancreatitis. *Pancreas* 2005; **30**: e46-e50 [PMID: 15714129]
76 **Zhang DL**, Li JS, Jiang ZW, Yu BJ, Tang XM, Zheng HM. Association of two polymorphisms of tumor necrosis factor gene with acute biliary pancreatitis. *World J Gastroenterol* 2003; **9**: 824-828 [PMID: 12679941]
77 **Yang Z**, Qi X, Wu Q, Li A, Xu P, Fan D. Lack of association between TNF-α gene promoter polymorphisms and pancreatitis: a meta-analysis. *Gene* 2012; **503**: 229-234 [PMID: 22579868 DOI: 10.1016/j.gene.2012.04.057]
78 **Zhao HF**, Ito T, Gibo J, Kawabe K, Oono T, Kaku T, Arita Y, Zhao QW, Usui M, Egashira K, Nawata H. Anti-monocyte chemoattractant protein 1 gene therapy attenuates experimental chronic pancreatitis induced by dibutyltin dichloride in rats. *Gut* 2005; **54**: 1759-1767 [PMID: 16284287 DOI: 10.1136/gut.2004.049403]
79 **Cavestro GM**, Zuppardo RA, Bertolini S, Sereni G, Frulloni L, Okolicsanyi S, Calzolari C, Singh SK, Sianesi M, Del Rio P, Leandro G, Franzè A, Di Mario F. Connections between genetics and clinical data: Role of MCP-1, CFTR, and SPINK-1 in the setting of acute, acute recurrent, and chronic pancreatitis. *Am J Gastroenterol* 2010; **105**: 199-206 [PMID: 19844201 DOI: 10.1038/ajg.2009.611]
80 **Nie JS**, Chen WC. [Relationship between genetic polymorphism of MCP-1 and acute pancreatitis in Han population of Suzhou in China]. *Zhonghua Yixue Yichuanxue Zazhi* 2007; **24**: 598-600 [PMID: 17922439]
81 **Sass DA**, Papachristou GI, Lamb J, Barmada MM, Brand RE, Money ME, Hawes RH, Cotton PB, Slivka A, Whitcomb DC. The MCP-1 -2518 A/G polymorphism is not a susceptibility factor for chronic pancreatitis. *Pancreatology* 2006; **6**: 297-300 [PMID: 16636603]
82 **Marrache F**, Tu SP, Bhagat G, Pendyala S, Osterreicher CH, Gordon S, Ramanathan V, Penz-Osterreicher M, Betz KS, Song Z, Wang TC. Overexpression of interleukin-1beta in the murine pancreas results in chronic pancreatitis. *Gastroenterology* 2008; **135**: 1277-1287 [PMID: 18789941 DOI: 10.1053/j.gastro.2008.06.078]
83 **Yin YW**, Sun QQ, Feng JQ, Hu AM, Liu HL, Wang Q. Influence of interleukin gene polymorphisms on development of acute pancreatitis: a systematic review and meta-analysis. *Mol Biol Rep* 2013; **40**: 5931-5941 [PMID: 24072654 DOI: 10.1007/s11033-013-2700-6]
84 **Benigni F**, Atsumi T, Calandra T, Metz C, Echtenacher B, Peng T, Bucala R. The proinflammatory mediator macrophage migration inhibitory factor induces glucose catabolism in muscle. *J Clin Invest* 2000; **106**: 1291-1300 [PMID: 11086030]
85 **Makhija R**, Kingsnorth A, Demaine A. Gene polymorphisms of the macrophage migration inhibitory factor and acute pancreatitis. *JOP* 2007; **8**: 289-295 [PMID: 17495357]
86 **Whitcomb DC**, LaRusch J, Krasinskas AM, Klei L, Smith JP, Brand RE, Neoptolemos JP, Lerch MM, Tector M, Sandhu BS, Guda NM, Orlichenko L, Alkaade S, Amann ST, Anderson MA, Baillie J, Banks PA, Conwell D, Coté GA, Cotton PB, DiSario J, Farrer LA, Forsmark CE, Johnstone M, Gardner TB, Gelrud A, Greenhalf W, Haines JL, Hartman DJ, Hawes

RA, Lawrence C, Lewis M, Mayerle J, Mayeux R, Melhem NM, Money ME, Muniraj T, Papachristou GI, Pericak-Vance MA, Romagnuolo J, Schellenberg GD, Sherman S, Simon P, Singh VP, Slivka A, Stolz D, Sutton R, Weiss FU, Wilcox CM, Zarnescu NO, Wisniewski SR, O'Connell MR, Kienholz ML, Roeder K, Barmada MM, Yadav D, Devlin B. Common genetic variants in the CLDN2 and PRSS1-PRSS2 loci alter risk for alcohol-related and sporadic pancreatitis. *Nat Genet* 2012; **44**: 1349-1354 [PMID: 23143602 DOI: 10.1038/ng.2466]

87 **Witt H**, Beer S, Rosendahl J, Chen JM, Chandak GR, Masamune A, Bence M, Szmola R, Oracz G, Macek M, Bhatia E, Steigenberger S, Lasher D, Bühler F, Delaporte C, Tebbing J, Ludwig M, Pilsak C, Saum K, Bugert P, Masson E, Paliwal S, Bhaskar S, Sobczynska-Tomaszewska A, Bak D, Balascak I, Choudhuri G, Nageshwar Reddy D, Rao GV, Thomas V, Kume K, Nakano E, Kakuta Y, Shimosegawa T, Durko L, Szabó A, Schnúr A, Hegyi P, Rakonczay Z, Pfützer R, Schneider A, Groneberg DA, Braun M, Schmidt H, Witt U, Friess H, Algül H, Landt O, Schuelke M, Krüger R, Wiedenmann B, Schmidt F, Zimmer KP, Kovacs P, Stumvoll M, Blüher M, Müller T, Janecke A, Teich N, Grützmann R, Schulz HU, Mössner J, Keim V, Löhr M, Férec C, Sahin-Tóth M. Variants in CPA1 are strongly associated with early onset chronic pancreatitis. *Nat Genet* 2013; **45**: 1216-1220 [PMID: 23955596 DOI: 10.1038/ng.2730]

88 **Ellis I**, Lerch MM, Whitcomb DC. Genetic testing for hereditary pancreatitis: guidelines for indications, counselling, consent and privacy issues. *Pancreatology* 2001; **1**: 405-415 [PMID: 12120217 DOI: 10.1159/000055840]

89 **Whitcomb DC**. Motion--genetic testing is useful in the diagnosis of nonhereditary pancreatic conditions: arguments for the motion. *Can J Gastroenterol* 2003; **17**: 47-52 [PMID: 12560855]

90 **Cohn JA**. Motion--genetic testing is useful in the diagnosis of nonhereditary pancreatic conditions: arguments against the motion. *Can J Gastroenterol* 2003; **17**: 53-55 [PMID: 12560856]

91 **Sweeney JT**, Ulrich CD. Genetics of pancreatic disease. *Clin Perspect Gastroenterol* 2002; **5**: 110-116

92 **Lowenfels AB**, Maisonneuve P, Whitcomb DC, Lerch MM, DiMagno EP. Cigarette smoking as a risk factor for pancreatic cancer in patients with hereditary pancreatitis. *JAMA* 2001; **286**: 169-170 [PMID: 11448279 DOI: 10.1001/jama.286.2.169]

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TOPIC HIGHLIGHT

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Molecular markers and imaging tools to identify malignant potential in Barrett's esophagus

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Abstract

Due to its rapidly rising incidence and high mortality, esophageal adenocarcinoma is a major public health concern, particularly in Western countries. The steps involved in the progression from its predisposing condition, gastroesophageal reflux disease, to its premalignant disorder, Barrett's esophagus, and to cancer, are incompletely understood. Current screening and surveillance methods are limited by the lack of population-wide utility, incomplete sampling of standard biopsies, and subjectivity of evaluation. Advances in endoscopic ablation have raised the hope of effective therapy for eradication of high-risk Barrett's lesions, but improvements are needed in determining when to apply this treatment and how to follow patients clinically. Researchers have evaluated numerous potential molecular biomarkers with the goal of detecting dysplasia, with varying degrees of success. The combination of biomarker panels with epidemiologic risk factors to yield clinical risk scoring systems is promising. New approaches to sample tissue may also be combined with these biomarkers for less invasive screening and surveillance. The development of novel endoscopic imaging tools in recent years has the potential to markedly improve detection of small foci of dysplasia *in vivo*. Current and future efforts will aim to determine the combination of markers and imaging modalities that will most effectively improve the rate of early detection of high-risk lesions in Barrett's esophagus.

Key words: Barrett's esophagus; Esophageal adenocarcinoma; Gastroesophageal reflux disease; Dysplasia; Biomarkers; Endoscopic imaging

Core tip: This review highlights recent advances and future directions in biomarker development and endoscopic imaging technology for identification of patients at risk of malignant progression of Barrett's esophagus.

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INTRODUCTION

Esophageal adenocarcinoma (EAC) has increased in incidence in the United States and other Western countries by at least six-fold in the past three decades, making it the cancer with the most rapid rise in incidence[1]. Prognosis is dismal at the time of diagnosis, with a five-year survival rate that remains below 20%[2]. This is particularly sobering in light of the longstanding recognition of Barrett's esophagus as a premalignant condition and of the technological advancements allowing for improved early detection and intervention.

Barrett's esophagus (BE) is defined as a replacement of normal squamous epithelium in the esophagus with columnar mucosa (endoscopic diagnosis), which is confirmed by biopsy as intestinal metaplasia (histologic diagnosis). Debate persists regarding the histologic requirement (such as presence of goblet cells) as well as the lack of distinction between short and long segment BE[3]. It is the leading risk factor for EAC, conferring a relative risk of 30-60 compared with that of the general population[4]. The pathophysiology of Barrett's metaplasia is incompletely understood but is related to chronic damage from gastric acid and bile reflux[5]. Strong association has been demonstrated between chronic gastroesophageal reflux disease (GERD) and both BE and EAC[6-8], but the nature of the progression from GERD to BE to EAC is less clear[9].

While BE is found in 5%-10% of patients with chronic GERD, most patients do not progress to EAC[10]. Moreover, most EAC are diagnosed incidentally, without a known history of GERD or BE[11], and quite often in advanced stages less amenable to cure. Thus from a public health standpoint, the key questions are: which members of the general population should be screened for BE, which patients with BE are likely to progress to EAC, and what surveillance program is appropriate[12]. In this review, we discuss the current understanding of Barrett's progression, recent advances in biomarker and endoscopic imaging development, and implications for future research and clinical practice.

EPIDEMIOLOGICAL MARKERS

In addition to chronic GERD, several risk factors for BE are well-established, including age over 50 years, male sex, white race, obesity, intra-abdominal fat distribution, and presence of hiatal hernia. Screening endoscopy may be appropriate for patients meeting several of these criteria[3,13]. Unfortunately, the vast majority of patients diagnosed with EAC have no prior diagnosis of BE, and many patients diagnosed with BE have no prior GERD symptoms[9].

CURRENT SURVEILLANCE PRACTICES

Current United States society guidelines recommend endoscopic surveillance of patients with documented BE[3,9,13] for the presence of EAC or its precursor lesions low-grade or high-grade dysplasia (LGD or HGD, respectively). This consists of regularly scheduled white light endoscopy with four-quadrant biopsies taken at 2 cm intervals, or 1 cm in patients with known or suspected dysplasia (Seattle protocol)[13]. Even when applied rigorously, this approach samples only a small fraction of the mucosal surface, and retrospective evidence suggests that in practice, the number of biopsies taken is often considerably lower than recommended, creating further sampling error[14]. This is especially problematic since early dysplastic lesions typically occur as small foci and can readily evade detection by the standard endoscopic biopsy practice regimen. Furthermore, these biopsy samples are typically not fully sectioned and examined; instead only a few sections from each sample are reviewed, which represents yet another order or two of magnitude decrease in actual tissue examined[15]. The present definitions of LGD and HGD are based on morphologic distinctions as graded by a pathologist; although interobserver reproducibility has been shown to be high at the ends of the spectrum (BE *vs* HGD or EAC), there appears to be considerable variation in separating nondysplastic BE from LGD or indeterminate dysplasia[12,16]. This non-concordance is even greater in the community setting, where a recent study demonstrated marked over-diagnosis of LGD following review of samples by a panel of expert pathologists[17].

These distinctions are important in practice because they have bearing on the likelihood of progression to EAC and consequently the need for close surveillance or intervention. For example, in the aforementioned study, patients with a consensus histologic diagnosis of LGD went on to develop HGD or EAC at a rate of 13% per year, whereas those downgraded to nondysplastic BE (NDBE) progressed at a rate of only 0.49% per year[17], although other studies suggest a lower incidence of LGD to HGD/EAC progression[18,19]. This is in keeping with data from recent large multicenter studies and meta-analyses, which estimate a low overall rate of progression from NDBE to EAC, on the order of 0.12%-0.38% per year, with very low mortality from EAC[19-21]. These findings, coupled with a lack of strong evidence showing mortality benefit, have led some health economists to argue that routine endoscopic surveillance of all patients with BE is likely not cost-effective[22], although at present it remains supported by guidelines[3,13].

ADVANCES IN THERAPY

Recent years have also seen the development and evaluation of endoscopic ablative techniques for dysplastic BE, which hold the promise of cancer prevention analogous to the current practice of polyp resection in the colon. Endoscopic mucosal resection has proven to be an effective therapeutic intervention in many patients with HGD or even intramucosal carcinoma and is associated with lower morbidity than surgical resection, although risk of cancer recurrence is higher in patients with lesions not strictly confined to the mucosa[23,24]. Radiofrequency ablation (RFA) has been shown to have high efficacy in the eradication of dysplasia and intestinal metaplasia as well as a good safety profile[25], and this effect appears to be durable[26]. In light of these encouraging findings and the high mortality of EAC, some experts have reintroduced the question of whether all BE should be ablated[27,28]. At present, while it appears to be cost-effective to ablate all HGD, it is less clear whether ablation of all LGD or NDBE is reasonable public health policy[29]. In addition, such efforts are complicated

by the presence of subsquamous intestinal metaplasia (SSIM), or "buried Barrett's," which can persist after ablation, is difficult to detect using current practice methods, and whose significance as a premalignant condition is as yet undetermined[30].

NEED FOR NEW BIOMARKERS

In this context, the main unresolved issue in BE management is to improve identification of those patients at highest risk for developing EAC.

The term "biomarker" broadly encompasses physiologic measurements, molecular analyses, or endoscopic or imaging findings[31]. The National Cancer Institute has established an Early Detection Research Network, which has developed a recommended biomarker validation pipeline encompassing a discovery phase, translational phase, and clinical implementation phase[32]. An ideal biomarker would objectively detect all dysplastic BE without significant false-positive results leading to unnecessary testing and intervention. As discoveries of such markers are few and far between, it is more realistic to expect that some combination of less-perfect markers will ultimately prove useful for the risk stratification of patients with BE. Many of the recent advances in biomarker research can be grouped into the categories of molecular markers and endoscopic imaging tools.

MOLECULAR MARKERS FOR DYSPLASTIC PROGRESSION

Much effort has been devoted in recent years to the search for a molecular marker that can serve as an adjunct to endoscopic and histologic surveillance in predicting malignant potential in BE. A recent comprehensive review of investigated and published molecular markers classifies them along the GERD-BE-EAC axis according to their potential usage as either diagnostic tools, indicators of progression, predictors of response to therapy, or aids in prognosis[33]. Of course, some markers span several of these denominations. Most of the hundreds of markers being evaluated are not yet approaching clinical utility, and another recent review article, using the same categories, discusses what requirements remain for clinical implementation of several of the more promising markers, such as larger prospective studies and external validation[31]. Since many of the molecular markers under investigation involve the differential expression of genes from normal to BE to dysplasia to EAC, another way to categorize these approaches could be where they fall along the axis of DNA to RNA to protein. Again, in some cases the same marker may be detected at multiple points along this axis.

Genetic coding

A hereditary component to BE and EAC has long been postulated[34] with reports of familial clustering, but most evidence has favored environmental rather than genetic risk factors[4]. A recent genome-wide association study, using large population-based epidemiological databases, compared patients with EAC to those with BE and normal controls. The authors report extensive polygenic overlap between BE and EAC and interpret this as evidence that the genetic basis for EAC is already present at the development of BE rather than occurring during progression. They identify several loci having strong association with both conditions, namely 19p13 in the oncogene-associated transcription coactivator gene *CRTC1*, 9q22 in esophageal speciation transcription factor gene *BARX1*, 3p14 near esophageal development transcription factor gene *FOXP1*, and 16p24 near the putative tumor suppressor gene *FOXF1*[35]. Further investigation will be needed to examine the clinical utility of genomic investigation as a screening or surveillance tool.

DNA content abnormalities are common among malignancies and preneoplastic states and involve all chromosomes. Several studies have demonstrated that such abnormalities, including aneuploidy, tetraploidy, and loss of heterozygosity at 17p and 9p loci, which affect the tumor suppressors p53 and p16, respectively, occur in EAC and may precede progression to cancer by up to 10 years[6,36]. Impressively, patients with all of these abnormalities in the setting of BE were found in one cohort to have a relative risk of EAC progression of 38.7 compared to patients with BE and none of the DNA abnormalities[36].

Epigenetics: DNA methylation

The role of epigenetics, defined as cellular information other than the DNA sequence itself that is heritable during cell division, in cancer development has been the subject of a growing body of literature since the 1980s[37]. An important epigenetic alteration is DNA methylation, which occurs almost exclusively at CpG nucleotides, found in high numbers in promoter regions, and is involved in the regulation of gene expression and silencing[37,38]. In malignancies, this may involve hypermethylation and consequent transcriptional repression of tumor suppressor genes or hypomethylation and increased expression of oncogenes[39].

Several recent studies have examined the role of DNA methylation in BE and EAC development. A genome-wide profiling, using microarray and hierarchical clustering analysis, of CpG methylation in esophageal tissue samples found that there was substantial difference in methylation pattern between normal esophagus samples and those with BE or EAC, but that the difference between BE and EAC was less clear[38]. This finding also suggests that the epigenetic, as well as genetic, alterations present in EAC may already be present in BE, thus suggesting potential markers for BE surveillance. However, a significant limitation of this study was that all of the BE samples were obtained from patients who developed EAC, as opposed to the vast majority of cases of BE that do not progress[38]. This weakness would become strength if future work demonstrates differences in methylation

patterns of these pre-malignant BE samples from those of nonprogressing, nondysplastic BE.

This was addressed by another recent study, which used DNA methylation arrays to differentiate between BE and EAC in tissue samples. This work delineated four genes (*SLC22A18*, *PIGR*, *GJA2*, and *RIN2*) which, when taken together, had an excellent receiver operating characteristic curve (AUC = 0.988) to distinguish BE from EAC. The authors applied this 4-gene methylation panel to a prospective multicenter study and presented evidence that it can detect nearby dysplasia or early neoplasia in endoscopic biopsies of BE even in the absence of visible histologic change in that particular sample, suggesting a field effect as observed in other types of malignancy. They proposed that patients with BE can be stratified into low, medium, and high risk of malignant progression using this panel as an adjunct to histopathologic evaluation but cautioned that follow-up data on its predictive power is not yet available[40].

Other publications focus on differential methylation of individual genes. As an example, endoglin, or ENG, is a transmembrane glycoprotein with a role in angiogenesis; hypermethylation of its encoding gene's promoter region has been associated with several cancers. Recently, this hypermethylation was found in human esophageal tissue, with frequency of 11.9% in normal esophagus and increasing sequentially to 13.3% in BE, 25% in dysplastic BE, and 26.9% in EAC. However, the frequency of ENG hypermethylation is greater in esophageal squamous cell carcinoma and thus may be more useful as a biomarker for this malignancy[41].

Epigenetics: microRNA

Another active field of research in cancer epigenetic markers is the use of microRNA (miRNA) signatures. MiRNAs are small, non-coding RNAs that regulate RNA translation including that of oncogenes and tumor suppressors; the current state of this research in esophageal cancer has recently been reviewed[42]. Based on analysis of multiple recent studies on miRNA in EAC and BE, the reviewers found that four miRNAs (miR-25, -99a, -133a, and -133b) have potential as diagnostic markers and five (miR-21, -27b, -126, -143, and -145) may have utility as both diagnostic and prognostic markers[42].

Two studies not included in the aforementioned review due to their very recent publication sought to assess the miRNA signature of BE and EAC using microarray analyses and hierarchical clustering, much like the DNA methylation studies described above and with similar results. A genome-wide analysis of miRNA expression levels showed clustering of BE and EAC signatures together as compared with that of normal esophageal tissue but interspersing of BE and EAC signals[43]. However, another study using microarray analysis showed a distinct pattern in EAC, with different patterns of up- and down-regulation seen in EAC compared with BE. This study also showed two miRNAs which were up-regulated in BE tissue adjacent to HGD lesions, again suggestive of a field effect for dysplasia that may be clinically useful alongside histologic surveillance[44].

Protein markers

The vast majority of molecular biomarker research in EAC has focused on differential expression of proteins in esophageal tissue. There are several recent review articles describing the state of this research, including a comprehensive list[33] and additional analysis[31], among others. Several promising and recently investigated classes of markers are described here.

One of the best-described cancer-associated proteins is the tumor suppressor p53. In a recently published large prospective case-control study, aberrant p53 expression by immunohistochemistry of biopsy samples was found to have a higher predictive value for neoplastic progression in BE than histologic diagnosis of LGD with strong inter-observer agreement among scoring pathologists. This association was seen with p53 overexpression, but even more strongly with loss of normal p53 expression[45]. This adds further support to prior studies using p53, including a case-control study which showed that using a combination of aneuploidy and overexpression of transcription factor Ki67 and p53 was predictive of neoplastic progression to HGD or EAC, independent of histology[46]. Another well-known protein in the cancer literature is human epidermal growth factor-2 (HER2), a proto-oncogene notorious for its role in predicting clinically aggressive breast cancers. A recent study using immunohistochemical and fluorescent in-situ hybridization methods on samples from patients with EAC showed a correlation between HER2 expression and p53 overexpression as well as early lesion protrusion[47].

Caudal homeobox transcription factor-2 (Cdx-2) is an intestine-specific transcription factor, but is expressed in BE, activated by acid and bile according to *in vitro* studies. It appears to help direct the development of intestinal metaplasia in BE[5]. Recent histologic and epigenetic research suggests that the encoding gene's promoter region is hypermethylated in HGD and intramucosal EAC; Cdx2 expression was correspondingly downregulated in dysplasia compared with BE metaplasia but restored in poorly differentiated invasive cancer, demonstrating gene silencing memory[48].

Stem cell markers have also received considerable attention as predictors of dysplasia and neoplasia in BE, in light of a newer theory of BE development and progression involving the activation of pluripotent esophageal stem cells to develop intestinal metaplasia in response to gastric acid and bile[5]. Leucine-rich repeat-containing G protein-coupled receptor 5 (Lgr5), a downstream target of the Wnt pathway and an intestinal stem cell marker, has been identified in immunohistochemical analyses of BE and shown to have increased expression in HGD and EAC as well as an apparent association with poor survival[49]. Doublecortin and CaM kinase-like-1, also a putative gastrointestinal stem cell marker, similarly has shown a progressive increase in expression from BE to dysplasia

to EAC by immunohistochemistry[50].

Cell signaling to control such processes as proliferation and apoptosis is tightly regulated by receptor tyrosine kinases (RTKs). Disruption of this balance is a common factor in various types of cancer[51]. A recent report showed increased expression and gene copy numbers of tyrosine kinase EPHB4 in both squamous cancer and adenocarcinoma of the esophagus, with corresponding supporting evidence in mouse and cell culture models[52]. Among the RTKs felt to be most promising as markers in EAC are EGFR, ErbB2, ErbB3, FGFR2, and Met, which have been shown to be up-regulated at early stages in dysplasia[53]. However, they have thus far met with mixed results as predictors of malignant progression, perhaps in part due to their heterogeneous expression among individual cancers[54]. A recent study described this heterogeneity using an RTK array; these differentially expressed proteins have great promise in therapeutics as targets of individualized therapy using different tyrosine kinase inhibitors depending on the RTK expressed *in vitro*[54]. For example, antibodies to EGFR and HER2 are promising therapeutic treatments for EACs expressing these particular RTKs[55-58]. The MAPK pathway, downstream of these individually varied RTKs, was frequently activated in pre-malignant and malignant states in human gene expression, representing another potential target for surveillance and treatment[54].

Another class of proteins known to have involvement in malignancy is that of mucins; these secreted and transmembrane glycoproteins function in limiting the activation of inflammatory responses and may become deregulated in states of chronic inflammation, leading to impaired epithelial repair and malignant transformation[59]. Based on initial immunohistochemistry analysis, regulation of different mucin proteins may be involved in BE progression, with decreased expression of the mucin aG1cNAc observed in Barrett's epithelium adjacent to EAC compared with nondysplastic BE when controlled for expression of the scaffold protein MUC6[60].

Literature on the use of NSAIDs including aspirin as therapy for BE has also evolved, and has included the use of prostaglandin E2 (PGE2) as a surrogate endpoint marker. PGE2 is associated with up-regulation of proliferation, resistance to apoptosis, angiogenesis, and increased cellular invasiveness and thus has a theoretically sound basis and utility in these research studies. However, it will need further validation for use as a clinical biomarker[61].

Molecular marker panels and associated conditions

Given the limitations and early phase trials of each of the above and many other candidate molecular markers when assessed alone, it is appealing to consider combining them as a panel and using with other associated risk factors to achieve better predictability of dysplastic progression. For example, given the known association between obesity and EAC has been shown[6], it stands to reason that markers of obesity may be predictive of malignant transformation. Indeed, in patients enrolled in the Seattle BE study (all with BE), increased levels of leptin and insulin resistance were associated with increased EAC risk, while increased high-molecular-weight adiponectin was inversely correlated with EAC[62].

A recent analysis of data from a nested case-control study assessed the utility of a panel of several established biomarkers (abnormal DNA content, p53, and cyclin A expression) and newer biomarkers (levels of sialyl Lewis-a, Lewis-x, and Aspergillus oryzae lectin (AOL) and binding of wheat germ agglutinin) on tissue samples from patients diagnosed with BE who either progressed or did not progress to EAC (cases and controls). A conditional logistic regression analysis was employed, which identified the best panel for risk prediction, consisting of LGD, abnormal DNA ploidy, and AOL. This panel of biomarkers conferred an odds ratio of 3.7 for EAC progression[63].

IMPROVING SAMPLING: NON-ENDOSCOPIC METHODS

A major limitation of the current molecular markers discussed above is that, no matter how sensitive or specific they may be in detecting dysplasia, they depend on adequate tissue sampling by random biopsies. Given the limitations of current endoscopic sampling practices as discussed above, a major remaining challenge is to improve the yield of tissue sampling. One approach relies on "field effect" of malignancies. This refers to the concept that genetic and environmental factors create a broad field of injury, upon which further insult leads to the formation of focal neoplasia[64]. As discussed above, some markers were present not only in areas of dysplasia or neoplasia but also in adjacent tissue, and the majority of genetic and epigenetic abnormalities were found to be already present in pre-dysplastic BE, illustrating this concept. A recent study investigated whether brushings from proximal squamous epithelium in patients with distal EAC exhibited intracellular nanoarchitectural changes as measured by partial wave spectroscopic microscopy, a technology that measures intracellular spatial distribution. Significant differences were observed using this technique, which is encouraging as it could allow for detection of distant malignancy with a minimally invasive approach[65]. However, by the time EAC is present it is often too late to intervene effectively, and it is presently unknown if a similar approach would detect earlier phases of dysplasia.

Several non-endoscopic techniques for screening and surveillance have garnered attention in recent years. One that has shown promise as a potential screening tool in the primary care setting is the Cytosponge. This is a sample acquisition technique in which a pill is swallowed following which a sponge expands in the stomach and is withdrawn *via* the esophagus, brushing off cells in the process. This is safe and well tolerated by patients in initial studies and has diagnostic potential when combined with a potential BE biomarker trefoil factor 3[66]. A microsimulation model predicts that screening 50-year-old

men with GERD using this technology would be cost-effective and reduce mortality[67]. A higher-tech approach to screening and perhaps surveillance is tethered capsule endomicroscopy, in which a pill-sized optical coherence tomography (OCT, see below) probe is swallowed and has the capability to obtain microstructure level imaging of the entire esophagus without requiring sedation[68].

SERUM BIOMARKERS

Although these less-invasive techniques show promise for reducing sampling error and achieving a broader screening population, they do not have the ease of use of a simple blood test. Researchers are working to find a biomarker that is present in the serum that could objectively aid in assessing risk of malignant transformation. Though such a marker has thus far proven elusive, several groups have demonstrated promising findings using antibodies to the well-described tumor protein p53. These antibodies form in response to overexpression of mutant p53 protein in patients with a variety of malignancies and are rare in serum from healthy control patients[69]. A study of serum samples of patients under endoscopic surveillance found a small number of patients who had detectable anti-p53 antibodies in serum samples taken before they were diagnosed with cancer[70]. A meta-analysis of 15 studies found that patients with esophageal cancer were approximately 7 times more likely to have serum p53 antibodies than those without cancer, but the marker was limited by poor and variable sensitivity[71]. A recent case report describes the post-operative surveillance of a patient with EAC over four years, showing lower titers of anti-p53 antibody in the serum after resection and suggesting utility of this marker to detect residual cancer in such patients[72]. These findings support the use of anti-p53 antibodies as a potential surveillance tool in patients with known BE or EAC, but its utility as a screening test in a broader population is not yet clear.

Panels including several biomarkers in combination may prove superior to individual markers alone in screening serum samples. Recently, use of serum biomarker panels was evaluated as a potential screening tool for the presence of BE in a VA population[73]. The best panel in this study included serum levels of several cytokines (IL 12p70, IL6, IL8, IL10), leptin, GERD frequency and duration, age, sex, race, waist-to-hip ratio, and *H. pylori* status. These were combined to give a biomarker risk score, with the highest equal to a 10-fold increase in risk of BE[73].

ENDOSCOPIC IMAGING TECHNIQUES

The mainstay of screening and surveillance of BE is standard white light endoscopy. Particularly with the increased resolution and high-definition monitors in current use, endoscopy is a successful screening modality as it allows for excellent visualization and the ability to sample tissue[6,74]. Dysplasia detection has been shown to increase with longer inspection time in patients with BE[75], a finding with clear relevance to the use of endoscopy as a surveillance tool. However, dependence on endoscopic surveillance with four-quadrant biopsies has to date not been successful in decreasing mortality from EAC and has raised concerns of cost-effectiveness, as mentioned above. Thus, a number of enhancements to conventional endoscopy are being explored to achieve more effective surveillance. An ideal imaging tool would improve objectivity, have a wide area of surveillance, produce results rapidly in real time, and have improved sensitivity and specificity for the detection of dysplasia compared to white light endoscopy. Current modalities in practice and under investigation were recently reviewed[74] and are discussed here.

Chromoendoscopy

The oldest and most "low-tech" of the available endoscopic image enhancements, chromoendoscopy involves the application of stains to mucosal surfaces during endoscopy to enhance visualization of musical surfaces. These stains are characterized as absorptive (*e.g.,* Lugol's iodine, methylene blue, toluidine blue), reactive (Congo read, phenol red), and contrast (indigo carmine)[76]. Methylene blue has been well studied in BE due to its propensity to stain intestinal metaplasia consistent with BE while sparing gastric mucosa, which may be useful for diagnosing short segment BE[74,77]. Widespread use of chromoendoscopy has been limited by variability of staining, laborious effort, and unclear correlation with dysplasia, and there is evidence demonstrating a lack of interobserver agreement or yield identifying early neoplasia in BE with the addition of indigo carmine or acetic acid to white light images[78]. More recent advances in endoscopic imaging have allowed for combination of chromoendoscopy with optical magnification, which has led to descriptions of characteristic relief patterns known as pit patterns[79,80]. While these patterns have shown to have good sensitivity for BE detection, a recent study found them to have low specificity, which may limit their clinical utility in targeting biopsies[81].

Optical enhancements

Improvement in digital endoscope technology has made endoscopic image enhancement possible without the mess of chromoendoscopy, earning the term "virtual chromoendoscopy." Narrow band imaging (NBI, Olympus) uses specific wavelengths of light to construct an enhanced image, and flexible spectral imaging color enhancement (Fujinon) and i-Scan EPKi processor (Pentax) apply digital filters to white light images[74]. NBI has been evaluated in BE. In the same study mentioned above for chromoendoscopy, NBI similarly failed to improve diagnostic yield or interobserver agreement[78]. On the other hand, a recent study demonstrates comparable or improved rates of BE detection but with fewer biopsies compared with standard methods[82], and a meta-analysis demonstrates high accuracy and precision in diagnos-

ing HGD in BE[83]. Thus this modality appears to have potential utility as both a screening and surveillance tool. Taken together, a meta-analysis and systematic review concluded that advanced imaging techniques using chromoendoscopy or virtual chromoendoscopy were found to improve diagnostic yield for dysplasia or cancer in patients with BE compared to white light endoscopy, but there was no significant difference in yield of detection between the two advanced imaging techniques[84].

Autofluorescence and trimodal imaging

Autofluorescence imaging takes advantage of endogenous fluorophores (*e.g.,* collagen, nicotinamide, adenine dinucleotide, flavin, and porphyrins), which can be stimulated by excitation (short-wavelength) light[85]. This has the advantage over white light endoscopy of producing real-time fluorescent images that may aid in detection, but initial systems have been limited by false positives from ulcers and inflammation rather than true dysplasia[85]. More recent efforts have combined autofluorescence with magnification endoscopy and narrow-band imaging ("trimodal imaging"), providing improved visualization of microvascular and microstructural architecture in malignant and premalignant gastrointestinal lesions[86]. Endoscopic trimodal imaging has been shown to be more effective in improving the targeted detection of HGD or EAC in BE[87].

However, this advantage seemed to no longer be present when trimodal imaging was evaluated in a community setting[88].

Fluorescent lectins

A more sophisticated adaptation of chromoendoscopy involves the targeted binding of markers, which are specific to areas of dysplasia. A recent study utilized the alteration in cell-surface glycans over the progression from BE to EAC. A fluorescently-tagged lectin, wheat germ agglutinin, was sprayed over the esophageal mucosa during endoscopy and was found to have specific binding permitting visualization of high-grade dysplastic lesions that were not visible by white light endoscopy alone[89]. This type of molecular imaging has considerable promise as a surveillance tool if findings are borne out in clinical trials.

Confocal laser endomicroscopy

A number of high-tech, high-resolution imaging modalities are currently under investigation. One of these is confocal laser endomicroscopy (CLE), which is in effect an endoscopic light microscope, enabling "optical biopsy" or near-histologic level of detail and tissue enhancement *via* the application of topical or IV contrast agents[74]. Existing commercial CLE systems are endoscope-based (Optiscan, Pentax) or probe-based (Cellvizio)[74]. A multicenter randomized-control trial using probe-based CLE showed significantly improved detection of neoplasia (HGD or EAC) compared with white light endoscopy[90]. Despite high specificity, there has been some concern about sensitivity of this method, which may be related to its limited field of view[91]. Early dysplastic changes are still being characterized, including pit patterns and possible vascular changes, but these remain largely subjective in interpretation. While this technology is promising and may have a role in specialized cases, application of this expensive, time-consuming, and operator-dependent modality in the community is unlikely to occur in the near future.

Optical coherence tomography

Another promising high-tech modality is optical coherence tomography (OCT), which is a high-resolution, cross-sectional imaging technique that utilizes backscattered light waves in a manner analogous to ultrasound with sound waves[92]. It has shown promising accuracy for detection of dysplasia and may help target biopsies[93]. OCT has several advantages as a surveillance tool – it has a wider field of view than confocal microscopy but similar resolution, does not require contrast administration, allows rapid image acquisition and 3-dimensional reconstruction, and can detect subsurface changes. This latter characteristic, the ability to visualize subsurface structures at greater depth than other modalities, enables accurate assessment of BE thickness and presence of SSIM before or after ablation, which in turn correlate with ability to achieve eradication of intestinal metaplasia using RFA[30,94,95]. Like other such modalities, though, OCT is presently costly and operator-dependent and likely has more of a future in tertiary centers. Given less distal optical requirements compared to confocal microendoscopy, however, OCT can be miniaturized for potential non-endoscopic screening of BE, as recently employed using a swallowed tethered capsule[68,96].

Elastic scatter spectroscopy

Elastic scatter spectroscopy (ESS) is related to optical scattering efficiency caused by optical index gradients of cellular and subcellular structures, allowing for detailed evaluation of microstructural features such as nuclear size, crowding and chromaticity, chromatin granularity, and mitochondrial and organellar size and density[97]. This technique has shown promise in preliminary studies, notably decreasing the number of biopsies required to diagnose dysplasia compared to the Seattle protocol[98], but more prospective data is needed.

Angle-resolved low-coherence interferometry

Another novel endoscopic imaging tool is angle-resolved low-coherence interferometry (a/LCI), which uses the distribution of elastically scattered light to make depth-resolved measurements of the size and index of refraction of cell nuclei. In BE, this can be employed to evaluate dysplasia up to significant depth, and preliminary studies indicate that it is accurate in doing so[99,100].

Raman spectroscopy

Finally, a tool that is being developed at present for en-

doscopic use is Raman spectroscopy (ERS), which relies on inelastic light scattering and can assess the biochemical components of its target, notably specific molecular constituents and signals. A recently published study reports high sensitivity and specificity of HGD and EAC detection and the ability to grade dysplasia, as well as the potential to combine ERS with narrow-band imaging for clinical application[101].

FUTURE DIRECTIONS: TOWARD A TARGETED AND OBJECTIVE APPROACH

Recent years have seen considerable research efforts devoted to the development of molecular markers and endoscopic imaging techniques to improve detection rates and diagnostic accuracy for esophageal adenocarcinoma and its premalignant conditions, BE and especially dysplastic BE. A great many molecular markers have been studied and are at varied phases of biomarker development using benchmarks established by the National Cancer Institute. Thus far, no single marker alone has shown sufficient improvement in accuracy of early detection compared with current guideline-based practice to warrant widespread clinical use. Perhaps the greatest promise has been shown by panels of several markers taken along with clinical risk factors and current endoscopic surveillance practices, which can be combined to yield risk scores similar to those used as predictive models in other disease states. Future biomarker research will likely focus on improving the predictive accuracy of these models.

A significant limitation to the ability to reliably detect small early foci of dysplasia on a background of metaplasia is the current reliance on random and limited, rather than targeted, sampling. Even a molecular marker with perfect sensitivity and specificity is only as good as the sample on which it is tested. Thus a major unmet need for improving detection will require improved endoscopic imaging modalities, likely used in combination, to locate such foci of dysplasia. This can be accomplished by improving visualization of the entire mucosal surface, using techniques such as microscopy, chromoendoscopy, optical enhancements, and fluorescence, or by using novel tools like CLE, OCT, ESS, a/LCI, or ERS to obtain an "optical biopsy" of subsurface structure and microstructure. Improvement in surface imaging may require combining imaging techniques, as has been illustrated by trimodal imaging, and further developments will likely validate and improve upon these methods. Subsurface imaging efforts will further confirm the correlations between optical findings and microstructural and biochemical composition. Optimal imaging tools will have the ability to evaluate broad areas of the esophagus, quickly hone in on those areas of highest significance, and have less dependence on subjective analysis when guided by simultaneously applied appropriate biomarkers.

Another way to mitigate the problem of sampling error is to take advantage of the field effect in malignant progression. This principle is relevant both for molecular marker and endoscopic imaging research. The prospect of using non-endoscopic sampling such as sponge or brush methods is appealing as a screening tool, if it can be combined with a sufficiently accurate marker. If field effect can be adequately demonstrated with a given marker on brush or biopsy samples, random sampling would be less troublesome for diagnostic purposes. Advanced optical imaging techniques have been investigated to detect ultrastructural cellular and vascular alterations suggestive of field effect in the colon cancer literature[64], and such efforts will likely be undertaken in the esophagus as well.

Even the most advanced endoscopic imaging techniques suffer from dependence on subjective interpretation by the endoscopist during examination, much as standard histologic evaluation of biopsy samples relies upon subjective determinations by the pathologist. Limiting this subjectivity in histopathology is a key goal of molecular marker development, and similar efforts should also be made in endoscopic imaging. Taking advantage of properties like autofluorescence and specific targeting of molecules to dysplastic foci *in vivo*, it may be possible to combine advanced imaging with molecular markers to achieve this goal. An ideal system would seamlessly integrate a marker of high predictive value with imaging technology allowing for microscopic level imaging of surface and subsurface structure, allowing for objective and targeted diagnosis and therapy.

As systems emerge that reliably demonstrate superiority to conventional approaches in the early detection of dysplasia and EAC, the degree to which they can be reasonably implemented as population-wide surveillance tools will become an important focus of investigation. These techniques require highly trained operators and at present are expensive and not widely available. At the outset, it can be expected that advanced modalities will be effective tools primarily at large academic centers, which may shift the responsibility of BE surveillance toward these institutions. As more providers become trained in the use of these systems and their cost decreases, their use in community settings should become more widespread.

CONCLUSION

Current screening and surveillance methods for the early detection of esophageal adenocarcinoma remain suboptimal given this cancer's increasing incidence and high mortality. Significant challenges include limitations in tissue sampling, lack of objectivity in describing premalignant states, and difficulties in targeting diagnostic and therapeutic modalities. Advances in biomarker development, from genetic and epigenetic characteristics to protein expression profiles, new approaches to sample acquisition, and novel endoscopic imaging tools allowing for improved surface and subsurface visualization, have shown considerable promise in addressing these issues. Future research endeavors will determine which

combination of markers and imaging techniques are most effective in detecting and decreasing mortality from esophageal adenocarcinoma.

REFERENCES

1 **Pohl H**, Welch HG. The role of overdiagnosis and reclassification in the marked increase of esophageal adenocarcinoma incidence. *J Natl Cancer Inst* 2005; **97**: 142-146 [PMID: 15657344 DOI: 10.1093/jnci/dji024]

2 **Siegel R**, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA Cancer J Clin* 2014; **64**: 9-29 [PMID: 24399786 DOI: 10.3322/caac.21208]

3 **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]

4 **Lagergren J**. Adenocarcinoma of oesophagus: what exactly is the size of the problem and who is at risk? *Gut* 2005; **54** Suppl 1: i1-i5 [PMID: 15711002 DOI: 10.1136/gut.2004.041517]

5 **Souza RF**, Krishnan K, Spechler SJ. Acid, bile, and CDX: the ABCs of making Barrett's metaplasia. *Am J Physiol Gastrointest Liver Physiol* 2008; **295**: G211-G218 [PMID: 18556417 DOI: 10.1152/ajpgi.90250.2008]

6 **di Pietro M**, Fitzgerald RC. Screening and risk stratification for Barrett's esophagus: how to limit the clinical impact of the increasing incidence of esophageal adenocarcinoma. *Gastroenterol Clin North Am* 2013; **42**: 155-173 [PMID: 23452636 DOI: 10.1016/j.gtc.2012.11.006]

7 **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]

8 **Winters C**, Spurling TJ, Chobanian SJ, Curtis DJ, Esposito RL, Hacker JF, Johnson DA, Cruess DF, Cotelingam JD, Gurney MS. Barrett's esophagus. A prevalent, occult complication of gastroesophageal reflux disease. *Gastroenterology* 1987; **92**: 118-124 [PMID: 3781178]

9 **Reid BJ**, Li X, Galipeau PC, Vaughan TL. Barrett's oesophagus and oesophageal adenocarcinoma: time for a new synthesis. *Nat Rev Cancer* 2010; **10**: 87-101 [PMID: 20094044]

10 **Phillips WA**, Lord RV, Nancarrow DJ, Watson DI, Whiteman DC. Barrett's esophagus. *J Gastroenterol Hepatol* 2011; **26**: 639-648 [PMID: 21166712 DOI: 10.1111/j.1440-1746.2010.06602.x]

11 **Dulai GS**, Guha S, Kahn KL, Gornbein J, Weinstein WM. Preoperative prevalence of Barrett's esophagus in esophageal adenocarcinoma: a systematic review. *Gastroenterology* 2002; **122**: 26-33 [PMID: 11781277]

12 **Mashimo H**, Wagh MS, Goyal RK. Surveillance and screening for Barrett esophagus and adenocarcinoma. *J Clin Gastroenterol* 2005; **39**: S33-S41 [PMID: 15758657]

13 **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]

14 **Peters FP**, Curvers WL, Rosmolen WD, de Vries CE, Ten Kate FJ, Krishnadath KK, Fockens P, Bergman JJ. Surveillance history of endoscopically treated patients with early Barrett's neoplasia: nonadherence to the Seattle biopsy protocol leads to sampling error. *Dis Esophagus* 2008; **21**: 475-479 [PMID: 18430186 DOI: 10.1111/j.1442-2050.2008.00813.x]

15 **Mashimo H**. The absolutely perfect biomarker for early detection of esophageal cancer and how it would affect morbidity and mortality. *Ann N Y Acad Sci* 2014; in press

16 **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 DOI: 10.1053/hupa.2001.23510]

17 **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]

18 **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]

19 **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]

20 **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 e26 [PMID: 21115133 DOI: 10.1016/j.cgh.2010.11.008]

21 **Sikkema M**, de Jonge PJ, Steyerberg EW, Kuipers EJ. Risk of esophageal adenocarcinoma and mortality in patients with Barrett's esophagus: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2010; **8**: 235-244; quiz e32 [PMID: 19850156 DOI: 10.1016/j.cgh.2009.10.010]

22 **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]

23 **Pech O**, Bollschweiler E, Manner H, Leers J, Ell C, Hölscher AH. Comparison between endoscopic and surgical resection of mucosal esophageal adenocarcinoma in Barrett's esophagus at two high-volume centers. *Ann Surg* 2011; **254**: 67-72 [PMID: 21532466 DOI: 10.1097/SLA.0b013e31821d4bf6]

24 **Wang KK**. Endoscopic vs surgical resection for Barrett's intramucosal adenocarcinoma: beyond a therapeutic equipoise. *Gastroenterology* 2012; **143**: 257-259 [PMID: 22633771 DOI: 10.1053/j.gastro.2012.05.022]

25 **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]

26 **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]

27 **Inadomi JM**. Time to burn? Endoscopic ablation for Barrett's esophagus. *Gastroenterology* 2011; **141**: 417-419 [PMID: 21708153 DOI: 10.1053/j.gastro.2011.06.025]

28 **May A**. Barrett's esophagus: should we burn it all? *Gastrointest Endosc* 2012; **76**: 1113-1115 [PMID: 23164511 DOI: 10.1016/j.gie.2012.09.010]

29 **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]

30 **Mashimo H**. Subsquamous intestinal metaplasia after ablation of Barrett's esophagus: frequency and importance. *Curr Opin Gastroenterol* 2013; **29**: 454-459 [PMID: 23674187 DOI: 10.1097/MOG.0b013e3283622796]

31 **Varghese S**, Lao-Sirieix P, Fitzgerald RC. Identification and clinical implementation of biomarkers for Barrett's esophagus. *Gastroenterology* 2012; **142**: 435-441.e2 [PMID: 22266150 DOI: 10.1053/j.gastro.2012.01.013]

32 **Pepe MS**, Etzioni R, Feng Z, Potter JD, Thompson ML, Thornquist M, Winget M, Yasui Y. Phases of biomarker development for early detection of cancer. *J Natl Cancer Inst* 2001; **93**: 1054-1061 [PMID: 11459866]

33 **Illig R**, Klieser E, Kiesslich T, Neureiter D. GERD-Barrett-Adenocarcinoma: Do We Have Suitable Prognostic and Predictive Molecular Markers? *Gastroenterol Res Pract* 2013; **2013**: 643084 [PMID: 23573078 DOI: 10.1155/2013/643084]

34 **Crabb DW**, Berk MA, Hall TR, Conneally PM, Biegel AA, Lehman GA. Familial gastroesophageal reflux and development of Barrett's esophagus. *Ann Intern Med* 1985; **103**: 52-54 [PMID: 4003988]

35 **Levine DM**, Ek WE, Zhang R, Liu X, Onstad L, Sather C, Lao-Sirieix P, Gammon MD, Corley DA, Shaheen NJ, Bird NC, Hardie LJ, Murray LJ, Reid BJ, Chow WH, Risch HA, Nyrén O, Ye W, Liu G, Romero Y, Bernstein L, Wu AH, Casson AG, Chanock SJ, Harrington P, Caldas I, Debiram-Beecham I, Caldas C, Hayward NK, Pharoah PD, Fitzgerald RC, Macgregor S, Whiteman DC, Vaughan TL. A genome-wide association study identifies new susceptibility loci for esophageal adenocarcinoma and Barrett's esophagus. *Nat Genet* 2013; **45**: 1487-1493 [PMID: 24121790]

36 **Galipeau PC**, Li X, Blount PL, Maley CC, Sanchez CA, Odze RD, Ayub K, Rabinovitch PS, Vaughan TL, Reid BJ. NSAIDs modulate CDKN2A, TP53, and DNA content risk for progression to esophageal adenocarcinoma. *PLoS Med* 2007; **4**: e67 [PMID: 17326708 DOI: 10.1371/journal.pmed.0040067]

37 **Feinberg AP**, Tycko B. The history of cancer epigenetics. *Nat Rev Cancer* 2004; **4**: 143-153 [PMID: 14732866]

38 **Xu E**, Gu J, Hawk ET, Wang KK, Lai M, Huang M, Ajani J, Wu X. Genome-wide methylation analysis shows similar patterns in Barrett's esophagus and esophageal adenocarcinoma. *Carcinogenesis* 2013; **34**: 2750-2756 [PMID: 23996928 DOI: 10.1093/carcin/bgt286]

39 **Sharma S**, Kelly TK, Jones PA. Epigenetics in cancer. *Carcinogenesis* 2010; **31**: 27-36 [PMID: 19752007 DOI: 10.1093/carcin/bgp220]

40 **Alvi MA**, Liu X, O'Donovan M, Newton R, Wernisch L, Shannon NB, Shariff K, di Pietro M, Bergman JJ, Ragunath K, Fitzgerald RC. DNA methylation as an adjunct to histopathology to detect prevalent, inconspicuous dysplasia and early-stage neoplasia in Barrett's esophagus. *Clin Cancer Res* 2013; **19**: 878-888 [PMID: 23243219 DOI: 10.1158/1078-0432.CCR-12-2880]

41 **Jin Z**, Zhao Z, Cheng Y, Dong M, Zhang X, Wang L, Fan X, Feng X, Mori Y, Meltzer SJ. Endoglin promoter hypermethylation identifies a field defect in human primary esophageal cancer. *Cancer* 2013; **119**: 3604-3609 [PMID: 23893879 DOI: 10.1002/cncr.28276]

42 **Sakai NS**, Samia-Aly E, Barbera M, Fitzgerald RC. A review of the current understanding and clinical utility of miRNAs in esophageal cancer. *Semin Cancer Biol* 2013; **23**: 512-521 [PMID: 24013023 DOI: 10.1016/j.semcancer.2013.08.005]

43 **Wu X**, Ajani JA, Gu J, Chang DW, Tan W, Hildebrandt MA, Huang M, Wang KK, Hawk E. MicroRNA expression signatures during malignant progression from Barrett's esophagus to esophageal adenocarcinoma. *Cancer Prev Res* (Phila) 2013; **6**: 196-205 [PMID: 23466817 DOI: 10.1158/1940-6207.CAPR-12-0276]

44 **Saad R**, Chen Z, Zhu S, Jia P, Zhao Z, Washington MK, Belkhiri A, El-Rifai W. Deciphering the unique microRNA signature in human esophageal adenocarcinoma. *PLoS One* 2013; **8**: e64463 [PMID: 23724052 DOI: 10.1371/journal.pone.0064463]

45 **Kastelein F**, Biermann K, Steyerberg EW, Verheij J, Kalisvaart M, Looijenga LH, Stoop HA, Walter L, Kuipers EJ, Spaander MC, Bruno MJ. Aberrant p53 protein expression is associated with an increased risk of neoplastic progression in patients with Barrett's oesophagus. *Gut* 2013; **62**: 1676-1683 [PMID: 23256952 DOI: 10.1136/gutjnl-2012-303594]

46 **Sikkema M**, Kerkhof M, Steyerberg EW, Kusters JG, van Strien PM, Looman CW, van Dekken H, Siersema PD, Kuipers EJ. Aneuploidy and overexpression of Ki67 and p53 as markers for neoplastic progression in Barrett's esophagus: a case-control study. *Am J Gastroenterol* 2009; **104**: 2673-2680 [PMID: 19638963 DOI: 10.1038/ajg.2009.437]

47 **Tanaka T**, Fujimura A, Ichimura K, Yanai H, Sato Y, Takata K, Okada H, Kawano S, Tanabe S, Yoshino T. Clinicopathological characteristics of human epidermal growth factor receptor 2-positive Barrett's adenocarcinoma. *World J Gastroenterol* 2012; **18**: 6263-6268 [PMID: 23180947 DOI: 10.3748/wjg.v18.i43.6263]

48 **Makita K**, Kitazawa R, Semba S, Fujiishi K, Nakagawa M, Haraguchi R, Kitazawa S. Cdx2 expression and its promoter methylation during metaplasia-dysplasia-carcinoma sequence in Barrett's esophagus. *World J Gastroenterol* 2013; **19**: 536-541 [PMID: 23382633 DOI: 10.3748/wjg.v19.i4.536]

49 **Becker L**, Huang Q, Mashimo H. Lgr5, an intestinal stem cell marker, is abnormally expressed in Barrett's esophagus and esophageal adenocarcinoma. *Dis Esophagus* 2010; **23**: 168-174 [PMID: 19549212 DOI: 10.1111/j.1442-2050.2009.00979.x]

50 **Vega KJ**, May R, Sureban SM, Lightfoot SA, Qu D, Reed A, Weygant N, Ramanujam R, Souza R, Madhoun M, Whorton J, Anant S, Meltzer SJ, Houchen CW. Identification of the putative intestinal stem cell marker doublecortin and CaM kinase-like-1 in Barrett's esophagus and esophageal adenocarcinoma. *J Gastroenterol Hepatol* 2012; **27**: 773-780 [PMID: 21916995 DOI: 10.1111/j.1440-1746.2011.06928.x]

51 **Sangwan V**, Park M. Receptor tyrosine kinases: role in cancer progression. *Curr Oncol* 2006; **13**: 191-193 [PMID: 22792017]

52 **Hasina R**, Mollberg N, Kawada I, Mutreja K, Kanade G, Yala S, Surati M, Liu R, Li X, Zhou Y, Ferguson BD, Nallasura V, Cohen KS, Hyjek E, Mueller J, Kanteti R, El Hashani E, Kane D, Shimada Y, Lingen MW, Husain AN, Posner MC, Waxman I, Villaflor VM, Ferguson MK, Varticovski L, Vokes EE, Gill P, Salgia R. Critical role for the receptor tyrosine kinase EPHB4 in esophageal cancers. *Cancer Res* 2013; **73**: 184-194 [PMID: 23100466 DOI: 10.1158/0008-5472.CAN-12-0915]

53 **Paterson AL**, O'Donovan M, Provenzano E, Murray LJ, Coleman HG, Johnson BT, McManus DT, Novelli M, Lovat LB, Fitzgerald RC. Characterization of the timing and prevalence of receptor tyrosine kinase expression changes in oesophageal carcinogenesis. *J Pathol* 2013; **230**: 118-128 [PMID: 22733579 DOI: 10.1002/path.4044]

54 **Paterson AL**, Shannon NB, Lao-Sirieix P, Ong CA, Peters CJ, O'Donovan M, Fitzgerald RC. A systematic approach to therapeutic target selection in oesophago-gastric cancer. *Gut* 2013; **62**: 1415-1424 [PMID: 22773546 DOI: 10.1136/gutjnl-2012-302039]

55 **Fichter CD**, Timme S, Braun JA, Gudernatsch V, Schöpflin A, Bogatyreva L, Geddert H, Faller G, Klimstra D, Tang L, Hauschke D, Werner M, Lassmann S. EGFR, HER2 and HER3 dimerization patterns guide targeted inhibition in two histotypes of esophageal cancer. *Int J Cancer* 2014; **135**:

1517-1530 [PMID: 24510732 DOI: 10.1002/ijc.28771]
56 **Guo XF**, Zhu XF, Yang WC, Zhang SH, Zhen YS. An EGFR/HER2-Bispecific and enediyne-energized fusion protein shows high efficacy against esophageal cancer. *PLoS One* 2014; **9**: e92986 [PMID: 24664246 DOI: 10.1371/journal.pone.0092986]
57 **Okines A**, Cunningham D, Chau I. Targeting the human EGFR family in esophagogastric cancer. *Nat Rev Clin Oncol* 2011; **8**: 492-503 [PMID: 21468131 DOI: 10.1038/nrclinonc.2011.45]
58 **Mohamed A**, El-Rayes B, Khuri FR, Saba NF. Targeted therapies in metastatic esophageal cancer: advances over the past decade. *Crit Rev Oncol Hematol* 2014; **91**: 186-196 [PMID: 24582516 DOI: 10.1016/j.critrevonc.2014.01.010]
59 **Kufe DW**. Mucins in cancer: function, prognosis and therapy. *Nat Rev Cancer* 2009; **9**: 874-885 [PMID: 19935676 DOI: 10.1038/nrc2761]
60 **Iwaya Y**, Hasebe O, Koide N, Kitahara K, Suga T, Shinji A, Muraki T, Yokosawa S, Yamada S, Arakura N, Tanaka E, Nakayama J. Reduced expression of αGlcNAc in Barrett's oesophagus adjacent to Barrett's adenocarcinoma--a possible biomarker to predict the malignant potential of Barrett's oesophagus. *Histopathology* 2014; **64**: 536-546 [PMID: 24117499 DOI: 10.1111/his.12296]
61 **Falk GW**, Buttar NS, Foster NR, Ziegler KL, Demars CJ, Romero Y, Marcon NE, Schnell T, Corley DA, Sharma P, Cruz-Correa MR, Hur C, Fleischer DE, Chak A, Devault KR, Weinberg DS, Della'Zanna G, Richmond E, Smyrk TC, Mandrekar SJ, Limburg PJ. A combination of esomeprazole and aspirin reduces tissue concentrations of prostaglandin E(2) in patients with Barrett's esophagus. *Gastroenterology* 2012; **143**: 917-926.e1 [PMID: 22796132 DOI: 10.1053/j.gastro.2012.06.044]
62 **Duggan C**, Onstad L, Hardikar S, Blount PL, Reid BJ, Vaughan TL. Association between markers of obesity and progression from Barrett's esophagus to esophageal adenocarcinoma. *Clin Gastroenterol Hepatol* 2013; **11**: 934-943 [PMID: 23466711 DOI: 10.1016/j.cgh.2013.02.017]
63 **Bird-Lieberman EL**, Dunn JM, Coleman HG, Lao-Sirieix P, Oukrif D, Moore CE, Varghese S, Johnston BT, Arthur K, McManus DT, Novelli MR, O'Donovan M, Cardwell CR, Lovat LB, Murray LJ, Fitzgerald RC. Population-based study reveals new risk-stratification biomarker panel for Barrett's esophagus. *Gastroenterology* 2012; **143**: 927-935.e3 [PMID: 22771507 DOI: 10.1053/j.gastro.2012.06.041]
64 **Backman V**, Roy HK. Advances in biophotonics detection of field carcinogenesis for colon cancer risk stratification. *J Cancer* 2013; **4**: 251-261 [PMID: 23459690 DOI: 10.7150/jca.5838]
65 **Konda VJ**, Cherkezyan L, Subramanian H, Wroblewski K, Damania D, Becker V, Gonzalez MH, Koons A, Goldberg M, Ferguson MK, Waxman I, Roy HK, Backman V. Nanoscale markers of esophageal field carcinogenesis: potential implications for esophageal cancer screening. *Endoscopy* 2013; **45**: 983-988 [PMID: 24019132 DOI: 10.1055/s-0033-1344617]
66 **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]
67 **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]
68 **Gora MJ**, Sauk JS, Carruth RW, Lu W, Carlton DT, Soomro A, Rosenberg M, Nishioka NS, Tearney GJ. Imaging the upper gastrointestinal tract in unsedated patients using tethered capsule endomicroscopy. *Gastroenterology* 2013; **145**: 723-725 [PMID: 23932950 DOI: 10.1053/j.gastro.2013.07.053]
69 **Wu M**, Mao C, Chen Q, Cu XW, Zhang WS. Serum p53 protein and anti-p53 antibodies are associated with increased cancer risk: a case-control study of 569 patients and 879 healthy controls. *Mol Biol Rep* 2010; **37**: 339-343 [PMID: 19693693 DOI: 10.1007/s11033-009-9744-7]
70 **Cawley HM**, Meltzer SJ, De Benedetti VM, Hollstein MC, Muehlbauer KR, Liang L, Bennett WP, Souza RF, Greenwald BD, Cottrell J, Salabes A, Bartsch H, Trivers GE. Anti-p53 antibodies in patients with Barrett's esophagus or esophageal carcinoma can predate cancer diagnosis. *Gastroenterology* 1998; **115**: 19-27 [PMID: 9649454]
71 **Zhang J**, Xv Z, Wu X, Li K. Potential diagnostic value of serum p53 antibody for detecting esophageal cancer: a meta-analysis. *PLoS One* 2012; **7**: e52896 [PMID: 23285221 DOI: 10.1371/journal.pone.0052896]
72 **Shimada H**, Nagata M, Cho A, Takiguchi N, Kainuma O, Soda H, Ikeda A, Nabeya Y, Yajima S, Yamamoto H, Sugiyama T, Itami M. Long-term monitoring of serum p53 antibody after neoadjuvant chemotherapy and surgery for esophageal adenocarcinoma: report of a case. *Surg Today* 2014; **44**: 1957-1961 [PMID: 24241479 DOI: 10.1007/s00595-013-0787-y]
73 **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]
74 **Urquhart P**, DaCosta R, Marcon N. Endoscopic mucosal imaging of gastrointestinal neoplasia in 2013. *Curr Gastroenterol Rep* 2013; **15**: 330 [PMID: 23771504 DOI: 10.1007/s11894-013-0330-8]
75 **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]
76 **Canto MI**. Vital staining and Barrett's esophagus. *Gastrointest Endosc* 1999; **49**: S12-S16 [PMID: 10049441]
77 **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]
78 **Curvers W**, Baak L, Kiesslich R, Van Oijen A, Rabenstein T, Ragunath K, Rey JF, Scholten P, Seitz U, Ten Kate F, Fockens P, Bergman J. Chromoendoscopy and narrow-band imaging compared with high-resolution magnification endoscopy in Barrett's esophagus. *Gastroenterology* 2008; **134**: 670-679 [PMID: 18242603 DOI: 10.1053/j.gastro.2008.01.003]
79 **Endo T**, Awakawa T, Takahashi H, Arimura Y, Itoh F, Yamashita K, Sasaki S, Yamamoto H, Tang X, Imai K. Classification of Barrett's epithelium by magnifying endoscopy. *Gastrointest Endosc* 2002; **55**: 641-647 [PMID: 11979244]
80 **Guelrud M**, Ehrlich EE. Endoscopic classification of Barrett's esophagus. *Gastrointest Endosc* 2004; **59**: 58-65 [PMID: 14722549]
81 **Wasielica-Berger J**, Baniukiewicz A, Wroblewski E, Chwiesko A, Dabrowski A. Magnification endoscopy and chromoendoscopy in evaluation of specialized intestinal metaplasia in Barrett's Esophagus. *Dig Dis Sci* 2011; **56**: 1987-1995 [PMID: 21225343 DOI: 10.1007/s10620-010-1551-4]
82 **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]
83 **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]
84 **Qumseya BJ**, Wang H, Badie N, Uzomba RN, Parasa S,

White DL, Wolfsen H, Sharma P, Wallace MB. Advanced imaging technologies increase detection of dysplasia and neoplasia in patients with Barrett's esophagus: a meta-analysis and systematic review. *Clin Gastroenterol Hepatol* 2013; **11**: 1562-1570.e1-2 [PMID: 23851020 DOI: 10.1016/j.cgh.2013.06.017]

85 **Uedo N**, Iishi H, Tatsuta M, Yamada T, Ogiyama H, Imanaka K, Sugimoto N, Higashino K, Ishihara R, Narahara H, Ishiguro S. A novel videoendoscopy system by using autofluorescence and reflectance imaging for diagnosis of esophagogastric cancers. *Gastrointest Endosc* 2005; **62**: 521-528 [PMID: 16185965 DOI: 10.1016/j.gie.2005.06.031]

86 **Filip M**, Iordache S, Săftoiu A, Ciurea T. Autofluorescence imaging and magnification endoscopy. *World J Gastroenterol* 2011; **17**: 9-14 [PMID: 21218078 DOI: 10.3748/wjg.v17.i1.9]

87 **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]

88 **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]

89 **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]

90 **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]

91 **Pohl H**, Rösch T, Vieth M, Koch M, Becker V, Anders M, Khalifa AC, Meining A. Miniprobe confocal laser microscopy for the detection of invisible neoplasia in patients with Barrett's oesophagus. *Gut* 2008; **57**: 1648-1653 [PMID: 18755886 DOI: 10.1136/gut.2008.157461]

92 **Poneros J**. Optical coherence tomography and the detection of dysplasia in Barrett's esophagus. *Gastrointest Endosc* 2005; **62**: 832-833 [PMID: 16301021 DOI: 10.1016/j.gie.2005.07.027]

93 **Isenberg G**, Sivak MV, Chak A, Wong RC, Willis JE, Wolf B, Rowland DY, Das A, Rollins A. Accuracy of endoscopic optical coherence tomography in the detection of dysplasia in Barrett's esophagus: a prospective, double-blinded study. *Gastrointest Endosc* 2005; **62**: 825-831 [PMID: 16301020 DOI: 10.1016/j.gie.2005.07.048]

94 **Tsai TH**, Zhou C, Tao YK, Lee HC, Ahsen OO, Figueiredo M, Kirtane T, Adler DC, Schmitt JM, Huang Q, Fujimoto JG, Mashimo H. Structural markers observed with endoscopic 3-dimensional optical coherence tomography correlating with Barrett's esophagus radiofrequency ablation treatment response (with videos). *Gastrointest Endosc* 2012; **76**: 1104-1112 [PMID: 22831857 DOI: 10.1016/j.gie.2012.05.024]

95 **Zhou C**, Tsai TH, Lee HC, Kirtane T, Figueiredo M, Tao YK, Ahsen OO, Adler DC, Schmitt JM, Huang Q, Fujimoto JG, Mashimo H. Characterization of buried glands before and after radiofrequency ablation by using 3-dimensional optical coherence tomography (with videos). *Gastrointest Endosc* 2012; **76**: 32-40 [PMID: 22482920 DOI: 10.1016/j.gie.2012.02.003]

96 **Gora MJ**, Sauk JS, Carruth RW, Gallagher KA, Suter MJ, Nishioka NS, Kava LE, Rosenberg M, Bouma BE, Tearney GJ. Tethered capsule endomicroscopy enables less invasive imaging of gastrointestinal tract microstructure. *Nat Med* 2013; **19**: 238-240 [PMID: 23314056]

97 Rodriguez-Diaz E, Bigio IJ, Singh SK. Integrated optical tools for minimally invasive diagnosis and treatment at gastrointestinal endoscopy. *Robot Comput Integr Manuf* 2011; **27**: 249-256 [PMID: 21152112 DOI: 10.1016/j.rcim.2010.06.006]

98 **Lovat LB**, Johnson K, Mackenzie GD, Clark BR, Novelli MR, Davies S, O'Donovan M, Selvasekar C, Thorpe SM, Pickard D, Fitzgerald R, Fearn T, Bigio I, Bown SG. Elastic scattering spectroscopy accurately detects high grade dysplasia and cancer in Barrett's oesophagus. *Gut* 2006; **55**: 1078-1083 [PMID: 16469795 DOI: 10.1136/gut.2005.081497]

99 **Wax A**, Terry NG, Dellon ES, Shaheen NJ. Angle-resolved low coherence interferometry for detection of dysplasia in Barrett's esophagus. *Gastroenterology* 2011; **141**: 443-447, 447.e1-2 [PMID: 21703265 DOI: 10.1053/j.gastro.2011.06.020]

100 **Terry NG**, Zhu Y, Rinehart MT, Brown WJ, Gebhart SC, Bright S, Carretta E, Ziefle CG, Panjehpour M, Galanko J, Madanick RD, Dellon ES, Trembath D, Bennett A, Goldblum JR, Overholt BF, Woosley JT, Shaheen NJ, Wax A. Detection of dysplasia in Barrett's esophagus with in vivo depth-resolved nuclear morphology measurements. *Gastroenterology* 2011; **140**: 42-50 [PMID: 20854820 DOI: 10.1053/j.gastro.2010.09.008]

101 **Almond LM**, Hutchings J, Lloyd G, Barr H, Shepherd N, Day J, Stevens O, Sanders S, Wadley M, Stone N, Kendall C. Endoscopic Raman spectroscopy enables objective diagnosis of dysplasia in Barrett's esophagus. *Gastrointest Endosc* 2014; **79**: 37-45 [PMID: 23886354 DOI: 10.1016/j.gie.2013.05.028]

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TOPIC HIGHLIGHT

WJGP 5th Anniversary Special Issues (4): Barrett's

Biomarkers of Barrett's esophagus

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Abstract

Barrett's esophagus is the strongest risk for esophageal adenocarcinoma (EAC). Metaplasia in patients with BE may progress to dysplasia and then invasive carcinoma. Well-defined diagnostic, progressive, predictive, and prognostic biomarkers are needed to identify the presence of the disease, estimate the risk of malignant transformation, and predict the therapeutic outcome and survival of EAC patients. There are many predictive and prognostic markers that lack substantial validation, and do not allow stratification of patients with gastroesophageal reflux disease in clinical practice for outcome and effectiveness of therapy. In this short review we summarize the current knowledge regarding possible biomarkers, focusing on the pathophysiologic mechanisms to improve prognostic and therapeutic approaches.

Key words: Barrett's esophagus; Esophageal adenocarcinoma; Biomarkers

Core tip: The importance of biomarkers of Barrett's esophagus is to provide identification of the disease, estimate the risk of malignant transformation, predict the response to therapy, and indicate the overall survival-prognosis for esophageal adenocarcinoma patients. Proposed predictive and prognostic markers do not allow stratification of gastroesophageal reflux disease patients for progression, outcome, and effectiveness of therapy in clinical practice. The aim of this short review is to discuss the current knowledge regarding proposed biomarkers to improve prognostic and predictive therapeutic approaches, with a focus on the pathophysiologic mechanisms.

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INTRODUCTION

Barrett's esophagus (BE) is characterized by the replacement of squamous epithelium in the esophagus by metaplastic columnar epithelium with goblet cells[1]. BE is a well-known risk factor for esophageal adenocarcinoma (EAC), a malignancy with the most rapid increase in incidence (approximately 500%) over the past 3 decades in the Western world, and with persistently poor outcomes when diagnosed after the onset of symptoms (survival less than 20% at 5 years)[2]. An important problem in treating the patients with BE is the absence of satisfactory surveillance programs in spite of the known stages of carcinogenesis from BE to adenocarcinoma. Over the past two decades, there have been many studies attempting to identify patients with BE and predict patients with a high risk of progression to adenocarcinoma[3-6].

In this review, the definition, mechanisms of produc-

Table 1 Phases of biomarker production

Phases of biomarker validation and development
Phase 1: Biomarkers of promise are identified based on application in other cancers and elucidation of novel pathways
Phase 2: Cross-sectional studies validate the biomarker of interest to be sufficiently discriminatory and biomarker assays are standardized
Phase 3: Case-control studies with a retrospective but longitudinal design confirm the biomarker is expressed before the development of cancer
Phase 4: Prospective longitudinal studies avoid biases associated with case-control studies
Phase 5: Population-based studies show the impact of biomarker detection on disease burden and cancer control

tion, and types of biomarker in patients with BE will be summarized.

DEFINITION OF BIOMARKERS

The biomarker

A biological marker affords an indication of the condition or disease, whether normal or abnormal. It is found in the blood, body fluids, and tissues. Moreover, a biomarker may be used for assessment of the response of the body to treatment of a disease or condition[7].

Phases of biomarker identification and validation

Biomarker discovery has to pass through 5 to 6 phases before clinical application (Table 1). Phases 4, 5 and 6 present a significant challenge because of the required large sample sizes, long follow-up and high costs[8].

TYPES OF BIOMARKERS IN PATIENTS WITH BE

Genomic instability

The similarity of the genetic patterns of BE and EAC demonstrated by DNA microarray studies supported the hypothesis that BE is a step preceding EAC. The genomic instability has been shown to be a poor prognostic marker in BE patients. Chromosomal alterations, deletions, point mutations, methylation abnormalities, and loss of heterozygosity (LOH) are the main indications of genomic instability in patients with BE[9-11].

DNA abnormalities

DNA abnormalities, *e.g.*, aneuploidy or tetraploidy, assessed by flow cytometry, can be used as predictive markers in patients with BE with no or low grade dysplasia[12,13]. LOH represents the loss of normal function of one allele of a gene in which the other allele was already inactivated. In a long-term follow-up study of BE patients, a panel combining 9p LOH, 17p LOH in addition to aneuploidy and tetraploidy was a strong predictor of EAC[14].

Abnormalities of tumor loci

An important predictor of risk of dysplasia and EAC in patients with BE is LOH for p53. LOH for p53 was shown to be associated with a 16-fold increase in the risk of progression to cancer[15]. However, in another study, in patients with non-dysplastic BE, only 32.4% of patients with progression showed overexpression of p53 in their initial biopsy[16]. Furthermore, alteration of APC, a regulator of the WNT pathway, by methylation[17] and LOH[18] were found in patients with BE with a positive predictive value.

Epigenetics

Epigenetics entails post-transcriptional silencing of specific genes without a change in the DNA sequence. A variety of mechanisms are involved, including methylation and acetylation. It has been shown that hypermethylation and loss of p16, are independently associated with an increased risk of progression from intestinal metaplasia (IM) to high-grade dysplasia (HGD)[19,20].

The p16 methylation was shown to be highly prevalent in patients with BE (34%-66%)[17,19,21]. Moreover, in a multicenter study, a panel of 8 genes (*p16*, *RUNX3*, *HPP1*, *NELL1*, *TAC1*, *SST*, *AKAP12*, and *CDH13*), was used to predict the risk of progression in patients with BE. In this study, 195 patients were included and sensitivities for prediction of progression approached 50%[22].

Cell cycle predictors

A dysregulated cell cycle may lead to accumulation of genetic aberrations in most cancer cells. Cyclins are cell cycle regulator proteins, and potentially useful biomarkers for progression. In patients with BE, cyclin D1 overexpression was shown to be associated with progression to EAC[23-26]. Further research in large groups of patients is needed to confirm the predictive values of cyclins.

Proliferation abnormalities

The association between increasing proliferation and worsening of dysplasia in BE was shown in many studies[26-28], while other studies found no association[29,30]. Researchers explained the discrepancies between there results by the use different techniques, the different histological pattern between columnar and squamous epithelium, and the use of different proliferative indices. One of the important markers of cellular proliferation is Ki67. However, in a long follow-up study, Ki67-positive proliferative fractions were not associated with risk of progression[31]. Further larger studies with standardized techniques are needed to measure proliferation.

Clonal diversity in BE

Genetic instabilities may lead to multiple distinct clones. The coexistence of multiple distinct clones is called clonal diversity. In patients with BE, clonal diversity measures

Table 2 Types of biomarkers in Barrett's esophagus

	Biomarker	Method	Remarks	Ref.
Diagnostic	TFF3	IHC	To screen asymptomatic patients for BE	[49,50]
	Chromosome 7 and 17 changes	IDKA and FISH	Early stages of BE	[52]
	8q24 (*C-MYC*), 17q12 (*HER2*), and 20q13 changes	FISH	Early stages of BE	[53]
	17q11.2 (*ERBB2*)	Microarray analysis	EAC	[54]
	Serum proteomic analysis	Mass spectrometry	EAC	[55]
Predictive	P16 allelic loss	FISH	Response to therapy	[56]
	DNA ploidy abnormalities	ICDA	Covariate value for recurrence	[57]
	HSP27	IHC	No response to therapy	[58]
	Ephrin B receptor	Microarray	Response to therapy in EAC	[59]
	Genetic polymorphism	qRT-PCR	Associated with clinical outcome	[60]
	P21	IHC	Correlated with better CTX response	[61]
	P53	IHC	Correlated with better CTX response	[62]
Progression markers	ERCC1	IHC	Predicts CTX resistance	[16]
	P53	IHC	Limited efficacy as a progression marker	[13,63]
	DNA abnormalities	Flow cytometry	High risk for progression to EAC	[13]
	LOH of 157p and 9p	Flow cytometry	Predict progression to EAC	[14]
	EGFR	IHC	Overexpression in HGD and EAC	[64]
	Cyclin A	IHC	Predicts progression to dysplasia	[65]
	Cyclin D1	IHC	Risk of Progression to EAC	[19]
	Hypermethylation of p16, RUNX2,HPP1	RT-PCR	Risk of progression to EAC/HGD	[22]
	8 gene methylation panel	RT-PCR	Predicts progression to EAC/HGD	[66]
Prognostic biomarkers	Cathepsin D,AKR1D10,AKR1C2 mRNA levels	Western blot, qRt-PCR	Dysregulation predicts progression to EAC/HGD	[67]
	DCK, PAPSS2, SIRT,TRIM44	RT-PCR, IHC	4 gene signature in EAC , predict 5 year survival	[56]
	P16 loss, C-MYC gain	FISH	Associated with therapy response	[68]
	ASS expression	Microarray	Low expression associated with metastases	[69]
	MicroRNA expression profile	Microarray, RT-PCR	Low level associated with worse prognosis in EAC	[70]
	Cyclin D1	IHC, FISH	Decreased survival	[71]
	EGFR	IHC	Decreased expression associated with decreased survival	[72]
	TGF-α	IHC, ISH	High level indicates progression and metastases	[73]
	TGF-β1	RT-PCR, ELISA	High expression associated with decreased survival	[73]
	APC	PCR	High level associated with decreased survival	[74]
	COX-2	IHC	Associated with metastases and recurrence	[75]
	Telomerase	Southern-blot and PCR	Associated with decreased survival	[76]
	VEGF	IHC	Associated with metastases and decreased survival	[77]
	Cadherin	IHC	Decreased level associated with decreased survival	[78]
	TIMP	IHC, PCR	Decreased level associated with decreased survival	[79]

ACIS: Automated cellular imaging system; ASS: Argininosuccinate synthase; APC: Adenomatous polyposis coli; BE: Barrett's esophagus; COX: Cyclooxygenase; DCK: Deoxycytidine kinase; DICM: Digital image cytometry; EAC: Esophageal adenocarcinoma; EGFR: Epidermal growth factor receptor; ELISA: Enzymelinked immunosorbent assay; FISH: Fluorescence in-situ-hybridization; ICDA: Image cytometric DNA analysis; HSP27: Heat-shock protein 27; IHC: Immunohistochemistry; LOH: Loss of heterozygosity; PAPSS2: 3'-phosphoadenosine 5'-phosphosulfate synthase 2; PCR: Polymerase chain reaction; qRT: Quantitative reverse transcriptase; MLPA: Multiplex ligation dependent probe amplification; NF-κB: Nuclear factor kappa B; SIRT2: Sirtuin 2; SNP: Single nucleotide polymorphism; TFF3: Trefoil factor 3; TGF: Transforming growth factor; TIMP: Tissue inhibitors of metalloproteinases; TRIM44: Tripartite motifcontaining 44; uPA: Urokinase-type plasminogen activator; VEGF: Vascular endothelial growth factor.

were strong predictors of progression[32]. However, the complicated methodology limited the use of clonal diversity as a predictive marker.

Mitochondrial DNA

Mitochondrial DNA (mtDNA) has been implicated in the process of carcinogenesis[33]. mtDNA mutations were found in 53% of patients with BE without dysplasia[32]. In patients with BE, deletion of the mitochondrial genome (4977 bp) was found in 15.4% in IM, 40% in low-grade dysplasia, 69.2% in HGD, and 90% in paratumoral tissue[34].

FLUORESCENCE *IN-SITU* HYBRIDIZATION

Fluorescence *in situ* hybridization (FISH) is a technique which detects DNA content and loci abnormalities in the cells by fluorescent-tagged DNA probes. FISH can detect aneusomy (abnormalities of chromosome copy number), deletion, duplication, amplification and translocation at tumor suppressor loci and protooncogene loci.

In patients with BE, FISH was used to detect genetic abnormalities by investigators in different studies from multiple centers[35-39]. Detection of dysplasia in BE and identification of HGD and EAC using the FISH 4-probe set has been shown to have a reasonable sensitivity (84%-93%) and specificity (93%)[39]. In another multicenter study, polysomy detected by FISH has been shown to predict risk of progression to HGD/EAC[40].

CLASSIFICATION OF BIOMARKERS OF BE

Biomarkers of BE can be classified into 4 groups: (1)

diagnostic biomarkers; (2) biomarkers of progression; (3) predictive biomarkers; and (4) prognostic biomarkers. This classification is based on the previous intensive research, and review articles[6,41-43] (Table 2).

Diagnostic biomarkers

Diagnostic biomarkers indicate the presence of disease. The histochemical analysis of biopsies of the gastro-esophageal junction remains the conventional approach for detection and diagnosis of BE. In patients with asymptomatic BE, trefoil factor 3 combined with a non-invasive diagnostic technique has been investigated with promising results in the screening of these patients[44,45]. Further validation and assessment are needed to confirm the results of these studies.

Progression biomarkers

The degree of dysplasia in obtained biopsies is the main marker of progression of BE, although there is much intra- and inter-observer errors[46-48]. The most promising biomarkers are minichromosome maintenance 2 (MCM2) expression pattern and LOH on distinct gene loci, especially at 17p. The cost and intensive laboratory time limit the use of these markers in clinical practice.

Predictive biomarkers

These biomarkers predict the response to therapy. A limited number of predictive biomarkers are available (Table 2) and this category is in need of further intensified research.

Prognostic biomarkers

These biomarkers indicate overall survival and prognosis of EAC. The majority of biomarkers are in this category. Prognostic biomarkers include growth signals, insensitivity to growth inhibitory signals, markers of evasion of programmed cell death, limitless replicative potential (telomerase), markers of sustained angiogenesis, markers of invasion and metastasis, marker of tumor differentiation, and cancer-related inflammation (Table 2).

Biomarkers in the clinical field: problems and obstacles

Much work is needed to set up clinical trials of biomarkers as this requires cooperation between clinical researchers and experts in molecular techniques. Moreover, the validation of a biomarker passes through 5 phases and requires multicenter studies, with prohibitive costs and long-term follow-up.

The method of specimen collection is another challenge. While microarray studies require special equipment and may not be easy to access by clinical scientists, molecular profiling using formalin-fixed paraffin-embedded specimens is interesting to researchers because of easy availability of specimens. In patients with hepatocellular carcinoma, the use of large scale (> 6000) gene profiling resulted in high quality data even from specimens archived for as long as 24 years[49].

The lack of prospective controlled trials is another important problem attributed to high costs and the need for large sample sizes. Moreover, the lack of reproducibility of assays between laboratories represent another obstacle for identification of clinically useful cancer biomarkers[50]. The reanalysis of DNA microarray studies showed that the selection of patients had an impact on the predictor role of genes in prognosis[51]. Careful interpretation of biomarker studies is needed by using large datasets such as DNA microarray repositories.

CONCLUSION

A biomarker for BE should help in population screening, improve the surveillance of patients with BE, and identify the prognostic groups and best therapy once EAC develops. Many biomarkers have been intensively studied and accurately predict the progress of BE to EAC. The MCM2 expression pattern, LOH on distinct gene loci, especially at 17p, hypermethylation of p16 and the expression pattern of P53 are promising markers especially for progression of the disease. Important prognostic biomarkers include cyclin D1, Ki-67, transforming growth factor-α, adenomatous polyposis coli, cyclooxygenase-2, telomerase and vascular endothelial growth factor. Till now, no biomarker has been able to replace the current gold standard of dysplasia in routine clinical practice. Panels of biomarkers seem to be better in predicting progression more accurately. The issue of costs and practicality of biomarkers should be considered before research is performed. A model incorporating clinical data and biomarkers will be promising and can accurately predict the risk of progression, prognosis or response to therapy. Similar models have been used in other cancers and diseases such as the Nottingham prognostic index for breast cancer and MELD score for liver disease. After generation and validation of such a model, it should then be rigorously validated in a large cohort of patients in a prospective fashion.

REFERENCES

1 **Sharma P**. Clinical practice. Barrett's esophagus. *N Engl J Med* 2009; **361**: 2548-2556 [PMID: 20032324 DOI: 10.1056/NEJMcp0902173]

2 **Pohl H**, Welch HG. The role of overdiagnosis and reclassification in the marked increase of esophageal adenocarcinoma incidence. *J Natl Cancer Inst* 2005; **97**: 142-146 [PMID: 15657344]

3 **Holmes RS**, Vaughan TL. Epidemiology and pathogenesis of esophageal cancer. *Semin Radiat Oncol* 2007; **17**: 2-9 [PMID: 17185192 DOI: 10.1016/j.semradonc.2006.09.003]

4 **Brown LM**, Devesa SS. Epidemiologic trends in esophageal and gastric cancer in the United States. *Surg Oncol Clin N Am* 2002; **11**: 235-256 [PMID: 12424848 DOI: 10.1016/S1055-3207(02)00002-9]

5 **Kerkhof M**, van Dekken H, Steyerberg EW, Meijer GA, Mulder AH, de Bruïne A, Driessen A, ten Kate FJ, Kusters JG, Kuipers EJ, Siersema PD. Grading of dysplasia in Barrett's oesophagus: substantial interobserver variation between general and gastrointestinal pathologists. *Histopathology* 2007; **50**: 920-927 [PMID: 17543082 DOI: 10.1111/j.1365-2559.2007.02706.x]

6 **Illig R**, Klieser E, Kiesslich T, Neureiter D. GERD-Barrett-Adenocarcinoma: Do We Have Suitable Prognostic and Predictive Molecular Markers? *Gastroenterol Res Pract* 2013; **2013**: 643084 [PMID: 23573078 DOI: 10.1155/2013/643084]
7 National cancer institute. Available from: URL: http://www.cancer.gov/dictionary?CdrID=45618)
8 **Jankowski JA**, Odze RD. Biomarkers in gastroenterology: between hope and hype comes histopathology. *Am J Gastroenterol* 2009; **104**: 1093-1096 [PMID: 19417749 DOI: 10.1038/ajg.2008.172]
9 **Paulson TG**, Maley CC, Li X, Li H, Sanchez CA, Chao DL, Odze RD, Vaughan TL, Blount PL, Reid BJ. Chromosomal instability and copy number alterations in Barrett's esophagus and esophageal adenocarcinoma. *Clin Cancer Res* 2009; **15**: 3305-3314 [PMID: 19417022 DOI: 10.1158/1078-0432.CCR-08-2494]
10 **Li X**, Galipeau PC, Sanchez CA, Blount PL, Maley CC, Arnaudo J, Peiffer DA, Pokholok D, Gunderson KL, Reid BJ. Single nucleotide polymorphism-based genome-wide chromosome copy change, loss of heterozygosity, and aneuploidy in Barrett's esophagus neoplastic progression. *Cancer Prev Res* (Phila) 2008; **1**: 413-423 [PMID: 19138988 DOI: 10.1158/1940-6207.CAPR-08-0121]
11 **Selaru FM**, Zou T, Xu Y, Shustova V, Yin J, Mori Y, Sato F, Wang S, Olaru A, Shibata D, Greenwald BD, Krasna MJ, Abraham JM, Meltzer SJ. Global gene expression profiling in Barrett's esophagus and esophageal cancer: a comparative analysis using cDNA microarrays. *Oncogene* 2002; **21**: 475-478 [PMID: 11821959 DOI: 10.1038/sj.onc.1205111]
12 **Reid BJ**, Levine DS, Longton G, Blount PL, Rabinovitch PS. Predictors of progression to cancer in Barrett's esophagus: baseline histology and flow cytometry identify low- and high-risk patient subsets. *Am J Gastroenterol* 2000; **95**: 1669-1676 [PMID: 10925966 DOI: 10.1111/j.1572-0241.2000.02196.x]
13 **Rabinovitch PS**, Longton G, Blount PL, Levine DS, Reid BJ. Predictors of progression in Barrett's esophagus III: baseline flow cytometric variables. *Am J Gastroenterol* 2001; **96**: 3071-3083 [PMID: 11721752 DOI: 10.1111/j.1572-0241.2001.05261.x]
14 **Galipeau PC**, Li X, Blount PL, Maley CC, Sanchez CA, Odze RD, Ayub K, Rabinovitch PS, Vaughan TL, Reid BJ. NSAIDs modulate CDKN2A, TP53, and DNA content risk for progression to esophageal adenocarcinoma. *PLoS Med* 2007; **4**: e67 [PMID: 17326708 DOI: 10.1371/journal.pmed.0040067]
15 **Reid BJ**, Prevo LJ, Galipeau PC, Sanchez CA, Longton G, Levine DS, Blount PL, Rabinovitch PS. Predictors of progression in Barrett's esophagus II: baseline 17p (p53) loss of heterozygosity identifies a patient subset at increased risk for neoplastic progression. *Am J Gastroenterol* 2001; **96**: 2839-2848 [PMID: 11693316 DOI: 10.1111/j.1572-0241.2001.04236.x]
16 **Murray L**, Sedo A, Scott M, McManus D, Sloan JM, Hardie LJ, Forman D, Wild CP. TP53 and progression from Barrett's metaplasia to oesophageal adenocarcinoma in a UK population cohort. *Gut* 2006; **55**: 1390-1397 [PMID: 16682429 DOI: 10.1136/gut.2005.083295]
17 **Bian YS**, Osterheld MC, Fontolliet C, Bosman FT, Benhattar J. p16 inactivation by methylation of the CDKN2A promoter occurs early during neoplastic progression in Barrett' s esophagus. *Gastroenterology* 2002; **122**: 1113-1121 [PMID: 11910361 DOI: 10.1053/gast.2002.32370]
18 **Zhuang Z**, Vortmeyer AO, Mark EJ, Odze R, Emmert-Buck MR, Merino MJ, Moon H, Liotta LA, Duray PH. Barrett's esophagus: metaplastic cells with loss of heterozygosity at the APC gene locus are clonal precursors to invasive adenocarcinoma. *Cancer Res* 1996; **56**: 1961-1964 [PMID: 8616831]
19 **Schulmann K**, Sterian A, Berki A, Yin J, Sato F, Xu Y, Olaru A, Wang S, Mori Y, Deacu E, Hamilton J, Kan T, Krasna MJ, Beer DG, Pepe MS, Abraham JM, Feng Z, Schmiegel W, Greenwald BD, Meltzer SJ. Inactivation of p16, RUNX3, and HPP1 occurs early in Barrett's-associated neoplastic progression and predicts progression risk. *Oncogene* 2005; **24**: 4138-4148 [PMID: 15824739 DOI: 10.1038/sj.onc.1208598]
20 **Mokrowiecka A**, Wierzchniewska-Ławska A, Smolarz B, Romanowicz-Makowska H, Małecka-Panas E. p16 gene mutations in Barrett's esophagus in gastric metaplasia - intestinal metaplasia - dysplasia - adenocarcinoma sequence. *Adv Med Sci* 2012; **57**: 71-76 [PMID: 22440936 DOI: 10.2478/v10039-012-0003-0]
21 **Maley CC**, Galipeau PC, Li X, Sanchez CA, Paulson TG, Blount PL, Reid BJ. The combination of genetic instability and clonal expansion predicts progression to esophageal adenocarcinoma. *Cancer Res* 2004; **64**: 7629-7633 [PMID: 15492292 DOI: 10.1158/0008-5472.CAN-04-1738]
22 **Jin Z**, Cheng Y, Gu W, Zheng Y, Sato F, Mori Y, Olaru AV, Paun BC, Yang J, Kan T, Ito T, Hamilton JP, Selaru FM, Agarwal R, David S, Abraham JM, Wolfsen HC, Wallace MB, Shaheen NJ, Washington K, Wang J, Canto MI, Bhattacharyya A, Nelson MA, Wagner PD, Romero Y, Wang KK, Feng Z, Sampliner RE, Meltzer SJ. A multicenter, double-blinded validation study of methylation biomarkers for progression prediction in Barrett's esophagus. *Cancer Res* 2009; **69**: 4112-4115 [PMID: 19435894 DOI: 10.1158/0008-5472.CAN-09-0028]
23 **Shi XY**, Bhagwandeen B, Leong AS. p16, cyclin D1, Ki-67, and AMACR as markers for dysplasia in Barrett esophagus. *Appl Immunohistochem Mol Morphol* 2008; **16**: 447-452 [PMID: 18665038 DOI: 10.1097/PAI.0b013e318168598b]
24 **van Dekken H**, Hop WC, Tilanus HW, Haringsma J, van der Valk H, Wink JC, Vissers KJ. Immunohistochemical evaluation of a panel of tumor cell markers during malignant progression in Barrett esophagus. *Am J Clin Pathol* 2008; **130**: 745-753 [PMID: 18854267 DOI: 10.1309/AJCPO31THGVEUIDH]
25 **Bani-Hani K**, Martin IG, Hardie LJ, Mapstone N, Briggs JA, Forman D, Wild CP. Prospective study of cyclin D1 overexpression in Barrett's esophagus: association with increased risk of adenocarcinoma. *J Natl Cancer Inst* 2000; **92**: 1316-1321 [PMID: 10944553 DOI: 10.1093/jnci/92.16.1316]
26 **Lao-Sirieix P**, Brais R, Lovat L, Coleman N, Fitzgerald RC. Cell cycle phase abnormalities do not account for disordered proliferation in Barrett's carcinogenesis. *Neoplasia* 2008; **6**: 751-760 [PMID: 15720801 DOI: 10.1593/neo.04280]
27 **Going JJ**, Keith WN, Neilson L, Stoeber K, Stuart RC, Williams GH. Aberrant expression of minichromosome maintenance proteins 2 and 5, and Ki-67 in dysplastic squamous oesophageal epithelium and Barrett's mucosa. *Gut* 2002; **50**: 373-377 [PMID: 11839717 DOI: 10.1136/gut.50.3.373]
28 **Hong MK**, Laskin WB, Herman BE, Johnston MH, Vargo JJ, Steinberg SM, Allegra CJ, Johnston PG. Expansion of the Ki-67 proliferative compartment correlates with degree of dysplasia in Barrett's esophagus. *Cancer* 1995; **75**: 423-429 [PMID: 7812911]
29 **Pellish LJ**, Hermos JA, Eastwood GL. Cell proliferation in three types of Barrett's epithelium. *Gut* 1980; **21**: 26-31 [PMID: 7364315 DOI: 10.1136/gut.21.1.26]
30 **Reid BJ**, Sanchez CA, Blount PL, Levine DS. Barrett's esophagus: cell cycle abnormalities in advancing stages of neoplastic progression. *Gastroenterology* 1993; **105**: 119-129 [PMID: 8514029]
31 **Chao DL**, Sanchez CA, Galipeau PC, Blount PL, Paulson TG, Cowan DS, Ayub K, Odze RD, Rabinovitch PS, Reid BJ. Cell proliferation, cell cycle abnormalities, and cancer outcome in patients with Barrett's esophagus: a long-term prospective study. *Clin Cancer Res* 2008; **14**: 6988-6995 [PMID: 18980994 DOI: 10.1158/1078-0432.CCR-07-5063]
32 **Merlo LM**, Shah NA, Li X, Blount PL, Vaughan TL, Reid BJ, Maley CC. A comprehensive survey of clonal diversity measures in Barrett's esophagus as biomarkers of progression to esophageal adenocarcinoma. *Cancer Prev Res* (Phila) 2010; **3**: 1388-1397 [PMID: 20947487 DOI: 10.1158/1940-6207.CAPR-10-0108]

33 **Ishikawa K**, Takenaga K, Akimoto M, Koshikawa N, Yamaguchi A, Imanishi H, Nakada K, Honma Y, Hayashi J. ROS-generating mitochondrial DNA mutations can regulate tumor cell metastasis. *Science* 2008; **320**: 661-664 [PMID: 18388260 DOI: 10.1126/science.1156906]

34 **Lee S**, Han MJ, Lee KS, Back SC, Hwang D, Kim HY, Shin JH, Suh SP, Ryang DW, Kim HR, Shin MG. Frequent occurrence of mitochondrial DNA mutations in Barrett's metaplasia without the presence of dysplasia. *PLoS One* 2012; **7**: e37571 [PMID: 22629421 DOI: 10.1371/journal.pone.0037571]

35 **Tan BH**, Skipworth RJ, Stephens NA, Wheelhouse NM, Gilmour H, de Beaux AC, Paterson-Brown S, Fearon KC, Ross JA. Frequency of the mitochondrial DNA 4977bp deletion in oesophageal mucosa during the progression of Barrett's oesophagus. *Eur J Cancer* 2009; **45**: 736-740 [PMID: 19211242 DOI: 10.1016/j.ejca.2009.01.013]

36 **Rossi E**, Grisanti S, Villanacci V, Della Casa D, Cengia P, Missale G, Minelli L, Buglione M, Cestari R, Bassotti G. HER-2 overexpression/amplification in Barrett's oesophagus predicts early transition from dysplasia to adenocarcinoma: a clinico-pathologic study. *J Cell Mol Med* 2009; **13**: 3826-3833 [PMID: 19292734 DOI: 10.1111/j.1582-4934.2008.00517]

37 **Rygiel AM**, Milano F, Ten Kate FJ, Schaap A, Wang KK, Peppelenbosch MP, Bergman JJ, Krishnadath KK. Gains and amplifications of c-myc, EGFR, and 20.q13 loci in the no dysplasia-dysplasia-adenocarcinoma sequence of Barrett's esophagus. *Cancer Epidemiol Biomarkers Prev* 2008; **17**: 1380-1385 [PMID: 18559552 DOI: 10.1158/1055-9965.EPI-07-2734]

38 **Falk GW**, Skacel M, Gramlich TL, Casey G, Goldblum JR, Tubbs RR. Fluorescence in situ hybridization of cytologic specimens from Barrett's esophagus: a pilot feasibility study. *Gastrointest Endosc* 2004; **60**: 280-284 [PMID: 15278064 DOI: 10.1016/S0016-5107(04)01687-6]

39 **Brankley SM**, Wang KK, Harwood AR, Miller DV, Legator MS, Lutzke LS, Kipp BR, Morrison LE, Halling KC. The development of a fluorescence in situ hybridization assay for the detection of dysplasia and adenocarcinoma in Barrett's esophagus. *J Mol Diagn* 2006; **8**: 260-267 [PMID: 16645214 DOI: 10.2353/jmoldx.2006.050118]

40 **Wang KK**, Barr Fritcher E, Halling KC. The use of FISH in a multicenter blinded study to predict development of neoplasia in Barrett's esophagus. *Gastroenterology* 2009; **136**: A 157

41 **Ong CA**, Lao-Sirieix P, Fitzgerald RC. Biomarkers in Barrett's esophagus and esophageal adenocarcinoma: predictors of progression and prognosis. *World J Gastroenterol* 2010; **16**: 5669-5681 [PMID: 21128316 DOI: 10.3748/wjg.v16.i45.5669]

42 **Fang D**, Das KM, Cao W, Malhotra U, Triadafilopoulos G, Najarian RM, Hardie LJ, Lightdale CJ, Beales IL, Felix VN, Schneider PM, Bellizzi AM. Barrett's esophagus: progression to adenocarcinoma and markers. *Ann N Y Acad Sci* 2011; **1232**: 210-229 [PMID: 21950815 DOI: 10.1111/j.1749-6632.2011.06053.x]

43 **Huang Q**, Hardie LJ. Biomarkers in Barrett's oesophagus. *Biochem Soc Trans* 2010; **38**: 343-347 [PMID: 20298180 DOI: 10.1042/BST0380343]

44 **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]

45 **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]

46 **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 DOI: 10.1016/S0046-8177(88)80344-7]

47 **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]

48 **Reid BJ**, Li X, Galipeau PC, Vaughan TL. Barrett's oesophagus and oesophageal adenocarcinoma: time for a new synthesis. *Nat Rev Cancer* 2010; **10**: 87-101 [PMID: 20094044]

49 **Hoshida Y**, Villanueva A, Kobayashi M, Peix J, Chiang DY, Camargo A, Gupta S, Moore J, Wrobel MJ, Lerner J, Reich M, Chan JA, Glickman JN, Ikeda K, Hashimoto M, Watanabe G, Daidone MG, Roayaie S, Schwartz M, Thung S, Salvesen HB, Gabriel S, Mazzaferro V, Bruix J, Friedman SL, Kumada H, Llovet JM, Golub TR. Gene expression in fixed tissues and outcome in hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 1995-2004 [PMID: 18923165 DOI: 10.1056/NEJMoa0804525]

50 **Wilson JF**. The rocky road to useful cancer biomarkers. *Ann Intern Med* 2006; **144**: 945-948 [PMID: 16785487 DOI: 10.7326/0003-4819-144-12-200606200-00022]

51 **Michiels S**, Koscielny S, Hill C. Prediction of cancer outcome with microarrays: a multiple random validation strategy. *Lancet* 2005; **365**: 488-492 [PMID: 15705458 DOI: 10.1016/S0140-6736(05)17866-0]

52 **Rygiel AM**, Milano F, Ten Kate FJ, de Groot JG, Peppelenbosch MP, Bergman JJ, Krishnadath KK. Assessment of chromosomal gains as compared to DNA content changes is more useful to detect dysplasia in Barrett's esophagus brush cytology specimens. *Genes Chromosomes Cancer* 2008; **47**: 396-404 [PMID: 18265409 DOI: 10.1002/gcc.20543]

53 **Fritcher EG**, Brankley SM, Kipp BR, Voss JS, Campion MB, Morrison LE, Legator MS, Lutzke LS, Wang KK, Sebo TJ, Halling KC. A comparison of conventional cytology, DNA ploidy analysis, and fluorescence in situ hybridization for the detection of dysplasia and adenocarcinoma in patients with Barrett's esophagus. *Hum Pathol* 2008; **39**: 1128-1135 [PMID: 18602665 DOI: 10.1016/j.humpath.2008.02.003]

54 **Dahlberg PS**, Jacobson BA, Dahal G, Fink JM, Kratzke RA, Maddaus MA, Ferrin LJ. ERBB2 amplifications in esophageal adenocarcinoma. *Ann Thorac Surg* 2004; **78**: 1790-1800 [PMID: 15511476]

55 **Hammoud ZT**, Dobrolecki L, Kesler KA, Rahmani E, Rieger K, Malkas LH, Hickey RJ. Diagnosis of esophageal adenocarcinoma by serum proteomic pattern. *Ann Thorac Surg* 2007; **84**: 384-392; discussion 392 [PMID: 17643604]

56 **Prasad GA**, Wang KK, Halling KC, Buttar NS, Wongkeesong LM, Zinsmeister AR, Brankley SM, Westra WM, Lutzke LS, Borkenhagen LS, Dunagan K. Correlation of histology with biomarker status after photodynamic therapy in Barrett esophagus. *Cancer* 2008; **113**: 470-476 [PMID: 18553366]

57 **Dunn JM**, Mackenzie GD, Oukrif D, Mosse CA, Banks MR, Thorpe S, Sasieni P, Bown SG, Novelli MR, Rabinovitch PS, Lovat LB. Image cytometry accurately detects DNA ploidy abnormalities and predicts late relapse to high-grade dysplasia and adenocarcinoma in Barrett's oesophagus following photodynamic therapy. *Br J Cancer* 2010; **102**: 1608-1617 [PMID: 20461081 DOI: 10.1038/sj.bjc.6605688]

58 **Langer R**, Ott K, Specht K, Becker K, Lordick F, Burian M, Herrmann K, Schrattenholz A, Cahill MA, Schwaiger M, Hofler H, Wester HJ. Protein expression profiling in esophageal adenocarcinoma patients indicates association of heat-shock protein 27 expression and chemotherapy response. *Clin Cancer Res* 2008; **14**: 8279-8287 [PMID: 19088045 DOI: 10.1158/1078-0432.CCR-08-0679]

59 **Wu X**, Gu J, Wu TT, Swisher SG, Liao Z, Correa AM, Liu J, Etzel CJ, Amos CI, Huang M, Chiang SS, Milas L, Hittelman WN, Ajani JA. Genetic variations in radiation and chemotherapy drug action pathways predict clinical outcomes in esophageal cancer. *J Clin Oncol* 2006; **24**: 3789-3798 [PMID:

16785472 DOI: 10.1200/JCO.2005.03.6640]
60 **Heeren PA**, Kloppenberg FW, Hollema H, Mulder NH, Nap RE, Plukker JT. Predictive effect of p53 and p21 alteration on chemotherapy response and survival in locally advanced adenocarcinoma of the esophagus. *Anticancer Res* 2004; **24**: 2579-2583 [PMID: 15330218]
61 **Nakashima S**, Natsugoe S, Matsumoto M, Kijima F, Takebayashi Y, Okumura H, Shimada M, Nakano S, Kusano C, Baba M, Takao S, Aikou T. Expression of p53 and p21 is useful for the prediction of preoperative chemotherapeutic effects in esophageal carcinoma. *Anticancer Res* 2000; **20**: 1933-1937 [PMID: 10928129]
62 **Kim MK**, Cho KJ, Kwon GY, Park SI, Kim YH, Kim JH, Song HY, Shin JH, Jung HY, Lee GH, Choi KD, Kim SB. ERCC1 predicting chemoradiation resistance and poor outcome in oesophageal cancer. *Eur J Cancer* 2008; **44**: 54-60 [PMID: 17976974 DOI: 10.1016/j.ejca.2007.09.006]
63 **Weston AP**, Banerjee SK, Sharma P, Tran TM, Richards R, Cherian R. p53 protein overexpression in low grade dysplasia (LGD) in Barrett's esophagus: immunohistochemical marker predictive of progression. *Am J Gastroenterol* 2001; **96**: 1355-1362 [PMID: 11374668 DOI: 10.1111/j.1572-0241.2001.03851.x]
64 **Cronin J**, McAdam E, Danikas A, Tselepis C, Griffiths P, Baxter J, Thomas L, Manson J, Jenkins G. Epidermal growth factor receptor (EGFR) is overexpressed in high-grade dysplasia and adenocarcinoma of the esophagus and may represent a biomarker of histological progression in Barrett's esophagus (BE). *Am J Gastroenterol* 2011; **106**: 46-56 [PMID: 21157443 DOI: 10.1038/ajg.2010.433]
65 **Lao-Sirieix P**, Lovat L, Fitzgerald RC. Cyclin A immunocytology as a risk stratification tool for Barrett's esophagus surveillance. *Clin Cancer Res* 2007; **13**: 659-665 [PMID: 17255290 DOI: 10.1158/1078-0432.CCR-06-1385]
66 **Breton J**, Gage MC, Hay AW, Keen JN, Wild CP, Donnellan C, Findlay JB, Hardie LJ. Proteomic screening of a cell line model of esophageal carcinogenesis identifies cathepsin D and aldo-keto reductase 1C2 and 1B10 dysregulation in Barrett's esophagus and esophageal adenocarcinoma. *J Proteome Res* 2008; **7**: 1953-1962 [PMID: 18396902 DOI: 10.1021/pr7007835]
67 **Peters CJ**, Rees JR, Hardwick RH, Hardwick JS, Vowler SL, Ong CA, Zhang C, Save V, O'Donovan M, Rassl D, Alderson D, Caldas C, Fitzgerald RC. A 4-gene signature predicts survival of patients with resected adenocarcinoma of the esophagus, junction, and gastric cardia. *Gastroenterology* 2010; **139**: 1995-2004.e15 [PMID: 20621683]
68 **Lagarde SM**, Ver Loren van Themaat PE, Moerland PD, Gilhuijs-Pederson LA, Ten Kate FJ, Reitsma PH, van Kampen AH, Zwinderman AH, Baas F, van Lanschot JJ. Analysis of gene expression identifies differentially expressed genes and pathways associated with lymphatic dissemination in patients with adenocarcinoma of the esophagus. *Ann Surg Oncol* 2008; **15**: 3459-3470 [PMID: 18825457 DOI: 10.1245/s10434-008-0165-y]
69 **Mathé EA**, Nguyen GH, Bowman ED, Zhao Y, Budhu A, Schetter AJ, Braun R, Reimers M, Kumamoto K, Hughes D, Altorki NK, Casson AG, Liu CG, Wang XW, Yanaihara N, Hagiwara N, Dannenberg AJ, Miyashita M, Croce CM, Harris CC. MicroRNA expression in squamous cell carcinoma and adenocarcinoma of the esophagus: associations with survival. *Clin Cancer Res* 2009; **15**: 6192-6200 [PMID: 19789312 DOI: 10.1158/1078-0432.CCR-09-1467]
70 **Izzo JG**, Wu TT, Wu X, Ensor J, Luthra R, Pan J, Correa A, Swisher SG, Chao CK, Hittelman WN, Ajani JA. Cyclin D1 guanine/adenine 870 polymorphism with altered protein expression is associated with genomic instability and aggressive clinical biology of esophageal adenocarcinoma. *J Clin Oncol* 2007; **25**: 698-707 [PMID: 17308274 DOI: 10.1200/JCO.2006.08.0283]
71 **Langer R**, Von Rahden BH, Nahrig J, Von Weyhern C, Reiter R, Feith M, Stein HJ, Siewert JR, Höfler H, Sarbia M. Prognostic significance of expression patterns of c-erbB-2, p53, p16INK4A, p27KIP1, cyclin D1 and epidermal growth factor receptor in oesophageal adenocarcinoma: a tissue microarray study. *J Clin Pathol* 2006; **59**: 631-634 [PMID: 16731604 DOI: 10.1136/jcp.2005.034298]
72 **D'Errico A**, Barozzi C, Fiorentino M, Carella R, Di Simone M, Ferruzzi L, Mattioli S, Grigioni WF. Role and new perspectives of transforming growth factor-alpha (TGF-alpha) in adenocarcinoma of the gastro-oesophageal junction. *Br J Cancer* 2000; **82**: 865-870 [PMID: 10732760 DOI: 10.1054/bjoc.1999.1013]
73 **von Rahden BH**, Stein HJ, Feith M, Pühringer F, Theisen J, Siewert JR, Sarbia M. Overexpression of TGF-beta1 in esophageal (Barrett's) adenocarcinoma is associated with advanced stage of disease and poor prognosis. *Mol Carcinog* 2006; **45**: 786-794 [PMID: 16921482 DOI: 10.1002/mc.20259]
74 **Kawakami K**, Brabender J, Lord RV, Groshen S, Greenwald BD, Krasna MJ, Yin J, Fleisher AS, Abraham JM, Beer DG, Sidransky D, Huss HT, Demeester TR, Eads C, Laird PW, Ilson DH, Kelsen DP, Harpole D, Moore MB, Danenberg KD, Danenberg PV, Meltzer SJ. Hypermethylated APC DNA in plasma and prognosis of patients with esophageal adenocarcinoma. *J Natl Cancer Inst* 2000; **92**: 1805-1811 [PMID: 11078757 DOI: 10.1093/jnci/92.22.1805]
75 **Buskens CJ**, Van Rees BP, Sivula A, Reitsma JB, Haglund C, Bosma PJ, Offerhaus GJ, Van Lanschot JJ, Ristimäki A. Prognostic significance of elevated cyclooxygenase 2 expression in patients with adenocarcinoma of the esophagus. *Gastroenterology* 2002; **122**: 1800-1807 [PMID: 12055587 DOI: 10.1053/gast.2002.33580]
76 **Gertler R**, Doll D, Maak M, Feith M, Rosenberg R. Telomere length and telomerase subunits as diagnostic and prognostic biomarkers in Barrett carcinoma. *Cancer* 2008; **112**: 2173-2180 [PMID: 18348304 DOI: 10.1002/cncr.23419]
77 **Saad RS**, El-Gohary Y, Memari E, Liu YL, Silverman JF. Endoglin (CD105) and vascular endothelial growth factor as prognostic markers in esophageal adenocarcinoma. *Hum Pathol* 2005; **36**: 955-961 [PMID: 16153457 DOI: 10.1016/j.humpath.2005.06.019]
78 **Falkenback D**, Nilbert M, Oberg S, Johansson J. Prognostic value of cell adhesion in esophageal adenocarcinomas. *Dis Esophagus* 2008; **21**: 97-102 [PMID: 18269642 DOI: 10.1111/j.1442-2050.2007.00749.x]
79 **Darnton SJ**, Hardie LJ, Muc RS, Wild CP, Casson AG. Tissue inhibitor of metalloproteinase-3 (TIMP-3) gene is methylated in the development of esophageal adenocarcinoma: loss of expression correlates with poor prognosis. *Int J Cancer* 2005; **115**: 351-358 [PMID: 15688381 DOI: 10.1002/ijc.20830]

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TOPIC HIGHLIGHT

WJGP 5th Anniversary Special Issues (6): Crohn's disease

Role of bowel ultrasound in the management of postoperative Crohn's disease

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Abstract

The use of biological and immunosuppressive therapy in Crohn's disease (CD) changed favorably the course of the disease and is currently suggested in the prevention of clinical recurrence. Symptomatic exacerbation is a feature of the natural course of the disease. Endoscopic recurrence may occur earlier than clinical manifestations and its rate is still high ever since the first year after surgery. The severity of mucosal lesions is highly predictive of a new flare of the disease so that the early detection of recurrence warrants strong therapeutic changes or a closer monitoring of the case. Endoscopy is at present the gold-standard technique for the diagnosis and grading of recurrence severity, but is poorly accepted by patients for its invasiveness. A simple and easy repeatable examination able to detect early signs of recurrence could be useful in the follow-up as an alternative or as a backing in the choice of the right timing for endoscopy in questionable cases. The use of bowel ultrasound (B-US) in the management of CD has grown in the past twenty years. Its accuracy in the real time detection of the disease and its complications, known since the 80's, together with the non-invasiveness, low cost and wide availability of the technique have influenced the extension of its clinical use in many referral centers in Europe. The latest generation of ultrasound scanners allows a precise and reproducible morphological assessment of the intestinal tract and the surrounding tissues and enables a complete evaluation of the disease. This review analyzes the literature history about B-US in the diagnosis of postoperative recurrence of CD and outlines the clinical implications of its use. Published works confirm a very good accuracy of B-US in the diagnosis of CD recurrence compared to endoscopy, also in the early phase. B-US shows a good correlation with Rutgeert's score grading, but does not prove significant association with C-reactive protein or CD Activity Index values. A wider use of B-US in the daily practice could allow to set a prompt diagnosis and an earlier and targeted treatment, probably sparing more invasive tests.

Key words: Ultrasound; Endoscopy; Postoperative; Crohn's disease; Recurrence

Core tip: In the recent years, after the introduction of new drugs, prevention of recurrence is one of the emerging issues in the management of Crohn's disease because a more aggressive and earlier therapy is supposed to change the clinical course of the disease. Endoscopy, that is presently the standard reference for the diagnosis, is not well tolerated by patients. To asses pre-clinical signs of recurrence a non-invasive alternative is needed. Magnetic resonance imaging shows accurate results but with high costs and low availability. Bowel ultrasound can detect early specific signs of recurrence. Advantages, limits and clinical implications of the technique are discussed below.

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INTRODUCTION

The therapeutic management of Crohn's disease (CD) patients is an open challenge. The correct use of steroids, antibiotics, immunomodulators and biological therapies requires an appropriate timing in the decision making process. From this point of view an early diagnosis of postoperative recurrence is extremely important in order to identify patients with a more aggressive course and to address the correct therapeutic choice. Recurrence is endoscopically present in around 70% of patients at 1 year after surgery. Early endoscopic signs of recurrence have been detected even three months after surgery and the severity of mucosal lesions is highly predictive of future clinical manifestations of the disease[1,2].

Endoscopy is at present the gold-standard for the diagnosis of recurrence but less invasive, repeatable techniques would fit better to follow the evolution of chronic disease if they showed comparable results. The use of computed tomography (CT) should be limited because of its biological invasiveness while magnetic resonance (MR) can not be carried out routinely for its substantial costs and inadequate availability.

Starting from the first reports in the 80's on the possibility of detecting inflammatory bowel diseases using ultrasounds, the role of this technique in characterizing inflammatory bowel disease (IBD) in terms of extension, activity and complications compared to radiology or endoscopy has steadily increased[3-8].

In the last decade the continuous improvement in ultrasound technology enabled a better definition of the bowel wall morphology. The addition of color-power doppler, oral or intravenous contrast to advanced ultrasound (US) technical equipment made it possible to distinguish fibrotic from inflammatory involvement of the intestinal tract, phlegmons from abscesses and to select a portion of patients at increased surgical risk or with optimal response to new pharmacological approaches[9-13].

Advantages and limits of the technique and the technical aspects of potential impact on clinical practice are discussed below.

LITERATURE ANALYSIS

All the studies available in literature define post-surgical US recurrence as an increased bowel wall thickness at the anastomosis level and the majority of them correlates US findings with endoscopy. Major obstacles to a correct interpretation of the literature are due to a significant heterogeneity in the studies' design (different reference standards and variability in the timing of procedures), in technical aspects (different cut-offs for bowel wall thickness, BWT) and in the use of additional technical equipment (Power Doppler, Enteral or Intravenous Contrast Agents).

Since 1986 DiCandio *et al*[14] described the possibility of detecting post-surgical recurrence using transabdominal US compared to contrast radiography and endoscopy. His pioneering work on 32 patients showed a good sensitivity (82%) and an excellent specificity (100%) of the technique with an overall accuracy of 93.7%. In this study the possibility to distinguish between inflammatory and neoplastic lesions is shown through a structural study of the bowel wall, paying particular attention to the integrity of its layers[14].

In 1998 Andreoli studied the US detection rate of CD recurrence in 47 patients who underwent terminal ileum resection for CD using endoscopy at the anastomotic site as the gold standard. Bowel US sensitivity was 81%, specificity 86% and the overall accuracy 83%. The authors suggest to perform US in case of clinical suspected recurrence, reserving ileocolonscopy to negative or uncertain cases[15].

In 2001 and 2004 two studies have been published on the role of ultrasonography in the detection of recurrence after conservative surgery (strictureplastic and/or miniresections)[16,17]. Thickness and echopattern (the sequence of layers that constitute the sonographic appearance of the intestinal wall) of the diseased wall were considered before and 6 mo after surgery in patients with ileal stictures in order to understand if these characteristics and their postoperative behavior have a prognostic value. Both thickness and echopattern, in different measure, are relevant in order to reliably predict recurrence (hazards ratio 8.8 and 4.1 respectively).

A possible role of US as a predictor of endoscopic recurrence has been evaluated by Orlando *et al*[18] in 2006. Looking for the best calprotectin cut-off to assess recurrence, 50 resected patients were studied with US and fecal calprotectin every three months after surgery. Endoscopy was performed at one year. US sensitivity with a 5 mm BWT cut-off was 26% and specificity 90%. The best calprotectin cut-off value to predict the highest number of endoscopic recurrences was > 200 mg/L (sensitivity 63% and specificity 75%). Considering such a high specificity of US, the authors suggest that a positive ultrasound 3 mo after surgery, may be an indication to colonoscopy. In case of US negative, faecal calprotectin with a cut-off value of 200 mg/L could be a useful tool in order to decide if performing colonoscopy in asymptomatic patients.

In the study of Biancone *et al*[19] Bowel US was performed with oral contrast (small intestine contrast ultrasound - SICUS). Twenty-two asymptomatic patients, prospectively followed after surgery, underwent clinical controls every 3 mo and SICUS, wireless capsule endoscopy (WCE) and colonoscopy 1 year after surgery. Seventeen patients underwent all the 3 procedures. SICUS showed 100% sensitivity, 0% specificity (16 TPs, 1 FP), whereas WCE 100% sensitivity, 100% specificity (16 TPs, 1 TN). The small serie was then split in smaller subgroups. Considering only neo-terminal ileum recurrence and excluding patients in which disease was limited to the anastomosis the sensibility was 86% and specificity 33 %. In a very small subgroup (10 patients) SICUS and WCE were performed at 3, 6 and 12 mo. SICUS identified four of the nine WCE positive at month 3. At month 6, eight of the nine WCE positive were detected by SICUS. No

significant correlation between BWT and Rutgeert's score was found.

A part of a long term prospective follow up study on severity of CD recurrence after ileal resection published in 2010 by Pallotta *et al*[20] reports on 58 CD patients scheduled to SICUS and ileocolonoscopy at 6 mo regular intervals after surgery. Ileocolonoscopy was performed within 2 wk from SICUS. Bowel wall thickness at the anastomosis site was measured and it correlated with the anastomotic recurrence degree sec. Rutgeerts. SICUS could detect extension of intramural lesions even in patients with tight anastomotic stenosis.

In 2010, Onali *et al*[21] performed a longitudinal prospective study in 25 patients 3 years after surgery using oral contrast US and obtaining a very good correlation between SICUS and endoscopy. The correspondence of SICUS detected lesions with Rutgeert's grade was moderate and the attempt of identifying a bowel wall thickness value predictive for clinical recurrence did not reach statistical significance[21].

Between 2006 and 2010 other four prospective studies comparing US performance with endoscopy have been published[22-25]. In these studies sensitivity varies from 79% to 92% and specificity from 20% to 95%. In two of them oral contrast was used[23,24]. For all of them ileocolonoscopy was the reference standard and bowel wall thickness (> 3 mm) the only pathological feature considered. In one paper Doppler findings were considered, slightly strengthening the accuracy only in moderate-severe recurrence and with no impact on recurrence detection[25]. Bowel wall thickness was compared with Rutgeerts' score obtaining a good correlation between ultrasonographic findings and endoscopic lesions. Using a cut-off of 5 mm for bowel wall thickness mild from severe disease can be distinguished. No significant correlations between CD activity index (CDAI) and SICUS where found[24], while SICUS showed a higher sensitivity and specificity in detecting recurrence compared to CRP and CDAI values[23].

The use of intravenous contrast enhancement ultrasonography (CEUS) to emphasize B-US findings was reported by Paredes *et al*[26] in a study on postoperative recurrence of CD. The sample size of the study is consistent (60 patients) and the interval between ileocolonoscopy and CEUS was 3 d only. The study considered bowel wall thickness (cut-off 3-5 mm recurrence present, > 5 mm moderate-severe), color doppler vascularity (subjectively graded) and CEUS. The authors quantify ultrasonographic activity, with a software processing of the difference in brightness of contrast enhancement maximum uptake and the baseline and worked out a US activity score that correlates with Rutgeert's degree of severity. B-US sensitivity rises with CEUS from 89.8% to 98% while specificity keeps 81%.

In the same year Cammarota *et al*[27] published the largest retrospective study on the subject and investigate in particular the possible predictive role of BWT on surgical recurrence. All the patients included (196) were followed for 114 mo on average and the rate of surgical recurrence was 20.4%. Bowel US was performed 6-15 mo after surgery; bowel wall thickness > 3 mm was predictive of surgical recurrence. Moreover the authors describe an increased percentage of surgical recurrence in higher values of BWT at 1 year after surgery[27].

CONCLUSION

Several studies have been performed on bowel ultrasound and post-surgical recurrence in CD. Although most of them have a small sample size and different study designs, a very good correspondence between US and ileocolonoscopy is reported even in the early stages after surgery[18,24]. Bowel wall thickness is the main US parameter in the detection of recurrence. The majority of the studies compare ultrasonografic with endoscopic findings and BWT values > 3 mm shows, except in two cases[18,19], high percentage of sensibility and specificity (until 100% both) in identifying recurrence[14,15,20-26]. Some studies demonstrate also a correlation between BWT values (> 5 mm) and Rutgeert's score severe disease grade[19-24].

Few studies consider the echopattern performance before and after surgery in addition to bowel wall thickness[16,17]. Morphological alterations of the echopattern are a relevant parameter in the follow-up of CD, and a good correspondence of different echopatterns with histologic findings has been shown[28]. Moreover the predictive value of different echopatterns on the relative risk of surgical treatment and the normalization of the echopattern after biologic therapy have been reported[9,13].

Despite the positive data supporting its use, this technique is not widespread and its use is substantially limited to some European countries. The main criticism raised by some authors is the supposed low reproducibility of the method.

Ultrasonography is by definition a subjective technique and its employement in the study of ileum and colon may be particularly difficult considering the scarcity of repere points, the high anatomical variability expecially in post-surgical patients and the presence of gas in the bowel which implies the use of graduated pressure to display the deepest loops. On this issue (the reproducibility of B-US in the evaluation of Crohn' disease) a multicenter study has been performed which brought together gastroenterologists sonographers and radiologists from six referral centers for inflammatory bowel diseases, including our group. We found in different clinical settings of CD a good k value concerning BWT (K = 0.72-1) and the presence of complications (K = 0.81-1)[29].

The performance of the examination, blinded, sequentially conducted by different operators, was preceded by a long theoretical comparison that led to the choice of parameters to be measured and methods of detection.

The results of this experience, combined with the well known positive characteristics of ultrasound (optimal tolerance, low invasiveness, low costs, wide availability) and the comparable accuracy values of B-US, CT and

MRI in different controlled settings of CD attest B-US as an added value in the clinical management of IBD[30-32].

In our opinion features needed for a correct use of B-US are an adequate learning curve, a good clinical knowledge in inflammatory bowel diseases and the basics of ultrasound technique. The use of B-US should be included in pathways of clinical management at different levels in the management of inflammatory bowel diseases (screening IBS-IBD, therapy monitoring, follow up of complications, emergency, young children) because it raises an efficient clinical work up and reduces the use of more expensive and invasive tests with similar results in terms of clinical impact[31,32].

It is conceivable that new technologies can improve the correspondence between imaging and the bowel wall morphology in intestinal inflammation. A wider confrontation among experienced operators on this and other interesting US parameters in B-US would be desirable.

REFERENCES

1 **Rutgeerts P**, Geboes K, Vantrappen G, Beyls J, Kerremans R, Hiele M. Predictability of the postoperative course of Crohn's disease. *Gastroenterology* 1990; **99**: 956-963 [PMID: 2394349]

2 **Olaison G**, Smedh K, Sjödahl R. Natural course of Crohn's disease after ileocolic resection: endoscopically visualised ileal ulcers preceding symptoms. *Gut* 1992; **33**: 331-335 [PMID: 1568651]

3 **Sonnenberg A**, Erckenbrecht J, Peter P, Niederau C. Detection of Crohn's disease by ultrasound. *Gastroenterology* 1982; **83**: 430-434 [PMID: 7084620]

4 **Hata J**, Haruma K, Suenaga K, Yoshihara M, Yamamoto G, Tanaka S, Shimamoto T, Sumii K, Kajiyama G. Ultrasonographic assessment of inflammatory bowel disease. *Am J Gastroenterol* 1992; **87**: 443-447 [PMID: 1553931]

5 **Maconi G**, Parente F, Bollani S, Cesana B, Bianchi Porro G. Abdominal ultrasound in the assessment of extent and activity of Crohn's disease: clinical significance and implication of bowel wall thickening. *Am J Gastroenterol* 1996; **91**: 1604-1609 [PMID: 8759670]

6 **Maconi G**, Bollani S, Bianchi Porro G. Ultrasonographic detection of intestinal complications in Crohn's disease. *Dig Dis Sci* 1996; **41**: 1643-1648 [PMID: 8769293]

7 **Parente F**, Maconi G, Bollani S, Anderloni A, Sampietro G, Cristaldi M, Franceschelli N, Bianco R, Taschieri AM, Bianchi Porro G. Bowel ultrasound in assessment of Crohn's disease and detection of related small bowel strictures: a prospective comparative study versus x ray and intraoperative findings. *Gut* 2002; **50**: 490-495 [PMID: 11889068]

8 **Fraquelli M**, Colli A, Casazza G, Paggi S, Colucci A, Massironi S, Duca P, Conte D. Role of US in detection of Crohn disease: meta-analysis. *Radiology* 2005; **236**: 95-101 [PMID: 15987966]

9 **Rigazio C**, Ercole E, Laudi C, Daperno M, Lavagna A, Crocella L, Bertolino F, Viganò L, Sostegni R, Pera A, Rocca R. Abdominal bowel ultrasound can predict the risk of surgery in Crohn's disease: proposal of an ultrasonographic score. *Scand J Gastroenterol* 2009; **44**: 585-593 [PMID: 19148846 DOI: 10.1080/00365520802705992]

10 **Castiglione F**, de Sio I, Cozzolino A, Rispo A, Manguso F, Del Vecchio Blanco G, Di Girolamo E, Castellano L, Ciacci C, Mazzacca G. Bowel wall thickness at abdominal ultrasound and the one-year-risk of surgery in patients with Crohn's disease. *Am J Gastroenterol* 2004; **99**: 1977-1983 [PMID: 15447760]

11 **Ripollés T**, Rausell N, Paredes JM, Grau E, Martínez MJ, Vizuete J. Effectiveness of contrast-enhanced ultrasound for characterisation of intestinal inflammation in Crohn's disease: a comparison with surgical histopathology analysis. *J Crohns Colitis* 2013; **7**: 120-128 [PMID: 22483566 DOI: 10.1016/j.crohns.2012.03.002]

12 **Calabrese E**, Zorzi F, Onali S, Stasi E, Fiori R, Prencipe S, Bella A, Petruzziello C, Condino G, Lolli E, Simonetti G, Biancone L, Pallone F. Accuracy of small-intestine contrast ultrasonography, compared with computed tomography enteroclysis, in characterizing lesions in patients with Crohn's disease. *Clin Gastroenterol Hepatol* 2013; **11**: 950-955 [PMID: 23375998 DOI: 10.1016/j.cgh.2013.01.015]

13 **Moreno N**, Ripollés T, Paredes JM, Ortiz I, Martínez MJ, López A, Delgado F, Moreno-Osset E. Usefulness of abdominal ultrasonography in the analysis of endoscopic activity in patients with Crohn's disease: changes following treatment with immunomodulators and/or anti-TNF antibodies. *J Crohns Colitis* 2014; **8**: 1079-1087 [PMID: 24613399 DOI: 10.1016/j.crohns.2014.02.008]

14 **DiCandio G**, Mosca F, Campatelli A, Bianchini M, D'Elia F, Dellagiovampaola C. Sonographic detection of postsurgical recurrence of Crohn disease. *AJR Am J Roentgenol* 1986; **146**: 523-526 [PMID: 3511636]

15 **Andreoli A**, Cerro P, Falasco G, Giglio LA, Prantera C. Role of ultrasonography in the diagnosis of postsurgical recurrence of Crohn's disease. *Am J Gastroenterol* 1998; **93**: 1117-1121 [PMID: 9672341]

16 **Maconi G**, Sampietro GM, Cristaldi M, Danelli PG, Russo A, Bianchi Porro G, Taschieri AM. Preoperative characteristics and postoperative behavior of bowel wall on risk of recurrence after conservative surgery in Crohn's disease: a prospective study. *Ann Surg* 2001; **233**: 345-352 [PMID: 11224621]

17 **Parente F**, Sampietro GM, Molteni M, Greco S, Anderloni A, Sposito C, Danelli PG, Taschieri AM, Gallus S, Bianchi Porro G. Behaviour of the bowel wall during the first year after surgery is a strong predictor of symptomatic recurrence of Crohn's disease: a prospective study. *Aliment Pharmacol Ther* 2004; **20**: 959-968 [PMID: 15521843]

18 **Orlando A**, Modesto I, Castiglione F, Scala L, Scimeca D, Rispo A, Teresi S, Mocciaro F, Criscuoli V, Marrone C, Platania P, De Falco T, Maisano S, Nicoli N, Cottone M. The role of calprotectin in predicting endoscopic post-surgical recurrence in asymptomatic Crohn's disease: a comparison with ultrasound. *Eur Rev Med Pharmacol Sci* 2006; **10**: 17-22 [PMID: 16494106]

19 **Biancone L**, Calabrese E, Petruzziello C, Onali S, Caruso A, Palmieri G, Sica GS, Pallone F. Wireless capsule endoscopy and small intestine contrast ultrasonography in recurrence of Crohn's disease. *Inflamm Bowel Dis* 2007; **13**: 1256-1265 [PMID: 17577246]

20 **Pallotta N**, Giovannone M, Pezzotti P, Gigliozzi A, Barberani F, Piacentino D, Hassan NA, Vincoli G, Tosoni M, Covotta A, Marcheggiano A, Di Camillo M, Corazziari E. Ultrasonographic detection and assessment of the severity of Crohn's disease recurrence after ileal resection. *BMC Gastroenterol* 2010; **10**: 69 [PMID: 20594291 DOI: 10.1186/1471-230X-10-69]

21 **Onali S**, Calabrese E, Petruzziello C, Zorzi F, Sica GS, Lolli E, Ascolani M, Condino G, Pallone F, Biancone L. Endoscopic vs ultrasonographic findings related to Crohn's disease recurrence: a prospective longitudinal study at 3 years. *J Crohns Colitis* 2010; **4**: 319-328 [PMID: 21122521]

22 **Rispo A**, Bucci L, Pesce G, Sabbatini F, de Palma GD, Grassia R, Compagna A, Testa A, Castiglione F. Bowel sonography for the diagnosis and grading of postsurgical recurrence of Crohn's disease. *Inflamm Bowel Dis* 2006; **12**: 486-490 [PMID: 16775492]

23 **Castiglione F**, Bucci L, Pesce G, De Palma GD, Camera L, Cipolletta F, Testa A, Diaferia M, Rispo A. Oral contrast-enhanced sonography for the diagnosis and grading of postsurgical recurrence of Crohn's disease. *Inflamm Bowel*

Dis 2008; **14**: 1240-1245 [PMID: 18398896 DOI: 10.1002/ibd.20469]

24 **Calabrese E**, Petruzziello C, Onali S, Condino G, Zorzi F, Pallone F, Biancone L. Severity of postoperative recurrence in Crohn's disease: correlation between endoscopic and sonographic findings. *Inflamm Bowel Dis* 2009; **15**: 1635-1642 [PMID: 19408327 DOI: 10.1002/ibd.20948]

25 **Paredes JM**, Ripollés T, Cortés X, Reyes MD, López A, Martínez MJ, Moreno-Osset E. Non-invasive diagnosis and grading of postsurgical endoscopic recurrence in Crohn's disease: usefulness of abdominal ultrasonography and (99m)Tc-hexamethylpropylene amineoxime-labelled leucocyte scintigraphy. *J Crohns Colitis* 2010; **4**: 537-545 [PMID: 21122557 DOI: 10.1016/j.crohns.2010.03.002]

26 **Paredes JM**, Ripollés T, Cortés X, Moreno N, Martínez MJ, Bustamante-Balén M, Delgado F, Moreno-Osset E. Contrast-enhanced ultrasonography: usefulness in the assessment of postoperative recurrence of Crohn's disease. *J Crohns Colitis* 2013; **7**: 192-201 [PMID: 22542055 DOI: 10.1016/j.crohns.2012.03.017]

27 **Cammarota T**, Ribaldone DG, Resegotti A, Repici A, Danese S, Fiorino G, Sarno A, Robotti D, Debani P, Bonenti G, Pellicano R, Andrealli A, Sapone N, Simondi D, Bresso F, Astegiano M. Role of bowel ultrasound as a predictor of surgical recurrence of Crohn's disease. *Scand J Gastroenterol* 2013; **48**: 552-555 [PMID: 23477675]

28 **Maconi G**, Carsana L, Fociani P, Sampietro GM, Ardizzone S, Cristaldi M, Parente F, Vago GL, Taschieri AM, Bianchi Porro G. Small bowel stenosis in Crohn's disease: clinical, biochemical and ultrasonographic evaluation of histological features. *Aliment Pharmacol Ther* 2003; **18**: 749-756 [PMID: 14510749]

29 **Fraquelli M**, Sarno A, Girelli C, Laudi C, Buscarini E, Villa C, Robotti D, Porta P, Cammarota T, Ercole E, Rigazio C, Senore C, Pera A, Malacrida V, Gallo C, Maconi G. Reproducibility of bowel ultrasonography in the evaluation of Crohn's disease. *Dig Liver Dis* 2008; **40**: 860-866 [PMID: 18583205 DOI: 10.1016/j.dld.2008.04.006]

30 **Maconi G**, Radice E, Greco S, Bezzio C, Bianchi Porro G. Transient small-bowel intussusceptions in adults: significance of ultrasonographic detection. *Clin Radiol* 2007; **62**: 792-797 [PMID: 17604770]

31 **Panés J**, Bouzas R, Chaparro M, García-Sánchez V, Gisbert JP, Martínez de Guereñu B, Mendoza JL, Paredes JM, Quiroga S, Ripollés T, Rimola J. Systematic review: the use of ultrasonography, computed tomography and magnetic resonance imaging for the diagnosis, assessment of activity and abdominal complications of Crohn's disease. *Aliment Pharmacol Ther* 2011; **34**: 125-145 [PMID: 21615440]

32 **Panes J**, Bouhnik Y, Reinisch W, Stoker J, Taylor SA, Baumgart DC, Danese S, Halligan S, Marincek B, Matos C, Peyrin-Biroulet L, Rimola J, Rogler G, van Assche G, Ardizzone S, Ba-Ssalamah A, Bali MA, Bellini D, Biancone L, Castiglione F, Ehehalt R, Grassi R, Kucharzik T, Maccioni F, Maconi G, Magro F, Martín-Comín J, Morana G, Pendsé D, Sebastian S, Signore A, Tolan D, Tielbeek JA, Weishaupt D, Wiarda B, Laghi A. Imaging techniques for assessment of inflammatory bowel disease: joint ECCO and ESGAR evidence-based consensus guidelines. *J Crohns Colitis* 2013; **7**: 556-585 [PMID: 23583097 DOI: 10.1016/j.crohns.2013.02.020]

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TOPIC HIGHLIGHT

WJGP 5th Anniversary Special Issues (6): Crohn's disease

Quality of care in Crohn's disease

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Abstract

Crohn's disease (CD) is a chronic and progressive inflammatory disease of the intestine. Overall, healthcare delivery for patients with CD is not optimal at the present time and therefore needs improvement. There are evidences which suggest that there is a variation in the care provided to patients with CD by the inflammatory bowel disease (IBD) experts and community care providers. The delivery of healthcare for patients with CD is often complex and requires coordination between gastroenterologists/IBD specialist, gastrointestinal surgeon, radiologists and IBD nurses. In order to improve the quality of health care for patients with CD, there is need that we focus on large-scale, system-wide changes including creation of IBD comprehensive care units, provision to provide continuous care, efforts to standardize care, and education of the community practitioners.

Key words: Inflammatory bowel disease; Quality assurance; Quality indicators; Outcome; Comprehensive care units; Quality improvement

Core tip: Crohn's disease (CD) is a progressive inflammatory disease of the intestine. Overall, healthcare delivery for patients with CD is not optimal at the present time and therefore needs improvement; Despite availability of national and international practice guidelines, there is a variation in the care provided to patients with CD; There is a need to develop well defined quality indicators which assures delivery of adequate care of the disease.

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INTRODUCTION

Crohn's disease (CD) is a chronic and progressive inflammatory disease of the intestine, which occurs because of interaction between, immunological factors, environmental factors and gut microbiome[1].

At the onset of the disease, the majority of patients with CD while have ulcerations in the intestine, the course of the disease gets complicated with patients developing strictures and fistula in the intestine[2]. In a study including 297 patients with CD over 25 years, Louis *et al*[3] reported a change in the behavior of the disease in 46% patients from non-stricturing, non-penetrating to either stricturing (27%) or penetrating (29%) disease in the first 10 years of follow-up. Because of the progressive nature of the disease, patients with CD are more likely to require not only repeated hospitalization but also surgical interventions[4,5].

While majority of patients with CD generally present in third and fourth decade of their lives, approximately one fifth of them become symptomatic during childhood and nearly 5% of them even before 10 years of their age[6]. Failure to thrive, retardation in the linear growth and defect in bone formation are the major issues in pediatric patients with active CD. Even puberty gets delayed in children patients with CD. Therefore, induction of re-

mission of the disease and maintenance of remission before the onset of the puberty is essential for children patients with CD. A good control of inflammatory activity is required to prevent or even minimize the consequences of a missed pubertal growth spurt and the maintenance of pre-pubertal levels of sex hormones. Since more than 90% of the bone mass is attained during childhood and adolescence, inflammatory diseases during this period can affect bone development and may ultimately lead to osteopenia and make them susceptible to fractures[7].

Till a few years back, control of symptoms has been considered to be an end point of treatment of CD; over the past years however, healing of mucosal ulcerations has emerged as a major therapeutic goal for patients with CD[8-10]. There are now evidences which suggest that healing of mucosal ulcerations with anti-inflammatory/immunomodulators or biologicals has a potential for changing the natural history of the disease and the available primitive evidences suggest that there is reduction in the rate of hospitalization and requirement for surgery in those patients who attains mucosal healing[11].

The treatment of CD depends upon the activity (active phase, remission phase), location, extent and behaviour (inflammatory, stricturing, fistulizing) of the disease[1]. The treatment needs to be tailored for each patient. The choice of treatment is also influenced by well-known negative prognostic predictors of CD such as young age of onset, presence of extensive disease, stricturing disease, and positive smoking history[12].

Chronic disease management has become a significant focus for providing a quality and continuous care of these diseases in order to decrease their morbidity and mortality[13]. Care of chronic diseases requires a continuous and optimal care including use of newly discovered with proven value diagnostic or therapeutic strategies. The question arises, are we providing a standard and quality care to patients having chronic diseases? In a landmark study from US, based on review of medical records and telephonic interviews, has shown that only 57% of patients attending the outpatients clinic regularly receive recommended standard of care for a variety of acute and chronic illnesses[14]. This study has raised an important concern and further highlights the importance of delivering an evidence based care and preventative measures to patients with chronic diseases in order to decrease complications, hospitalizations, and death.

QUALITY OF CARE IN INFLAMMATORY BOWEL DISEASE IS SUBOPTIMAL

Since there is no definite cure for most patients with CD, the main objectives of treatment therefore include induction of remission and maintenance of remission; minimization of complications of the disease such as strictures, fistulae, osteoporosis, short-and long-term toxicities of the drugs; improvement in quality of life; decrease in number of hospitalizations and surgeries; and maintenance of linear growth in pediatric patients. The practice of many chronic diseases is generally guided by evidence based literature and on the guidelines of both International and national societies[12,15-17]. While there is some degree of variability amongst the guidelines, the essential components remain more or less similar, since such recommendations are based on the available evidences derived from a body of published literature. Since CD is a disease with heterogeneous characteristics, treatment is generally tailored or individualized for a particular patient[12,15-18].

There is a variability in the treatment provided by an expert and a general practitioner especially for diseases, which are heterogeneous in their clinical behavior and where treatment options and guidelines are still emerging[19]. A variability in the care of a particular disease provided by various physicians is regarded as an index for poor quality of care. In inflammatory bowel disease (IBD), there is evidence of a high degree of variation of care for both patients with UC and CD[19]. In order to develop quality indicators for care, it is therefore, critical to understand the current status of care of such diseases. If current practice varies widely and is not well standardized, it calls for standardization of treatment protocols.

In a survey on the management of CD by IBD experts and community care providers, Esrailian *et al*[19] reported that there was good agreement in the decision making of diagnostic testing between community care providers and the IBD experts. In the management decisions, there was significant disagreement between community care providers and IBD experts[19]. While most community care providers in this study believed that 5-aminosalicylate products were appropriate across a variety of presentations of CD, IBD experts were significantly less likely to endorse 5-ASA use in patients with CD. In contrast to 5-ASA results, experts and community providers generally agreed with each other on the use of immunomodulators, infliximab and antibiotics in CD. Furthermore, the differences existed not only between community care providers and IBD experts; there was marked differences in the management decisions taken by various IBD experts, especially with the use of immunomodulators in newly diagnosed CD and perianal fistulizing CD[19].

Another study including patients with CD and UC also suggested that patients with IBD often do not receive optimal medical therapy. The main points include suboptimal dosing of 5-ASA and immunosuppressive therapy, prolonged use of corticosteroids, underuse of immunosuppressive drugs, non-compliance to use of calcium and vitamin D, and inadequate screening for colorectal cancer[20].

QUALITY IMPROVEMENT AND QUALITY ASSURANCE

Quality improvement (QI) and quality assurance (QA) are now becoming essential components of public services including delivery of healthcare services. While quality improvement is used to describe the process of

Table 1 Measures to provide quality care to patient with Crohn's disease

Delivery of high quality, safe and integrated clinical care for IBD patients based on multi-disciplinary team called IBD Comprehensive Care Unit
Delivery of care at the local center and if needed with rapid access to more specialized IBD care center
Patient education and support
Care for IBD patients that is patient-centered, responsive to individual needs
Regular audit of the care provided and outcomes

IBD: Inflammatory bowel disease.

implementing evidence-based interventions to bridge the disparities currently present in various healthcare systems; quality assurance is defined as planned, systematic activities that are implemented to ensure that a level of performance is attained[21]. In any chronic disease process the three main objective of care include improvement in population health, improvement in patient's experience of care, and at the mimimal cost; all three together are defined as Triple Aim of the disease[22].

The essential building blocks for quality improvement efforts are the proper identification and implementation of effective quality indicators. These quality indicators are measurable elements of practice performance for which there is evidence or consensus that they may be applied to assess and improve the quality provided[23-25]. The types of quality indicators have been broadly categorized as structural measures, process measures and outcome measures. Structural measures are indicators to do with the structure of the health system such as staffing, equipment, and electronic medical records. Process indicators are the processes of providing care such as investigations, treatment, and interactions with patients. Outcomes indicators assess the outcome of patients such as quality of life, patient satisfaction, prophylactic vaccines, mortality and morbidity. While improvement in all categories of indicators is desirable, process measures have garnered the majority of the attention, as they are most easily modifiable.

EFFORTS TO IMPROVE QUALITY OF CARE

Health care measures such as use of electronic medical record systems, automated entry of diagnostic and therapeutic orders, decision support tool at the point of care, and routine measurement of and reporting on quality have been shown to improve the quality of care[14]. In 2004, with funding from the American Board of Pediatrics, a group of care providers started a "research and improvement network", focused on improving care for children and teens with CD[26]. ImproveCareNow (ICN) network invited care providers to form collaboration to record information from all the patient visits and the care they were providing to children with IBD[27]. With insitution of protocal based recording of care, the group observed an increase in the proportion of visits with complete disease classification, measurement of thiopurine methyltransferase (TPMT) before initiation of thiopurines, and patients receiving an initial thiopurine dose appropriate to their TPMT status. Furthermore, an increase in the proportion of patients either CD or UC having inactive disease on follow up was observed, suggesting a better care. The number of patients taking prednisolone also decreased[28]. With the similar changes in the practice at IBD center at Cincinnati Children's Hospital Medical Center, there was an increase in the clinical remission rate from 59% to 76% ($P < 0.05$), decrease in frequency of steroid use from 17% to 10% and an increase in patients having Short Pediatric Crohn's Disease Activity Index < 15 from 60% to 77%[29].

These preliminary studies from ICN are testimony that a large-scale pediatric IBD quality improvement network can change practice and improve the quality of care. The key measures required for the delivery of quality care to patients with CD is summarized in Table 1.

QUALITY INDICATORS FOR IBD

There is a lack of definitive guidelines on the measurement of quality indices in IBD. The American Gastroenterology Association has recommended 10 indices as a measurement of quality of care in IBD[30] (Table 2). Similarly, the Crohn's and Colitis Foundation of America have also proposed a questionnaire for the assessment of quality of care of patients with IBD[31,32] (Table 2).

In order to identify a set of quality indices, Calvet *et al*[33] conducted a two-round web-based survey including an expert panel of patient representatives ($n = 4$), nurses ($n = 7$), surgeons ($n = 2$) and physicians ($n = 18$) using Delphi consensus-based approach. The expert panel selected a core set of 56 QIs (including 12 structure, 20 process and 24 outcome related). Structure and process quality indicators highlighted the need for multidisciplinary management and continuity of care. The key outcome quality indices focused on the adequate prophylaxis of disease complication and drug adverse events, the need to monitor appropriateness of treatment and the need to reinforce patient autonomy by providing adequate information and facilitating the patients' participation in their own care. The panel also suggested that there should be an IBD team and the team should be consisted of gastroenterologists, radiologists, surgeons, endoscopists, IBD nurse, and stoma management specialists.

HOW TO IMPROVE QUALITY OF CARE: A CONCEPT OF IBD COMPREHENSIVE CARE UNIT

The care of CD requires a coordinated action of a number of health care professionals such as a gastroenterologists/IBD expert, gastrointestinal surgeon, radiologist, stoma care personel and well trained nurses. All of them can

Table 2 Quality of care indicators in inflammatory bowel disease

	Quality of care indicators
10 quality of care indicators by American Gastroenterology Association	IBD: type, anatomic location and activity all assessed IBD preventive care: corticosteroid sparing therapy IBD preventive care: corticosteroid related iatrogenic injury - bone loss assessment IBD preventive care: influenza immunization IBD preventive care: pneumococcal immunization Testing for latent tuberculosis before initiating anti-TNF therapy Assessment of hepatitis B virus before initiating anti-TNF therapy Testing for Clostridium difficile - inpatient measure Prophylaxis for venous thromboembolism - inpatient measure IBD preventive care: tobacco user - screening and cessation intervention
CCFA top 10 quality outcome indicators of IBD	Corticosteroid use Proportion of patients with steroid-free clinical remission for a 12-mo period Proportion of patients currently taking prednisone Number of days per month and year lost from school or work because of IBD Number of days hospitalized per year because of IBD Number of emergency room visits per year for IBD Proportion of patients with malnutrition Proportion of patients with anemia Proportion of patients with normal disease-targeted health-related quality of life Proportion of patients currently taking narcotic analgesics Proportion of patients with nighttime bowel movements or leakage Proportion of patients with incontinence in the past month

IBD: Inflammatory bowel disease; TNF: Tumor necrosis factor; TB: Tuberculosis.

form a IBD Comprehensive Care Unit (ICCU). While it is commonly accepted that ICCUs facilitate the provision of quality care to patients with IBD, a structure of ICCU is still not well defined[33].

The cost of implementing some of these quality measures is modest suggesting that substantial improvement is possible. Individuals at all levels from senior clinicians to administrative staff should be encouraged to identify areas of potential improvement in the quality of care. In all settings, quality indicators should be seen as a team effort of the practice as a whole. One of the important features of chronic disease care is to provide continuous care, such as from clinic to home, interval reminder and also in between appointment care.

ANTAGONISTIC VIEW

While most supports the view that providing a quality care is a essential element of healthcare delivery system, a few believes that the imposition of quality measures may disrupt the art of medicine and the precious minutes at an office visit may be lost in documentation rather than spending time in thoughtfully delivered health care[22,34].

PROVIDING QUALITY OF CARE IN RESOURCE LIMITED COUNTRIES

Providing quality care in resource limited countries is a real challenge. The barrier to impart quality of CD care in resource limited countries may mainly be structure related such lack of optimal number of IBD experts, lack of diagnostic facilities, and affordability and non-referral of patients to tertiary care centers.

CONCLUSION

The delivery of healthcare for patients with CD is often complex and requires coordination between gastroenterologists/IBD specialist, gastrointestinal surgeon, radiologists and IBD nurses. Overall, healthcare delivery for patients with CD may not be the optimal at the present time and therefore needs improvement. There are evidences which suggests that there is a variation in the care provided by the IBD expert and general practitioner. To make substantial improvements in the quality of health care available to all patients, there is need of making large-scale, system-wide changes.

REFERENCES

1 **Baumgart DC**, Sandborn WJ. Crohn's disease. *Lancet* 2012; **380**: 1590-1605 [PMID: 22914295 DOI: 10.1016/S0140-6736(12)60026-9]
2 **Lichtenstein GR**. Emerging prognostic markers to determine Crohn's disease natural history and improve management strategies: a review of recent literature. *Gastroenterol Hepatol* (N Y) 2010; **6**: 99-107 [PMID: 20567551]
3 **Louis E**, Collard A, Oger AF, Degroote E, Aboul Nasr El Yafi FA, Belaiche J. Behaviour of Crohn's disease according to the Vienna classification: changing pattern over the course of the disease. *Gut* 2001; **49**: 777-782 [PMID: 11709511]
4 **Bewtra M**, Su C, Lewis JD. Trends in hospitalization rates for inflammatory bowel disease in the United States. *Clin Gastroenterol Hepatol* 2007; **5**: 597-601 [PMID: 17382602]
5 **Cosnes J**, Nion-Larmurier I, Beaugerie L, Afchain P, Tiret E, Gendre JP. Impact of the increasing use of immunosuppressants in Crohn's disease on the need for intestinal surgery. *Gut* 2005; **54**: 237-241 [PMID: 15647188]
6 **Rogers BH**, Clark LM, Kirsner JB. The epidemiologic and demographic characteristics of inflammatory bowel disease: an analysis of a computerized file of 1400 patients. *J Chronic Dis* 1971; **24**: 743-773 [PMID: 5146188]
7 **Sauer CG**, Kugathasan S. Pediatric inflammatory bowel disease: highlighting pediatric differences in IBD. *Gastroenterol Clin North Am* 2009; **38**: 611-628 [PMID: 19913205 DOI: 10.1016/j.gtc.2009.07.010]
8 **Iacucci M**, Ghosh S. Looking beyond symptom relief: evolution of mucosal healing in inflammatory bowel disease. *Therap Adv Gastroenterol* 2011; **4**: 129-143 [PMID: 21694814 DOI: 10.1177/1756283X11398930]
9 **Peyrin-Biroulet L**, Ferrante M, Magro F, Campbell S, Franchimont D, Fidder H, Strid H, Ardizzone S, Veereman-Wauters G, Chevaux JB, Allez M, Danese S, Sturm A. Results from the 2nd Scientific Workshop of the ECCO. I: Impact of mucosal healing on the course of inflammatory bowel dis-

ease. *J Crohns Colitis* 2011; **5**: 477-483 [PMID: 21939925 DOI: 10.1016/j.crohns.2011.06.009]
10 **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]
11 **Johnson LA**, Luke A, Sauder K, Moons DS, Horowitz JC, Higgins PD. Intestinal fibrosis is reduced by early elimination of inflammation in a mouse model of IBD: impact of a "Top-Down" approach to intestinal fibrosis in mice. *Inflamm Bowel Dis* 2012; **18**: 460-471 [PMID: 21761511 DOI: 10.1002/ibd.21812]
12 **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]
13 **Chassin MR**, Galvin RW. The urgent need to improve health care quality. Institute of Medicine National Roundtable on Health Care Quality. *JAMA* 1998; **280**: 1000-1005 [PMID: 9749483]
14 **Asch SM**, McGlynn EA, Hogan MM, Hayward RA, Shekelle P, Rubenstein L, Keesey J, Adams J, Kerr EA. Comparison of quality of care for patients in the Veterans Health Administration and patients in a national sample. *Ann Intern Med* 2004; **141**: 938-945 [PMID: 15611491]
15 **Bernstein CN**, Fried M, Krabshuis JH, Cohen H, Eliakim R, Fedail S, Gearry R, Goh KL, Hamid S, Khan AG, LeMair AW, Malfertheiner Q, Rey JF, Sood A, Steinwurz F, Thomsen OO, Thomson A, Watermeyer G. World Gastroenterology Organization Practice Guidelines for the diagnosis and management of IBD in 2010. *Inflamm Bowel Dis* 2010; **16**: 112-124 [PMID: 19653289 DOI: 10.1002/ibd.21048]
16 **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]
17 **Ueno F**, Matsui T, Matsumoto T, Matsuoka K, Watanabe M, Hibi T. Evidence-based clinical practice guidelines for Crohn's disease, integrated with formal consensus of experts in Japan. *J Gastroenterol* 2013; **48**: 31-72 [PMID: 23090001 DOI: 10.1007/s00535-012-0673-1]
18 **Ordás I**, Feagan BG, Sandborn WJ. Early use of immunosuppressives or TNF antagonists for the treatment of Crohn's disease: time for a change. *Gut* 2011; **60**: 1754-1763 [PMID: 21997558 DOI: 10.1136/gutjnl-2011-300934]
19 **Esrailian E**, Spiegel BM, Targownik LE, Dubinsky MC, Targan SR, Gralnek IM. Differences in the management of Crohn's disease among experts and community providers, based on a national survey of sample case vignettes. *Aliment Pharmacol Ther* 2007; **26**: 1005-1018 [PMID: 17877507]
20 **Reddy SI**, Friedman S, Telford JJ, Strate L, Ookubo R, Banks PA. Are patients with inflammatory bowel disease receiving optimal care? *Am J Gastroenterol* 2005; **100**: 1357-1361 [PMID: 15929770]
21 **Brook RH**, McGlynn EA, Cleary PD. Quality of health care. Part 2: measuring quality of care. *N Engl J Med* 1996; **335**: 966-970 [PMID: 8782507]
22 **Berwick DM**, Nolan TW, Whittington J. The triple aim: care, health, and cost. *Health Aff* (Millwood) 1996; **27**: 759-769 [PMID: 18474969 DOI: 10.1377/hlthaff.27.3.759]
23 **Weizman AV**, Nguyen GC. Interventions and targets aimed at improving quality in inflammatory bowel disease ambulatory care. *World J Gastroenterol* 2013; **19**: 6375-6382 [PMID: 24151356 DOI: 10.3748/wjg.v19.i38.6375]
24 **Quach P**, Nguyen GC, Benchimol EI. Quality improvement in pediatric inflammatory bowel disease: moving forward to improve outcomes. *World J Gastroenterol* 2013; **19**: 6367-6374 [PMID: 24151355 DOI: 10.3748/wjg.v19.i38.6367]
25 **Weizman AV**, Nguyen GC. Quality of care delivered to hospitalized inflammatory bowel disease patients. *World J Gastroenterol* 2013; **19**: 6360-6366 [PMID: 24151354 DOI: 10.3748/wjg.v19.i38.6360]
26 ImproveCareNow. Available from: URL: http://improvecarenow.org/about/how-we-started. Accessed on January 20, 2014
27 **Crandall WV**, Boyle BM, Colletti RB, Margolis PA, Kappelman MD. Development of process and outcome measures for improvement: lessons learned in a quality improvement collaborative for pediatric inflammatory bowel disease. *Inflamm Bowel Dis* 2011; **17**: 2184-2191 [PMID: 21456033 DOI: 10.1002/ibd.21702]
28 **Crandall WV**, Margolis PA, Kappelman MD, King EC, Pratt JM, Boyle BM, Duffy LF, Grunow JE, Kim SC, Leibowitz I, Schoen BT, Colletti RB. Improved outcomes in a quality improvement collaborative for pediatric inflammatory bowel disease. *Pediatrics* 2012; **129**: e1030-e1041 [PMID: 22412030 DOI: 10.1542/peds.2011-1700]
29 **Samson CM**, Morgan P, Williams E, Beck L, Addie-Carson R, McIntire S, Booth A, Mendez E, Luzader C, Tomer G, Saeed S, Donovan E, Bucuvalas J, Denson LA. Improved outcomes with quality improvement interventions in pediatric inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 2012; **55**: 679-688 [PMID: 22699837 DOI: 10.1097/MPG.0b013e318262de16]
30 American Gastroenterology Association: Adult inflammatory bowel disease physician performance measure set. Accessed on January 20, 2014. Available from: URL: http://www.gastro.org/practice/quality-initiatives/IBD_Measures.pdf
31 **Melmed GY**, Siegel CA, Spiegel BM, Allen JI, Cima R, Colombel JF, Dassopoulos T, Denson LA, Dudley-Brown S, Garb A, Hanauer SB, Kappelman MD, Lewis JD, Lynch I, Moynihan A, Rubin DT, Sartor RB, Schwartz RM, Wolf DC, Ullman TA. Quality indicators for inflammatory bowel disease: development of process and outcome measures. *Inflamm Bowel Dis* 2013; **19**: 662-668 [PMID: 23388547 DOI: 10.1097/mib.0b013e31828278a2]
32 **Melmed GY**, Siegel CA. Quality improvement in inflammatory bowel disease. *Gastroenterol Hepatol* (N Y) 2013; **9**: 286-292 [PMID: 23943663]
33 **Calvet X**, Panés J, Alfaro N, Hinojosa J, Sicilia B, Gallego M, Pérez I, Lázaro y de Mercado P, Gomollón F, Aldeguera X, Alós R, Andreu M, Barreiro M, Bermejo F, Casis B, Domenech E, Espín E, Esteve M, García-Sánchez V, López-Sanromán A, Martínez-Montiel P, Luis Mendoza J, Gisbert JP, Vera M, Dosal A, Sánchez E, Marín L, Sanromán L, Pinilla P, Murciano F, Torrejón A, Ramón García J, Ortega M, Roldán J. Delphi consensus statement: Quality Indicators for Inflammatory Bowel Disease Comprehensive Care Units. *J Crohns Colitis* 2014; **8**: 240-251 [PMID: 24295646 DOI: 10.1016/j.crohns.2013.10.010]
34 **Siegel CA**, Allen JI, Melmed GY. Translating improved quality of care into an improved quality of life for patients with inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2013; **11**: 908-912 [PMID: 23747710 DOI: 10.1016/j.cgh.2013.05.027]

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TOPIC HIGHLIGHT

WJGP 5th Anniversary Special Issues (9): Gastrointestinal bleeding

Diagnosis of gastrointestinal bleeding: A practical guide for clinicians

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Abstract

Gastrointestinal bleeding is a common problem encountered in the emergency department and in the primary care setting. Acute or overt gastrointestinal bleeding is visible in the form of hematemesis, melena or hematochezia. Chronic or occult gastrointestinal bleeding is not apparent to the patient and usually presents as positive fecal occult blood or iron deficiency anemia. Obscure gastrointestinal bleeding is recurrent bleeding when the source remains unidentified after upper endoscopy and colonoscopic evaluation and is usually from the small intestine. Accurate clinical diagnosis is crucial and guides definitive investigations and interventions. This review summarizes the overall diagnostic approach to gastrointestinal bleeding and provides a practical guide for clinicians.

Key words: Gastrointestinal hemorrhage; Diagnostic techniques; Endoscopy; Colonoscopy; Capsule endoscopy; Enteroscopy; Computed tomography; Angiography

Core tip: This review provides a practical diagnostic guide for clinicians who encounter patients with suspected gastrointestinal bleeding in the hospital and primary health care settings. Clinical presentations of gastrointestinal bleeding are classified as overt (acute), occult (chronic) or obscure and the corresponding diagnostic algorithms are illustrated through review of the key evidence and consensus guidelines. Upper endoscopy and colonoscopy are the mainstay of initial investigations. Angiography and radionuclide imaging are best suited for acute overt gastrointestinal (GI) bleeding. Capsule endoscopy and deep enteroscopy play significant roles in the diagnosis of obscure GI bleeding, usually from the small bowel.

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INTRODUCTION

Gastrointestinal (GI) bleeding is a common problem medical practitioners encounter in the emergency department and in the primary care setting[1]. Annual hospital admissions for GI bleeding in the United States and United Kingdom have been estimated at up to 150 patients per 100000 population with a mortality rate of 5%-10%[2-5]. While GI bleeding can be potentially life-threatening, it has been shown that many cases can be safely managed on an outpatient basis[6]. The accurate diagnosis of GI bleeding relies on prompt resuscitation, initial risk evaluation, provisional clinical diagnosis followed by appropriate definitive investigation which enables specific interventions. This review provides a practical diagnostic guide for clinicians who may encounter patients with suspected GI bleeding.

DEFINITIONS

Overt (acute) vs occult (chronic) vs obscure

Although GI bleeding can be a result of benign pathology, life-threatening hemorrhage, varices, ulceration and malignant neoplasms need to be considered and carefully excluded[7,8]. Given the wide range of underlying pathology and the differences in their appropriate diagnostic approach, it is crucial for clinicians to define the type of GI bleeding based on clinical presentation.

Depending on the rate of blood loss, GI bleeding can manifest in several forms and can be classified as overt, occult or obscure. Overt GI bleeding, otherwise known as acute GI bleeding, is visible and can present in the form of hematemesis, "coffee-ground" emesis, melena, or hematochezia. Occult or chronic GI bleeding as a result of microscopic hemorrhage can present as Hemoccult-positive stools with or without iron deficiency anemia[9,10]. The American Gastroenterological Association defines occult GI bleeding as the initial presentation of a positive fecal occult blood test (FOBT) result and/or iron-deficiency anemia when there is no evidence of visible blood loss to the patient or clinician[11]. Obscure GI bleeding refers to recurrent bleeding in which a source is not identified after upper endoscopy and colonoscopy. Obscure bleeding may be either overt or occult[10-12].

Upper vs lower

Upper GI bleeding includes hemorrhage originating from the esophagus to the ligament of Treitz, at the duodenojejunal flexure[13]. Lower GI bleeding is defined as bleeding that originates from a site distal to the ligament of Treitz[14]. In recent years upper GI bleeding has been redefined as bleeding above the ampulla of Vater within reach of an upper endoscopy; lower GI bleeding has been further subdivided into mid GI bleeding coming from the small bowel between the ampulla of Vater to the terminal ileum, and lower GI bleeding coming from the colon[11].

OVERT (ACUTE) GI BLEEDING

Epidemiology

Acute GI bleeding is a major cause of hospital admissions in the United States, which is estimated at 300000 patients annually[15]. Upper GI bleeding has an annual incidence that ranges from 40-150 episodes per 100000 persons and a morality rate of 6%-10%[16-18]; compared with lower GI bleeding which has an annual incidence ranging from 20-27 episodes per 100000 persons and a mortality rate of 4%-10%[19,20]. Acute GI bleeding is more common in men than women and its prevalence increases with age[13,21].

Etiology and pathophysiology

Acute upper GI bleeding may originate in the esophagus, stomach, and duodenum. Upper GI bleeding can be categorized based upon anatomic and pathophysiologic factors: ulcerative, vascular, traumatic, iatrogenic, tumors, portal hypertension. The commonest causes of acute upper GI bleeding are peptic ulcer disease including from the use of aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs), variceal hemorrhage, Mallory-Weiss tear and neoplasms including gastric cancers[8]. Other relatively common causes include esophagitis, erosive gastritis/duodenitis, vascular ectasias and Dieulafoy's lesions[22]. Significant geographical variations in pathophysiology exist for esophageal varices and peptic ulceration between the East and the West, with East Asians having a stronger association with non-alcoholic cirrhosis and helicobacter pylori as their respective etiologies which generally have a more favorable prognosis[23,24]. However, esophageal varices and peptic ulcer disease are nevertheless major causes of upper GI bleeding in both Eastern and Western societies[24,25].

Acute lower GI bleeding may originate in the small bowel, colon or rectum[21]. The causes of acute lower GI bleeding may also be grouped into categories based on the pathophysiology: vascular, inflammatory, neoplastic, traumatic and iatrogenic. Common causes of lower GI bleeding are diverticular disease, angiodysplasia or angiectasia, neoplasms including colorectal cancer, colitis including Crohn's disease and ulcerative colitis, and benign anorectal lesions such as hemorrhoids, anal fissures and rectal ulcers[8].

In the special setting where the patient is known to have an abdominal aortic aneurysm or an aortic graft, acute GI bleeding should be considered secondary to aortoenteric fistula until proven otherwise[26].

Initial evaluation

Rapid assessment and resuscitation should precede diagnostic evaluation in unstable patients with acute severe bleeding[27]. Once hemodynamic stability is assured, patients should be evaluated for the immediate risk of rebleeding and complications, as well as the underlying source of bleeding. For acute upper GI bleeding, risk scores such as the Rockall Score and Glasgow Blatch-

ford Score (GBS) have been developed and validated[6,28]. Patients with minimal or intermittent bleeding who are stratified as low risk can be evaluated in an outpatient setting, allowing more effective utilization of limited hospital in-patient resources[19]. While the Rockall score uses endoscopic findings, the GBS is based upon the patient' s clinical presentation such as systolic blood pressure, pulse, presence of melena, syncope, hepatic disease, cardiac failure and laboratory parameters such as blood urea nitrogen and hemoglobin. A meta-analysis found that a GBS of zero decreases the likelihood of requiring urgent intervention (likelihood ratio 0.02, 95%CI: 0-0.05)[4]. Therefore, the GBS may be best suited for initial risk evaluation of suspected acute upper GI bleeding, such as in the emergency department setting.

As in the diagnosis of any disease, the clinical history, physical examination and initial laboratory findings are crucial in determining the likely sources of bleeding which would help direct the appropriate definitive investigation and intervention. A medication history here is particularly important, especially on the use of aspirin and other NSAIDs.

Clinical presentation

Upper GI bleeding usually presents with hematemesis (vomiting of fresh blood), "coffee-ground" emesis (vomiting of dark altered blood), and/or melena (black tarry stools). Hematochezia (passing of red blood from rectum) usually indicates bleeding from the lower GI tract, but can occasionally be the presentation for a briskly bleeding upper GI source[9]. The presence of frank bloody emesis suggests more active and severe bleeding in comparison to coffee-ground emesis[29]. Variceal hemorrhage is life threatening and should be a major consideration in diagnosis as it accounts for up to 30% of all cases of acute upper GI bleeding and up to 90% in patients with liver cirrhosis[30].

Lower GI bleeding classically presents with hematochezia, however bleeding from the right colon or the small intestine can present with melena[31]. Bleeding from the left side of the colon tends to present bright red in color, whereas bleeding from the right side of the colon often appears dark or maroon-colored and may be mixed with stool[31].

Other presentations which can accompany both upper and lower GI bleeding include hemodynamic instability, abdominal pain and symptoms of anemia such as lethargy, fatigue, syncope and angina[21]. Patients with acute bleeding usually have normocytic red blood cells. Microcytic red blood cells or iron deficiency anemia suggests chronic bleeding. In contrast to patients with acute upper GI bleeding, patients with acute lower GI bleeding and normal renal perfusion usually have a normal blood urea nitrogen-to-creatinine or urea-to-creatinine ratio[32]. In general, anatomic and vascular causes of bleeding present with painless, large-volume blood loss, whereas inflammatory causes of bleeding are associated with diarrhoea and abdominal pain[33].

When patients with known abdominal aortic aneurysm or aortic graft present with above symptoms of GI bleeding, aortoenteric fistula most commonly at the duodenum should be strongly suspected. In this case, urgent computed tomography (CT) abdomen or CT angiogram is indicated to look for loss of tissue plane between the aorta and duodenum, contrast extravasation and the presence of gas indicating graft infection. Upper endoscopy prior to surgical intervention may help exclude other diagnoses when CT findings are not definitive[26,34]. The details of these investigations are discussed later in this review.

Investigations

Options for the investigation of acute GI bleeding include upper endoscopy and/or colonoscopy, nuclear scintigraphy, CT angiogram and catheter angiography. The investigation of choice would be guided by the suspected location of bleeding (upper *vs* lower GI) based on clinical presentation. In most circumstances, the standard of care for the initial diagnostic evaluation of suspected acute GI bleeding is urgent upper endoscopy and/or colonoscopy, as recommended by guidelines from the American College of Gastroenterology and the 2010 International Consensus Recommendations[20,27]. As investigations are being planned, infusions of proton pump inhibitor or octreotide should be initiated for suspected bleeding peptic ulcer and varices respectively[27,30].

Upper endoscopy

In patients with acute upper GI bleeding, upper endoscopy is considered the investigation of choice[35]. Early upper endoscopy within 24 h of presentation is recommended in most patients with acute upper GI bleeding to confirm diagnosis and has the benefit of targeted endoscopic treatment (Figure 1), resulting in reduced morbidity, hospital length of stay, risk of recurrent bleeding and the need for surgery[27]. Endoscopic evacuation of hematoma or blood clot may enable visualization of underlying pathology such as a visible vessel in a peptic ulcer and allows directed endoscopic hemostatic therapy[36,37]. The reported sensitivity and specificity of endoscopy for upper gastroduodenal bleeding are 92%-98% and 30%-100%, respectively[38]. Risks of upper endoscopy include aspiration, side-effects from sedation, perforation, and increased bleeding while attempting therapeutic intervention. The airway should be secured by endotracheal intubation in the case of massive upper GI bleeding.

The use of nasogastric-tube insertion and gastric lavage in all patients with suspected upper GI bleeding is controversial and studies have failed to demonstrate a benefit in clinical outcomes[39,40]. The use of prokinetics such as erythromycin and metoclopramide as a single dose before upper endoscopy promotes gastric emptying and clearance of blood, clots and food. Two meta-analyses have demonstrated the use of a prokinetic agent improved visibility at endoscopy and significantly reduced the need for repeat endoscopy[41,42]. In particular, the use

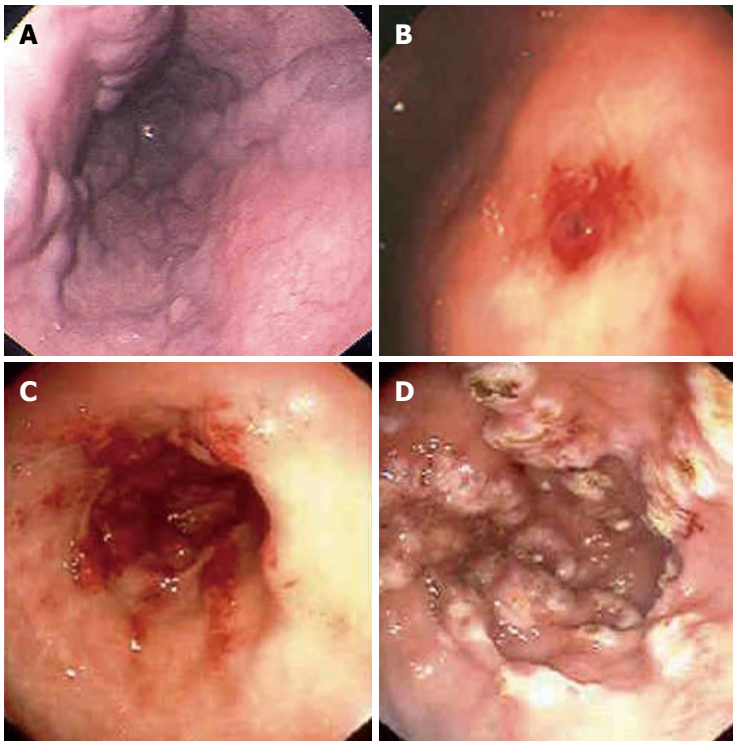

Figure 1 Upper endoscopic findings in patients with suspected upper gastrointestinal bleeding. Esophageal varices (A), Dieulafoy's lesion in the stomach (B), gastric antral vascular ectasia (watermelon stomach) in the antrum of the stomach pre and post argon plasma coagulation therapy (C, D).

of erythromycin was associated with a decrease in the amount of blood in the stomach, reduced amount of blood transfusion and shorter length of hospital stay[42]. Therefore prokinetics such as erythromycin before upper endoscopy should be recommended for patients with major bleeding who are expected to have large amount of blood in the stomach.

The practice of routine second look endoscopy after hemostasis is achieved on first endoscopy remains controversial. Two meta-analyses of randomized controlled trials have shown that second look endoscopy significantly reduced peptic ulcer rebleeding but did not improve overall mortality[43,44]. Due to the relatively small number of subjects studied, suboptimal hemostatic measures used and the lack of proton pump inhibitor use in those trials, the 2010 International Consensus Recommendations did not recommend routine use of second look endoscopy but stated it may be useful in selected patients with high risk of re-bleeding[27]. This should be considered particularly when there are concerns of suboptimal prior endoscopy and potential missed lesions.

In cases of acute upper GI bleeding where upper endoscopy is non-diagnostic in which a bleeding site cannot be identified or treated, the next investigation depends on the patient's hemodynamic stability. If the patient is unstable with large volume upper GI blood loss, patient should proceed to urgent surgery, such as an exploration and partial gastrectomy for uncontrolled bleeding gastric ulcer[9]. Intraoperative endoscopy may be a useful adjunct during surgery to help localize the source of bleeding[45,46]. If the patient is hemodynamically stable with low volume bleeding, repeat endoscopy may be considered. Colonoscopy should also be considered in the setting of melena to exclude a right-sided colonic source of bleeding, as discussed later.

Further imaging should be considered after non-diagnostic upper endoscopy with or without colonoscopy and the options include CT angiography, catheter angiography and nuclear scintigraphy[38], all of which are discussed separately in later sections of this review. Upper GI barium studies are contraindicated in the setting of acute upper GI bleeding because they may interfere with subsequent investigations or surgery[22], and due to the risk of barium peritonitis if there is a pre-existing perforation of the bowel wall[47].

Colonoscopy

In acute lower GI bleeding, the diagnostic approach is somewhat more variable. Colonoscopy and CT angiogram are the two diagnostic tools of choice for evaluation of acute lower GI bleeding[15]. The American College of Gastroenterology guidelines suggest that colonoscopy should be the first-line diagnostic modality for evaluation and treatment of lower GI bleeding[20]. Studies have indicated that colonoscopy identifies definitive bleeding sites (Figure 2) in 45%-90% of patients[48]. Advantages of colonoscopy include direct visualization, access to tissue biopsy and endoscopic hemostatic therapy, and as an initial diagnostic test has a higher sensitivity[15,49]. However, there are several limitations to colonoscopy in the setting of acute lower GI bleeding, including potential inadequate bowel preparation, the inability to evaluate most of the small bowel, as well as risks associated with sedation, perforation and bleeding similar to upper endoscopy[50]. In patients with inadequate bowel preparation, the sensitivity drops significantly and successful treatment may only be possible in as few as 21% of patients in the acute setting[51]. It has been advocated that urgent colonoscopy

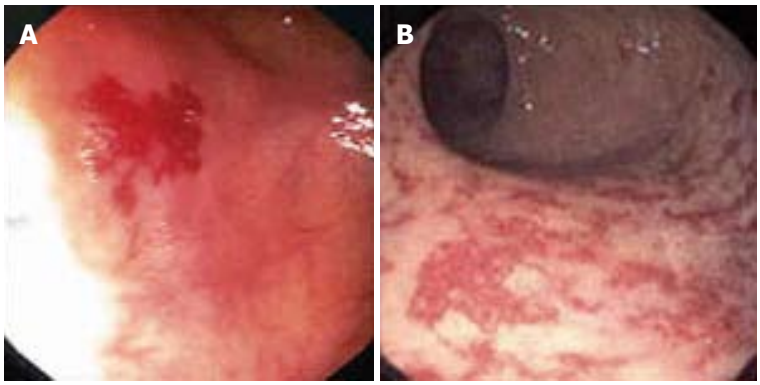

Figure 2 Colonoscopic findings in patients with suspected lower gastrointestinal bleeding. Colonic angiodysplasia (A) and radiation proctopathy (B).

in this setting should be preceded by a rapid purge with isotonic colonic lavage 4-6 liters orally until the effluent passed is diluted pink in color. This rapid purge may require the use of a nasogastric tube and a prokinetic agent such as metoclopramide. This is based on the findings that blood or stool in the colon can obscure the bleeding source during urgent colonoscopy[51,52].

It is recommended by the American College of Radiology that colonoscopy be utilized as the initial modality in hemodynamically stable patients (allowing for adequate bowel preparation) and angiography in those are who are hemodynamically unstable with massive lower GI bleeding[53]. It should be noted that colonoscopy is also indicated in the evaluation of patients presenting with melena who have negative upper endoscopy to exclude a right-sided colonic source of bleeding.

In cases where the source of bleeding is unidentified after upper endoscopy and/or colonoscopy, the utilization of subsequent diagnostic modalities should be guided by clinical presentation, hemodynamic stability and local expertise with the individual tests. No large randomized trials have demonstrated superiority of a particular strategy. The next section will outline the diagnostic use of CT angiography, catheter angiography and radionuclide imaging in acute GI bleeding.

CT angiography

CT angiography requires the rate of ongoing arterial bleeding to be at least 0.5 mL/min to reliably show extravasation of contrast into the bowel lumen to identify a bleeding site[54]. A systematic review of the diagnostic accuracy of CT angiography demonstrated a sensitivity of 86% and specificity of 95% in the evaluation of patients with acute GI bleeding[55]. The potential advantages of CT angiogram in diagnosis of acute GI bleeding include its minimally invasive nature and its wider availability in comparison to catheter angiography[38]. It can also demonstrate neoplasms or vascular malformations and provide evidence of recent bleeding, such as hyperdense blood in bowel lumen[38,56]. Active GI bleeding is diagnosed by extravasation of contrast into the bowel lumen, which appears as an area of high attenuation on the arterial phase scan which increases on the venous phase scan (Figure 3A-D). By demonstrating the precise site of bleeding and the underlying etiology, CT angiography is useful for directing and planning definitive treatment whether it be through endoscopy, catheter angiography or surgery[57]. If the gastrointestinal bleeding is intermittent and the initial CT is negative, a repeat CT angiogram can be performed when rebleeding occurs[58].

Disadvantages of CT angiography is the lack of therapeutic capability, risk of contrast induced nephropathy in patients with renal impairment and contrast allergy[59]. It has been suggested that the role of CT angiography in evaluation of patients with acute GI bleeding is in those who are stable and when upper endoscopy or colonoscopy is unable to locate the site of bleeding. Patients with massive GI hemorrhage with hemodynamic instability are recommended to proceed directly to catheter angiography or urgent surgery[38].

Catheter angiography

Catheter angiography can detect bleeding at rates of 0.5 to 1.5 mL/min[60,61]. It is used often in suspected acute lower GI bleeding due to anatomical availability of end arteries and is more challenging in acute upper GI bleeding due to the presence of multiple collateral vessels[62]. In comparison to other imaging modalities it offers the advantages of being both a diagnostic and therapeutic tool allowing for infusion of vasoconstrictive drugs and/or embolization (Figure 3E and F). It also does not require bowel preparation. The sensitivity for a diagnosis of acute GI bleeding is 42%-86% with the specificity close to 100%[63]. Other factors that may affect the sensitivity of angiography include intermittent bleeding, procedural delays, atherosclerotic anatomy, and venous or small vessel bleeding[64,65].

Complications include access-site hematoma or pseudoaneurysm, arterial dissection or spasm, bowel ischemia, and contrast-induced nephropathy or allergic reaction. Complications occur in 0%-10% of patients undergoing angiography, with the incidence of serious complications occurring in < 2% of patients[48,66]. It is recommended that catheter angiography be reserved for patients in whom endoscopy is not feasible due to severe bleeding with hemodynamic instability, or in those with persistent

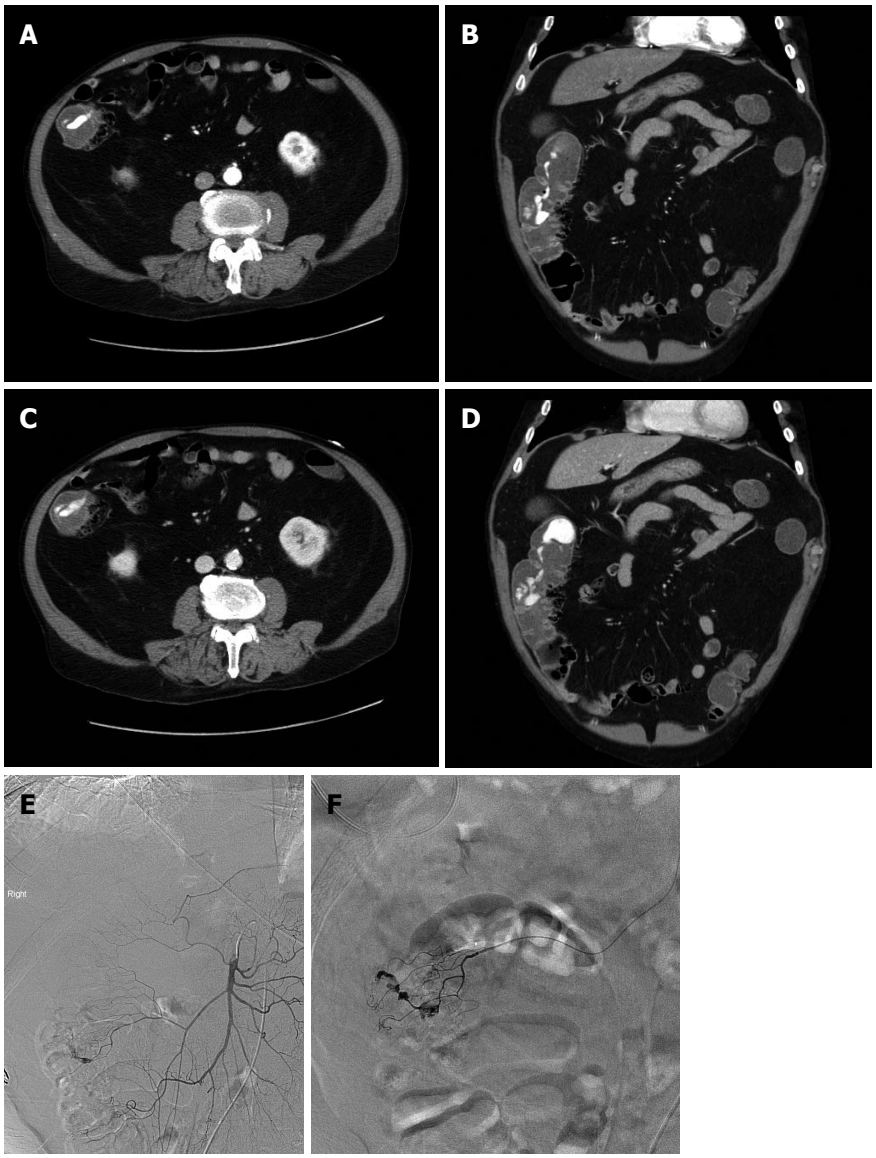

Figure 3 73-year-old man with per rectal bleeding and active gastrointestinal hemorrhage. Contrast enhanced computed tomography (CT) angiogram images show extravasation of contrast into the lumen of the ascending colon, with pooling of contrast which increases from the arterial phase (A, B) to the delayed venous phase (C, D). Diverticula are seen arising from the medial wall of the ascending colon indicating the etiology of bleeding. Following the CT angiogram, the patient underwent catheter angiography, which demonstrated blush of contrast from the right colic branch of the superior mesenteric artery (E). Selective catheterization of the right colic artery demonstrates the bleeding focus more clearly (F). Gelfoam and coil embolization was subsequently performed.

or recurrent GI bleeding and a non-diagnostic upper endoscopy and/or colonoscopy[20].

Radionuclide imaging

The threshold rate of GI bleeding for localization with radionuclide scanning is 0.1 mL/min, and this is the most sensitive imaging modality for GI bleeding[67]. Nuclear scans are either technetium-99m (^{99m}Tc) sulphur colloid or ^{99m}Tc pertechnetate-labelled autologous red blood cells. The short half-life of ^{99m}Tc sulphur colloid is a limitation as this means that patients must be actively bleeding during the few minutes the label is present in the intravascular space, and repeat scanning for intermittent bleeding is not possible without reinjection. ^{99m}Tc pertechnetate-labelled red blood cell scan allows for frequent abdominal images up to 24 h if necessary and is more commonly utilized for investigation of patients with obscure, intermittent bleeding. The main disadvantage of this test is poor anatomic localization of the bleeding site, and this poorly predicts subsequent angiogram results[68,69]. Furthermore, radionuclide only provides functional data, and is unable to diagnose the pathological cause of GI bleeding. Although advocated as a guide for surgical resection, surgical planning should not be based on only a positive nuclear scan[70].

All imaging studies have the advantage of allowing the clinician to identify the location of bleeding throughout the GI tract, especially those originating from the small bowel. However, their use is often limited by the need for active bleeding at the time of investigation. Other diagnostic modalities such as push enteroscopy, deep small bowel enteroscopy and capsule endoscopy may be

of value when the above described investigations prove to be non-diagnostic and when patients are hemodynamically stable with low volume bleeding. These studies will be discussed in the subsequent section evaluating chronic occult GI bleeding.

OCCULT (CHRONIC) GI BLEEDING

Epidemiology

Chronic occult GI bleeding occurs in the setting of a positive FOBT and/or iron deficiency anemia. Iron deficiency is the most common cause of anemia worldwide. In developed countries the major cause of iron deficiency is secondary to chronic blood loss[71]. In the United States, it is estimated that 5%-11% of women and 1%-4% of men are iron deficient and 5% and 2% of adult women and men have iron deficiency anemia, respectively[72]. Iron deficiency anemia has traditionally been attributed to chronic occult GI bleeding, especially in groups other than premenopausal women, and warrants further investigation of the gastrointestinal tract, including for colorectal cancer[12].

Etiology and pathophysiology

Chronic occult GI bleeding may occur anywhere in the GI tract, from the oral cavity to the anorectum[73]. In a systematic review of five prospective studies, 29%-56% of patients had an upper GI source and 20%-30% of patients had a colorectal source of occult GI bleeding diagnosed by the means of upper endoscopy and colonoscopy. These studies were unable to identify a source in 29%-52% of patients[74]. Causes of chronic occult GI bleeding can be broadly categorized into mass lesions, inflammatory, vascular, and infectious[12]. More common causes include colorectal cancer (especially right-sided colon), severe esophagitis, gastric or duodenal ulcers including from the use of aspirin and other NSAIDs, inflammatory bowel disease, gastric cancer, celiac disease, vascular ectasias (any site), diverticula, and portal hypertensive gastropathy. Non-GI sources of blood loss such as hemoptysis and oropharyngeal bleeding can also cause a positive FOBT[75]. A small bowel source accounts for a high percentage of patients with chronic occult GI bleeding and negative findings on upper endoscopy and colonoscopy[10], which is classified as obscure GI bleeding.

Clinical presentation

Patients with iron deficiency anemia may or may not be symptomatic. Rockey[75] recommended that initial investigation be directed towards the location of specific symptoms if possible. In the absence of symptoms, particularly in the elderly, the colon should be evaluated first, and if this is negative, upper GI tract is further investigated[75]. A targeted history is of value to discern symptoms of unintentional weight loss (suggestive of malignancy), use of aspirin or other NSAIDs (ulcerative mucosal injury), antiplatelet or anticoagulant use, family history, liver disease, and previous gastrointestinal tract surgery[76]. Physical signs could indicate presence of an underlying condition such as celiac disease, inflammatory bowel disease, Plummer-Vinson syndrome, and Peutz-Jeghers syndrome[74].

Investigations

Once a patient has been identified as having positive FOBT and/or iron deficiency anemia, multiple diagnostic procedures are available for investigation of the GI tract. The choice and sequence of procedures will depend on clinical suspicion and symptoms[10]. Endoscopic measures include upper endoscopy, colonoscopy, deep enteroscopy, or capsule endoscopy. CT colonography, CT and magnetic resonance (MR) enterography are some of the radiographic investigations utilized in the evaluation of patients with chronic occult GI bleeding. The role of barium enema, small bowel series, enteroclysis, standard CT or MR imaging and nuclear scans have substantially declined due to their low diagnostic yield and the advent of capsule endoscopy[11]. The choice of investigation should also incorporate consideration of patient risk factors and preference. In general, colonoscopy and upper endoscopy are the initial investigations of choice for chronic occult GI bleeding[11].

Colonoscopy and upper endoscopy

The 2007 American Gastroenterological Association guidelines on obscure GI bleeding recommended that the evaluation of a patient with a positive FOBT depends upon whether iron deficiency anemia is present. Patients with positive FOBT and no anemia should first be investigated with a colonoscopy (if upper GI symptoms present then also upper endoscopy) whereas patients with iron deficiency anemia should undergo both upper endoscopy and colonoscopy[11]. Patients with negative findings on upper endoscopy and colonoscopy without anemia do not require further investigations, but those with anemia should be referred for further investigation of the small bowel. The initial small bowel investigation of choice, when available, is wireless capsule endoscopy[11].

Capsule endoscopy, push enteroscopy and deep enteroscopy

Wireless capsule endoscopy is a simple, non-invasive method to study the small intestine for evaluation of small intestinal occult GI bleeding (Figure 4). The diagnostic yield in patients with chronic occult and obscure GI bleeding (after negative upper endoscopy and colonoscopy) ranges from 55%-92% for capsule endoscopy[77,78] in comparison to 25%-30% for push endoscopy[79,80]. A meta-analysis of 14 studies demonstrated that the diagnostic yield of capsule endoscopy was superior to push enteroscopy (63% *vs* 28%) and barium studies (42% *vs* 6%)[81]. Capsule endoscopy also avoids the higher rates of morbidity and mortality associated with push enteroscopy[82]. Capsule endoscopy is less useful in evaluating colonic sources of bleeding because of retained stool, battery life and poor field of vision due

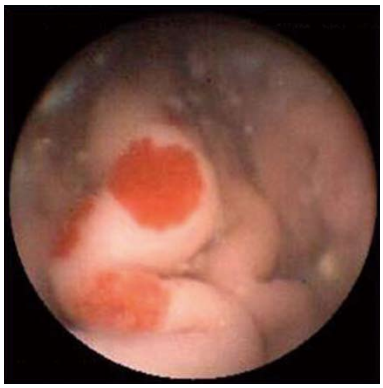

Figure 4 Jejunal angiodysplasia as seen on capsule endoscopy.

to the colon's large diameter[48]. Complications related to the procedure are rare and include capsule retention and obstruction[83].

Push enteroscopy can evaluate the GI tract to 60-80 cm of the proximal jejunum. However, with the availability of deep enteroscopy, which can reach to the distal small bowel, the use of push enteroscopy has diminished. Three systems widely used are: the double balloon endoscopy system, the single balloon enteroscope system, and the Endo-Ease Discovery SB small bowel enteroscope or spiral enteroscope, and may be performed *via* the oral or anal route[10]. Studies comparing the three different modalities are lacking. The advantage of deep enteroscopy over capsule endoscopy is that it can also be a therapeutic modality. The diagnostic yield of double-balloon enteroscopy varies from 40%-80% and therapeutic success ranging between 15%-55%[84,85].

Radiographic imaging modalities

Historically, an upper GI series with small bowel follow-through and/or enteroclysis was the next test performed, but in recent years, where available, CT and MR enterography have superseded these older radiographic modalities.

CT enterography involves ingestion of a neutral contrast agent to distend the small bowel which enables better evaluation of the small bowel wall in comparison to barium solutions. The alternative is MR enterography which has the advantage of not using ionizing radiation allowing serial imaging of the small bowel.

Compared to capsule endoscopy, CT enterography provides better visualization of the entire small bowel wall and shows extra-enteric complications of small bowel disease, whereas capsule endoscopy allows direct visualization of the small bowel mucosa and has a higher sensitivity for mucosal processes[86].

OBSCURE GI BLEEDING

Obscure GI bleeding accounts for 5% of patients of all cases of GI bleeding, both acute overt and chronic occult[12,76]. It is defined as recurrent bleeding when the source remains unidentified after endoscopic procedures and is most commonly caused by bleeding from the small intestine. The commonest causes of obscure GI bleeding include small bowel tumors, vascular anomalies such as angiodysplasias and varices, diverticula and Celiac disease. The emphasis in diagnosis of obscure GI bleeding is the investigation of the small bowel[76].

Repeat upper endoscopy and/or colonoscopy should be considered as one study using double-balloon enteroscopy showed that 24.3% of obscure GI bleed were of non-small bowel origin and within the reach of conventional upper and lower endoscopes[87]. The already mentioned small bowel investigations using capsule endoscopy and deep enteroscopy techniques (including double-balloon enteroscopy, single-balloon enteroscopy and spiral enteroscopy) have enabled the diagnosis of substantially more cases of obscure GI bleeding. Independent series showed that capsule endoscopy had a diagnostic yield of 53%-68% in obscure GI bleeding, led to a specific intervention in the majority of patients and was associated with a significant reductions in hospitalizations and blood transfusions[88,89]. In a randomized controlled trial in patients with iron deficiency anemia and obscure GI bleeding, capsule endoscopy identified a bleeding source significantly more than push enteroscopy (50% *vs* 24%, $P = 0.02$)[90]. Double-balloon enteroscopy was shown in a systematic review to have a diagnostic yield of approximately 68% in obscure GI bleeding[91]. A meta-analysis of studies comparing capsule endoscopy and double-balloon enteroscopy concluded comparable diagnostic yield (60% *vs* 57%, $P = 0.42$) in small bowel disease and obscure GI bleeding[92]. Capsule endoscopy has the major advantage of being less invasive than deep enteroscopy but the major advantage of deep enteroscopy techniques is their ability to perform treatment at the same time. The choice between capsule endoscopy and deep enteroscopy should be individualized for each patient and one approach may be initial capsule endoscopy followed by a directed deep enteroscopy as directed intervention[76].

CT or MR enterography may be considered as an alternative investigation for small bowel disease due to its ability to visualize the small bowel wall and extra-enteric complications, especially when capsule endoscopy and deep enteroscopy are non-diagnostic. In patients with signs of active bleeding, the above mentioned technetium-99 radionuclide scan, CT angiography and catheter angiography should be considered to help locate the lesion prior to intervention.

CONCLUSION

GI bleeding can be caused by a wide range of pathologies and they differ in onset, location, risk and clinical presentation. In patients with active GI bleeding who are unstable, acute resuscitation should precede any investigations. Accurate clinical diagnosis is crucial in determining the investigation of choice and specific treatment interventions. The correct diagnostic algorithm (Figure 5) relies on a good understanding of the type of GI bleeding, risk

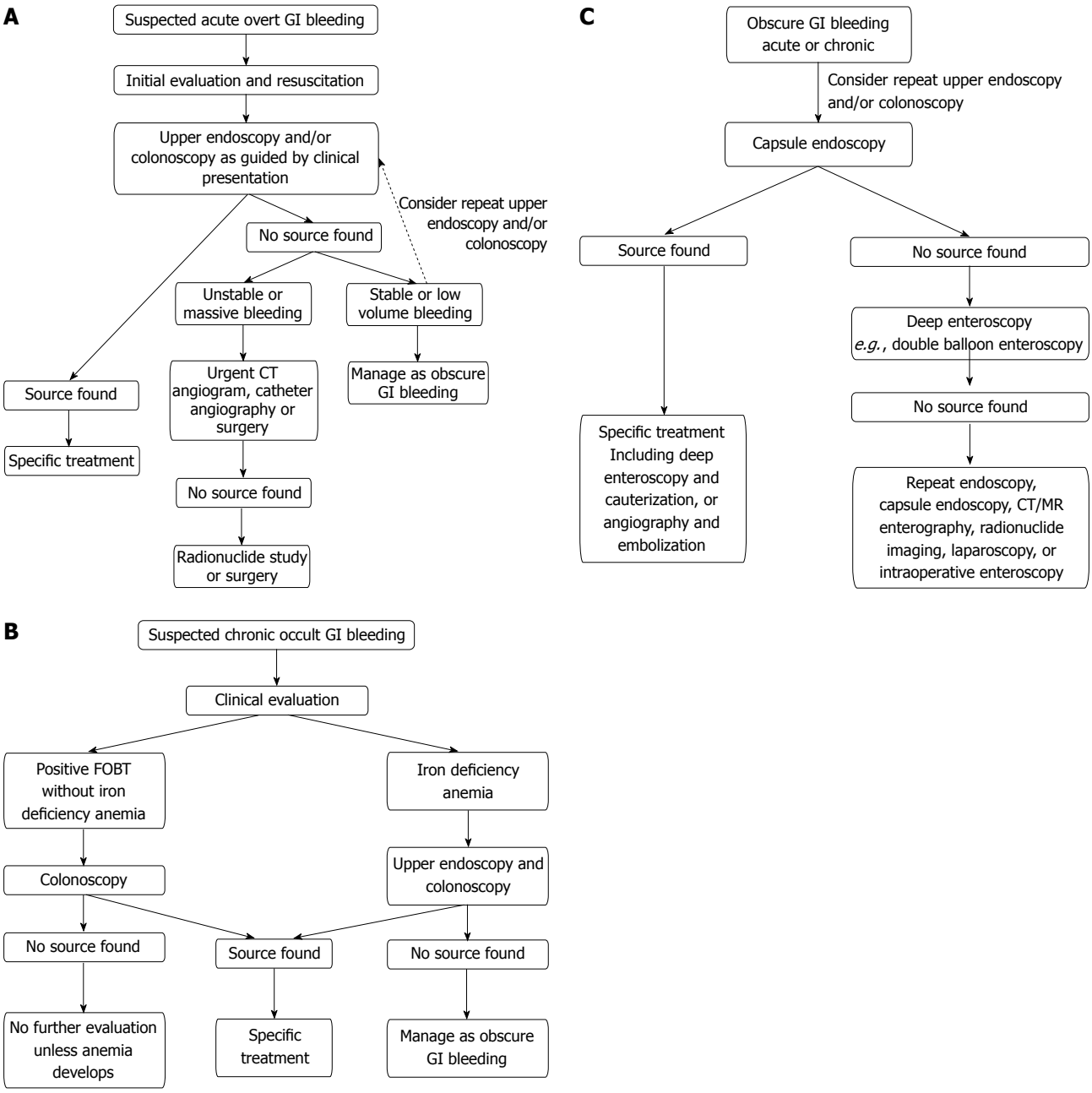

Figure 5 Diagnostic algorithms. A: Acute overt; B: Chronic occult; C: Obscure. CT: Computed tomography; MR: Magnetic resonance; GI: Gastrointestinal; FOBT; Fecal occult blood test.

evaluation and clinical presentation which may indicate the nature and source of bleeding. Upper endoscopy and colonoscopy are the mainstay of initial investigations. Angiography and radionuclide imaging are best suited for acute overt GI bleeding. Capsule endoscopy and deep enteroscopy play significant roles in the diagnosis of obscure GI bleeding, usually from the small bowel.

REFERENCES

1 **Cutler JA**, Mendeloff AI. Upper gastrointestinal bleeding. Nature and magnitude of the problem in the U.S. *Dig Dis Sci* 1981; **26**: 90S-96S [PMID: 6985341]
2 **Hilsden RJ**, Shaffer EA. Management of gastrointestinal hemorrhage. *Can Fam Physician* 1995; **41**: 1931-1936, 1939-1941 [PMID: 8563510]
3 **Blatchford O**, Davidson LA, Murray WR, Blatchford M, Pell J. Acute upper gastrointestinal haemorrhage in west of Scotland: case ascertainment study. *BMJ* 1997; **315**: 510-514 [PMID: 9329304]
4 **Srygley FD**, Gerardo CJ, Tran T, Fisher DA. Does this patient have a severe upper gastrointestinal bleed? *JAMA* 2012; **307**: 1072-1079 [PMID: 22416103 DOI: 10.1001/jama.2012.253]
5 **Lanas A**, García-Rodríguez LA, Polo-Tomás M, Ponce M, Alonso-Abreu I, Perez-Aisa MA, Perez-Gisbert J, Bujanda L, Castro M, Muñoz M, Rodrigo L, Calvet X, Del-Pino D, Garcia S. Time trends and impact of upper and lower gastrointestinal bleeding and perforation in clinical practice. *Am J Gastroenterol* 2009; **104**: 1633-1641 [PMID: 19574968 DOI: 10.1038/ajg.2009.164]
6 **Stanley AJ**, Ashley D, Dalton HR, Mowat C, Gaya DR, Thompson E, Warshow U, Groome M, Cahill A, Benson G,

Blatchford O, Murray W. Outpatient management of patients with low-risk upper-gastrointestinal haemorrhage: multicentre validation and prospective evaluation. *Lancet* 2009; **373**: 42-47 [PMID: 19091393 DOI: 10.1016/s0140-6736(08)61769-9]
7 **Lewis JD**, Brown A, Localio AR, Schwartz JS. Initial evaluation of rectal bleeding in young persons: a cost-effectiveness analysis. *Ann Intern Med* 2002; **136**: 99-110 [PMID: 11790061]
8 **Lee EW**, Laberge JM. Differential diagnosis of gastrointestinal bleeding. *Tech Vasc Interv Radiol* 2004; **7**: 112-122 [PMID: 16015555]
9 **British Society of Gastroenterology Endoscopy Committee**. Non-variceal upper gastrointestinal haemorrhage: guidelines. *Gut* 2002; **51** Suppl 4: iv1-iv6 [PMID: 12208839]
10 **Bull-Henry K**, Al-Kawas FH. Evaluation of occult gastrointestinal bleeding. *Am Fam Physician* 2013; **87**: 430-436 [PMID: 23547576]
11 **Raju GS**, Gerson L, Das A, Lewis B. American Gastroenterological Association (AGA) Institute medical position statement on obscure gastrointestinal bleeding. *Gastroenterology* 2007; **133**: 1694-1696 [PMID: 17983811 DOI: 10.1053/j.gastro.2007.06.008]
12 **Rockey DC**. Occult gastrointestinal bleeding. *N Engl J Med* 1999; **341**: 38-46 [PMID: 10387941 DOI: 10.1056/nejm199907013410107]
13 **Longstreth GF**. Epidemiology of hospitalization for acute upper gastrointestinal hemorrhage: a population-based study. *Am J Gastroenterol* 1995; **90**: 206-210 [PMID: 7847286]
14 **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.gie.2005.07.032]
15 **Manning-Dimmitt LL**, Dimmitt SG, Wilson GR. Diagnosis of gastrointestinal bleeding in adults. *Am Fam Physician* 2005; **71**: 1339-1346 [PMID: 15832537]
16 **Vreeburg EM**, Snel P, de Bruijne JW, Bartelsman JF, Rauws EA, Tytgat GN. Acute upper gastrointestinal bleeding in the Amsterdam area: incidence, diagnosis, and clinical outcome. *Am J Gastroenterol* 1997; **92**: 236-243 [PMID: 9040198]
17 **Wilcox CM**, Clark WS. Causes and outcome of upper and lower gastrointestinal bleeding: the Grady Hospital experience. *South Med J* 1999; **92**: 44-50 [PMID: 9932826]
18 **Yavorski RT**, Wong RK, Maydonovitch C, Battin LS, Furnia A, Amundson DE. Analysis of 3,294 cases of upper gastrointestinal bleeding in military medical facilities. *Am J Gastroenterol* 1995; **90**: 568-573 [PMID: 7717312]
19 **Hussain H**, Lapin S, Cappell MS. Clinical scoring systems for determining the prognosis of gastrointestinal bleeding. *Gastroenterol Clin North Am* 2000; **29**: 445-464 [PMID: 10836189]
20 **Zuccaro G**. Management of the adult patient with acute lower gastrointestinal bleeding. American College of Gastroenterology. Practice Parameters Committee. *Am J Gastroenterol* 1998; **93**: 1202-1208 [PMID: 9707037 DOI: 10.1111/j.1572-0241.1998.00395.x]
21 **Peura DA**, Lanza FL, Gostout CJ, Foutch PG. The American College of Gastroenterology Bleeding Registry: preliminary findings. *Am J Gastroenterol* 1997; **92**: 924-928 [PMID: 9177503]
22 **Jutabha R**, Jensen DM. Management of upper gastrointestinal bleeding in the patient with chronic liver disease. *Med Clin North Am* 1996; **80**: 1035-1068 [PMID: 8804374]
23 **Sarin SK**, Kumar A, Angus PW, Baijal SS, Baik SK, Bayraktar Y, Chawla YK, Choudhuri G, Chung JW, de Franchis R, de Silva HJ, Garg H, Garg PK, Helmy A, Hou MC, Jafri W, Jia JD, Lau GK, Li CZ, Lui HF, Maruyama H, Pandey CM, Puri AS, Rerknimitr R, Sahni P, Saraya A, Sharma BC, Sharma P, Shiha G, Sollano JD, Wu J, Xu RY, Yachha SK, Zhang C. Diagnosis and management of acute variceal bleeding: Asian Pacific Association for Study of the Liver recommendations. *Hepatol Int* 2011; **5**: 607-624 [PMID: 21484145 DOI: 10.1007/s12072-010-9236-9]
24 **Terblanche J**, Krige JE, Bornman PC. The treatment of esophageal varices. *Annu Rev Med* 1992; **43**: 69-82 [PMID: 1580607 DOI: 10.1146/annurev.me.43.020192.000441]
25 **Lam SK**. Differences in peptic ulcer between East and West. *Baillieres Best Pract Res Clin Gastroenterol* 2000; **14**: 41-52 [PMID: 10749088]
26 **Busuttil SJ**, Goldstone J. Diagnosis and management of aortoenteric fistulas. *Semin Vasc Surg* 2001; **14**: 302-311 [PMID: 11740838]
27 **Barkun AN**, Bardou M, Kuipers EJ, Sung J, Hunt RH, Martel M, Sinclair P. International consensus recommendations on the management of patients with nonvariceal upper gastrointestinal bleeding. *Ann Intern Med* 2010; **152**: 101-113 [PMID: 20083829 DOI: 10.7326/0003-4819-152-2-201001190-00009]
28 **Rockall TA**, Logan RF, Devlin HB, Northfield TC. Selection of patients for early discharge or outpatient care after acute upper gastrointestinal haemorrhage. National Audit of Acute Upper Gastrointestinal Haemorrhage. *Lancet* 1996; **347**: 1138-1140 [PMID: 8609747]
29 **Cappell MS**, Friedel D. Initial management of acute upper gastrointestinal bleeding: from initial evaluation up to gastrointestinal endoscopy. *Med Clin North Am* 2008; **92**: 491-509, xi [PMID: 18387374 DOI: 10.1016/j.mcna.2008.01.005]
30 **Sharara AI**, Rockey DC. Gastroesophageal variceal hemorrhage. *N Engl J Med* 2001; **345**: 669-681 [PMID: 11547722 DOI: 10.1056/NEJMra003007]
31 **Zuckerman GR**, Trellis DR, Sherman TM, Clouse RE. An objective measure of stool color for differentiating upper from lower gastrointestinal bleeding. *Dig Dis Sci* 1995; **40**: 1614-1621 [PMID: 7648958]
32 **Mortensen PB**, Nøhr M, Møller-Petersen JF, Balslev I. The diagnostic value of serum urea/creatinine ratio in distinguishing between upper and lower gastrointestinal bleeding. A prospective study. *Dan Med Bull* 1994; **41**: 237-240 [PMID: 8039439]
33 **Edelman DA**, Sugawa C. Lower gastrointestinal bleeding: a review. *Surg Endosc* 2007; **21**: 514-520 [PMID: 17294304 DOI: 10.1007/s00464-006-9191-7]
34 **Hagspiel KD**, Turba UC, Bozlar U, Harthun NL, Cherry KJ, Ahmed H, Bickston SJ, Angle JF. Diagnosis of aortoenteric fistulas with CT angiography. *J Vasc Interv Radiol* 2007; **18**: 497-504 [PMID: 17446540 DOI: 10.1016/j.jvir.2007.02.009]
35 **Adang RP**, Vismans JF, Talmon JL, Hasman A, Ambergen AW, Stockbrügger RW. Appropriateness of indications for diagnostic upper gastrointestinal endoscopy: association with relevant endoscopic disease. *Gastrointest Endosc* 1995; **42**: 390-397 [PMID: 8566625]
36 **Jensen DM**, Kovacs TO, Jutabha R, Machicado GA, Gralnek IM, Savides TJ, Smith J, Jensen ME, Alofaituli G, Gornbein J. Randomized trial of medical or endoscopic therapy to prevent recurrent ulcer hemorrhage in patients with adherent clots. *Gastroenterology* 2002; **123**: 407-413 [PMID: 12145792]
37 **Bleau BL**, Gostout CJ, Sherman KE, Shaw MJ, Harford WV, Keate RF, Bracy WP, Fleischer DE. Recurrent bleeding from peptic ulcer associated with adherent clot: a randomized study comparing endoscopic treatment with medical therapy. *Gastrointest Endosc* 2002; **56**: 1-6 [PMID: 12085028]
38 **Jaskolka JD**, Binkhamis S, Prabhudesai V, Chawla TP. Acute gastrointestinal hemorrhage: radiologic diagnosis and management. *Can Assoc Radiol J* 2013; **64**: 90-100 [PMID: 23245297 DOI: 10.1016/j.carj.2012.08.001]
39 **Huang ES**, Karsan S, Kanwal F, Singh I, Makhani M, Spiegel BM. Impact of nasogastric lavage on outcomes in acute GI bleeding. *Gastrointest Endosc* 2011; **74**: 971-980 [PMID: 21737077 DOI: 10.1016/j.gie.2011.04.045]
40 **Pallin DJ**, Saltzman JR. Is nasogastric tube lavage in patients with acute upper GI bleeding indicated or antiquated? *Gastrointest Endosc* 2011; **74**: 981-984 [PMID: 22032314 DOI:

10.1016/j.gie.2011.07.007]
41 **Barkun AN**, Bardou M, Martel M, Gralnek IM, Sung JJ. Prokinetics in acute upper GI bleeding: a meta-analysis. *Gastrointest Endosc* 2010; **72**: 1138-1145 [PMID: 20970794 DOI: 10.1016/j.gie.2010.08.011]
42 **Bai Y**, Guo JF, Li ZS. Meta-analysis: erythromycin before endoscopy for acute upper gastrointestinal bleeding. *Aliment Pharmacol Ther* 2011; **34**: 166-171 [PMID: 21615438 DOI: 10.1111/j.1365-2036.2011.04708.x]
43 **El Ouali S**, Barkun AN, Wyse J, Romagnuolo J, Sung JJ, Gralnek IM, Bardou M, Martel M. Is routine second-look endoscopy effective after endoscopic hemostasis in acute peptic ulcer bleeding? A meta-analysis. *Gastrointest Endosc* 2012; **76**: 283-292 [PMID: 22695209 DOI: 10.1016/j.gie.2012.04.441]
44 **Tsoi KK**, Chan HC, Chiu PW, Pau CY, Lau JY, Sung JJ. Second-look endoscopy with thermal coagulation or injections for peptic ulcer bleeding: a meta-analysis. *J Gastroenterol Hepatol* 2010; **25**: 8-13 [PMID: 20136971 DOI: 10.1111/j.1440-1746.2009.06129.x]
45 **Zmora O**, Dinnewitzer AJ, Pikarsky AJ, Efron JE, Weiss EG, Nogueras JJ, Wexner SD. Intraoperative endoscopy in laparoscopic colectomy. *Surg Endosc* 2002; **16**: 808-811 [PMID: 11997827 DOI: 10.1007/s00464-001-8226-3]
46 **Bowden TA**, Hooks VH, Mansberger AR. Intraoperative gastrointestinal endoscopy. *Ann Surg* 1980; **191**: 680-687 [PMID: 6966910]
47 **Grobmyer AJ**, Kerlan RA, Peterson CM, Dragstedt LR. Barium peritonitis. *Am Surg* 1984; **50**: 116-120 [PMID: 6703517]
48 **Strate LL**. Lower GI bleeding: epidemiology and diagnosis. *Gastroenterol Clin North Am* 2005; **34**: 643-664 [PMID: 16303575 DOI: 10.1016/j.gtc.2005.08.007]
49 **Chaudhry V**, Hyser MJ, Gracias VH, Gau FC. Colonoscopy: the initial test for acute lower gastrointestinal bleeding. *Am Surg* 1998; **64**: 723-728 [PMID: 9697900]
50 **Lhewa DY**, Strate LL. Pros and cons of colonoscopy in management of acute lower gastrointestinal bleeding. *World J Gastroenterol* 2012; **18**: 1185-1190 [PMID: 22468081 DOI: 10.3748/wjg.v18.i11.1185]
51 **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]
52 **Jensen DM**, Machicado GA. Diagnosis and treatment of severe hematochezia. The role of urgent colonoscopy after purge. *Gastroenterology* 1988; **95**: 1569-1574 [PMID: 3263294]
53 **Millward SF**. ACR Appropriateness Criteria on treatment of acute nonvariceal gastrointestinal tract bleeding. *J Am Coll Radiol* 2008; **5**: 550-554 [PMID: 18359441 DOI: 10.1016/j.jacr.2008.01.010]
54 **Kuhle WG**, Sheiman RG. Detection of active colonic hemorrhage with use of helical CT: findings in a swine model. *Radiology* 2003; **228**: 743-752 [PMID: 12954894 DOI: 10.1148/radiol.2283020756]
55 **Chua AE**, Ridley LJ. Diagnostic accuracy of CT angiography in acute gastrointestinal bleeding. *J Med Imaging Radiat Oncol* 2008; **52**: 333-338 [PMID: 18811756 DOI: 10.1111/j.1440-1673.2008.01964.x]
56 **Ernst O**, Bulois P, Saint-Drenant S, Leroy C, Paris JC, Sergent G. Helical CT in acute lower gastrointestinal bleeding. *Eur Radiol* 2003; **13**: 114-117 [PMID: 12541118 DOI: 10.1007/s00330-002-1442-y]
57 **Martí M**, Artigas JM, Garzón G, Alvarez-Sala R, Soto JA. Acute lower intestinal bleeding: feasibility and diagnostic performance of CT angiography. *Radiology* 2012; **262**: 109-116 [PMID: 22084211 DOI: 10.1148/radiol.11110326]
58 **Geffroy Y**, Rodallec MH, Boulay-Coletta I, Jullès MC, Ridereau-Zins C, Zins M. Multidetector CT angiography in acute gastrointestinal bleeding: why, when, and how. *Radiographics* 2011; **31**: E35-E46 [PMID: 21721196 DOI: 10.1148/rg.313105206]
59 **Yoon W**, Jeong YY, Shin SS, Lim HS, Song SG, Jang NG, Kim JK, Kang HK. Acute massive gastrointestinal bleeding: detection and localization with arterial phase multi-detector row helical CT. *Radiology* 2006; **239**: 160-167 [PMID: 16484350 DOI: 10.1148/radiol.2383050175]
60 **Steer ML**, Silen W. Diagnostic procedures in gastrointestinal hemorrhage. *N Engl J Med* 1983; **309**: 646-650 [PMID: 6604219 DOI: 10.1056/nejm198309153091106]
61 **Zuckerman DA**, Bocchini TP, Birnbaum EH. Massive hemorrhage in the lower gastrointestinal tract in adults: diagnostic imaging and intervention. *AJR Am J Roentgenol* 1993; **161**: 703-711 [PMID: 8372742 DOI: 10.2214/ajr.161.4.8372742]
62 **Cherian MP**, Mehta P, Kalyanpur TM, Hedgire SS, Narsinghpura KS. Arterial interventions in gastrointestinal bleeding. *Semin Intervent Radiol* 2009; **26**: 184-196 [PMID: 21326563 DOI: 10.1055/s-0029-1225661]
63 **Vernava AM**, Moore BA, Longo WE, Johnson FE. Lower gastrointestinal bleeding. *Dis Colon Rectum* 1997; **40**: 846-858 [PMID: 9221865]
64 **Browder W**, Cerise EJ, Litwin MS. Impact of emergency angiography in massive lower gastrointestinal bleeding. *Ann Surg* 1986; **204**: 530-536 [PMID: 3094466]
65 **Pennoyer WP**, Vignati PV, Cohen JL. Mesenteric angiography for lower gastrointestinal hemorrhage: are there predictors for a positive study? *Dis Colon Rectum* 1997; **40**: 1014-1018 [PMID: 9293927]
66 **Walker TG**, Salazar GM, Waltman AC. Angiographic evaluation and management of acute gastrointestinal hemorrhage. *World J Gastroenterol* 2012; **18**: 1191-1201 [PMID: 22468082 DOI: 10.3748/wjg.v18.i11.1191]
67 **Dusold R**, Burke K, Carpentier W, Dyck WP. The accuracy of technetium-99m-labeled red cell scintigraphy in localizing gastrointestinal bleeding. *Am J Gastroenterol* 1994; **89**: 345-348 [PMID: 8122642]
68 **Hunter JM**, Pezim ME. Limited value of technetium 99m-labeled red cell scintigraphy in localization of lower gastrointestinal bleeding. *Am J Surg* 1990; **159**: 504-506 [PMID: 2334015]
69 **Levy R**, Barto W, Gani J. Retrospective study of the utility of nuclear scintigraphic-labelled red cell scanning for lower gastrointestinal bleeding. *ANZ J Surg* 2003; **73**: 205-209 [PMID: 12662227]
70 **Schuetz A**, Jauch KW. Lower gastrointestinal bleeding: therapeutic strategies, surgical techniques and results. *Langenbecks Arch Surg* 2001; **386**: 17-25 [PMID: 11405084]
71 **Cook JD**, Skikne BS. Iron deficiency: definition and diagnosis. *J Intern Med* 1989; **226**: 349-355 [PMID: 2681511]
72 **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]
73 **Mitchell SH**, Schaefer DC, Dubagunta S. A new view of occult and obscure gastrointestinal bleeding. *Am Fam Physician* 2004; **69**: 875-881 [PMID: 14989574]
74 **Zuckerman GR**, Prakash C, Askin MP, Lewis BS. AGA technical review on the evaluation and management of occult and obscure gastrointestinal bleeding. *Gastroenterology* 2000; **118**: 201-221 [PMID: 10611170]
75 **Rockey DC**. Occult gastrointestinal bleeding. *Gastroenterol Clin North Am* 2005; **34**: 699-718 [PMID: 16303578 DOI: 10.1016/j.gtc.2005.08.010]
76 **Rockey DC**. Occult and obscure gastrointestinal bleeding: causes and clinical management. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 265-279 [PMID: 20351759 DOI: 10.1038/nrgastro.2010.42]
77 **Pennazio M**, Santucci R, Rondonotti E, Abbiati C, Beccari G, Rossini FP, De Franchis R. Outcome of patients with obscure gastrointestinal bleeding after capsule endoscopy: report of 100 consecutive cases. *Gastroenterology* 2004; **126**: 643-653 [PMID: 14988816]
78 **Rastogi A**, Schoen RE, Slivka A. Diagnostic yield and clinical

outcomes of capsule endoscopy. *Gastrointest Endosc* 2004; **60**: 959-964 [PMID: 15605012]

79 **Adler DG**, Knipschield M, Gostout C. A prospective comparison of capsule endoscopy and push enteroscopy in patients with GI bleeding of obscure origin. *Gastrointest Endosc* 2004; **59**: 492-498 [PMID: 15044884]

80 **Ell C**, Remke S, May A, Helou L, Henrich R, Mayer G. The first prospective controlled trial comparing wireless capsule endoscopy with push enteroscopy in chronic gastrointestinal bleeding. *Endoscopy* 2002; **34**: 685-689 [PMID: 12195324 DOI: 10.1055/s-2002-33446]

81 **Triester SL**, Leighton JA, Leontiadis GI, Fleischer DE, Hara AK, Heigh RI, Shiff AD, Sharma VK. A meta-analysis of the yield of capsule endoscopy compared to other diagnostic modalities in patients with obscure gastrointestinal bleeding. *Am J Gastroenterol* 2005; **100**: 2407-2418 [PMID: 16279893 DOI: 10.1111/j.1572-0241.2005.00274.x]

82 **Hartmann D**, Schmidt H, Bolz G, Schilling D, Kinzel F, Eickhoff A, Huschner W, Möller K, Jakobs R, Reitzig P, Weickert U, Gellert K, Schultz H, Guenther K, Hollerbuhl H, Schoenleben K, Schulz HJ, Riemann JF. A prospective two-center study comparing wireless capsule endoscopy with intraoperative enteroscopy in patients with obscure GI bleeding. *Gastrointest Endosc* 2005; **61**: 826-832 [PMID: 15933683]

83 **Sears DM**, Avots-Avotins A, Culp K, Gavin MW. Frequency and clinical outcome of capsule retention during capsule endoscopy for GI bleeding of obscure origin. *Gastrointest Endosc* 2004; **60**: 822-827 [PMID: 15557969]

84 **Di Caro S**, May A, Heine DG, Fini L, Landi B, Petruzziello L, Cellier C, Mulder CJ, Costamagna G, Ell C, Gasbarrini A. The European experience with double-balloon enteroscopy: indications, methodology, safety, and clinical impact. *Gastrointest Endosc* 2005; **62**: 545-550 [PMID: 16185969 DOI: 10.1016/j.gie.2005.04.029]

85 **Mehdizadeh S**, Ross A, Gerson L, Leighton J, Chen A, Schembre D, Chen G, Semrad C, Kamal A, Harrison EM, Binmoeller K, Waxman I, Kozarek R, Lo SK. What is the learning curve associated with double-balloon enteroscopy? Technical details and early experience in 6 U.S. tertiary care centers. *Gastrointest Endosc* 2006; **64**: 740-750 [PMID: 17055868 DOI: 10.1016/j.gie.2006.05.022]

86 **Boriskin HS**, Devito BS, Hines JJ, Scarmato VJ, Friedman B. CT enterography vs. capsule endoscopy. *Abdom Imaging* 2009; **34**: 149-155 [PMID: 18446400 DOI: 10.1007/s00261-008-9404-8]

87 **Fry LC**, Bellutti M, Neumann H, Malfertheiner P, Mönkemüller K. Incidence of bleeding lesions within reach of conventional upper and lower endoscopes in patients undergoing double-balloon enteroscopy for obscure gastrointestinal bleeding. *Aliment Pharmacol Ther* 2009; **29**: 342-349 [PMID: 19035975 DOI: 10.1111/j.1365-2036.2008.03888.x]

88 **Carey EJ**, Leighton JA, Heigh RI, Shiff AD, Sharma VK, Post JK, Fleischer DE. A single-center experience of 260 consecutive patients undergoing capsule endoscopy for obscure gastrointestinal bleeding. *Am J Gastroenterol* 2007; **102**: 89-95 [PMID: 17100969 DOI: 10.1111/j.1572-0241.2006.00941.x]

89 **Estévez E**, González-Conde B, Vázquez-Iglesias JL, de Los Angeles Vázquez-Millán M, Pértega S, Alonso PA, Clofent J, Santos E, Ulla JL, Sánchez E. Diagnostic yield and clinical outcomes after capsule endoscopy in 100 consecutive patients with obscure gastrointestinal bleeding. *Eur J Gastroenterol Hepatol* 2006; **18**: 881-888 [PMID: 16825907]

90 **de Leusse A**, Vahedi K, Edery J, Tiah D, Fery-Lemonnier E, Cellier C, Bouhnik Y, Jian R. Capsule endoscopy or push enteroscopy for first-line exploration of obscure gastrointestinal bleeding? *Gastroenterology* 2007; **132**: 855-862; quiz 1164-1165 [PMID: 17324401 DOI: 10.1053/j.gastro.2006.12.002]

91 **Xin L**, Liao Z, Jiang YP, Li ZS. Indications, detectability, positive findings, total enteroscopy, and complications of diagnostic double-balloon endoscopy: a systematic review of data over the first decade of use. *Gastrointest Endosc* 2011; **74**: 563-570 [PMID: 21620401 DOI: 10.1016/j.gie.2011.03.1239]

92 **Pasha SF**, Leighton JA, Das A, Harrison ME, Decker GA, Fleischer DE, Sharma VK. Double-balloon enteroscopy and capsule endoscopy have comparable diagnostic yield in small-bowel disease: a meta-analysis. *Clin Gastroenterol Hepatol* 2008; **6**: 671-676 [PMID: 18356113 DOI: 10.1016/j.cgh.2008.01.005]

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TOPIC HIGHLIGHT

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Evaluation and outcomes of patients with obscure gastrointestinal bleeding

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Abstract

Obscure gastrointestinal bleeding (OGIB) is defined as recurrent or persistent bleeding or presence of iron deficiency anaemia after evaluation with a negative bidirectional endoscopy. OGIB accounts for 5% of gastrointestinal bleeding and presents a diagnostic challenge. Current modalities available for the investigation of OGIB include capsule endoscopy, balloon assisted enteroscopy, spiral enteroscopy and computed tomography enterography. These modalities overcome the limitations of previous techniques. Following a negative bidirectional endoscopy, capsule endoscopy and double balloon enteroscopy remain the cornerstone of investigation in OGIB given their high diagnostic yield. Long-term outcome data in patients with OGIB is limited, but is most promising for capsule endoscopy. This article reviews the current literature and provides an overview of the clinical evaluation of patients with OGIB, available diagnostic and therapeutic modalities and long-term clinical outcomes.

Key words: Obscure gastrointestinal bleeding; Capsule endoscopy; Double balloon enteroscopy; Outcomes; Anaemia

Core tip: This article examines the role of current diagnostic modalities for the investigation of obscure gastrointestinal bleeding (OGIB) and outcomes in patients undergoing these investigations. Capsule endoscopy and double balloon enteroscopy remain the cornerstone of diagnostic and therapeutic management. The diagnostic and therapeutic capabilities of certain modalities are influenced by the nature of bleeding in OGIB. Long-term outcome data in patients with OGIB is limited but is most promising for capsule endoscopy.

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INTRODUCTION

Obscure gastrointestinal bleeding (OGIB) is defined as recurrent or persistent bleeding or presence of iron deficiency anaemia (IDA) after negative evaluation with oesophagogastroduodenoscopy (OGD) and colonoscopy[1]. OGIB can be categorised further into overt or occult obscure gastrointestinal (GI) bleeding. Overt GI bleeding refers to patients with clinically evident bleeding (haematemesis, melaena or haematochezia) whereas occult GI bleeding occurs in the setting of persistent IDA or a positive faecal occult blood test.

OGIB accounts for approximately 5% of GI bleeding. In more than 80% of cases, the bleeding arises from the small bowel distal to the Ampulla of Vater and proximal to the ileocaecal valve rendering it relatively inaccessible to traditional endoscopy[2-4]. Patients with OGIB

Table 1 Aetiology of obscure gastrointestinal bleeding

Vascular	Inflammatory	Neoplastic	Extraluminal	Rare causes
Angioectasias	Inflammatory bowel disease	Carcinoid	Haemobilia	Hereditary Haemorrhagic Telangiectasias
Dieulafoy's Lesion	Peptic ulcer disease	Gastrointestinal stromal tumour	Aortoenteric fistula	Von Willebrand disease
Gastric antral vascular ectasia	Oesophagitis	Adenocarcinoma	Haemosuccus pancreaticus	Amyloidosis
Portal hypertensive gastropathy	Cameron erosions	Metastases (melanoma)		Henoch Schonlein Purpura
Varices	Meckel's diverticulum	Lymphoma		
Radiation enteritis	NSAID related gastropathy/ enteropathy	Ampullary carcinoma		
Haemorrhoids				

NSAID: Non-steroidal anti-inflammatory drugs.

undergo more investigations, have longer duration of hospitalisation, require more blood transfusions and generate higher healthcare expenditures than patients with upper or lower gastrointestinal bleeding[1]. This is largely due to difficulty accessing the small bowel endoscopically which presents a diagnostic challenge[4].

Current modalities to investigate for OGIB include both endoscopic and radiological techniques. The role of radiological modalities in the evaluation of OGIB has declined substantially as a result of their low diagnostic yield[2]. In this article, we review the clinical evaluation and outcomes of patients presenting with OGIB.

EVALUATION OF OGIB

The clinical history may suggest the possible cause and location of OGIB but it is rarely diagnostic. Endoscopic evaluation remains the cornerstone of diagnosis and management in OGIB[5]. A careful history is key and should include the nature (occult or overt) and clinical presentation of GI bleeding (haematemesis, melaena, haematochezia). Further history regarding other gastrointestinal symptoms (weight loss, obstructive symptoms), medications (anticoagulants, non-steroidal anti-inflammatory drugs), comorbidities (haematological disease, valvular heart disease), prior surgeries (abdominal aortic aneurysm repair, bowel surgery), and family history (inflammatory bowel disease, malignancies, familial telangiectasias) may give clues to the underlying cause[6]. While haematemesis reliably localises the bleeding proximal to the ligament of Treitz, stool colour is a less reliable indicator as it is dependent upon intestinal transit time. Elderly patients, patients with valvular heart disease, renal disease or connective tissue disease are at high risk of vascular lesions. Use of non-steroidal anti-inflammatory drugs (NSAIDs) increases the risk of small bowel ulceration[7]. Physical examination may be useful in detecting systemic syndromes such as hereditary haemorrhagic telangiectasias or Coeliac disease[6].

The most common causes of OGIB vary according to age (Table 1). In patients younger than 40 years of age, small intestinal tumours, Crohn's disease, Meckel's diverticulum, polyposis syndromes and angiodysplasias predominate, whereas patients older than 40 years of age are more likely to bleed from vascular causes (*e.g.,* angiodysplasias) and NSAID enteropathy[8,9]. Causes of OGIB are mainly vascular in the Western population and ulcerations or erosions in the Asian population[10]. Patients who present with IDA without gastrointestinal symptoms, may have gastrointestinal diseases that cause iron malabsorption such as Coeliac disease, atrophic gastritis and Helicobacter Pylori gastritis[11].

Availability of procedures, patient preferences, physician expertise, costs and risks are important determinants of investigation and management[12].

CAPSULE ENDOSCOPY

Capsule endoscopy (CE) has revolutionised the ability to image the small bowel. It is commonly used as a first-line diagnostic tool for investigation of OGIB.[1] This is due to its non-invasiveness, patient tolerance, high negative predictive value (80%-100%) and high diagnostic yield[3,13,14]. CE enables direct visualisation of the small bowel mucosa and has a high sensitivity for detecting flat lesions, such as angiodysplasias, ulcers and arteriovenous malformations which are not easily detectable on radiological modalities[15].

The reported diagnostic yield in literature ranges from 58.4% to 86.8%[9,14,16-21]. The wide range is attributable to different definitions of a positive finding on CE. The diagnostic yield is not affected by age, rendering it a useful test across all age groups[22]. However, it is affected by patient factors including ongoing bleeding, low haemoglobin and ongoing transfusion requirements[23].

Pennazio *et al*[16] reported that the diagnostic yield of CE was significantly higher in patients with ongoing overt OGIB (92.3%), intermediate in patients with occult OGIB (44.2%) and lowest in patients with previous overt OGIB (12.9%). In the overt OGIB group, the diagnostic yield was inversely proportional to the length of time since the last bleeding episode, as delay in the use of CE allows for healing of the bleeding site[24]. CE thus has its highest diagnostic yield in patients with ongoing and overt bleeding[16,25].

CE has been shown to be superior to other modalities

including computed tomography, and small bowel barium studies[26-29]. When compared to push enteroscopy (PE), two meta-analyses have confirmed the superiority of CE, one of which demonstrated a diagnostic yield 30% higher than PE[29,30].

When comparing CE with double balloon enteroscopy (DBE), the literature is inconsistent due to small sample sizes[6]. Teshima *et al*[31]'s meta-analysis comparing CE and DBE in OGIB revealed a similar diagnostic yield (62% *vs* 56%), a finding supported by 2 other meta-analyses[31-33]. CE has a higher diagnostic yield than either anterograde or retrograde DBE alone (OR = 1.61, 95%CI: 1.07-2.43) but not when both approaches are used together (OR = 0.12, 95%CI: 0.03-0.52). This highlights the importance of a total enteroscopy in patients with a high clinical suspicion of small bowel pathology[33]. However, the completion rate of DBE is highly variable (16%-86%)[34,35].

CE has other distinct advantages since it allows the patient to remain ambulatory and requires minimal preparation without sedation[36]. Its main limitation is that it is solely a diagnostic tool lacking therapeutic capacity and the ability to obtain histology[37,38]. It has limited effectiveness in detecting small bowel submucosal tumours, with a false-negative rate up to 19%[39,40]. Other limitations include the inability to precisely locate the bleeding lesions and a small (but significant) risk of capsule retention (0.75% to 5.8%)[3,41,42].

ENTEROSCOPY

PE

PE can visualise the proximal small bowel up to 100cm distal to the ligament of Trietz[6]. It has diagnostic and therapeutic (biopsy, electrocautery, injection, polypectomy) capabilities[4]. An important advantage of PE is that it facilitates a second look for missed lesions within reach of an OGD which is seen in 25%-40% of cases[43,44].

The reported diagnostic yield is between 3%-70%[2,45-47]. The main limitation is its inability to reach lesions beyond the middle jejunum, patient discomfort and its time-consuming nature[4,48]. Complications are rare and include pancreatitis and mucosal injuries[43]. It has largely been replaced by CE for diagnosis and DBE for small bowel endoscopic treatment. Its role mainly lies in the treatment of proximal small bowel lesions found on CE[6].

Double balloon enteroscopy

Double balloon enteroscopy facilitates examination of the entire small bowel[4]. It is considered the gold standard for therapeutic intervention of many small bowel disorders in OGIB[49]. The diagnostic yield and treatment success of DBE for OGIB in published literature ranges from 60%-81% and 43%-84% respectively[10,50-60]. The variation in diagnostic yield is a result of differences in DBE timing, inclusion criteria and definitions of a significant finding[61]. Like CE, DBE has a higher diagnostic yield in patients with overt-ongoing OGIB than overt previous and occult OGIB, suggesting that the time interval between the last bleeding episode and the DBE examination is a key factor in diagnosing the causative lesion in OGIB[10].

The approach of a targeted DBE (after a prior CE) has been shown to increase both its diagnostic (73%-93%) and therapeutic yield (53%-73%)[38,62,63]. DBE can change or improve the diagnosis in a significant number of patients in whom CE is performed beforehand. In a study by Kaffes *et al*[38], DBE after CE clarified or made a new diagnosis in 20% of patients. A CE guided DBE is likely to diminish the need for total enteroscopy in most patients, as demonstrated by Gay *et al*[62] who showed a high positive predictive value for CE to correctly predict the DBE approach. The targeted approach is also useful in confirming indeterminate findings from CE. Hence, it is strongly suggested that CE is the initial screening modality in OGIB and that these two investigations should be viewed as complementary[20,64].

Not surprisingly, when compared with PE, a controlled prospective trial on patients with suspected small bowel bleeding, confirmed that anterograde DBE is significantly superior to PE in regards to the detection of pathological lesions (63% *vs* 44%) and the length of small bowel visualised (230 cm *vs* 80 cm)[65].

DBE is restricted by its limited availability, prolonged procedural times and sedation requirements[37]. The complication rate is 0.8% for diagnostic procedures and up to 4% for therapeutics such as polypectomy, electrocautery or dilatation[6]. Complications include bleeding, ileus, intestinal perforation, pancreatitis or those related to sedation[49]. For these reasons, DBE is a second-line investigation in OGIB, reserved for patients with a positive CE who require therapeutic intervention or biopsy[2].

Current guidelines recommend CE as the preferred initial modality in OGIB given its diagnostic yield, outcome data, safety and non-invasive nature. DBE should be viewed as a complementary procedure. It plays an important therapeutic role following diagnostic CE and diagnostic role following negative CE in patients with ongoing bleeding or high suspicion of small bowel pathology. Other scenarios for initial use of DBE are where CE is not available or affordable and in patients with overt OGIB who may benefit from early DBE[64]. More prospective randomised controlled clinical studies are required to determine the most efficient and cost effective use of CE and DBE[61].

Spiral enteroscopy

Spiral enteroscopy utilises a spiral shaped overtube with a raised helix at the distal end. It allows for advancement and withdrawal of the enteroscope through the small bowel by using clockwise and anticlockwise movements respectively[6]. It offers the same diagnostic and therapeutic capabilities as DBE. Initial studies comparing DBE and spiral enteroscopy have suggested that the two procedures have similar diagnostic yields[66-68]. Further studies

comparing spiral enteroscopy to other modalities such as CE and DBE are required.

Intraoperative enteroscopy

Intraoperative enteroscopy (IOE) was previously considered the gold standard of small intestinal imaging. It has the highest sensitivity in detecting bleeding small bowel lesions with a diagnostic yield of 80%-100%[69,70]. This is at the expense of extreme invasiveness making this modality a last resort in the investigation OGIB[4]. Indications of IOE include when small bowel lesions cannot be managed by angiographic embolisation or endoscopic treatment or when surgery is required[70].

REPEAT UPPER AND LOWER ENDOSCOPY

Bleeding sources within reach of upper and lower endoscopy may be missed as a result of small size, atypical location, inadequate endoscopy investigation, slow or intermittent bleeding, or compromised visualisation (due to presence of blood or poor colonic preparation)[6].

Numerous studies demonstrate that a significant proportion of patients with negative initial investigations have a bleeding source on repeat OGD in 35%-75% or repeat colonoscopy in 6% of cases[45,71-76]. Thus a re-look endoscopy may be recommended as a cost-effective first step before further evaluation[7]. Factors associated with increased yield on repeat OGD include large hiatus hernias, history of NSAID use, and haematemesis[45].

Common missed lesions include colonic angiodysplasias, peptic ulcers, Cameron's lesions, gastric antral vascular ectasia and radiation proctitis[49].

The American Gastroenterological Association recommend repeating OGD and colonoscopy if there is suspicion of an overlooked lesion before proceeding to CE or DBE[2]. Repeat OGD and/or colonoscopy should also be considered if suboptimal equipment was used or in the setting of inadequate mucosal visualisation secondary to poor bowel preparation[49].

COMPUTED TOMOGRAPHY ENTEROGRAPHY

Computed tomography enterography (CTE) is a readily available, non-invasive, operator independent method for visualising the small bowel. It can detect extraluminal pathology which is not possible with CE. The overall sensitivity of CTE is low (50%), however it is effective for detecting small bowel tumours (sensitivity exceeding 90%)[77-79]. The diagnostic yield of CE following negative CTE is high, 57% in one study[25]. Small bowel ulcers are the most commonly missed lesions with CTE which are readily detected by CE[1]. However, in patients less than 40 years of age where small bowel tumours are the most common cause of OGIB, CTE should be strongly considered given the aforementioned false negative rate of CE for detecting small bowel neoplasms[80,81].

OUTCOMES

Capsule endoscopy

Although many studies demonstrate a high diagnostic yield of CE for detecting a cause of OGIB, its impact on patient outcomes is more important[82]. With regards to rebleeding rates, Endo *et al*[18] found that among patients with significant CE findings, the rebleeding rate at a mean of 11.6 mo follow up of the patients who underwent therapeutic intervention was significantly lower than that of those without intervention (9.5% *vs* 40.0%, $P = 0.046$). This is supported by other studies[83,84]. Hence, aggressive intervention of patients with significant CE findings reduces risk of rebleeding. Patients with insignificant findings (erosions, small ulcers, red spots, small polyps) or a negative CE, had a significantly higher rate of re-bleeding than those with significant findings on CE. These patients should have careful follow up, whilst being mindful that the bleeding may not be originating from the small bowel[18]. Viazis *et al*[85] found that 65% of patients with a negative initial CE continued to have OGIB after a mean follow up period of 24 mo. Development of overt bleeding and a haemoglobin drop of 4 g/dL or more were significant predictive factors for a diagnostic repeat CE. Similar to its influence on diagnostic yield, the nature of bleeding in OGIB also impacts on rebleeding rates. In the Pennazio *et al*[16] study, complete resolution of bleeding occurred significantly more often in patients with ongoing overt and occult OGIB than with previous OGIB.

In regards to other outcome measures, Leighton *et al*[36] demonstrated significant reductions in the requirement for blood transfusions, gastrointestinal procedures and hospitalisation as well as significant improvements in haemoglobin levels at 1 year follow-up of 20 patients undergoing CE for investigation of OGIB. Hindryckx *et al*[86] also confirmed favourable outcomes in 66.3% of their patients after CE guided therapy which led to a decrease in the need for blood transfusions and significantly higher haemoglobin levels after a mean follow up of 635.5 d.

DBE

Kaffes *et al*[38] reported significant reductions in further bleeding (80%), blood transfusions and iron requirements in a prospective cohort study of 60 patients with positive CE findings undergoing DBE treatment after 10 ± 5.2 mo follow up. Seventy-seven percent of patients maintained a normal haemoglobin. Hsu *et al*[59] similarly found significantly less rebleeding in patients who were treated for an identified lesion when compared to patients in whom no lesion was found (20% *vs* 80%).

Byeon *et al*[87] found that repeat DBE in the same direction may detect a source of bleeding in 53% of recurrent OGIB patients, particularly in patients with a previous positive DBE (81% yield). Angiodysplasias were the most common cause of OGIB in both DBEs. An-

giodysplasia has been identified as a common source of rebleeding in studies exploring outcome in patients with OGIB after PE, CE and or DBE[88,89].

Most studies follow up patients for up to 12 mo. Larger prospective studies with longer follow up are required to evaluate long term outcomes of OGIB patients following DBE.

Push enteroscopy

Several small studies suggest that patient outcomes are improved after PE[4]. In one study of 105 patients with OGIB with a mean follow up of 29 mo, resolution of bleeding occurred in 69% of patients[90]. PE impacts upon clinical management in 40%-50% of patients with OGIB[74,91]. Decreased transfusion requirements and improvement in functional status one year post treatment have been found with PE[92].

Other modalities

There are limited data on outcomes of OGIB patients after investigation with other modalities. However, similar to data from CE, DBE and PE, patients who underwent endoscopic treatment for an identified lesion had better outcomes than those without treatment.

Williamson *et al*[37] followed up 61 patients undergoing spiral enteroscopy for OGIB. The mean time to recurrent overt bleeding was 10.4 months. Patients who had endoscopic treatment for bleeding lesions during spiral enteroscopy were significantly less likely to have further overt bleeding (26% *vs* 64%). Increased haemoglobin levels and reduced requirements for blood transfusions, iron supplementation and additional procedures were all observed after spiral enteroscopy.

A retrospective study of IOE demonstrated, at 32 mo follow up, bleeding had resolved in 52% of patients with OGIB in whom a lesion was detected and treated during IOE. Bleeding persisted in 20% and recurred in 8% of patients[93]. Angiodysplasias were responsible for the majority of patients with ongoing bleeding[4].

In a retrospective study, Shin *et al*[1] showed that CTE discovered the source of bleeding in only 26.7% of patients with OGIB. The overall re-bleeding rate was 21.7% during a mean follow up of 17.6 mo. Again, patients with positive CTE who were treated endoscopically had significantly reduced rebleeding rates. A negative CTE did not predict lower long term rebleeding, and thus these patients should be closely observed and have further diagnostic work up (such as with CE or DBE) if there is a high clinical suspicion of small bowel bleeding.

CONCLUSION

Obscure gastrointestinal bleeding is a common problem and remains a diagnostic challenge to gastroenterologists. Various endoscopic, radiological and surgical modalities exist for the investigation of OGIB each with their own advantages, disadvantages and indications in which they should be used. Both CE and DBE remain the cornerstone of investigation and management of OGIB, with other modalities assuming a more selective role. Ultimately patient factors and resource availability determine the modality used. The short-term outcomes of OGIB patients with a treated lesion are good; however rebleeding is common especially in patients where no source of bleeding was found. Further studies are required to evaluate long-term outcomes. With ongoing development and experience in new techniques, the clinical conundrum that is OGIB may no longer be so obscure.

REFERENCES

1 **Shin JK**, Cheon JH, Lim JS, Park JJ, Moon CM, Jeon SM, Lee JH, Hong SP, Kim TI, Kim WH. Long-term outcomes of obscure gastrointestinal bleeding after CT enterography: does negative CT enterography predict lower long-term rebleeding rate? *J Gastroenterol Hepatol* 2011; **26**: 901-907 [PMID: 21073673 DOI: 10.1111/j.1440-1746.2010.06577]

2 **Raju GS**, Gerson L, Das A, Lewis B. American Gastroenterological Association (AGA) Institute technical review on obscure gastrointestinal bleeding. *Gastroenterology* 2007; **133**: 1697-1717 [PMID: 17983812 DOI: 10.1053/j.gastro.2007.06.007]

3 **Keum B**, Chun HJ. Capsule endoscopy and double balloon enteroscopy for obscure gastrointestinal bleeding: which is better? *J Gastroenterol Hepatol* 2011; **26**: 794-795 [PMID: 21488944 DOI: 10.1111/j.1440-1746.2011.06708]

4 **Carey EJ**, Fleischer DE. Investigation of the small bowel in gastrointestinal bleeding--enteroscopy and capsule endoscopy. *Gastroenterol Clin North Am* 2005; **34**: 719-734 [PMID: 16303579 DOI: 10.1016/j.gtc.2005.08.009]

5 **Concha R**, Amaro R, Barkin JS. Obscure gastrointestinal bleeding: diagnostic and therapeutic approach. *J Clin Gastroenterol* 2007; **41**: 242-251 [PMID: 17426461 DOI: 10.1097/01.mcg.0000225616.79223.75]

6 **Pasha SF**, Hara AK, Leighton JA. Diagnostic evaluation and management of obscure gastrointestinal bleeding: a changing paradigm. *Gastroenterol Hepatol* (N Y) 2009; **5**: 839-850 [PMID: 20567529]

7 **Lin S**, Rockey DC. Obscure gastrointestinal bleeding. *Gastroenterol Clin North Am* 2005; **34**: 679-698 [PMID: 16303577 DOI: 10.1016/j.gtc.2005.08.005]

8 **Mujica VR**, Barkin JS. Occult gastrointestinal bleeding. General overview and approach. *Gastrointest Endosc Clin N Am* 1996; **6**: 833-845 [PMID: 8899413]

9 **Estévez E**, González-Conde B, Vázquez-Iglesias JL, de Los Angeles Vázquez-Millán M, Pértega S, Alonso PA, Clofent J, Santos E, Ulla JL, Sánchez E. Diagnostic yield and clinical outcomes after capsule endoscopy in 100 consecutive patients with obscure gastrointestinal bleeding. *Eur J Gastroenterol Hepatol* 2006; **18**: 881-888 [PMID: 16825907 DOI: 10.1097/00042737-200608000-00014]

10 **Tanaka S**, Mitsui K, Yamada Y, Ehara A, Kobayashi T, Seo T, Tatsuguchi A, Fujimori S, Gudis K, Sakamoto C. Diagnostic yield of double-balloon endoscopy in patients with obscure GI bleeding. *Gastrointest Endosc* 2008; **68**: 683-691 [PMID: 18561920 DOI: 10.1016/j.gie.2008.03.1062]

11 **Annibale B**, Capurso G, Chistolini A, D'Ambra G, DiGiulio E, Monarca B, DelleFave G. Gastrointestinal causes of refractory iron deficiency anemia in patients without gastrointestinal symptoms. *Am J Med* 2001; **111**: 439-445 [PMID: 11690568 DOI: 10.1016/S0002-9343(01)00883-X]

12 **Rondonotti E**, Marmo R, Petracchini M, de Franchis R, Pennazio M. The American Society for Gastrointestinal Endoscopy (ASGE) diagnostic algorithm for obscure gastrointestinal bleeding: eight burning questions from everyday clinical practice. *Dig Liver Dis* 2013; **45**: 179-185 [PMID: 22921043

DOI: 10.1016/j.dld.2012.07.012]
13 **Delvaux M**, Fassler I, Gay G. Clinical usefulness of the endoscopic video capsule as the initial intestinal investigation in patients with obscure digestive bleeding: validation of a diagnostic strategy based on the patient outcome after 12 months. *Endoscopy* 2004; **36**: 1067-1073 [PMID: 15578296 DOI: 10.1055/s-2004-826034]
14 **Hartmann D**, Schmidt H, Bolz G, Schilling D, Kinzel F, Eickhoff A, Huschner W, Möller K, Jakobs R, Reitzig P, Weickert U, Gellert K, Schultz H, Guenther K, Hollerbuhl H, Schoenleben K, Schulz HJ, Riemann JF. A prospective two-center study comparing wireless capsule endoscopy with intraoperative enteroscopy in patients with obscure GI bleeding. *Gastrointest Endosc* 2005; **61**: 826-832 [PMID: 15933683 DOI: 10.1016/S0016-5107(05)00372-X]
15 **Soyer P**. Obscure gastrointestinal bleeding: difficulties in comparing CT enterography and video capsule endoscopy. *Eur Radiol* 2012; **22**: 1167-1171 [PMID: 22447355 DOI: 10.1007/s00330-012-2398-1]
16 **Pennazio M**, Santucci R, Rondonotti E, Abbiati C, Beccari G, Rossini FP, De Franchis R. Outcome of patients with obscure gastrointestinal bleeding after capsule endoscopy: report of 100 consecutive cases. *Gastroenterology* 2004; **126**: 643-653 [PMID: 14988816 DOI: 10.1053/j.gastro.2003.11.057]
17 **Apostolopoulos P**, Liatsos C, Gralnek IM, Kalantzis C, Giannakoulopoulou E, Alexandrakis G, Tsibouris P, Kalafatis E, Kalantzis N. Evaluation of capsule endoscopy in active, mild-to-moderate, overt, obscure GI bleeding. *Gastrointest Endosc* 2007; **66**: 1174-1181 [PMID: 18061718 DOI: 10.1016/j.gie.2007.06.058]
18 **Endo H**, Matsuhashi N, Inamori M, Akimoto K, Ohya T, Yanagawa T, Asayama M, Hisatomi K, Teratani T, Fujita K, Yoneda M, Nakajima A. Rebleeding rate after interventional therapy directed by capsule endoscopy in patients with obscure gastrointestinal bleeding. *BMC Gastroenterol* 2008; **8**: 12 [PMID: 18430253 DOI: 10.1186/1471-230X-8-12]
19 **Kim JB**, Ye BD, Song Y, Yang DH, Jung KW, Kim KJ, Byeon JS, Myung SJ, Yang SK, Kim JH. Frequency of rebleeding events in obscure gastrointestinal bleeding with negative capsule endoscopy. *J Gastroenterol Hepatol* 2013; **28**: 834-840 [PMID: 23425190 DOI: 10.1111/jgh.12145]
20 **Li X**, Dai J, Lu H, Gao Y, Chen H, Ge Z. A prospective study on evaluating the diagnostic yield of video capsule endoscopy followed by directed double-balloon enteroscopy in patients with obscure gastrointestinal bleeding. *Dig Dis Sci* 2010; **55**: 1704-1710 [PMID: 19672712 DOI: 10.1007/s10620-009-0911-4]
21 **Zakaria MS**, El-Serafy MA, Hamza IM, Zachariah KS, El-Baz TM, Tacheci JB. The role of capsule endoscopy in obscure gastrointestinal bleeding. *Arab J Gastroenterol* 2009; **10**: 57–62 [DOI: 10.1016/j.ajg.2009.05.004]
22 **Orlando G**, Luppino IM, Lerose MA, Gervasi R, Amato B, Silecchia G, Puzziello A. Feasibility of capsule endoscopy in elderly patients with obscure gastrointestinal bleeding. An up-to-date report. *BMC Surg* 2012; **12** Suppl 1: S30 [PMID: 23173943 DOI: 10.1186/1471-2482-12-S1-S30]
23 **Rockey DC**. Occult and obscure gastrointestinal bleeding: causes and clinical management. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 265-279 [PMID: 20351759 DOI: 10.1038/nrgastro.2010.42]
24 **Esaki M**, Matsumoto T, Yada S, Yanaru-Fujisawa R, Kudo T, Yanai S, Nakamura S, Iida M. Factors associated with the clinical impact of capsule endoscopy in patients with overt obscure gastrointestinal bleeding. *Dig Dis Sci* 2010; **55**: 2294-2301 [PMID: 19957038 DOI: 10.1007/s10620-009-1036-5]
25 **Heo HM**, Park CH, Lim JS, Lee JH, Kim BK, Cheon JH, Kim TI, Kim WH, Hong SP. The role of capsule endoscopy after negative CT enterography in patients with obscure gastrointestinal bleeding. *Eur Radiol* 2012; **22**: 1159-1166 [PMID: 22270143 DOI: 10.1007/s00330-011-2374-1]
26 **Voderholzer WA**, Ortner M, Rogalla P, Beinhölzl J, Lochs H. Diagnostic yield of wireless capsule enteroscopy in comparison with computed tomography enteroclysis. *Endoscopy* 2003; **35**: 1009-1014 [PMID: 14648412 DOI: 10.1055/s-2003-44583]
27 **Voderholzer WA**, Beinhoelzl J, Rogalla P, Murrer S, Schachschal G, Lochs H, Ortner MA. Small bowel involvement in Crohn's disease: a prospective comparison of wireless capsule endoscopy and computed tomography enteroclysis. *Gut* 2005; **54**: 369-373 [PMID: 15710985 DOI: 10.1136/gut.2004.040055]
28 **Hara AK**, Leighton JA, Sharma VK, Fleischer DE. Small bowel: preliminary comparison of capsule endoscopy with barium study and CT. *Radiology* 2004; **230**: 260-265 [PMID: 14617764 DOI: 10.1148/radiol.2301021535]
29 **Triester SL**, Leighton JA, Leontiadis GI, Fleischer DE, Hara AK, Heigh RI, Shiff AD, Sharma VK. A meta-analysis of the yield of capsule endoscopy compared to other diagnostic modalities in patients with obscure gastrointestinal bleeding. *Am J Gastroenterol* 2005; **100**: 2407-2418 [PMID: 16279893 DOI: 10.1111/j.1572-0241.2005.00274.x]
30 **Leighton JA**, Triester SL, Sharma VK. Capsule endoscopy: a meta-analysis for use with obscure gastrointestinal bleeding and Crohn's disease. *Gastrointest Endosc Clin N Am* 2006; **16**: 229-250 [PMID: 16644453 DOI: 10.1016/j.giec.2006.03.004]
31 **Teshima CW**, Kuipers EJ, van Zanten SV, Mensink PB. Double balloon enteroscopy and capsule endoscopy for obscure gastrointestinal bleeding: an updated meta-analysis. *J Gastroenterol Hepatol* 2011; **26**: 796-801 [PMID: 21155884 DOI: 10.1111/j.1440-1746.2010.06530.x]
32 **Pasha SF**, Leighton JA, Das A, Harrison ME, Decker GA, Fleischer DE, Sharma VK. Double-balloon enteroscopy and capsule endoscopy have comparable diagnostic yield in small-bowel disease: a meta-analysis. *Clin Gastroenterol Hepatol* 2008; **6**: 671-676 [PMID: 18356113 DOI: 10.1016/j.cgh.2008.01.005]
33 **Chen X**, Ran ZH, Tong JL. A meta-analysis of the yield of capsule endoscopy compared to double-balloon enteroscopy in patients with small bowel diseases. *World J Gastroenterol* 2007; **13**: 4372-4378 [PMID: 17708614]
34 **Gross SA**, Stark ME. Initial experience with double-balloon enteroscopy at a U.S. center. *Gastrointest Endosc* 2008; **67**: 890-897 [PMID: 18178204 DOI: 10.1016/j.gie.2007.07.047]
35 **Yamamoto H**, Sekine Y, Sato Y, Higashizawa T, Miyata T, Iino S, Ido K, Sugano K. Total enteroscopy with a nonsurgical steerable double-balloon method. *Gastrointest Endosc* 2001; **53**: 216-220 [PMID: 11174299 DOI: 10.1067/mge.2001.112181]
36 **Leighton JA**, Sharma VK, Hentz JG, Musil D, Malikowski MJ, McWane TL, Fleischer DE. Capsule endoscopy versus push enteroscopy for evaluation of obscure gastrointestinal bleeding with 1-year outcomes. *Dig Dis Sci* 2006; **51**: 891-899 [PMID: 16758305 DOI: 10.1007/s10620-006-9350-7]
37 **Williamson JB**, Judah JR, Gaidos JK, Collins DP, Wagh MS, Chauhan SS, Zoeb S, Buscaglia JM, Yan H, Hou W, Draganov PV. Prospective evaluation of the long-term outcomes after deep small-bowel spiral enteroscopy in patients with obscure GI bleeding. *Gastrointest Endosc* 2012; **76**: 771-778 [PMID: 22771101 DOI: 10.1016/j.gie.2012.05.025]
38 **Kaffes AJ**, Siah C, Koo JH. Clinical outcomes after double-balloon enteroscopy in patients with obscure GI bleeding and a positive capsule endoscopy. *Gastrointest Endosc* 2007; **66**: 304-309 [PMID: 17643704 DOI: 10.1016/j.gie.2007.02.044]
39 **Lewis BS**, Eisen GM, Friedman S. A pooled analysis to evaluate results of capsule endoscopy trials. *Endoscopy* 2005; **37**: 960-965 [PMID: 16189768 DOI: 10.1055/s-2005-870353]
40 **Pennazio M**, Rondonotti E, de Franchis R. Capsule endoscopy in neoplastic diseases. *World J Gastroenterol* 2008; **14**: 5245-5253 [PMID: 18785274 DOI: 10.3748/wjg.14.5245]
41 **Barkin JS**, Friedman S. Wireless capsule endoscopy requiring surgical intervention: the world's experience [abstract].

Am J Gastroenterol 2002; **97**: A83 [DOI: 10.1016/S0002-9270(02)05390-X]
42 **Sears DM**, Avots-Avotins A, Culp K, Gavin MW. Frequency and clinical outcome of capsule retention during capsule endoscopy for GI bleeding of obscure origin. *Gastrointest Endosc* 2004; **60**: 822-827 [PMID: 15557969 DOI: 10.1016/S0016-5107(04)02019-X]
43 **Berner JS**, Mauer K, Lewis BS. Push and sonde enteroscopy for the diagnosis of obscure gastrointestinal bleeding. *Am J Gastroenterol* 1994; **89**: 2139-2142 [PMID: 7977229]
44 **Parry SD**, Welfare MR, Cobden I, Barton JR. Push enteroscopy in a UK district general hospital: experience of 51 cases over 2 years. *Eur J Gastroenterol Hepatol* 2002; **14**: 305-309 [PMID: 11953697 DOI: 10.1097/00042737-200203000-00016]
45 **Chak A**, Koehler MK, Sundaram SN, Cooper GS, Canto MI, Sivak MV. Diagnostic and therapeutic impact of push enteroscopy: analysis of factors associated with positive findings. *Gastrointest Endosc* 1998; **47**: 18-22 [PMID: 9468418 DOI: 10.1016/S0016-5107(98)70293-7]
46 **Lin S**, Branch MS, Shetzline M. The importance of indication in the diagnostic value of push enteroscopy. *Endoscopy* 2003; **35**: 315-321 [PMID: 12664388 DOI: 10.1055/s-2003-38144]
47 **Chak A**, Cooper GS, Canto MI, Pollack BJ, Sivak MV. Enteroscopy for the initial evaluation of iron deficiency. *Gastrointest Endosc* 1998; **47**: 144-148 [PMID: 9512279 DOI: 10.1016/S0016-5107(98)70347-5]
48 **Ge ZZ**, Chen HY, Gao YJ, Hu YB, Xiao SD. Best candidates for capsule endoscopy for obscure gastrointestinal bleeding. *J Gastroenterol Hepatol* 2007; **22**: 2076-2080 [PMID: 18031363 DOI: 10.1111/j.1440-1746.2006.04724.x]
49 **Fry LC**, Bellutti M, Neumann H, Malfertheiner P, Mönkemüller K. Incidence of bleeding lesions within reach of conventional upper and lower endoscopes in patients undergoing double-balloon enteroscopy for obscure gastrointestinal bleeding. *Aliment Pharmacol Ther* 2009; **29**: 342-349 [PMID: 19035975 DOI: 10.1111/j.1365-2036.2008.03888.x]
50 **Hadithi M**, Heine GD, Jacobs MA, van Bodegraven AA, Mulder CJ. A prospective study comparing video capsule endoscopy with double-balloon enteroscopy in patients with obscure gastrointestinal bleeding. *Am J Gastroenterol* 2006; **101**: 52-57 [PMID: 16405533 DOI: 10.1111/j.1572-0241.2005.00346.x]
51 **Arakawa D**, Ohmiya N, Nakamura M, Honda W, Shirai O, Itoh A, Hirooka Y, Niwa Y, Maeda O, Ando T, Goto H. Outcome after enteroscopy for patients with obscure GI bleeding: diagnostic comparison between double-balloon endoscopy and videocapsule endoscopy. *Gastrointest Endosc* 2009; **69**: 866-874 [PMID: 19136098 DOI: 10.1016/j.gie.2008.06.008]
52 **Yamamoto H**, Kita H, Sunada K, Hayashi Y, Sato H, Yano T, Iwamoto M, Sekine Y, Miyata T, Kuno A, Ajibe H, Ido K, Sugano K. Clinical outcomes of double-balloon endoscopy for the diagnosis and treatment of small-intestinal diseases. *Clin Gastroenterol Hepatol* 2004; **2**: 1010-1016 [PMID: 15551254 DOI: 10.1016/S1542-3565(04)00453-7]
53 **Di Caro S**, May A, Heine DG, Fini L, Landi B, Petruzziello L, Cellier C, Mulder CJ, Costamagna G, Ell C, Gasbarrini A. The European experience with double-balloon enteroscopy: indications, methodology, safety, and clinical impact. *Gastrointest Endosc* 2005; **62**: 545-550 [PMID: 16185969 DOI: 10.1016/j.gie.2005.04.029]
54 **May A**, Nachbar L, Ell C. Double-balloon enteroscopy (push-and-pull enteroscopy) of the small bowel: feasibility and diagnostic and therapeutic yield in patients with suspected small bowel disease. *Gastrointest Endosc* 2005; **62**: 62-70 [PMID: 15990821 DOI: 10.1016/S0016-5107(05)01586-5]
55 **Heine GD**, Hadithi M, Groenen MJ, Kuipers EJ, Jacobs MA, Mulder CJ. Double-balloon enteroscopy: indications, diagnostic yield, and complications in a series of 275 patients with suspected small-bowel disease. *Endoscopy* 2006; **38**: 42-48 [PMID: 16429354]
56 **Zhong J**, Ma T, Zhang C, Sun B, Chen S, Cao Y, Wu Y. A retrospective study of the application on double-balloon enteroscopy in 378 patients with suspected small-bowel diseases. *Endoscopy* 2007; **39**: 208-215 [PMID: 17385105 DOI: 10.1055/s-2007-966190]
57 **Byeon JS**, Chung JW, Choi KD, Choi KS, Kim B, Myung SJ, Yang SK, Kim JH. Clinical features predicting the detection of abnormalities by double balloon endoscopy in patients with suspected small bowel bleeding. *J Gastroenterol Hepatol* 2008; **23**: 1051-1055 [PMID: 18086108 DOI: 10.1111/j.1440-1746.2007.05270.x]
58 **Choi H**, Choi KY, Eun CS, Jang HJ, Park DI, Chang DK, Kim JO, Ko BM, Lee MS, Huh KC, Han DS, Byeon JS, Yang SK, Kim JH. Korean experience with double balloon endoscopy: Korean Association for the Study of Intestinal Diseases multi-center study. *Gastrointest Endosc* 2007; **66**: S22-S25 [PMID: 17709024 DOI: 10.1016/j.gie.2007.06.048]
59 **Hsu CM**, Chiu CT, Su MY, Lin WP, Chen PC, Chen CH. The outcome assessment of double-balloon enteroscopy for diagnosing and managing patients with obscure gastrointestinal bleeding. *Dig Dis Sci* 2007; **52**: 162-166 [PMID: 17160468 DOI: 10.1007/s10620-006-9554-x]
60 **Manabe N**, Tanaka S, Fukumoto A, Nakao M, Kamino D, Chayama K. Double-balloon enteroscopy in patients with GI bleeding of obscure origin. *Gastrointest Endosc* 2006; **64**: 135-140 [PMID: 16813826 DOI: 10.1016/j.gie.2005.12.020]
61 **Pennazio M**. Diagnostic and Therapeutic Utility of Double-Balloon Endoscopy in Small-Bowel Bleeding. *Tech Gastrointest Endosc* 2008; **10**: 77-82 [DOI: 10.1016/j.tgie.2007.12.007]
62 **Gay G**, Delvaux M, Fassler I. Outcome of capsule endoscopy in determining indication and route for push-and-pull enteroscopy. *Endoscopy* 2006; **38**: 49-58 [PMID: 16429355 DOI: 10.1055/s-2005-921176]
63 **Hendel JW**, Vilmann P, Jensen T. Double-balloon endoscopy: who needs it? *Scand J Gastroenterol* 2008; **43**: 363-367 [PMID: 18266178 DOI: 10.1080/00365520701799468]
64 **John S**, Appleyard M. Role of double balloon enteroscopy in obscure gastrointestinal bleeding. *J Gastroenterol Hepatol* 2008; **23**: 994-996 [PMID: 18707594 DOI: 10.1111/j.1440-1746.2008.05515.x]
65 **May A**, Nachbar L, Schneider M, Ell C. Prospective comparison of push enteroscopy and push-and-pull enteroscopy in patients with suspected small-bowel bleeding. *Am J Gastroenterol* 2006; **101**: 2016-2024 [PMID: 16968508 DOI: 10.1111/j.1572-0241.2006.00745.x]
66 **Messer I**, May A, Manner H, Ell C. Prospective, randomized, single-center trial comparing double-balloon enteroscopy and spiral enteroscopy in patients with suspected small-bowel disorders. *Gastrointest Endosc* 2013; **77**: 241-249 [PMID: 23043851 DOI: 10.1016/j.gie.2012.08.020]
67 **Frieling T**, Heise J, Sassenrath W, Hülsdonk A, Kreysel C. Prospective comparison between double-balloon enteroscopy and spiral enteroscopy. *Endoscopy* 2010; **42**: 885-888 [PMID: 20803420 DOI: 10.1055/s-0030-1255714]
68 **Rahmi G**, Samaha E, Vahedi K, Ponchon T, Fumex F, Filoche B, Gay G, Delvaux M, Lorenceau-Savale C, Malamut G, Canard JM, Chatellier G, Cellier C. Multicenter comparison of double-balloon enteroscopy and spiral enteroscopy. *J Gastroenterol Hepatol* 2013; **28**: 992-998 [PMID: 23488827 DOI: 10.1111/jgh.12188]
69 **Swain P**, Fritscher-Ravens A. Role of video endoscopy in managing small bowel disease. *Gut* 2004; **53**: 1866-1875 [PMID: 15542530 DOI: 10.1136/gut.2003.035576]
70 **Bonnet S**, Douard R, Malamut G, Cellier C, Wind P. Intraoperative enteroscopy in the management of obscure gastrointestinal bleeding. *Dig Liver Dis* 2013; **45**: 277-284 [PMID: 22877794 DOI: 10.1016/j.dld.2012.07.003]
71 **Descamps C**, Schmit A, Van Gossum A. "Missed" upper gastrointestinal tract lesions may explain "occult" bleeding. *Endoscopy* 1999; **31**: 452-455 [PMID: 10494684 DOI: 10.1055/s-1999-151]

72 **Zuckerman GR**, Prakash C. Acute lower intestinal bleeding. Part II: etiology, therapy, and outcomes. *Gastrointest Endosc* 1999; **49**: 228-238 [PMID: 9925703 DOI: 10.1016/S0016-5107(99)70491-8]

73 **Zaman A**, Katon RM. Push enteroscopy for obscure gastrointestinal bleeding yields a high incidence of proximal lesions within reach of a standard endoscope. *Gastrointest Endosc* 1998; **47**: 372-376 [PMID: 9609429 DOI: 10.1016/S0016-5107(98)70221-4]

74 **Hayat M**, Axon AT, O'Mahony S. Diagnostic yield and effect on clinical outcomes of push enteroscopy in suspected small-bowel bleeding. *Endoscopy* 2000; **32**: 369-372 [PMID: 10817173 DOI: 10.1055/s-2000-9003]

75 **Spiller RC**, Parkins RA. Recurrent gastrointestinal bleeding of obscure origin: report of 17 cases and a guide to logical management. *Br J Surg* 1983; **70**: 489-493 [PMID: 6603248 DOI: 10.1002/bjs.1800700812]

76 **Leaper M**, Johnston MJ, Barclay M, Dobbs BR, Frizelle FA. Reasons for failure to diagnose colorectal carcinoma at colonoscopy. *Endoscopy* 2004; **36**: 499-503 [PMID: 15202045 DOI: 10.1055/s-2004-814399]

77 **Huprich JE**. Multi-phase CT enterography in obscure GI bleeding. *Abdom Imaging* 2009; **34**: 303-309 [PMID: 18493814 DOI: 10.1007/s00261-008-9412-8]

78 **Lee SS**, Oh TS, Kim HJ, Chung JW, Park SH, Kim AY, Ha HK. Obscure gastrointestinal bleeding: diagnostic performance of multidetector CT enterography. *Radiology* 2011; **259**: 739-748 [PMID: 21460027 DOI: 10.1148/radiol.11101936]

79 **Hakim FA**, Alexander JA, Huprich JE, Grover M, Enders FT. CT-enterography may identify small bowel tumors not detected by capsule endoscopy: eight years experience at Mayo Clinic Rochester. *Dig Dis Sci* 2011; **56**: 2914-2919 [PMID: 21735085 DOI: 10.1007/s10620-011-1773-0]

80 **Soyer P**, Boudiaf M, Fishman EK, Hoeffel C, Dray X, Manfredi R, Marteau P. Imaging of malignant neoplasms of the mesenteric small bowel: new trends and perspectives. *Crit Rev Oncol Hematol* 2011; **80**: 10-30 [PMID: 21035353 DOI: 10.1016/j.critrevonc.2010.09.010]

81 **Filippone A**, Cianci R, Milano A, Valeriano S, Di Mizio V, Storto ML. Obscure gastrointestinal bleeding and small bowel pathology: comparison between wireless capsule endoscopy and multidetector-row CT enteroclysis. *Abdom Imaging* 2008; **33**: 398-406 [PMID: 17619098 DOI: 10.1007/s00261-007-9271-8]

82 **Mazzarolo S**, Brady P. Small bowel capsule endoscopy: a systematic review. *South Med J* 2007; **100**: 274-280 [PMID: 17396731 DOI: 10.1097/SMJ.0b013e31802fa05a]

83 **García-Compean D**, Armenta JA, Marrufo C, Gonzalez JA, Maldonado H. Impact of therapeutic interventions induced by capsule endoscopy on long term outcome in chronic obscure GI bleeding. *Gastroenterol Clin Biol* 2007; **31**: 806-811 [PMID: 18166857 DOI: 10.1016/S0399-8320(07)73969-6]

84 **Park JJ**, Cheon JH, Kim HM, Park HS, Moon CM, Lee JH, Hong SP, Kim TI, Kim WH. Negative capsule endoscopy without subsequent enteroscopy does not predict lower long-term rebleeding rates in patients with obscure GI bleeding. *Gastrointest Endosc* 2010; **71**: 990-997 [PMID: 20304392 DOI: 10.1016/j.gie.2009.12.009]

85 **Viazis N**, Papaxoinis K, Vlachogiannakos J, Efthymiou A, Theodoropoulos I, Karamanolis DG. Is there a role for second-look capsule endoscopy in patients with obscure GI bleeding after a nondiagnostic first test? *Gastrointest Endosc* 2009; **69**: 850-856 [PMID: 18950762 DOI: 10.1016/j.gie.2008.05.053]

86 **Hindryckx P**, Botelberge T, De Vos M, De Looze D. Clinical impact of capsule endoscopy on further strategy and long-term clinical outcome in patients with obscure bleeding. *Gastrointest Endosc* 2008; **68**: 98-104 [PMID: 18291382 DOI: 10.1016/j.gie.2007.09.042]

87 **Byeon JS**, Mann NK, Jamil LH, Lo SK. Is a repeat double balloon endoscopy in the same direction useful in patients with recurrent obscure gastrointestinal bleeding? *J Clin Gastroenterol* 2013; **47**: 496-500 [PMID: 23388844 DOI: 10.1097/MCG.0b013e318275dabd]

88 **Gerson LB**, Batenic MA, Newsom SL, Ross A, Semrad CE. Long-term outcomes after double-balloon enteroscopy for obscure gastrointestinal bleeding. *Clin Gastroenterol Hepatol* 2009; **7**: 664-669 [PMID: 19514115 DOI: 10.1016/j.cgh.2009.01.021]

89 **May A**, Friesing-Sosnik T, Manner H, Pohl J, Ell C. Long-term outcome after argon plasma coagulation of small-bowel lesions using double-balloon enteroscopy in patients with mid-gastrointestinal bleeding. *Endoscopy* 2011; **43**: 759-765 [PMID: 21544778 DOI: 10.1055/s-0030-1256388]

90 **Landi B**, Cellier C, Gaudric M, Demont H, Guimbaud R, Cuillerier E, Couturier D, Barbier JP, Marteau P. Long-term outcome of patients with gastrointestinal bleeding of obscure origin explored by push enteroscopy. *Endoscopy* 2002; **34**: 355-359 [PMID: 11972265 DOI: 10.1055/s-2002-25276]

91 **Bezet A**, Cuillerier E, Landi B, Marteau P, Cellier C. Clinical impact of push enteroscopy in patients with gastrointestinal bleeding of unknown origin. *Clin Gastroenterol Hepatol* 2004; **2**: 921-927 [PMID: 15476156 DOI: 10.1016/S1542-3565(04)00397-0]

92 **Vakil N**, Huilgol V, Khan I. Effect of push enteroscopy on transfusion requirements and quality of life in patients with unexplained gastrointestinal bleeding. *Am J Gastroenterol* 1997; **92**: 425-428 [PMID: 9068462]

93 **Kendrick ML**, Buttar NS, Anderson MA, Lutzke LS, Peia D, Wang KK, Sarr MG. Contribution of intraoperative enteroscopy in the management of obscure gastrointestinal bleeding. *J Gastrointest Surg* 2001; **5**: 162-167 [PMID: 11331479 DOI: 10.1016/S1091-255X(01)80029-9]

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REVIEW

Contemporary surgical management of rectovaginal fistula in Crohn's disease

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Abstract

Rectovaginal fistula is a disastrous complication of Crohn's disease (CD) that is exceedingly difficult to treat. It is a disabling condition that negatively impacts a women's quality of life. Successful management is possible only after accurate and complete assessment of the entire gastrointestinal tract has been performed. Current treatment algorithms range from observation to medical management to the need for surgical intervention. A wide variety of success rates have been reported for all management options. The choice of surgical repair methods depends on various fistula and patient characteristics. Before treatment is undertaken, establishing reasonable goals and expectations of therapy is essential for both the patient and surgeon. This article aims to highlight the various surgical techniques and their outcomes for repair of CD associated rectovaginal fistula.

Key words: Rectovaginal fistula; Crohn's disease; Fistula; Advancement flap; Sleeve advancement; Episioproctotomy

Core tip: Rectovaginal fistula secondary to Crohn's disease is a devastating and disabling condition with a significant negative impact on quality of life. Furthermore, these fistulae pose an extremely challenging dilemma for the clinician with unique and often frustrating management challenges. Medical management is often futile and surgery may offer the only chance for cure. In this article, we aim to review the various treatment options to close these difficult to treat fistulae, with an emphasis on surgical technique and complex decision making.

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INTRODUCTION

Fistula-in-ano is the most common perianal manifestation of Crohn's disease (CD) and was first reported by Gabriel[1] in 1921, nine years before Crohn *et al*[2] identified regional enteritis as a clinical entity. These fistulae are classified by their relationship to the sphincter complex as either high (supra- or extra-sphincteric *vs* low (inter- or trans-sphincteric). Low fistulae that transverse the anal sphincter are more appropriately named anovaginal fistulae, but by convention, all such fistulae are termed rectovaginal fistula (RVF). After obstetrical trauma, CD is the most common etiological factor for RVF, and will occur in up to 10% of women with CD[3,4].

Rectovaginal fistulae secondary to CD are associated with significant morbidity and carry an increased risk for proctectomy[5,6]. It is a devastating and disabling condition and is a source of considerable social embarrassment

and has a significant negative impact on quality of life. Furthermore, CD associated RVF are an extremely challenging dilemma for the clinician and present unique and often frustrating management challenges. In this article, we aim to review the various treatment options to close these difficult to treat fistulae, with an emphasis on surgical technique and decision making.

PRESENTATION AND DIAGNOSIS

The presence of a fistulous tract between the gastrointestinal tract and the vagina can be distressing and embarrassing for the patient. The most common symptoms includes passage of either gas and/or stool *via* the vagina. Women may also report purulence from the vagina, dyspareunia, perineal pain and tenderness, along with vaginal irritation and recurrent genitourinary tract infections[3,7]. Physical examination may demonstrate the fistulous opening on inspection of the lower anorectum and vagina, but often, the RVF is not visible on inspection. The clinician must have a high index of suspicion when a women presents with signs and symptoms consistent with a RVF. These patients are best evaluated with an examination under anesthesia for definitive elucidation of the RVF[3].

Several other studies are available to help identify and delineate RVF including computed tomography (CT) scan, magnetic resonance imaging (MRI), fistulography, and endoluminal ultrasound (EUS). The use of EUS with hydrogen peroxide enhancement has been advocated in the evaluation of complex fistula disease to visualize side tracts and areas of fluid collection[3,8-10]. Sloots *et al*[10] reported on this modality in 41 patients with CD related fistula-in-ano (32% with RVF), and found that 78% of the patients had a more complex fistula found during EUS. An added benefit of using EUS to evaluate the fistula tract, is the ability to identify any anal sphincter defects.

After a comprehensive workup and evaluation of the perineum, the remainder of the small and large bowel, rectum, and anal canal must be investigated. It is important to identify any other active areas of CD in order to plan both medical and potential surgical management. Work-up may include a colonoscopy, esophagogastroduodenoscopy, small bowel series, CT or MRI enterography. If proximal CD is found, optimization with medical and/or surgical management should be strongly considered before any attempt to repair the RVF.

TREATMENT OPTIONS

There are several disease characteristics that guide treatment recommendations for patients with RVF. These include the location of the fistula (high, low, or transsphincteric), anal canal disease (ulcerations or stricturing), the presence of active inflammation in the rectum, and rectal compliance. The presence and severity of symptoms, discomfort, and quality of life also weigh heavily in regards to treatment type and timing. Because there is considerable debate with regards to the best treatment options for these notoriously difficult to close fistulae, a frank discussion setting realistic goals and expectations of treatment is the initial step. Patients with no or minimal symptoms may actually be advised to have no treatment at all[7,11-12]. For patients with intolerable symptoms, a logical and stepwise approach to management begins with conservative medical therapy and advances to surgical intervention when indicated[3]. It should be noted that there are currently no prospective, randomized, controlled trials for the surgical correction of CD related RVF. The factors previously discussed along with personal experience, surgical judgment and a critical appraisal of the available literature should be used to formulate an optimal and tailored treatment plan for women with CD associated RVF[3].

MEDICAL MANAGEMENT

Traditionally treatment of CD associated RVF has been mostly surgical as medical treatments were fraught with failure[3]. Medical treatment has centered on pharmacological therapy aimed at alleviating and treating the underlying active CD along with medication to alter stool consistency and control diarrhea. Current medications targeting CD include antibiotics, corticosteroids, immunomodulators, and biologics. Metronidazole has been reported to successfully treat RVF, although most surgeons will use this and other antibiotics as an adjunct to surgical treatment[3,13]. Present *et al*[14-16] have written extensively on various medical modalities for the treatment of all types of CD related fistulae, including, cyclosporine, 6-mercatopurine, and infliximab. A randomized, double-blinded, multicenter study by Present *et al*[16] studied infliximab for the treatment of both abdominal and perianal fistulae from CD. After 18 wk of infliximab treatment the authors found significant reduction in the number of fistulae with complete closure occurring in 46% *vs* 13% of placebo. The follow-up was relatively short (4.5 mo) and the study included all enterocutaneous fistulae, not specifically RVF, making generalizability to RVF somewhat limited.

In the ACCENT II study by Sands *et al*[17], the authors evaluated the effect of infliximab in patients with RVF secondary to CD. Twenty-five patients were enrolled and received infliximab infusions at weeks zero, two, and six. Initial responders (those who showed a 50% reduction in their fistula in the first ten weeks) were then randomized to continue receiving infliximab or placebo. At 54 wk follow-up, 44% of the initial responders healed their fistulae and alternatively 56% had RVF recurrence, regardless of infliximab treatment. Essentially, the women who initially responded to the infliximab had a 50% chance of fully healing their RVF.

It is unclear which RVF will respond to infliximab nor is there evidence that infliximab will reduce fistula recurrence rates. At our institution we tend to recommend infliximab (or other biologic therapy) as initial treatment when surrounding tissues are inflamed or ulcerated such

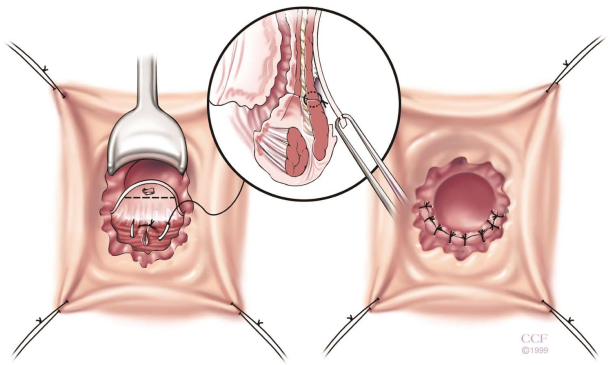

Figure 1 Rectal advancement flap. The rectal advancement flap begins with a 180 degree curvilinear incision starting just distal to fistula opening and extends 4-5 cm cephalad, encompassing mucosa, submucosa and the rectal wall is dissected from the rectovaginal septum. After mobilization, the fistula tract is cored out and the opening is closed with absorbable sutures. The diseased distal portion of the flap is trimmed before and the flap is advanced distally and sutured to the cut edge with absorbable sutures. The vaginal or perineal external opening is left open for drainage. Reprinted with permission, Cleveland Clinic Center for Medical Art and Photography © 1999-2014. All Rights Reserved.

that any attempt at surgical closure will uniformly fail. In some patients, the RVF may close with this therapy, but if it persists and the active inflammation becomes quiescent, then surgical correction may be attempted.

SURGICAL MANAGEMENT

Local repair of RVF secondary to CD can be successfully accomplished when optimal conditions exist. The approaches to local repair include transperineal, transvaginal, and transanal (with or without transabdominal mobilization) techniques. The choice of technique depends on the experience of the surgeon, location of the RVF, and the status and extent of local and distant inflammatory bowel disease activity. Additionally, anal sphincter integrity in women after vaginal delivery must be considered as some patients may require a sphincter repair along with the fistula repair.

An important aspect of RVF repair is initial drainage of perianal sepsis before consideration of surgical closure. Often, the use of loose draining setons is required for adequate sepsis control. The addition of antibiotics may also benefit selected patients with significant purulence. There is a group of women that benefit from a diverting stoma to facilitate sepsis eradication. Typically a stoma is helpful if stool consistency is loose and/or frequent. An added benefit of the stoma procedure is that is allows surgical treatment of intestinal CD at the same operation. Consideration of each of these steps is mandatory before any definitive attempt at RVF repair is undertaken. A waiting period of at least 3-6 mo is needed for all local inflammation and infection to be cleared.

It should be noted that in women with active anorectal disease, the use of a draining seton may be used indefinitely as a sphincter-saving procedure. Seton use in this situation has been shown to successfully preserve fecal continence and delay or avoid a permanent stoma in those women who cannot undergo local surgical repair[3,7,18,19].

Simple fistulotomy

Low and superficial (anovaginal) fistulas can be layed open or excised with a simple fistulotomy in very few select cases with successful healing. These circumstances are rare and virtually no sphincter muscle must be involved for this technique to be considered. If there is any anal canal deformity after fistulotomy (keyhole deformity), some degree of fecal incontinence will undoubtedly result[3,7].

Anocutaneous flap

The anocutaneous flap technique is rarely utilized but may be considered in situations where anal stenosis is present. The technique consists of mobilizing an island of skin and subcutaneous tissue from the anal margin or verge and advancing this flap into the anal canal to cover the RVF. This procedure is only possible if the anal skin is soft and pliable, which is not common in perianal CD patients. Hesterberg *et al*[20] reported a 70% healing rate at median follow-up of 18 mo with this technique.

Transrectal approaches

Most authors believe that repairing RVF from the high pressure (rectal) side of these low pressure fistulas is advantageous. This allows for the source of the fistula to be excised and closed. Then a healthy layer of tissue (flap) is used to cover the repair[7,21,22]. There is a wide variety of flap configurations, but the standard curvilinear rectal advancement flap is the most commonly performed flap procedure for RVF.

Rectal advancement flap

The rectal advancement flap (RAF) repair has been somewhat successful in healing RVF secondary to CD and should be considered in women with favorable anorectal anatomy[3]. Patients with minimally diseased or normal rectum and a normal anal canal are ideal candidates for this type of repair. However, this technique is contraindicated in women with extensive ulceration or stricturing of the anal canal and transitional zone as well as women with an anterior sphincter defect[3]. It should also be used with caution in woman with fecal incontinence. The technique has been well described in the literature, but briefly, it consists of making a curvilinear incision nearly 180 degrees just distal to the fistula opening in the anal canal. The mucosa of the cephalad anal canal is removed and then a flap of mucosa, submucosa and the rectal wall is dissected from the rectovaginal septum cephalad for approximately 4-5 cm. After sufficient mobilization to avoid tension when advancing the flap, that fistula is cored out and the fistula opening closed with absorbable suture. The flap is then trimmed and advanced distally to the cut edge. Then using absorbable sutures it is sewn to this cut edge with deep bites. The vaginal or perineal external opening is left open for drainage (Figure 1).

Hull and Fazio[23] reviewed forty-eight women who had an anovaginal fistula secondary to CD, with 35 undergoing one of 3 types of flap repairs. Twenty-four women underwent RAF with the standard curvilinear

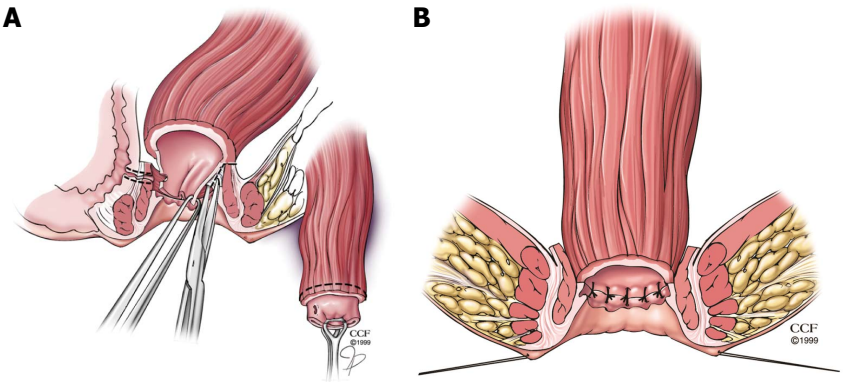

Figure 2 Rectal sleeve advancement flap. A: Dissection begins at the dentate line with a 90%-100% circumferential mucosectomy of ulcerated mucosa and submucosa of the anal canal and is carried cephalad until the supralevator space is breeched. After sufficient rectal mobilization has been accomplished, the fistula tract is cored out and then closed with absorbable suture and the vaginal mucosa is left open; B: The diseased distal margin of tissue is trimmed and the cuff of rectum is advanced down and sutured to the ridge of anoderm using absorbable sutures. Reprinted with permission, Cleveland Clinic Center for Medical Art and Photography © 1999-2014. All Rights Reserved.

incision and six patients with a long and high RVF or the presence of anal ulceration, underwent a linear flap procedure. The initial healing rate of all repair types was 54%, with an ultimate healing rate of 68% after additional surgical procedures were performed. The authors concluded that surgical intervention for low RVF secondary CD is advocated in properly selected patients by using an individualized approach based on the nature of the anovaginal fistula.

In another study by Kodner *et al*[24], endorectal advancement flaps were created in 24 patients with CD and a relatively normal rectum. Seventeen out of twenty-four (71%) patients achieved primary healing after initial flap repair and a total of 22/24 patients had healing after further repairs. Similarly, Makowiec *et al*[25] and Crim *et al*[26] reported successful healing of RVF secondary to CD in 5/12 and 10/14 patients, respectively with this technique.

Ruffolo *et al*[27] stress that the advantages of a flap procedures are a low chance of: producing a keyhole deformity, worsening fecal incontinence, or aggravation of patient's symptoms in case of failure. Additionally, there is no perineal wound and the presence of a stoma is not mandatory.

Rectal sleeve advancement flap

When an endorectal advancement flap is not an acceptable choice for RVF repair due to extensive ulceration or stricturing in the anal canal and transition zone, the rectal sleeve advancement flap may be considered. This technique also requires a normal or near normal rectum. First reported in the literature by Berman in 1991, the rectal sleeve advancement flap removes all of the diseased tissue in the anal canal and allows for a more 'normal' sleeve of rectal tissue to be sutured to the neodentate line[3,28,29]. The rectum should also be distensible and not exhibit any significant scarring from quiescent Crohn's proctitis. Starting at the dentate line, a mucosectomy of the ulcerated mucosa and submucosa of the anal canal is performed. The mobilization is 90%-100% circumferentially. Next the dissection breaches into the supralevator space and the rectal mobilization is continued cephalad until sufficient mobility is achieved so the rectum can be advanced within the sleeve of the internal sphincter to the neodentate line without tension. Anteriorly the dissection is in the rectovaginal septum. The fistula tract is then cored out and sutured, leaving the vaginal mucosa open as was discussed in the RAF (Figure 2A). The diseased distal margin of tissue is trimmed, and the cuff of rectum is advanced down and sutured to the ridge of anoderm, again using absorbable sutures as was described for the RAF (Figure 2B)[23]. In the event that sufficient mobility cannot be obtained to bring the sleeve of tissue to the cut distal edge without tension, the patient and the surgeon must be prepared to convert the operation to a transabdominal approach, with full mobilization of rectum, descending colon, and splenic flexure. Additionally, if a tension -free anastomosis cannot be achieved, proctectomy with end stoma may be necessary so the patient must be appraised of this possibility during the informed consent[3].

In a study from our institution, Marchesa *et al*[29] reviewed 13 patients (12 women) with severe perianal CD (11 with RVF, 1 rectourethral fistula, 1 anal canal ulceration) who underwent sleeve advancement as an alternative to proctectomy. All patients had been previously treated with a rectal advancement flap without success. Eight patients had proximal fecal diversion, with six having concomitant bowel resection with a protective stoma at the same time of sleeve advancement. A 60% success rate was achieved by using the sleeve advancement flap in this carefully selected population of patients. Additionally, Simmang *et al*[30] reported successful healing in two patients with RVF secondary to CD using the sleeve advancement flap.

Patient selection and preparation are keys to achieve a satisfactory outcome with the sleeve advancement flap, therefore careful patient selection is crucial[29]. Fecal diversion is a controversial option with this technique and the majority of patients in Marchesa's study appeared to have improved success rates for RVF closure

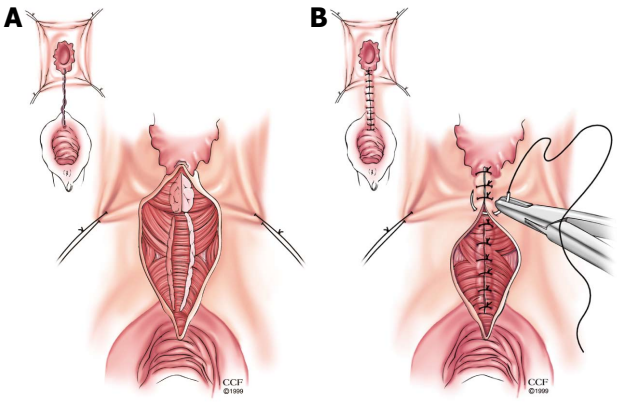

Figure 3 Episioproctotomy. A: Episioproctotomy begins with fistulotomy and division of all tissue overlying the fistula, including sphincter muscles and rectal and vaginal walls. Complete debridement of the granulation tissue of the fistula tract is carried out along with the lateral identification and mobilization of the sphincter muscles; B: The rectal mucosa is repaired followed by an overlap repair of the sphincter muscles. The repair is completed by closing the vaginal mucosa. Reprinted with permission, Cleveland Clinic Center for Medical Art and Photography © 1999-2014. All Rights Reserved.

when they were proximally diverted[29]. It is our practice currently to strongly consider diversion what performing a sleeve. This is typically an ileostomy. Meticulous surgical technique and adherence to principles such as hemostasis, gentle handling of tissues, and debridement of all diseased tissue is of paramount importance in this potentially technically demanding procedure. This repair is typically considered in a patient where the only other alternatives may be total proctocolectomy or permanent fecal diversion[29].

Transperineal approaches

Episioproctotomy: When an anterior sphincter defect coexists in women with an RVF, the surgeon should strongly consider repairing the sphincter defect with the RVF repair[3]. This can be accomplished with either a rectal advancement flap performed in concurrence with an anterior sphincteroplasty[31] or as the case at our institution, an episioproctotomy is performed[32]. An episioproctotomy entails performing a fistulotomy and creating of a defect similar to a fourth degree perineal laceration during vaginal childbirth[32]. A compete debridement of the granulation tissue of the fistula tract is carried out along with lateral identification and mobilization of the sphincter muscles (Figure 3A). The rectal mucosa is repaired initially. Then an overlap of the sphincter muscles is accomplished. Finally the vaginal mucosa is approximated which completes the repair (Figure 3B). El-Gazzaz *et al*[6] from our institution reported their results of various methods of Crohn's related RVF repair. There were 8 women who had an episioproctotomy with a healing rate of 71.4%.

Transverse transperineal repair: Particularly in the gynecological literature, an incision transversely through the perineal body is advocated to repair RVF. Dissection is carried out cephalad to the fistula tract and then the tract is transected with sharp dissection. The posterior vaginal and anterior rectal walls are mobilized and the cicatrix is excised. The vaginal and rectal walls are closed in 2 layers along with a levatoroplasty. Athanasiadis *et al*[33] reviewed various surgical techniques for CD RVF in 37 women undergoing 57 procedures with a mean follow-up of 7.1 years. Twenty women underwent transverse transperineal repair with a 70% overall success rate.

Transvaginal approaches

Vaginal advancement flap: The technique of repairing a RVF *via* the vaginal approach is considered by some surgeons a superior method due to the fact that the operation occurs not in the confines of the anal canal but in the vagina where the tissue is non-diseased, soft and pliable[27]. By avoiding the rectum, there is minimal to no manipulation or instrumentation in the potentially diseased and inflamed bowel. The vaginal advancement flap (VAF) consists of raising a posterior flap of vaginal tissue around the fistula. The rectal and vaginal orifices of the fistula are identified and repaired with absorbable sutures and the levator ani muscle is approximated in the midline. The vaginal flap is then advanced over the repair and sutured to the perineal skin.

Sher *et al*[34] reviewed their experience with 14 VAF for RVF in the setting of CD. They reported 13/14 patients achieved fistula closure. The authors attribute their success with using healthy tissue and also using the levator ani interposition to lend added support and further separation of suture lines. Of note, all 14 patients either had proximal diversion before or at the time of VAF with a loop ileostomy, which the authors felt to be an essential part of their success[34]. Furthermore, in a systematic review of eleven observational studies by Ruffolo *et al*[27], VAF was compared to RAF, with the primary end point of successful RVF closure rate. A total of 219 flap procedures (175 RAF *vs* 49 VAF) were reviewed and the authors noted a 54.2% closure rate after RAF and a 69.4% closure rate for VAF. This review suggests no significant differences in terms of outcome between VAF and RAF in CD. The VAF may be a good surgical option when there is anorectal stenosis or after a failed RVF repair.

Inversion of fistula: If the fistula is low and small, inversion may be an option. A circular incision is made around the vaginal os, and the surrounding flap of vaginal mucosa is mobilized. Several concentric purse-string sutures are placed to invert the fistula into the rectum. The vaginal mucosa is then reapproximated. All surrounding tissue must be soft and pliable for this approach to be considered. It should be noted that there is no reported data on this technique in Crohn's related RVF.

Abdominal approaches

Coloanal anastomosis and turbull-cutait procedure: As mentioned previously, when performing the rectal sleeve advancement, a tension-free anastomosis may not be possible. In this scenario, a transabdominal approach is then used to complete the repair. This can be

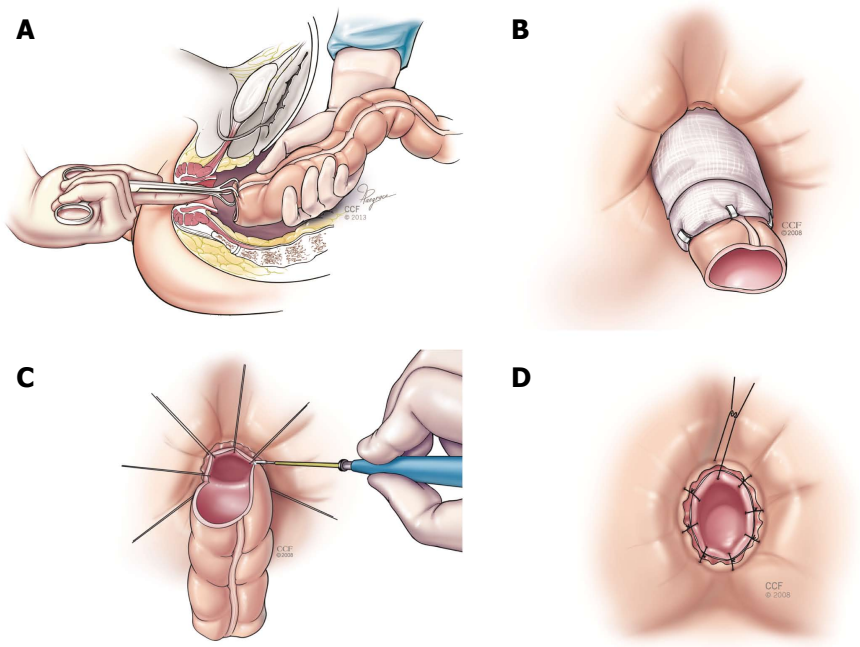

Figure 4 Turnbull-Cutait abdominalperineal pull-through procedure (A-D). Reprinted with permission, Cleveland Clinic Center for Medical Art and Photography © 1999-2014. All Rights Reserved.

done with two main techniques: an immediate hand-sewn coloanal anastomosis or a delayed coloanal anastomosis (Turnbull-Cutait). After complete rectal (and if needed) descending and splenic flexure mobilization, the colon is passed transanally. If the local conditions in the anus are satisfactory, a standard hand-sewn coloanal anastomosis is performed immediately. When there are other fistulous tracts close to the neodentate line or the internal opening of the fistula is close to the suture line, a delayed coloanal anastomosis as described by Cutait and Turnbull[35-37] should be considered. The highlight of this procedure relies on placing 8 sutures around the anus through the neodentate line. Then the proximal bowel is extruded out the anus and (along with the sutures with needles) wrapped in gauze and stabilized. Then after 5-7 d the extruded bowel is amputated and using the already placed sutures, the coloanal anastomosis is completed (Figure 4). This delayed maturation allows the portion of the bowel in the anal canal to adhere to the denuded surface and seal prior to amputation. El-Gazzaz *et al*[6] reported on this technique in 7 patients with CD associated RVF, with a 57.1% healing rate.

Miscellaneous repairs

Tissue interposition: Tissue interposition achieves bringing healthy, well vascularized tissue between the rectal and vaginal walls and acts as a buttress to suture lines as was mentioned in the transverse perineal approach above. Successful use of the gracilis muscle interposition has been reported for Crohn's RVF repairs, especially after other failed repairs. Zmora *et al*[38] reported on their use of the gracilis flap in nine patients for various causes of fistula, including 3 rectourethral fistulae, 1 pouch-vaginal fistula, and 5 RVF (2 CD associated RVF). All patients underwent fecal diversion. Seven patients achieved successful closure with this technique, with 1 CD associated fistulae achieving closure. The authors emphasized the importance of fecal diversion, performing a tension-free rectal repair, and the use of a well-vascularized muscle pedicle. They recommend the gracilis interposition in failed RVF repairs and noted that even though the rate of success in CD is not as high as a surgeon would prefer, a gracilis transposition can be attempted and should be considered. Similarly, in a study by Lefèvre *et al*[39], 4/5 women with Crohn's RVF were successfully closed at a 28 month follow-up, with the use of a gracilis muscle interposition.

The Martius (bulbocavernosus) flap may be used as an adjunct to transperineal repairs with anal sphincter reconstruction (Figure 5). The Martius flap has been reported to improve closure rates and possibly lead to better functional outcomes as well[40]. McNevin *et al*[40] reported on 16 patients with complex anovaginal fistulae, including 2 with CD. They reported success in 15 women and concluded that the Martius flap can be combined with an anterior sphincter repair for complex RVF with minimal morbidity.

Overall there are few studies utilizing the gracilis or Martius flaps in CD RVF. These studies have limited numbers of patients. Therefore it is not clear if the use of gracilis or Martius flaps improves outcomes after RVF repair.

Bioprosthetics: A bioprosthetic fistula plug made from lyophilized porcine intestinal submucosa is a technically feasible option in closing RVF, but the data on its use in Crohn's-related RVF is limited. Schwandner *et al*[41] reported using Surgisis™ mesh in 21 patients with RVF, 9 with Crohn's RVF. After a mean follow up of 12 mo, they achieved a 78% closure rate in the Crohn's group

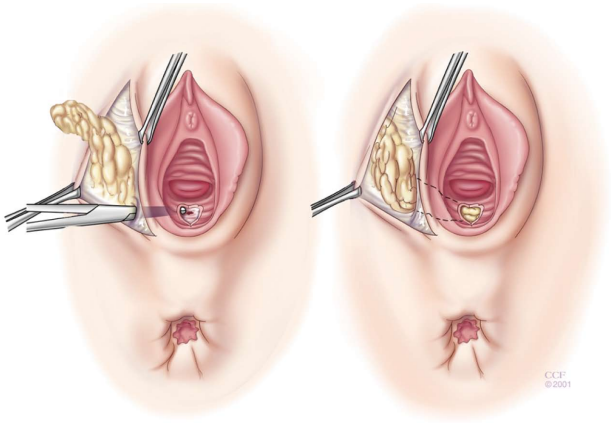

Figure 5 Martius graft. The martius graft begins standard perineal dissection followed by longitudinal incision over the labia majora. Skin flaps are raised medially and laterally until entire fat pad with bulbocavernosus muscle is mobilized. A subcutaneous, subvaginal tunnel is made and the flap is pulled through the tunnel after the anterior end is divided and then sutured to the posterior vaginal wall. Reprinted with permission, Cleveland Clinic Center for Medical Art and Photography © 1999-2014. All Rights Reserved.

and an 83% closure rate in the non-Crohn's RVF. The authors concluded that the mesh plug could be used as an adjunct to traditional advancement flap repair or muscle interposition, or possibly it could be used as an initial operation[41]. In a study by O'Connor *et al*[42], the use of the fistula plug was studied in patients with Crohn's fistulae (2 with RVF) with an 80% success rate. The success of the two RVF was not specifically addressed. Alternatively, in a report from our institution, a retrospective review of 49 plug insertions in thirty-three patients was conducted (13 CD with 2 RVF; 19 cryptogenic origin). The authors reported an 84.6% failure rate for CD associated fistulae (including both RVF) and a 68.4% failure rate for fistulae from cryptogenic origin. These results were much lower than previous reports and the authors concluded that septic complications were the most common cause of failure[43].

Currently, there is little data to support the routine use of bioprosthetics in Crohn's RVF, but the procedure carries a low morbidity and does not preclude further treatments. Further studies are required to determine the role of bioprosthetics in repair of CD associated RVF.

Stem cell transplantation: Adult stem cells extracted from certain tissues can differentiate into different tissue lines, including muscle. In a recent study by García-Olmo *et al*[44] from Spain, a female with Crohn's associated RVF received autologous adipose stem cells that were injected into her RVF. At three month follow up, the patient achieved successful closure of the fistula[44]. This is an exciting potential therapy where further research is needed.

Fecal diversion

As previously mentioned, the use of fecal diversion in repair of Crohn's associated RVF remains controversial. Proximal diversion does control symptomatology and likely improves the condition of the anorectum before subsequent repairs are undertaken. However, equivocal results are obtained whether or not proximal diversion is used in conjunction with RVF repair, regardless of the technique utilized[3]. There are no set criteria regarding when and in whom proximal diversion should be performed. Furthermore, a stoma does not ensure a successful repair. The literature is mixed with recommendations as some authors recommend all patients receive a loop ileostomy before or during repair and others recommend fecal diversion only in select situations. Without any randomized, prospective data, the creation of a stoma remains controversial and surgeons must use their best judgment in making the decision regarding diversion. Our institution recommends the construction of a proximal stoma in the following circumstances: re-do repairs, technically difficult repairs, and suboptimal tissue conditions[3].

Proctectomy

Traditionally, proctectomy has been the definitive treatment of Crohn's related RVF. In an early paper by Tuxen and Castro, total proctocolectomy (TPC) with an end ileostomy was the procedure of choice, due to shortcomings in medical treatment and proximal diversion to heal Crohn's RVF[11]. Over the last several decades, successful repairs with sphincter and rectum sparing techniques have been widely published. Despite published studies of successful repairs for Crohn's RVF, there are still subsets of patients who will require TPC. Patients with extensive colonic involvement or extensive anorectal involvement may not be candidates for definitive repair and proctectomy would be recommended as their initial step in treatment. It should be noted that proctectomy is not without its own complications, as delayed perineal wound healing and the potential for chronic perineal sinuses can be seen in up to 50% of patients in some series[5,22].

Surgical outcomes

Long-term success after repair is not guaranteed regardless of the method used. Crohn's related RVF have a high propensity to recur with a published range between 25%-50%[3,6,45-47]. Most studies have only reported short-term outcomes. Makowiec *et al*[25] evaluated perianal Crohn's fistulae in 32 patients who underwent RAF (12 patients had a RVF). Mean follow-up was 19.5 mo. A recurrence rate in the women with RVF was 58%. The authors analyzed their results which showed a cumulative risk of recurrence at one year of 46% and 72% at 2 years.

Ruffolo *et al*[48] evaluated surgical outcomes in women with Crohn's associated RVF over a 14 year period as well as assessing the effect of anti-TNF-α treatment on healing rates. With various techniques utilized, the authors found a fistula closure rate of 81% in 52 women. The cumulative closure rates after the first, second, third, and fourth attempt at repair was 56%, 75%, 78%, and 81%, respectively[48,49]. Furthermore, primary healing rates were found to be similar in patients receiving ant-TNF-alpha treatment *vs* those who did not.

In a long-term follow-up study from our institution,

El-Gazzaz *et al*[6], studied potential variables that may influence success or failure of fistula closure in Crohn's RVF. We also reported on quality of life and sexual function. With a median follow up of 44.6 mo, 30/65 (46.2%) had successful closure. Repair techniques were as follows: advancement flap (n = 47), episioproctotomy (n = 8), coloanal/Turnbull-Cutait (n = 7), and fibrin glue/plug (n = 3). The authors found that sexual function and quality of life were similar in healed *vs* unhealed women. Predictors of failure included smoking and steroids. The use of immunomodulator medications within 3 mo of repair showed a higher rate of fistula closure[6].

A retrospective study by Athanasiadis *et al*[33] reviewed rates of closure and functional outcomes in Crohn's RVF repair techniques over a 7 year period. Thirty-seven women with RVF underwent 57 operations with various repair techniques. The authors found that techniques with a low degree of tissue mobilization had higher success rates and less postoperative functional problems.

Repair of recurrent fistulae or re-repair of a failed repair is plausible. A review of all methods of repair over nine years for recurrent RVF secondary to all etiologies was undertaken at our institution. An overall success rate of 79% was accomplished after a median of 2 operations. When looking specifically at Crohn's associated recurrent RVF, 6/12 healed after a combined total of 21 operations. The authors noted that the most significant factor to influence outcome of repeat repairs was the duration of time between repairs. Patients re-operated within 3 mo of the original repair had lower healing rate compared to those treated after 3 mo. The authors highlighted that proper patient selection and optimization of clinical conditions is paramount in order to achieve the best possible outcome[46].

CONCLUSION

Rectovaginal fistulae are the most difficult manifestation of perianal CD to treat. They are a source of frustration for the patient and for the treating clinician. A thorough investigational work-up of the entire gastrointestinal tract, the anal sphincters, and the anorectum must be performed before any treatment attempt can be undertaken. Only after failed medical management and when local conditions are suitable, can surgical intervention be contemplated. Initial control of perianal sepsis with drainage and possible seton placement is paramount and may be the only treatment required. Medical treatments are indicated to control both local and distant active CD. Immunomodulators and anti-TNF-α therapy may play a role in primary correction of fistulae or may be used as an adjunct to surgical repairs. The surgical management of RVF can be complex and the treatment plan must be individualized. The chosen technique is based on the anatomy of the fistula, patient symptoms, and quality of life. The experience of the surgeon also influences the choice of repair and multiple options must be in one's armamentarium. Often, repairs fail and reoperative intervention is necessary, with acceptable results. Maintaining realistic treatment goals and expectations is essential.

REFERENCES

1 **Gabriel WB**. Results of an Experimental and Histological Investigation into Seventy-five Cases of Rectal Fistulae. *Proc R Soc Med* 1921; **14**: 156-161 [PMID: 19982086]
2 **Crohn BB**, Ginzburg L, Oppenheimer GD. Landmark article Oct 15, 1932. Regional ileitis. A pathological and clinical entity. By Burril B. Crohn, Leon Ginzburg, and Gordon D. Oppenheimer. *JAMA* 1984; **251**: 73-79 [PMID: 6361290]
3 **Hannaway CD**, Hull TL. Current considerations in the management of rectovaginal fistula from Crohn's disease. *Colorectal Dis* 2008; **10**: 747-755; discussion 755-756 [PMID: 18462243 DOI: 10.1111/j.1463-1318.2008.01552.x]
4 **Radcliffe AG**, Ritchie JK, Hawley PR, Lennard-Jones JE, Northover JM. Anovaginal and rectovaginal fistulas in Crohn's disease. *Dis Colon Rectum* 1988; **31**: 94-99 [PMID: 3338350]
5 **Heyen F**, Winslet MC, Andrews H, Alexander-Williams J, Keighley MR. Vaginal fistulas in Crohn's disease. *Dis Colon Rectum* 1989; **32**: 379-383 [PMID: 2714128 DOI: 10.1007/BF02563688]
6 **El-Gazzaz G**, Hull T, Mignanelli E, Hammel J, Gurland B, Zutshi M. Analysis of function and predictors of failure in women undergoing repair of Crohn's related rectovaginal fistula. *J Gastrointest Surg* 2010; **14**: 824-829 [PMID: 20232172 DOI: 10.1007/s11605-010-1167-1]
7 **Andreani SM**, Dang HH, Grondona P, Khan AZ, Edwards DP. Rectovaginal fistula in Crohn's disease. *Dis Colon Rectum* 2007; **50**: 2215-2222 [PMID: 17846837 DOI: 10.1007/s10350-007-9057-7]
8 **Maconi G**, Parente F, Bianchi Porro G. Hydrogen peroxide enhanced ultrasound- fistulography in the assessment of enterocutaneous fistulas complicating Crohn's disease. *Gut* 1999; **45**: 874-878 [PMID: 10562586 DOI: 10.1136/gut.45.6.874]
9 **Poen AC**, Felt-Bersma RJ, Eijsbouts QA, Cuesta MA, Meuwissen SG. Hydrogen peroxide-enhanced transanal ultrasound in the assessment of fistula-in-ano. *Dis Colon Rectum* 1998; **41**: 1147-1152 [PMID: 9749499 DOI: 10.1007/BF02239437]
10 **Sloots CE**, Felt-Bersma RJ, Poen AC, Cuesta MA, Meuwissen SG. Assessment and classification of fistula-in-ano in patients with Crohn's disease by hydrogen peroxide enhanced transanal ultrasound. *Int J Colorectal Dis* 2001; **16**: 292-297 [PMID: 11686526 DOI: 10.1007/s003840100308]
11 **Tuxen PA**, Castro AF. Rectovaginal fistula in Crohn's disease. *Dis Colon Rectum* 1979; **22**: 58-62 [PMID: 421652 DOI: 10.1007/BF02586761]
12 **Morrison JG**, Gathright JB, Ray JE, Ferrari BT, Hicks TC, Timmcke AE. Results of operation for rectovaginal fistula in Crohn's disease. *Dis Colon Rectum* 1989; **32**: 497-499 [PMID: 2791787 DOI: 10.1007/BF02554505]
13 **Bernstein LH**, Frank MS, Brandt LJ, Boley SJ. Healing of perineal Crohn's disease with metronidazole. *Gastroenterology* 1980; **79**: 357-365 [PMID: 7399243]
14 **Present DH**, Korelitz BI, Wisch N, Glass JL, Sachar DB, Pasternack BS. Treatment of Crohn's disease with 6-mercaptopurine. A long-term, randomized, double-blind study. *N Engl J Med* 1980; **302**: 981-987 [PMID: 6102739 DOI: 10.1056/NEJM198005013021801]
15 **Present DH**, Rutgeerts P, Targan S, Hanauer SB, Mayer L, van Hogezand RA, Podolsky DK, Sands BE, Braakman T, DeWoody KL, Schaible TF, van Deventer SJ. Infliximab for the treatment of fistulas in patients with Crohn's disease. *N Engl J Med* 1999; **340**: 1398-1405 [PMID: 10228190 DOI: 10.1056/NEJM199905063401804]
16 **Present DH**, Lichtiger S. Efficacy of cyclosporine in treatment of fistula of Crohn's disease. *Dig Dis Sci* 1994; **39**:

374-380 [PMID: 8313821 DOI: 10.1007/BF02090211]
17 **Sands BE**, Blank MA, Patel K, van Deventer SJ. Long-term treatment of rectovaginal fistulas in Crohn's disease: response to infliximab in the ACCENT II Study. *Clin Gastroenterol Hepatol* 2004; **2**: 912-920 [PMID: 15476155 DOI: 10.1053/S1542-3565(04)00414-8]
18 **Faucheron JL**, Saint-Marc O, Guibert L, Parc R. Long-term seton drainage for high anal fistulas in Crohn's disease--a sphincter-saving operation? *Dis Colon Rectum* 1996; **39**: 208-211 [PMID: 8620789 DOI: 10.1007/BF02068077]
19 **Thornton M**, Solomon MJ. Long-term indwelling seton for complex anal fistulas in Crohn's disease. *Dis Colon Rectum* 2005; **48**: 459-463 [PMID: 15719188]
20 **Hesterberg R**, Schmidt WU, Müller F, Röher HD. Treatment of anovaginal fistulas with an anocutaneous flap in patients with Crohn's disease. *Int J Colorectal Dis* 1993; **8**: 51-54 [PMID: 8492045]
21 **Greenwald JC**, Hoexter B. Repair of rectovaginal fistulas. *Surg Gynecol Obstet* 1978; **146**: 443-445 [PMID: 625688]
22 **Cohen JL**, Stricker JW, Schoetz DJ, Coller JA, Veidenheimer MC. Rectovaginal fistula in Crohn's disease. *Dis Colon Rectum* 1989; **32**: 825-828 [PMID: 2791765]
23 **Hull TL**, Fazio VW. Surgical approaches to low anovaginal fistula in Crohn's disease. *Am J Surg* 1997; **173**: 95-98 [PMID: 9074371]
24 **Kodner IJ**, Mazor A, Shemesh EI, Fry RD, Fleshman JW, Birnbaum EH. Endorectal advancement flap repair of rectovaginal and other complicated anorectal fistulas. *Surgery* 1993; **114**: 682-689; discussion 689-690 [PMID: 8211682]
25 **Makowiec F**, Jehle EC, Becker HD, Starlinger M. Clinical course after transanal advancement flap repair of perianal fistula in patients with Crohn's disease. *Br J Surg* 1995; **82**: 603-606 [PMID: 7613925 DOI: 10.1002/bjs.1800820509]
26 **Crim RW**, Fazio VW, Lavery IC. Rectal advancement flap repair in Crohn's disease. Factors predictive of failure. *Dis Colon Rectum* 1990; **33**: 3
27 **Ruffolo C**, Scarpa M, Bassi N, Angriman I. A systematic review on advancement flaps for rectovaginal fistula in Crohn's disease: transrectal vs transvaginal approach. *Colorectal Dis* 2010; **12**: 1183-1191 [PMID: 19674019 DOI: 10.1111/j.1463-1318.2009.02029.x]
28 **Berman IR**. Sleeve advancement anorectoplasty for complicated anorectal/vaginal fistula. *Dis Colon Rectum* 1991; **34**: 1032-1037 [PMID: 1935468]
29 **Marchesa P**, Hull TL, Fazio VW. Advancement sleeve flaps for treatment of severe perianal Crohn's disease. *Br J Surg* 1998; **85**: 1695-1698 [PMID: 9876077]
30 **Simmang CL**, Lacey SW, Huber PJ. Rectal sleeve advancement: repair of rectovaginal fistula associated with anorectal stricture in Crohn's disease. *Dis Colon Rectum* 1998; **41**: 787-789 [PMID: 9645750]
31 **Khanduja KS**, Padmanabhan A, Kerner BA, Wise WE, Aguilar PS. Reconstruction of rectovaginal fistula with sphincter disruption by combining rectal mucosal advancement flap and anal sphincteroplasty. *Dis Colon Rectum* 1999; **42**: 1432-1437 [PMID: 10566531]
32 **Hull TL**, El-Gazzaz G, Gurland B, Church J, Zutshi M. Surgeons should not hesitate to perform episioproctotomy for rectovaginal fistula secondary to cryptoglandular or obstetrical origin. *Dis Colon Rectum* 2011; **54**: 54-59 [PMID: 21160314 DOI: 10.1097/01.dcr.0000388926.29548.36]
33 **Athanasiadis S**, Yazigi R, Köhler A, Helmes C. Recovery rates and functional results after repair for rectovaginal fistula in Crohn's disease: a comparison of different techniques. *Int J Colorectal Dis* 2007; **22**: 1051-1060 [PMID: 17404747]
34 **Sher ME**, Bauer JJ, Gelernt I. Surgical repair of rectovaginal fistulas in patients with Crohn's disease: transvaginal approach. *Dis Colon Rectum* 1991; **34**: 641-648 [PMID: 1855419]
35 **Kirwan WO**, Turnbull RB, Fazio VW, Weakley FL. Pullthrough operation with delayed anastomosis for rectal cancer. *Br J Surg* 1978; **65**: 695-698 [PMID: 709078]
36 **CUTAIT DE**, FIGLIOLINI FJ. A new method of colorectal anastomosis in abdominoperineal resection. *Dis Colon Rectum* 1961; **4**: 335-342 [PMID: 13882795]
37 **Cutait DE**, Cutait R, Ioshimoto M, Hyppólito da Silva J, Manzione A. Abdominoperineal endoanal pull-through resection. A comparative study between immediate and delayed colorectal anastomosis. *Dis Colon Rectum* 1985; **28**: 294-299 [PMID: 3996144]
38 **Zmora O**, Tulchinsky H, Gur E, Goldman G, Klausner JM, Rabau M. Gracilis muscle transposition for fistulas between the rectum and urethra or vagina. *Dis Colon Rectum* 2006; **49**: 1316-1321 [PMID: 16752191]
39 **Lefèvre JH**, Bretagnol F, Maggiori L, Alves A, Ferron M, Panis Y. Operative results and quality of life after gracilis muscle transposition for recurrent rectovaginal fistula. *Dis Colon Rectum* 2009; **52**: 1290-1295 [PMID: 19571707 DOI: 10.1007/DCR.0b013e3181a74700]
40 **McNevin MS**, Lee PY, Bax TW. Martius flap: an adjunct for repair of complex, low rectovaginal fistula. *Am J Surg* 2007; **193**: 597-599; discussion 599 [PMID: 17434363]
41 **Schwandner O**, Fuerst A, Kunstreich K, Scherer R. Innovative technique for the closure of rectovaginal fistula using Surgisis mesh. *Tech Coloproctol* 2009; **13**: 135-140 [PMID: 19484346]
42 **O'Connor L**, Champagne BJ, Ferguson MA, Orangio GR, Schertzer ME, Armstrong DN. Efficacy of anal fistula plug in closure of Crohn's anorectal fistulas. *Dis Colon Rectum* 2006; **49**: 1569-1573 [PMID: 16998638]
43 **El-Gazzaz G**, Zutshi M, Hull T. A retrospective review of chronic anal fistulae treated by anal fistulae plug. *Colorectal Dis* 2010; **12**: 442-447 [PMID: 19220379 DOI: 10.1111/j.1463-1318.2009.01802.x]
44 **García-Olmo D**, García-Arranz M, García LG, Cuellar ES, Blanco IF, Prianes LA, Montes JA, Pinto FL, Marcos DH, García-Sancho L. Autologous stem cell transplantation for treatment of rectovaginal fistula in perianal Crohn's disease: a new cell-based therapy. *Int J Colorectal Dis* 2003; **18**: 451-454 [PMID: 12756590]
45 **Joo JS**, Weiss EG, Nogueras JJ, Wexner SD. Endorectal advancement flap in perianal Crohn's disease. *Am Surg* 1998; **64**: 147-150 [PMID: 9486887]
46 **Halverson AL**, Hull TL, Fazio VW, Church J, Hammel J, Floruta C. Repair of recurrent rectovaginal fistulas. *Surgery* 2001; **130**: 753-757; discussion 757-758 [PMID: 11602908]
47 **Ozuner G**, Hull TL, Cartmill J, Fazio VW. Long-term analysis of the use of transanal rectal advancement flaps for complicated anorectal/vaginal fistulas. *Dis Colon Rectum* 1996; **39**: 10-14 [PMID: 8601343]
48 **Ruffolo C**, Penninckx F, Van Assche G, Vermeire S, Rutgeerts P, Coremans G, D'Hoore A. Outcome of surgery for rectovaginal fistula due to Crohn's disease. *Br J Surg* 2009; **96**: 1190-1195 [PMID: 19688772 DOI: 10.1002/bjs.6661]
49 **Zhu YF**, Tao GQ, Zhou N, Xiang C. Current treatment of rectovaginal fistula in Crohn's disease. *World J Gastroenterol* 2011; **17**: 963-967 [PMID: 21448347 DOI: 10.3748/wjg.v17.i8.963]

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REVIEW

Zinc and gastrointestinal disease

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Abstract

This review is a current summary of the role that both zinc deficiency and zinc supplementation can play in the etiology and therapy of a wide range of gastrointestinal diseases. The recent literature describing zinc action on gastrointestinal epithelial tight junctions and epithelial barrier function is described. Zinc enhancement of gastrointestinal epithelial barrier function may figure prominently in its potential therapeutic action in several gastrointestinal diseases.

Key words: Zinc; Tight junction; Nutrition; Nutraceutical; Micronutrient

Core tip: This is an overview of the role that both zinc deficiency and zinc supplementation can play in the etiology and therapy of a wide range of gastrointestinal diseases.

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INTRODUCTION

When considering the topic of zinc and gastrointestinal (GI) disease, several general biomedical and nutritional situations must be considered. The first, and perhaps most obvious, is that of dietary zinc deficiency (ZD) - whether this arises out of generalized diet insufficiency, genetically-based zinc malabsorption, or dietary interference with zinc absorption (*e.g.*, phytates in the diet) - and resultant diseases from said deficiency. The second consideration is that diseases, such as the inflammatory bowel diseases (IBDs), whose morbidity *generates* GI malabsorption issues, ultimately giving rise to ZD. Finally, one must consider the *supplementation* of zinc to the diet, and the positive role that may play in certain GI diseases, especially those characterized by impairment of barrier function. The connection among all three situations is perhaps that ZD, from whatever source appears to lead to GI barrier compromise, an eventuality that is self perpetuating (Figure 1).

This is, then, a very broad topic, and one in which numerous excellent reviews have been written concerning the above individual situations. Duggan *et al*[1] (2002) did a thorough reporting of zinc and other "functional foods" for maintaining GI mucosal function. In terms of barrier function per se, Hering *et al*[2] (2009) have recently published on this from a more cellular perspective. Semrad[3] (1999) reported on the general role of zinc in intestinal function, particularly in diarrhea. Goh *et al*[4] (2003) deal with both ZD arising out of IBDs as well as the role zinc and other nutraceuticals may play in providing an alterna-

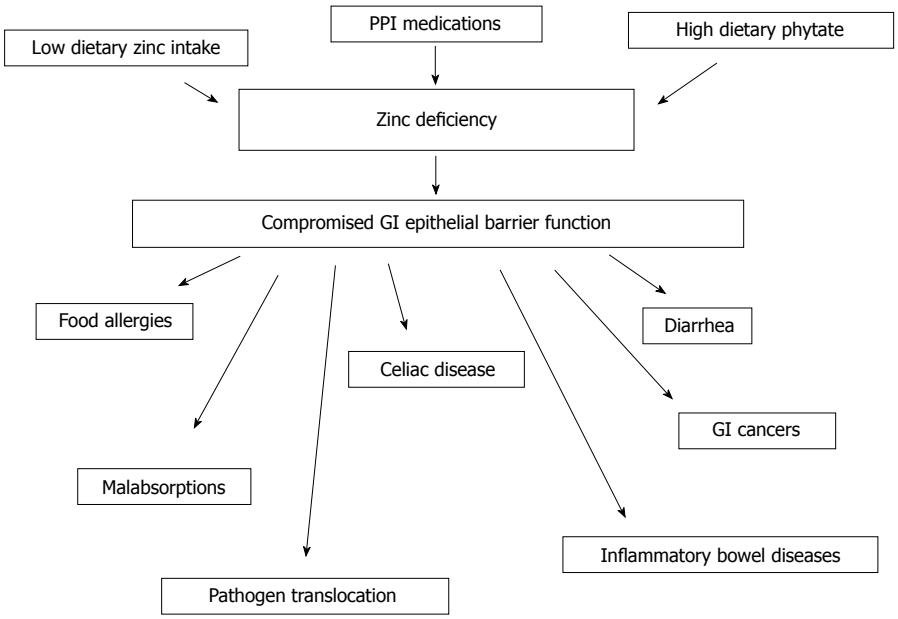

Figure 1 Zinc deficiency can arise from several sources, and a major physiological effect of zinc deficiency will be to induce leakiness in tight junctional seals and consequently epithelial cell layers. This figure diagrammatically shows the conditions/diseases that could be promoted by this eventuality arising in the gastrointestinal mucosa; GI: Gastrointestinal; PPI: Proton pump inhibitor.

tive to the use of steroids and anti-tumor necrosis factor (TNF) modalities in IBD therapy. Treatment *via* zinc supplementation of GI disease incited by ZD may in fact be the first (though inadvertent) clinical summary of supplemental zinc effects on GI barrier compromise[5]. The very concept of ZD as well as the myriad roles played by zinc in cellular and systemic function, are discussed comprehensively by Tuerk *et al*[6] (2009) and Wapnir[7] (2000). The singular issue of zinc in parenteral feeding, an important medical area for which zinc (and epithelial barrier function) may be highly important, is something that we do not consider here in any depth, but has been well investigated by Jeejeebhoy[8] (2009). The critical area of zinc "physiology", *i.e.*, its transport and binding in cells in general, and enterocytes in particular [ZIPs, ZnTs, metallothioneins (MTs), *etc.*], as well as the issue of zinc homeostasis systemically is discussed thoroughly by King[9] (2010), King *et al*[10] (2000) and Cousins[11] (2010). Finally, a very comprehensive review of zinc's role in disease generally has just recently been produced[12].

Our review generally does not address the enormous literature surrounding zinc finger proteins and zinc metalloproteinases, except where these proteins specifically deal with a deficiency or supplementation. There are numerous reviews covering these various topics touching on zinc[13-16]. Both protein classes have almost certain relevance toward all of the diseases we discuss in this review, but we largely avoid that literature because of its focus on the protein rather than specifically on zinc.

As alluded to above, one can be ZD for three reasons: (1) the diet is simply too low in zinc content, as can be true for certain diets poor in meat or fish protein; or (2) and (3) there can be two situations of ZD in the face of diets replete with zinc. The latter two reasons can be brought about through the chronic use of Proton Pump Inhibitor (PPI) medications, whose inhibition of gastric acid production (and elevation of gastroduodenal lumen pH) can render luminal zinc non-absorbable[17]. In addition, diets high in the form of phosphorous known as phytate (found significantly in whole grains, nuts and seeds), can result in zinc complexing with phytates and a resultant non-absorbable species[18,19]. It is worth considering that the average American, especially those of higher socioeconomic status, may be the victim of one or both outcomes, as PPIs are taken by over 20 million Americans yearly, and phytates exist at high levels in the very foods that are prominently featured in "healthy diet choices". The now common use of PPIs to treat gastroesophageal reflux in neonates makes this group particularly worrisome in this regard. Additionally, the body has no cell/tissue zinc stores (unlike, *e.g.*, iron or calcium) therefore its daily zinc needs are heavily dependent on satisfactory daily zinc intake and at least short-term states of zinc deficiency could be quite likely[20].

The government recommended daily intake of zinc is 11 mg/d[21], however studies have shown that, at least in the relatively short term, higher doses of zinc are safe. For example, our recent study in patients with Barrett's esophagus showed no adverse effects on a total dose of 52 mg/d for 14 d, with positive changes on intracellular signal transduction in the Barrett's epithelia[22]. It is clear that a dose of 150 mg/d over an extended period can be toxic, as is evidenced by the development of severe copper deficiency and anemia[23,24], but zinc lozenges are only effective in reducing the duration of the common cold at a minimum dose of 75 mg/d[25]. At daily doses above 50 mg/d, periodic measurements of blood copper levels (which can be driven lower by high zinc intake) and cho-

Table 1 Gastrointestinal morbidities associated with Zinc deficiency

Condition	Model used	Reversibility with Zn supplementation	Ref.
Esophageal cancer	Rodent	Yes	Fong *et al*[27], 2011
Diarrhea	Human	Yes	Hambidge[72], 1992
Inflammatory bowel diseases	Porcine	Yes	Sturniolo *et al*[82], 2002
Celiac disease	Human	Not determined	Wierdsma *et al*[143], 2013
Alcoholic liver disease	Mouse	Yes	Lambert *et al*[198], 2003
Malnutrition	Guinea pig	Yes	Rodriguez *et al*[65], 1996

lesterol levels would be prudent[26].

The use of zinc as a potential therapeutic in these various diseases and disorders should be treated realistically. No one would suggest, *e.g.*, that zinc-induced potentiation of GI barrier function might "cure" any associated illnesses. However, considering the issue quantitatively rather than qualitatively, there is a quite reasonable possibility that zinc administration could reduce morbidity by a meaningful extent, and that would be highly useful. This is especially true given that zinc could readily be used together with existing medications for these conditions.

The role that zinc may exert in specific GI diseases is now discussed below, along with a final consideration of zinc's role in maintaining GI epithelial barrier function. The compromise of that barrier function can be a catalyst for several of these diseases, and zinc-induced barrier enhancement may prove to be a means of reducing morbidity in some of these conditions (Table 1).

An important consideration in reading this review and interpreting the findings that are presented, is that one needs to always consider the chemical form of the administered zinc, its dosage/concentration, and its vehicle (tablet, capsule, emulsion, lozenge, *etc.*), in the interpretation of any given result/finding. Not all zinc salts are equally soluble and therefore permissive for zinc delivery to cells. In addition, in the discussion presented below on zinc-mediated inhibition of esophageal cancer in rodents, it is critical that the zinc was administered in drinking water[27]. If it was administered in capsule or tablet form, it would result in no topical delivery to the target tissue. This is further discussed in Valenzano *et al*[22] (2014) for delivery to humans. It is not really feasible in a review to discuss the mode of zinc administration in every citation, and so we encourage the reader to refer to specific references for chemistry, drug delivery and concentration information.

ZINC AND GASTROINTESTINAL CANCER

While there is a minimal amount of published literature regarding zinc's effect on transformed cells in culture, zinc - at a concentration that is otherwise not seen to be toxic or a hindrance to normal cell growth - has been shown to negatively impact growth in transformed human tumor cells[28]. Further evidence of tumor growth inhibition by zinc was observed in mice inoculated with sarcoma 180 cells into the peritoneal cavity. Mice treated with zinc sulfate injections experienced a suppression in the number of tumors produced; however, the treatment was unable to prevent the subsequent growth of a tumor once it developed[29]. Similarly, when mice inoculated by intraperitoneal injection with L1210 leukemia cells were treated with zinc acetate injections, they exhibited a reduction in tumor cell growth[30]. The mechanism by which zinc is capable of producing this effect may relate to its ability to prevent oxidative stress that causes DNA damage. Additionally, it is noteworthy that ZD impairs DNA repair by compromising the DNA binding activity of the tumor suppressor protein, p53, disrupting its function[31,32]. In a study on the treatment of the human colon cancer cell line, HCT-116, with zinc chloride, zinc inhibited cell proliferation by stabilizing adenomatous polyposis coli (APC) protein and arresting cell growth[33]. Zinc dysregulation and its subsequent effect on the development of cancer has been suggested to be cell type specific. For example, breast tumor biopsies have higher zinc levels than normal tissue whereas cellular zinc levels in prostate cancer and ovarian cancer tumor tissue are significantly lower than benign tissue[34].

Esophageal and oral cancers are significant upper aerodigestive tract cancers. Esophageal cancer is the eighth most common cancer and the sixth most common cause of death from cancer worldwide, while oral cancers, particularly those of the tongue, have a high mortality rate in part due to the risk of developing a second primary tumor, usually in the esophagus[35,36]. Esophageal cancers can be either squamous cell carcinoma (SCC) or adenocarcinoma (AC). Abnet *et al*[37] (2005) performed a 16-year observational study on participants in a nutrition intervention trial and found that the subjects who had a high zinc concentration in their esophageal biopsy specimens also had a reduced risk of developing esophageal SCC, indicating that dietary ZD associates strongly with SCC. Although studies examining dietary zinc intake and its relationship to cancer risk have reported conflicting results, a meta-analysis of 19 studies involving an estimated 400000 participants found that the level of zinc intake was inversely associated with digestive tract cancers, especially colorectal cancer[38]. In addition to these cancers of the GI tract, low serum zinc levels are also associated with prostate, ovarian, lung, gallbladder, and head and neck cancers[34].

There is strong evidence that ZD in rats and mice enhances carcinogenesis. When control and ZD rats were intragastrically administered 2 mg/kg body weight doses of the carcinogen methylbenzylnitrosamine the ZD rats

had a higher frequency of esophageal tumor development[39]. Similar outcomes ensued when ZD and zinc-sufficient (ZS) Sprague-Dawley rats were exposed to N-nitrosodimethylamine. The control rats did not suffer epithelial irregularities; however, 63% of the rats deficient in zinc developed squamous papillomas in the forestomach[40]. ZD and ZS Sprague-Dawley rats, exposed through their drinking water to precursors of the carcinogen N-nitro-N-benzylmethylamine (benzylmethylamine and $NaNO_2$) manifested like results[41]. Similarly, when C57BL/6 mice were given N-nitrosomethylbenzylamine (NMBA), after 46 wk the ZD mice had significantly more esophageal and forestomach tumors than ZS mice[42]. In an oral cancer study, ZD and ZS rats were treated with the carcinogen 4-nitroquinoline 1-oxide (NQO) to induce lingual tumors. A greater incidence of lingual squamous cell carcinomas was found in the ZD rats[43]. This is not a phenomenon limited to squamous cell cancers, as zinc supplementation (in combination with aspirin or vitamin C) showed dramatic reduction of colon tumors in dimethylhydrazine-treated rodents[44].

The mechanisms by which ZD creates a pro-tumor environment may relate to its ability to induce cell proliferation. In a study on the relationship between cell proliferation and tumor incidence, ZD and ZS rats either received intragastric doses of NMBA or were left untreated. At various time points, *in vivo* bromodeoxyuridine (BrDU) labeling and immunohistochemical detection of cells in S-phase were used to assess esophageal cell proliferation. In both NMBA-treated and untreated rats, the ZD condition showed a significantly higher labeling index than the ZS condition. In NMBA-treated animals, 100% of the ZD ad libitum rats, 23% of the ZS ad libitum fed rats, and 6% of the ZS rats pair-fed to the ZD rats developed tumors. After about 10 wk of the ZD diet, two rats not exposed to NMBA developed esophageal papillomas[45]. In an alternate study, BrDU labeling of ZD and ZS mice given doses of NMBA intragastrically showed that the labeling index and number of labeled cells were also increased in the ZD mice[42].

Dietary ZD also alters gene expression. Liu *et al*[46] (2005) identified 33 genes that were differentially expressed in a hyperplastic ZD *vs* a ZS esophagus. Key factors are the upregulation of the cyclooxygenase (COX-2) inflammatory gene and the induction of an overexpression of the proinflammatory mediators, S100A8 and S100A9. In the hyperplastic esophagus and tongue of ZD rats, the expression levels of both COX-2 protein and mRNA were between 8 and 14.6 fold higher than their ZS counterparts[43]. Treating these rats with an inhibitor of the COX-2 pathway, celecoxib, led to a reduction in cell proliferation but not a prevention of carcinogenesis, suggesting that there must be an additional process involved[43,47]. Celecoxib was found not to be an efficient treatment because it did not show a real effect on S100A8 overexpression. The expression of S100A8 and S100A9 in hyperplastic ZD esophagi was upregulated 57 and 5 fold, respectively[48]. Combining ZD-induced inflammation with low levels of NMBA resulted in a 66.7% incidence of esophageal SCC[49].

ZD in collaboration with other factors, such as p53 deficiency and cyclin D1 overexpression, can produce an accelerated progression towards malignancy[50-52]. p53 is a tumor suppressor protein responsible for the prevention of uncontrolled cell proliferation. Both p53 deficiency ($p53^{-/-}$) and insufficiency ($p53^{+/-}$) in combination with ZD leaves mice more susceptible to carcinogens, increasing the tumor incidence in the esophagus and tongue[50,52]. This rapid rate of tumor progression was accompanied by nearly 20% of ZD and p53-deficient rats developing esophageal Barrett's metaplasia[50]. Cyclin D1 overexpression in conjunction with ZD disrupts the cell cycle leading to uncontrolled cell proliferation and consequently a substantial increase in tumor incidence. At 77 d, 14% of mice with both cyclin D1 overexpression and ZD developed esophageal intestinal metaplasia[52].

Several experiments have investigated the ability of zinc replenishment (ZR) to prevent esophageal cancer. ZR was shown to begin reversing the inflammatory signature and reduce COX-2 overexpression to only 3-fold of that seen in ZS rats[43,49]. ZR also stimulated apoptosis, increased the expression of the proapoptotic Bax protein[53], returned 29 of the 33 altered genes back to normal expression levels[54], decreased cell proliferation[55], restored the expression of S100A8 and S100A9[48], and consequently began to reverse the pre-cancerous phenotype condition. Furthermore, animals with an already ZS diet were found to benefit from zinc supplementation. Additional zinc in the diet of these rats limited cell proliferation and stimulated apoptosis causing a reduced incidence of tumors and tumor progression induced by both low and high doses of NQO[27]. This finding is critical support for the value of zinc supplementation in cancer chemoprevention.

Dietary ZD may be creating a pro-tumor environment in the GI tract, enhancing carcinogenesis by inducing cell proliferation, altering gene expression, and promoting inflammation. Zinc as a dietary nutrient is essential because it plays a role in a variety of important functions, including DNA repair, apoptosis, cell cycle progression, p53 activation, and the prevention of oxidative stress that causes DNA damage. This in conjunction with several research studies suggesting zinc replenishment and supplementation results in a positive effect against carcinogens, supports the thesis that zinc supplementation has the potential to be efficacious in the prevention of several GI cancers.

ZINC AND DIARRHEA

It is well described that ZD can lead to diarrhea. Acrodermatitis enteropathica, a hereditary disorder of zinc metabolism, was reported in infants with skin lesions and diarrhea in the early 1970s[56], and shortly thereafter a similar syndrome was found in certain adult patients placed on total parenteral nutrition (TPN). These patients presented with diarrhea, depression and dermatitis, and

were found to have acute ZD[57]. Zinc supplementation resulted in rapid improvement of diarrhea and dermatitis in both of the above groups[56-61]. Very recent meta analyses of multiple studies supports this conclusion, showing decreased diarrhea duration with zinc therapy[62,63]. A recent review on infectious diarrhea supports this[64]. It is noteworthy that even in control, non disease states, zinc supplementation can positively affect multiple aspects of GI mucosa (on a molecular and cellular level) that would likely act to enhance GI barrier function[46]. ZD can cause intestinal hyperpermeability ("leaky gut"), which itself can be secondary to increased nitric oxide and oxidative stress, thereby leading to diarrhea[65-67]. In rats, ZD has been shown to upregulate expression of intestinal uroguanylin (a peptide that triggers Cl^- secretion and subsequent water secretion)[67,68], decrease absorption of triglycerides by altering chylomicron formation[69], and decrease absorption of proteins by altering enterocyte peptidase activity[70,71], all of which can potentiate diarrhea.

Later it was discovered that not only can ZD cause diarrhea but also chronic diarrhea conditions can cause ZD, thereby promoting even more diarrhea[72]. This has led to multiple studies of zinc supplementation in infants and children with diarrheal illnesses, mostly in developing nations where malnutrition often results in ZD. Children with acute diarrhea treated with zinc show a decrease in the rate and duration of diarrhea as well as decreased need for antibiotics when compared to controls (who were typically treated with oral rehydration and/or vitamin supplements)[73-76]. Zinc supplementation in healthy children in developing countries has decreased the prevalence, morbidity and mortality of diarrhea[77]. The World Health Organization currently recommends treating diarrhea in children with zinc tablets along with oral rehydration solutions as part of a first-line approach[78].

ZD can also decrease human immune function, increasing the risk for infection[74,79]. In infectious diarrhea there is typically increased intestinal permeability (seen as an increase in the lactulose/mannitol ratio)[80] which can sometimes be improved by zinc supplementation[81-83]. ZD leads to decreased electrolyte and water absorption, and can exacerbate diarrhea caused by *Vibrio cholera* (*V. cholera*)[84,85]. *V. cholera* causes diarrhea by increasing cyclic adenosine monophosphate (cAMP) production, inducing the intestine to secrete water and chloride, and inhibiting the absorption of sodium[85]. Interestingly, zinc supplementation actually impedes cAMP-regulated secretion of chloride *via* basal-lateral K^+ channels, explaining its efficacy in reducing the duration of cholera-induced diarrhea, an effect that may involve basal-lateral zinc action on basal-lateral membrane K^+ channels[86,87]. Zinc supplementation decreases expression of certain genes linked to immune function in piglets infected with enterotoxigenic *E. coli* (ETEC) as well decreasing ETEC-induced diarrhea and inflammation. This action is possibly due in part to a decrease in MUC4 expression, which might be an ETEC K88 receptor[88]. In treating ETEC-related diarrhea the mode of delivery in zinc supplementation can matter significantly[89]. In CACO-2 cells, zinc supplementation inhibits Ca^{++} and nitric oxide-mediated ion secretion, both of which are known pathways for pathogen-induced diarrhea. However the same may not be true for Ca^{++}-mediated ion secretion in rat ileum[86,90]. It should be noted however that Bzik *et al*[87] (2012) did observe an inhibitory effect of zinc on carbachol-stimulated short circuit current. Zinc has also been shown to have a direct antimicrobial effect on infectious enteric bacteria such as *E. coli*, *Shigella*, and various strains of *Salmonella in vitro*[91].

In summary, zinc is useful in the treatment of diarrhea of various etiologies. Its role in decreasing fluid secretion to the intestinal lumen (directly or indirectly) requires further investigation but cannot be disputed. Its impact on intestinal paracellular leak, another potential source of diarrhea, will be addressed in a later section of this review.

ZINC AND CROHN'S DISEASE

ZD has been well established in Crohn's disease (CD) and can arise in part due to poor zinc absorption in the small intestine (even if the jejunum appears normal) as well as from chronic dietary intolerances and restrictions[92-96]. In a large cohort of patients with IBD, it was estimated that 8.5% of patients had inadequate intake of zinc. The prevalence of low serum zinc levels was 29.3%[97]. ZD has even been documented in Crohn's patients while in remission[98]. Crohn's patients on TPN can develop acute ZD resulting in acrodermatitis enteropathica and decreased vision[99]. More commonly, ZD in CD contributes to stunted growth in children and manifests as decreased taste sensation, visual acuity, and immune function[100-102].

One very important element of CD pathophysiology is a defect in mucosal barrier function. Increased mucosal permeability correlates with disease activity as evidenced by increased uptake of large molecular markers (such as lactulose) from the GI lumen into the bloodstream[103]. While in remission, increased transmucosal permeability (leak) has been used as a marker for predicting relapse[104]. In the human intestinal cell line, CACO-2/T7, zinc depletion in conjunction with TNFα exposure (common in the inflamed mucosa in CD) increases apoptosis, ultimately compromising the organization of tight junctions (TJs) and epithelial barrier integrity[105]. This could explain why zinc supplementation has been reported to decrease transmucosal leak in CD[106], although this finding has been challenged[107]. CD pathophysiology involves a persistent recruitment of leukocytes into intestinal mucosa and submucosa followed by an unregulated granulomatous inflammatory response. Epithelial barrier dysfunction may facilitate this passage of leukocytes and allow for an unregulated leakage of luminal antigens across the epithelial barrier, a leak that normally does not take place. This is evidenced by a high frequency of neutrophils and crypt abscesses in the intestinal mucosa[108]. ZD may exacerbate CD morbidity by increasing gut permeability,

allowing for increased neutrophil transmigration and luminal antigen permeation[109]. Biologic agents such as Natalizumab have taken advantage of this persistent recruitment mechanism by blocking leukocyte transmigration at the level of the vascular endothelium[110,111].

Zinc supplementation in CD, whether the Crohn's is active or in remission, may be beneficial. It had been postulated that zinc supplementation in patients with IBD would increase serum and mucosal levels of the zinc-dependent free radical scavengers superoxide dismutase (SOD) and MT, however their rise in concentrations was not statistically significant[112]. According to Brignola *et al*[113] (1993), dietary supplementation with zinc in CD patients with active disease significantly improved serum zinc levels in addition to levels of zinc-dependent hormones like thymulin, which can potentiate T-cell differentiation and natural killer (NK) cell activity[113]. The effect of zinc supplementation on NK cell activity of IBD patients, varies in the literature. A small randomized trial by Van de Wal *et al*[114] (1993) showed a significantly reduced level of NK cell activity with zinc supplementation. Despite the current evidence in the literature for their benefits, zinc products have not undergone the scrutiny and validation of the Food and Drug Administration for this disease. For this reason, there are no specific guidelines for zinc supplementation beyond emphasizing the importance of adequate and balanced nutritional intake in CD. As described in the sections on zinc and GI cancers, and zinc and TJs, there may well be medical benefit of zinc daily intake above the current RDA.

ZINC AND ULCERATIVE COLITIS

Zinc is absorbed from the GI lumen principally in the small intestine at the distal duodenum and proximal jejunum[115]. This leads to obvious implications for zinc malabsorption in diseases targeting and damaging the small intestine, such as CD and Celiac sprue. However, ulcerative colitis (UC) does not typically manifest in the small bowel proximal to the ileum, and numerous studies have shown that well nourished UC patients are not ZD. In fact, when patients with moderate and severe (active) UC are compared to healthy controls and/or UC patients in remission, they have shown normal or even elevated serum zinc concentrations[112,116,117]. Similarly, serum zinc levels in children with UC were no different than controls[118].

In active UC flares, elevated serum zinc concentrations are correlated with increased levels of complement component C3C (part of the innate immune system) and elevated antinuclear antibodies, a known predictor of UC flares and a marker of steroid dependence in UC[119,120]. This may be indicative of zinc being released locally in response to the activated inflammatory cascade in active UC. However, an additional study showed no correlation between serum zinc and elevated erythrocyte sedimentation rate or C-Reactive protein, both standard predictors of high grade inflammation in UC[121]. Patients with refractory UC who underwent colectomies with ileal pouch anal anastomosis or a Brooke ileostomy had either elevated or normal serum zinc levels[122-124]. In fact, ZD in UC patients most likely stems from malnutrition deriving from poor oral intake due to acute (systemic) illness associated with active UC and not the damaged colonic tissue *per se*[125,126]. There is little evidence that the colon itself normally has any major role in zinc metabolism or homeostasis.

On a molecular level, the reactive oxygen species (ROS) produced from respiratory burst during neutrophilic invasion in UC cause significant mucosal damage[127]. SOD, vitamin E and ascorbic acid are all known to reduce ROS species produced in this process[128]. Zinc in various ionic forms and as a part of various moieties in proteins is also important in ROS reduction. For example, the copper/zinc isoform of SOD (Cu/Zn-SOD) removes ROS and indirectly prevents lipid peroxidation[129]. Interestingly, Cu/Zn-SOD is decreased in the epithelium of active UC[130]. Another family of zinc-cytoprotective enzymes are MTs. These are zinc-binding proteins that likely play a role in alleviating acute inflammation as ROS scavengers. The literature shows variability in MT expression in IBD. In ZD individuals, mucosal layers of active UC patients had decreased concentrations of MTs in their colonic mucosa and had increased concentrations of ROS intermediates[131-133]. Inducing ZD in animal models of UC increases the disease activity index (DAI) and serum TNFα levels, exacerbates weight loss, and leads to further shortening of colon length[134]. MT concentrations are increased in UC-associated colorectal carcinoma but are decreased in active UC flares at the inflamed mucosa[116,135]. In actively inflamed UC in both human and animal models, supplementing zinc has been shown to either slightly increase or have no effect on the tissue level of MTs[112,136-138]. There have been many studies on the effects of zinc supplementation on inflammation in UC. Di Leo *et al*[136] (2001) reported improvements in diarrhea and weight gain in a rat colitis model when supplemented with zinc but found no effect on neutrophil infiltration or visible inflammation. Mulder *et al*[112] (1994) reported no change in DAI or inflammation in human UC intestinal biopsies after zinc supplementation. More recently, however, Tran *et al*[137] (2007) found that zinc supplementation suppressed colitis in a mouse model as evidenced by decreased DAI and histological severity scores as well as reduced myeloperoxidase activity. Intrarectal zinc also has been found to be beneficial at the microscopic level with reduced inflammation in rat models in as little as 96 h, with other studies also reporting similar decreases in inflammation in both rat and mouse UC models[139-142].

In summary, in UC patients, systemic ZD does not arise in response to colonic tissue inflammation, but if a patient has decreased nutritional intake due to associated illness in ongoing UC, they may develop insufficient levels of serum zinc. This decreased serum zinc concentration does appear to have important implications at the cellular level and therefore may diminish the body's ability

to reduce active inflammation. More research is required to further examine the benefits of zinc supplementation in UC patients, specifically its effect, if any, on epithelial barrier function.

ZINC AND CELIAC DISEASE

Celiac disease (CeD) is an immunologic enteropathy triggered by the intake of gluten (and more specifically gliadin) which causes, among other outcomes, the loss of brush border proteins, enzymes needed for both digestion and absorption. CeD patients are deficient in many vitamins and minerals, including zinc[143]. Decreased plasma zinc has been observed in both untreated CeD patients and as well as those in clinical remission[144]. The prevalence of ZD in newly diagnosed adult CeD patients has been reported to be as high as 67%, and up to 64% of children with CeD are also ZD[143,145-148]. Moreover, ZD is known to correlate with villous atrophy, with one study finding 60% of patients with partial villous atrophy, 80% of patients with subtotal villous atrophy, and 92% of patients with total villous atrophy to be deficient in zinc[149-152].

Adherence to a gluten free diet (GFD) typically induces clinical and histological remission of CeD. While serum zinc levels are significantly lower in children with untreated CeD with enteropathy, levels normalize after following a GFD[147,153,154]. Interestingly, Jones *et al*[155] (2010) found that supplementing zinc along with a GFD provided no additional rise in plasma zinc levels. A small study in patients with non-responsive CeD showed a rise in serum and plasma zinc after oral zinc supplementation as well as a slight increase in brush border enzyme activity, but there was no improvement in the patients' clinical status[155]. Both Crofton *et al*[156] (1990) and Tran *et al*[157] (2011) found no difference in zinc absorption between untreated CeD patients and controls, however there was evidence of impaired zinc homeostasis. Crofton *et al*[156] (1990) reported an "increased turnover and loss of endogenous zinc" that improved on a GFD, and Tran *et al*[157] (2011) found the exchangeable zinc pool (EZP), *i.e.*, the total of the zinc pools in the body that are able to exchange with serum zinc, to be significantly decreased in CeD patients. Since the size of the EZP directly correlates with zinc nutritional status, patients with CeD can be ZD despite normal zinc absorption[157-159].

Stenberg *et al*[160] (2008) suggest that ZD, specifically in the intestinal mucosa (perhaps due to recruitment of zinc to a site of inflammation elsewhere in the body), activates the enzyme transglutaminase-2 (TG2) (normally *inhibited* by the presence of zinc) in the intestine. They theorize that a TG2-thioester intermediate-deamidated gliadin complex acts as a "neo-antigen," activating T-cells in persons genetically susceptible to CeD. These activated T-cells trigger B-lymphocytes to make antibodies against both TG2 and gliadin with the resultant inflammation causing villous atrophy (2008). Possibly contributing to this inflammatory phenomenon is an increased baseline level of paracellular permeability to gliadin *via* the TJs of the gut[161,162]. As the inflammation response intensifies, enterocyte apoptosis occurs and the TJs become increasingly disorganized, further amplifying paracellular permeability[163-165].

It remains to be investigated whether the known ability of zinc to enhance intestinal epithelial TJs[166] (see below) could be a therapeutic modality in CeD by way of reducing gluten and other luminal antigen permeation across the small bowel epithelium of the CeD patient.

GASTRIC CYTOPROTECTION AND INHIBITION OF ACID SECRETION BY ZINC

The observation of zinc affording protection of the gastric mucosal surface was actually reviewed over 20 years ago[167]. Bandyopadhyay *et al*[168] (1997) reported in animal studies that orally-administered zinc protects against chemically-induced ulceration, presumably in part by inhibiting gastric H^+ secretion. This followed published observations of zinc-mediated suppression of gastric acid output as well as zinc-derived improvement of gastric ulcer healing in the 1970s and 1980s[169-171]. Kirchhoff *et al*[172] (2011) more recently reported in humans that low-dose zinc administration abolishes secretagogue-induced gastric acid secretion, raising luminal pH as effectively as PPI medications. It remains to be determined if orally-administered zinc can be as effective as PPIs in treating, *e.g.*, gastroesophageal reflux disease without at least some of the side effects (liver cytochrome P450 inhibition) of the omeprazole class of drugs. These actions of zinc may trace in part to zinc's observed stabilizing effect on secretory cells and lysosomal organelles in general[173].

GASTRIC ACID SUPPRESSION AND ZINC DEFICIENCY

Omeprazole-induced inhibition of gastric acid production and elevation of gastroduodenal luminal pH has been observed to reduce small intestinal zinc absorption in humans[17,174], although not all studies agree[175]. Joshaghani *et al*[176] (2012) reported lower serum stores of zinc in males after 8 wk of omeprazole use and decreased zinc absorption was also observed with inhibition of gastric acid production by H-2 blockers[177]. The ability of omeprazole to increase pH only extends to the lumen of the stomach and upper small intestine[178]. It should be noted that the duodenum is not the only site for zinc absorption, as the cecum and colon have been observed to contribute significantly to zinc absorption by the GI tract if small intestine absorption is impaired, though the extent of absorption is not as great[179].

ZINC AND EPITHELIAL BARRIER FUNCTION

In most of the GI morbidities discussed in this review, the role of transepithelial barrier leak specifically at the site of the epithelial TJ, is very prominent. The recently documented ability of zinc to reduce TJ leak is very likely involved in zinc supplementation's ability to alleviate these morbidities, and the exacerbation of the morbidities in periods of ZD. For this reason, we present here an expanded description of zinc action on the tight junctional complex.

Examining the impact of ZD or supplementation on epithelial barrier function requires one to consider the molecular structure of the barrier. Epithelial and endothelial TJs (zonula occludens) selectively seal the space between adjacent cells, preventing unregulated paracellular exchange across the epithelial or endothelial barrier. The TJs are comprised of various proteins including the tetraspan transmembrane proteins occludin, tricellulin, and the 24+ members of the claudin family, as well as the single span transmembrane protein JAM-A, scaffolding proteins zona occuldens (ZO)-1, ZO-2, ZO-3, and ZAK and various peripheral membrane proteins[180,181]. Additionally, the TJ is regulated by the actin cytoskeleton through its interactions with scaffolding proteins[182]. The TJ's primary role is to regulate the paracellular permeability of the epithelial or endothelial barrier. Once thought to be a completely passive structure, it is now known that the TJ is actually quite dynamic, constantly adapting to stimuli[183]. Claudins are thought to interact in both homophilic and heterophilic patterns. Certain interactions are likely to have a sealing function while others can form pores that can be anion or cation specific[184,185]. Occludin likely plays a regulatory role at the TJ based on its phosphorylation state[186]. Exposure to different environmental stimuli such as pathogenic bacteria, foods or micronutrients can have a drastic positive or negative effect on the TJ's ability to regulate permeation of nutrients, water, and electrolytes[183].

The presence or absence of zinc has been shown to impact the barrier on its own, in conjunction with other molecules, and in various disease states. Zinc is a divalent cation that plays an important role in many mechanisms in the body. It is involved in DNA replication and transcription to RNA (*via* zinc-finger transcription factors), metalloproteinase catalysis, protection from oxidative stress (when bound to MTs), regulation of apoptosis, cell homeostasis, and immune function[187-191]. Both zinc supplementation and zinc deprivation have been found to impact epithelial barrier function with some evidence indicating this effect is at least partially due to TJ modifications[109,192]. In addition to TJ modification however, epithelial barrier leak can be induced more simply and dramatically by induction of cell death and detachment. Zinc has been shown to be quite able to induce apoptosis at certain concentrations in certain cell types[193,194]. However it has also been shown that epithelial barrier function can withstand the onset of cellular apoptosis (on an individual cell basis) by phagocytosis of the apoptotic cell by its epithelial neighbors, a process that ensues throughout with preservation of tight junctional seals[195].

The following disease situations illustrate zinc's ability to modify TJs in medically relevant scenarios.

Alcohol toxicity

Chronic alcohol exposure has drastic consequences for the liver. Alcohol induces leakage of endotoxins from naturally occurring Gram negative bacteria across the epithelial barrier of the gut into the bloodstream[196]. Alcohol-fed mice have impaired barrier function of the ileal epithelia (as measured by plasma endotoxin levels and increased leak of fluorescein-labeled dextrans) and a correlating decrease in levels of certain TJ proteins such as occludin and ZO-1[197]. Zinc supplementation has been shown to improve and even reverse alcohol induced epithelial barrier permeability and liver damage. Pre-treatment with zinc (prior to alcohol consumption) of a mouse model that mimics binge drinking in humans keeps serum levels of endotoxin at levels close to normal, resulting in significantly less elevation of serum ALT, AST and liver TNF-α, thereby inhibiting liver necrosis[198].

Alcohol exposure is also known to increase ROS in the ileum which is itself associated with decreased ileal zinc concentration. CACO-2 (human GI epithelia) cells exposed to alcohol display a time-dependent increase in barrier permeability (as shown by decreased R_t and increased flux of fluorescein-labeled dextrans) accompanied by an increase in ROS. When zinc deprivation is induced in CACO-2 cells by the zinc chelator, TPEN, there is significant downregulation of claudin-1, occludin and ZO-1. The combination of mild zinc deprivation and alcohol exposure to CACO-2 cells results in even greater decreases in expression of claudin-1, occludin, and ZO-1 as well as impairment of barrier function (as measured by increased dextran leak and decreased R_t)[192]. hepatic nuclear factor α (HNFα) is a zinc finger nuclear transcription factor regulating the expression of certain genes in the kidney, intestine, and liver that is highly expressed in the ileum. The alcohol-induced increase in ROS inactivates HNFα which negatively impacts CACO-2 epithelial barrier function by downregulation of claudin-1, occludin and ZO-1 at the transcriptional level[197].

Alcohol exposure also negatively affects alveolar epithelial barrier function in rats by decreasing expression of claudins -1 and -7, both known to be important in type I pneumocyte TJs in this animal model[199]. It also decreases claudin-3 expression as well as increasing expression of claudin-5, both known to be changes associated with leakier alveolar epithelia[199-201]. Supplementing alcohol-consumption with zinc acetate decreases alcohol-induced alveolar epithelial cell permeability to sucrose in a dose dependent manner and significantly increases expression and localization of occludin and ZO-1 at aveolar epithelial TJs.

Depleting zinc in alveolar epithelia in the presence of proinflammatory cytokines causes paracellular leak simultaneously with breakdown of E-cadherin and beta-catenin, induction of apoptosis, and activation of caspase-3 activation[202].

It is known that neutrophils can migrate across the TJ during immune response[203] which is initially beneficial, however accumulation of neutrophils causes prolonged inflammation[204]. In zinc-depleted CACO-2 cells, TJ barrier function was disrupted as evidenced by decreased R_t and disruption of occludin and ZO-1 expression (attributed to change in phosphorylation state). F-actin and beta-tubulin disorganization were also seen. This allowed increased transmigration of neutrophils across the cell layer[109].

Chronic Fatigue Syndrome

There is evidence that a leaky gut is a factor in Chronic Fatigue Syndrome (CFS) since endotoxin levels in CFS patients (as measured by increased IgM and IgA serum levels against LPS) are elevated. A case study showed a complete remission of CFS symptoms after being put on a leaky gut diet (low carbohydrate, gluten and milk free), in conjunction with intravenous immunoglobulins (IVIGs) and NAIOs (natural anti-inflammatory and anti-oxidative substances, namely N-acetyl-cysteine, glutamine and zinc, among others) which resulted in normalization of IgM and IgA levels and normalization of LPS translocation[205]. Presumably this was due to a tightening of the intestinal barrier. In a follow up study, CFS patients followed only the leaky gut diet and took NAIOs without the addition of IVIGs, and had similar results, meaning that these two therapies alone tightened leaky TJs in the gut, reducing translocation of endotoxin into the bloodstream[206].

Cadmium toxicity

Cadmium is an environmental hazard found, for example, in fossil fuels and fertilizers, and it can accumulate in the human body causing nephrotoxicity and other morbidities[207]. When exposed to the renal epithelial cell culture, LLC-PK$_1$, cadmium disrupted TJs, resulting in decreased R_t[208]. Zinc significantly inhibits changes to inulin U/P (urinary to plasma ratio), GFR (glomerular filtration rate), and urinary flow rate in cadmium-exposed rats and, given enough time, it can completely inhibit the negative effect of cadmium on renal function. Zinc treatment also protected against cadmium-induced disorganization of claudins -2 and -5 at the TJs in preparations of rat kidney tubules in a time-dependent manner[207].

Zinc likewise exerts a protective function against copper or iron toxicity. This can occur by both induction of metallothioneins as well as simply competition at the same ligand sites[209]. Zinc has been shown to prevent copper- and iron-related cirrhosis in rats[210] as well as iron accumulation in a simpler cell culture model[211].

Malnutrition

Malnutrition is also known to negatively impact intestinal barrier function. Rodriguez *et al*[65] (1996) found that malnourishment causes increased ionic conductance, mannitol and Na^+ permeability of the jejunal epithelium of guinea pigs, but zinc supplementation kept these permeability levels comparable to controls. Additionally, whereas freeze fracture electron microscopy showed malnourished guinea pigs' jejunal epithelia had 10% less TJ strands, this was not the case when malnourished animals were supplemented with zinc (1996). Treatment with zinc appears to alleviate small intestine paracellular leak due to malnutrition and protects TJs from degradation.

Inflammatory bowel diseases

When zinc is enterally delivered to rats with TNBS-induced colitis, the increase in paracellular permeability to Na^+ and mannitol in the distal colon is partially ameliorated[212]. In a related study, rats treated with $ZnSO_4$ prior to TNBS-induced colitis, weighed more, had less diarrhea, and showed increased MT synthesis, but $ZnSO_4$ did not affect inflammation, neutrophil invasion of the colonic mucosa, distension of the colon, or zinc concentrations[136]. Interestingly, rats pre-treated for 3 d with a high dose of zinc (30 mg/kg) before colitis was induced *via* intrarectal DNBS (dinitrobenzene sulfonic acid) showed significantly fewer TJs that were leaky to lanthanum and gained more weight than controls, but a lower dose of zinc did not show this effect (despite still being 10 times the daily requirement for humans)[82]. The level at which zinc conveys the maximal benefit without detrimental effects must be investigated more thoroughly.

In human studies, CD patients who were in remission but displayed increased permeability of the small intestine had a significantly lower lactulose/mannitol ratio when given $ZnSO_4$ supplements. Ten of the 12 patients reached normal permeability levels.

Chemotherapy and NSAIDs

Further studies of zinc's effect on barrier function in specific GI-related disease states have also been promising. When rats with methotrexate (MTX)-induced mucositis (a state mimicking intestinal mucosal injury in chemotherapy) were administered oral zinc alone or in conjunction with bovine whey-derived growth factor extract (WGFE) they showed decreased permeability of their intestine to ^{51}Cr-EDTA[213]. The combination of zinc + WGFE was the most effective in recovery of the intestine post-MTX injury, providing further evidence that zinc can enhance the beneficial properties of other treatments. In a human study examining the effects of the easily available natural food supplement, zinc carnosine (ZnC), on NSAID-induced intestinal leakiness, ZnC protected against the typical increase in small intestine permeability from indomethacin as well as indomethacin-induced gut injury[214].

Infectious disease

Bacterial infection can distinctly target epithelial barrier function. ETEC increases paracellular flux of inulin and

decreases R_t in CACO-2 cells. While ZnO treatment of *healthy* CACO-2 cells did not affect TJ permeability, ZnO treatment of ETEC-infected cells inhibited the ETEC-induced increase in inulin paracellular leak while maintaining R_t comparable to control cell layers in a time and dose-dependent manner[215]. Given that TNFα is known to cause breakdown in epithelial barrier function[216] whereas TGFβ can enhance TJ barrier function[217], it is also of note that TNFα expression was elevated and TGFβ was drastically reduced in ETEC-infected cells. Increasing ZnO concentrations counteracted these effects[215]. It should be noted that this study chose to use ZnO rather than $ZnSO_4$ because ZnO is more effective in skin epithelial wound healing[218]. Further investigation is warranted into the most effective zinc source, keeping in mind that it could be different depending on the tissue and physiology.

Mycotoxins

Ochratoxin A (OTA) is a mycotoxin that is both a food contaminant and potential human carcinogen. Although it takes over 24 h of exposure, OTA negatively affects barrier function in CACO-2/TC7 cell layers by reducing claudins -3 and -4 in TJs and increasing TJ permeability[219,220]. Zinc-depleted CACO-2/TC7 cells exposed to OTA showed increased TJ permeability (as measured by decreased R_t) and increased apoptosis. Zinc depletion significantly enhanced the deleterious effects of OTA on CACO-2/TC7 cells[219].

Hyperthermia

Hyperthermia increases intestinal mucosal permeability[221] and is known to affect the expression of TJ proteins[222,223]. Heat stress is known to negatively affect porcine growth and production during the warmer part of the year. When varying concentrations of zinc amino acid complex (ZnAA) were fed to growing pigs exposed to heat stress (HS), those exposed to ZnAA showed improved intestinal barrier function on day 7, although the lower level of ZnAA (+100 ppm over control levels) showed greater improvement than the higher level (+220 ppm over controls)[224]. This could be because higher concentrations are known to negatively affect the pancreas and other organs[225].

Diarrhea

Post-weaning diarrhea is a common cause of death in piglets and the resulting increase in gut permeability - likely due at least in part to alterations in the TJs - could be the reason[226,227]. Piglets fed either tetrabasic zinc chloride or ZnO showed decreased lactulose/mannitol ratios in their urine as well as increased expression of the TJ proteins occludin and ZO-1 in the ileum[228]. Piglets fed diosmectite-zinc oxide (DS-ZnO) showed comparable results[229]. These studies suggest that diarrhea is improved by zinc specifically *via* alleviation of intestinal barrier compromise[228].

Cell culture studies

In epithelial cell culture studies, zinc's effects on barrier function are promising. Zinc supplementation decreases expression of claudins -2 and -7 in CACO-2 cell layer TJs and improves barrier function to electrolytes (as shown *via* increased R_t), although paracellular permeability to small nonelectrolytes (mannitol) is actually increased[166]. In the renal epithelial cell culture line LLC-PK1 zinc increases R_t and significantly decreases transepithelial mannitol leak. Although changes in claudin levels in whole cell lysates were not seen with zinc supplementation, increases in cytosolic levels of claudins -2 and -4 were observed[166]. A combination of the micronutrients, zinc and quercetin, had an even stronger positive effect on R_t, but still did not significantly affect mannitol leak. The zinc/quercetin combination also seemed to further increase claudin-7 expression in the cells[230]. Apical exposure of the renal epithelial cell line MDCK II to zinc chloride, conveys high R_t to the normally leaky cell line[231]. MDCK cell layer conductance is predominantly paracellular, so the increase in R_t (*i.e.*, decrease in conductance) due to zinc chloride treatment likely involves a paracellular pathway[232]. Claudin-2 confers cation-selective pores in leaky epithelia such as MDCK[233]. In zinc-treated cells, claudin-2 internalization and degradation was increased, quite possibly being the reason for the tighter barrier. It should be noted that application of zinc chloride to both the basal-lateral and apical sides of MDCKII cells (simultaneously) causes a drop in R_t[231]. The barrier properties of the colon epithelial cell line, T84, however, showed no change when incubated in Ussing chambers with zinc for short time periods (30 min or less)[234].

Summary

Zinc supplementation has been shown to have a protective effect on the epithelial barrier - specifically at the level of the TJ - in cell lines, animal models, and humans in a variety of pathologies including chronic alcohol exposure, heat stress, diarrhea, CFS, colitis, other GI ailments, and even some neurological conditions. Often it can enhance the effects of other beneficial molecules, such as WGFE and quercetin. Whereas in a biological system at homeostasis there is evidence that zinc can improve baseline barrier function, zinc can also protect against both pathophysiologically-generated TJ and general barrier leak. Zinc deprivation, on the other hand, can have catastrophic effects and aggravate disease states *via* induced barrier leak. It should be mentioned, however, that one electron microscopy-based study of severe ZD in rats did not show junctional leak (to the electron dense tracer, lanthanum ion)[235]. The optimal level of zinc and the best form for delivery to distinct target tissues still require further investigation, as do the precise mechanisms involved in its regulation of TJs. However, its positive impact on epithelial barrier function is undeniable.

CONCLUSION

The state of ZD is very favorable to the development of various GI diseases, deriving in large part through its negative effect on GI epithelial barrier function. ZD in the United States seems counterintuitive, but with extensive use of PPI drugs, diets abundant in phytate-rich foods, and decreasing consumption of meat and fish in general, lower zinc body stores are not out of the question. Zinc supplementation could be a highly inexpensive and, within well-described daily dosage limits, quite safe, prophylactic measure against several distinct classes of GI disease. Zinc could also serve to possibly reduce the morbidity of certain established diseases. The utility of zinc as an adjuvant to certain current pharmacologic treatments may have real merit. Ironically if one refrains from expecting unrealistic effects of zinc, *i.e.*, out and out cures of certain GI diseases, its real merit and value comes into focus.

REFERENCES

1 **Duggan C**, Gannon J, Walker WA. Protective nutrients and functional foods for the gastrointestinal tract. *Am J Clin Nutr* 2002; **75**: 789-808 [PMID: 11976152]
2 **Hering NA**, Schulzke JD. Therapeutic options to modulate barrier defects in inflammatory bowel disease. *Dig Dis* 2009; **27**: 450-454 [PMID: 19897959 DOI: 10.1159/000233283]
3 **Semrad CE**. Zinc and intestinal function. *Curr Gastroenterol Rep* 1999; **1**: 398-403 [PMID: 10980978]
4 **Goh J**, O'Morain CA. Review article: nutrition and adult inflammatory bowel disease. *Aliment Pharmacol Ther* 2003; **17**: 307-320 [PMID: 12562443 DOI: 10.1046/j.1365-2036.2003.01482.x]
5 **Scrimgeour AG**, Condlin ML. Zinc and micronutrient combinations to combat gastrointestinal inflammation. *Curr Opin Clin Nutr Metab Care* 2009; **12**: 653-660 [PMID: 19684516]
6 **Tuerk MJ**, Fazel N. Zinc deficiency. *Curr Opin Gastroenterol* 2009; **25**: 136-143 [PMID: 19528881]
7 **Wapnir RA**. Zinc deficiency, malnutrition and the gastrointestinal tract. *J Nutr* 2000; **130**: 1388S-1392S [PMID: 10801949]
8 **Jeejeebhoy K**. Zinc: an essential trace element for parenteral nutrition. *Gastroenterology* 2009; **137**: S7-12 [PMID: 19874952 DOI: 10.1053/j.gastro.2009.08.014]
9 **King JC**. Does zinc absorption reflect zinc status? *Int J Vitam Nutr Res* 2010; **80**: 300-306 [PMID: 21462113 DOI: 10.1024/0300-9831/a000037]
10 **King JC**, Shames DM, Woodhouse LR. Zinc homeostasis in humans. *J Nutr* 2000; **130**: 1360S-1366S [PMID: 10801944]
11 **Cousins RJ**. Gastrointestinal factors influencing zinc absorption and homeostasis. *Int J Vitam Nutr Res* 2010; **80**: 243-248 [PMID: 21462106 DOI: 10.1024/0300-9831/a000030]
12 **Maret W**. Zinc and human disease. *Met Ions Life Sci* 2013; **13**: 389-414 [PMID: 24470098 DOI: 10.1007/978-94-007-7500-8_12]
13 **Bode W**. Structural basis of matrix metalloproteinase function. *Biochem Soc Symp* 2003; **(70)**: 1-14 [PMID: 14587278 DOI: 10.1073/pnas.0505489102]
14 **Jacobsen JA**, Major Jourden JL, Miller MT, Cohen SM. To bind zinc or not to bind zinc: an examination of innovative approaches to improved metalloproteinase inhibition. *Biochim Biophys Acta* 2010; **1803**: 72-94 [PMID: 19712708 DOI: 10.1016/j.bbamcr.2009.08.006]
15 **Urnov FD**. A feel for the template: zinc finger protein transcription factors and chromatin. *Biochem Cell Biol* 2002; **80**: 321-333 [PMID: 12123285]
16 **Pieler T**, Bellefroid E. Perspectives on zinc finger protein function and evolution--an update. *Mol Biol Rep* 1994; **20**: 1-8 [PMID: 7531280]
17 Farrell CP, Morgan M, Rudolph DS, Hwang A, Albert NE, Valenzano MC, Wang X, Mercogliano G, Mullin JM. Proton pump inhibitors interfere with zinc absorption and zinc body stores. *Gastroent Res* 2011; **4**: 243-251 [DOI: 10.4021/gr379w]
18 **Prasad AS**. Discovery of human zinc deficiency: its impact on human health and disease. *Adv Nutr* 2013; **4**: 176-190 [PMID: 23493534]
19 **Hambidge KM**, Miller LV, Westcott JE, Sheng X, Krebs NF. Zinc bioavailability and homeostasis. *Am J Clin Nutr* 2010; **91**: 1478S-1483S [PMID: 20200254 DOI: 10.3945/ajcn.2010.28674I]
20 **King JC**, Shames DM, Lowe NM, Woodhouse LR, Sutherland B, Abrams SA, Turnlund JR, Jackson MJ. Effect of acute zinc depletion on zinc homeostasis and plasma zinc kinetics in men. *Am J Clin Nutr* 2001; **74**: 116-124 [PMID: 11451726]
21 **Institute of Medicine**. Dietary reference intakes: vitamin a, vitamin k, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Food and Nutrition Board. Washington, D.C.: National Academy Press, 2002
22 **Valenzano MC**, Mercado JM, Wang X, Zurbach EP, Raines J, McDonnell E, Morgan M, Farrell C, Rudolph D, Hwang A, Barr M, Cherian D, Bailey R, Raile B, Albert N, Thornton J, Zitin M, Abramson J, Newman G, Daum G, Mercogliano G, Mullin JM. Drug delivery of zinc to Barrett's metaplasia by oral administration to Barrett's esophagus patients. *Ther Deliv* 2014; **5**: 257-264 [PMID: 24592952]
23 **Porter KG**, McMaster D, Elmes ME, Love AH. Anaemia and low serum-copper during zinc therapy. *Lancet* 1977; **2**: 774 [PMID: 71592]
24 **Prasad AS**, Brewer GJ, Schoomaker EB, Rabbani P. Hypocupremia induced by zinc therapy in adults. *JAMA* 1978; **240**: 2166-2168 [PMID: 359844]
25 **Hemilä H**. Zinc lozenges may shorten the duration of colds: a systematic review. *Open Respir Med J* 2011; **5**: 51-58 [PMID: 21769305 DOI: 10.2174/1874306401105010051]
26 **Fosmire GJ**. Zinc toxicity. *Am J Clin Nutr* 1990; **51**: 225-227 [PMID: 2407097]
27 **Fong LY**, Jiang Y, Rawahneh ML, Smalley KJ, Croce CM, Farber JL, Huebner K. Zinc supplementation suppresses 4-nitroquinoline 1-oxide-induced rat oral carcinogenesis. *Carcinogenesis* 2011; **32**: 554-560 [PMID: 21245412 DOI: 10.1093/carcin/bgr004]
28 **Epstein J**. SV40-transformed human cells fail to grow in zinc concentrations which permit normal human fibroblast proliferation. *J Cell Physiol* 1982; **110**: 17-22 [PMID: 6279677 DOI: 10.1002/jcp.1041100104]
29 **Woster AD**, Failla ML, Taylor MW, Weinberg ED. Zinc suppression of initiation of sarcoma 180 growth. *J Natl Cancer Inst* 1975; **54**: 1001-1003 [PMID: 1127712]
30 **Phillips JL**, Sheridan PJ. Effect of zinc administration on the growth of L1210 and BW5147 tumors in mice. *J Natl Cancer Inst* 1976; **57**: 361-363 [PMID: 1003517]
31 **Ho E**, Ames BN. Low intracellular zinc induces oxidative DNA damage, disrupts p53, NFkappa B, and AP1 DNA binding, and affects DNA repair in a rat glioma cell line. *Proc Natl Acad Sci USA* 2002; **99**: 16770-16775 [PMID: 12481036 DOI: 10.1073/pnas.222679399]
32 **Song Y**, Chung CS, Bruno RS, Traber MG, Brown KH, King JC, Ho E. Dietary zinc restriction and repletion affects DNA integrity in healthy men. *Am J Clin Nutr* 2009; **90**: 321-328 [PMID: 19515738 DOI: 10.3945/ajcn.2008.27300]
33 **Jaiswal AS**, Narayan S. Zinc stabilizes adenomatous polyposis coli (APC) protein levels and induces cell cycle arrest in colon cancer cells. *J Cell Biochem* 2004; **93**: 345-357 [PMID: 15368361 DOI: 10.1002/jcb.20156]

34 **Alam S**, Kelleher SL. Cellular mechanisms of zinc dysregulation: a perspective on zinc homeostasis as an etiological factor in the development and progression of breast cancer. *Nutrients* 2012; **4**: 875-903 [PMID: 23016122 DOI: 10.3390/nu4080875]

35 **Herszényi L**, Tulassay Z. Epidemiology of gastrointestinal and liver tumors. *Eur Rev Med Pharmacol Sci* 2010; **14**: 249-258 [PMID: 20496531]

36 **Makuuchi H**, Machimura T, Shimada H, Mizutani K, Chino O, Kise Y, Nishi T, Tanaka H, Mitomi T, Horiuchi M, Sakai M, Gotoh J, Sasaki J, Osamura Y. Endoscopic screening for esophageal cancer in 788 patients with head and neck cancers. *Tokai J Exp Clin Med* 1996; **21**: 139-145 [PMID: 9239817]

37 **Abnet CC**, Lai B, Qiao YL, Vogt S, Luo XM, Taylor PR, Dong ZW, Mark SD, Dawsey SM. Zinc concentration in esophageal biopsy specimens measured by x-ray fluorescence and esophageal cancer risk. *J Natl Cancer Inst* 2005; **97**: 301-306 [PMID: 15713965 DOI: 10.1093/jnci/dji042]

38 **Li P**, Xu J, Shi Y, Ye Y, Chen K, Yang J, Wu Y. Association between zinc intake and risk of digestive tract cancers: a systematic review and meta-analysis. *Clin Nutr* 2014; **33**: 415-420 [PMID: 24148607 DOI: 10.1016/j.clnu.2013.10.001]

39 **Fong LY**, Sivak A, Newberne PM. Zinc deficiency and methylbenzylnitrosamine-induced esophageal cancer in rats. *J Natl Cancer Inst* 1978; **61**: 145-150 [PMID: 276623]

40 **Fong LY**, Ng WL, Newberne PM. N-nitrosodimethylamine-induced forestomach tumours in male Sprague-Dawley rats fed a zinc-deficient diet. *IARC Sci Publ* 1984; **(57)**: 543-546 [PMID: 6533046]

41 **Fong LY**, Lee JS, Chan WC, Newberne PM. Zinc deficiency and the development of esophageal and forestomach tumors in Sprague-Dawley rats fed precursors of N-nitroso-N-benzylmethylamine. *J Natl Cancer Inst* 1984; **72**: 419-425 [PMID: 6582327]

42 **Fong LY**, Magee PN. Dietary zinc deficiency enhances esophageal cell proliferation and N-nitrosomethylbenzylamine (NMBA)-induced esophageal tumor incidence in C57BL/6 mouse. *Cancer Lett* 1999; **143**: 63-69 [PMID: 10465339 DOI: 10.1016/S0304-3835(99)00191-3]

43 **Fong LY**, Zhang L, Jiang Y, Farber JL. Dietary zinc modulation of COX-2 expression and lingual and esophageal carcinogenesis in rats. *J Natl Cancer Inst* 2005; **97**: 40-50 [PMID: 15632379 DOI: 10.1093/jnci/dji006]

44 **Christudoss P**, Selvakumar R, Pulimood AB, Fleming JJ, Mathew G. Protective role of aspirin, vitamin C, and zinc and their effects on zinc status in the DMH-induced colon carcinoma model. *Asian Pac J Cancer Prev* 2013; **14**: 4627-4634 [PMID: 24083715 DOI: 10.7314/APJCP.2013.14.8.4627]

45 **Fong LY**, Li JX, Farber JL, Magee PN. Cell proliferation and esophageal carcinogenesis in the zinc-deficient rat. *Carcinogenesis* 1996; **17**: 1841-1848 [PMID: 8824504 DOI: 10.1093/carcin/17.9.1841]

46 **Liu P**, Pieper R, Rieger J, Vahjen W, Davin R, Plendl J, Meyer W, Zentek J. Effect of dietary zinc oxide on morphological characteristics, mucin composition and gene expression in the colon of weaned piglets. *PLoS One* 2014; **9**: e91091 [PMID: 24609095]

47 **Fong LY**, Jiang Y, Riley M, Liu X, Smalley KJ, Guttridge DC, Farber JL. Prevention of upper aerodigestive tract cancer in zinc-deficient rodents: inefficacy of genetic or pharmacological disruption of COX-2. *Int J Cancer* 2008; **122**: 978-989 [PMID: 17985342 DOI: 10.1002/ijc.23221]

48 **Taccioli C**, Wan SG, Liu CG, Alder H, Volinia S, Farber JL, Croce CM, Fong LY. Zinc replenishment reverses overexpression of the proinflammatory mediator S100A8 and esophageal preneoplasia in the rat. *Gastroenterology* 2009; **136**: 953-966 [PMID: 19111725 DOI: 10.1053/j.gastro.2008.11.039]

49 **Taccioli C**, Chen H, Jiang Y, Liu XP, Huang K, Smalley KJ, Farber JL, Croce CM, Fong LY. Dietary zinc deficiency fuels esophageal cancer development by inducing a distinct inflammatory signature. *Oncogene* 2012; **31**: 4550-4558 [PMID: 22179833]

50 **Fong LY**, Ishii H, Nguyen VT, Vecchione A, Farber JL, Croce CM, Huebner K. p53 deficiency accelerates induction and progression of esophageal and forestomach tumors in zinc-deficient mice. *Cancer Res* 2003; **63**: 186-195 [PMID: 12517797]

51 **Fong LY**, Jiang Y, Farber JL. Zinc deficiency potentiates induction and progression of lingual and esophageal tumors in p53-deficient mice. *Carcinogenesis* 2006; **27**: 1489-1496 [PMID: 16543248 DOI: 10.1093/carcin/bgl012]

52 **Fong LY**, Mancini R, Nakagawa H, Rustgi AK, Huebner K. Combined cyclin D1 overexpression and zinc deficiency disrupts cell cycle and accelerates mouse forestomach carcinogenesis. *Cancer Res* 2003; **63**: 4244-4252 [PMID: 12874033]

53 **Fong LY**, Nguyen VT, Farber JL. Esophageal cancer prevention in zinc-deficient rats: rapid induction of apoptosis by replenishing zinc. *J Natl Cancer Inst* 2001; **93**: 1525-1533 [PMID: 11604475 DOI: 10.1093/jnci/93.20.1525]

54 **Liu CG**, Zhang L, Jiang Y, Chatterjee D, Croce CM, Huebner K, Fong LY. Modulation of gene expression in precancerous rat esophagus by dietary zinc deficit and replenishment. *Cancer Res* 2005; **65**: 7790-7799 [PMID: 16140947 DOI: 10.1158/0008-5472.CAN-05-1345]

55 **Fong LY**, Farber JL, Magee PN. Zinc replenishment reduces esophageal cell proliferation and N-nitrosomethylbenzylamine (NMBA)-induced esophageal tumor incidence in zinc-deficient rats. *Carcinogenesis* 1998; **19**: 1591-1596 [PMID: 9771929 DOI: 10.1093/carcin/19.9.1591]

56 **Barnes PM**, Moynahan EJ. Zinc deficiency in acrodermatitis enteropathica: multiple dietary intolerance treated with synthetic diet. *Proc R Soc Med* 1973; **66**: 327-329 [PMID: 4577330]

57 **Kay RG**, Tasman-Jones C, Pybus J, Whiting R, Black H. A syndrome of acute zinc deficiency during total parenteral alimentation in man. *Ann Surg* 1976; **183**: 331-340 [PMID: 817677]

58 **Kay RG**, Tasman-Jones C. Acute zinc deficency in man during intravenous alimentation. *Aust N Z J Surg* 1975; **45**: 325-330 [PMID: 813623]

59 **Arakawa T**, Tamura T, Igarashi Y, Suzuki H, Sandstead HH. Zinc deficiency in two infants during total parenteral alimentation for diarrhea. *Am J Clin Nutr* 1976; **29**: 197-204 [PMID: 814805]

60 **Der Kaloustian VM**, Musallam SS, Sanjad SA, Murib A, Hammad WD, Idriss ZH. Oral treatment of acrodermatitis enteropathica with zinc sulfate. *Am J Dis Child* 1976; **130**: 421-423 [PMID: 946739]

61 **Leupold D**, Poley JR, Meigel WN. Zinc therapy in acrodermatitis enteropathica. *Helv Paediatr Acta* 1976; **31**: 109-115 [PMID: 955933]

62 **Zou TT**, Mou J, Zhan X. Zinc Supplementation in Acute Diarrhea. *Indian J Pediatr* 2014; Epub ahead of print [PMID: 24954892]

63 **Lamberti LM**, Walker CL, Chan KY, Jian WY, Black RE. Oral zinc supplementation for the treatment of acute diarrhea in children: a systematic review and meta-analysis. *Nutrients* 2013; **5**: 4715-4740 [PMID: 24284615 DOI: 10.3390/nu5114715]

64 **Dickinson B**, Surawicz CM. Infectious diarrhea: an overview. *Curr Gastroenterol Rep* 2014; **16**: 399 [PMID: 25064318 DOI: 10.1007/s11894-014-0399-8]

65 **Rodriguez P**, Darmon N, Chappuis P, Candalh C, Blaton MA, Bouchaud C, Heyman M. Intestinal paracellular permeability during malnutrition in guinea pigs: effect of high dietary zinc. *Gut* 1996; **39**: 416-422 [PMID: 8949647]

66 **Cui L**, Takagi Y, Wasa M, Sando K, Khan J, Okada A. Nitric oxide synthase inhibitor attenuates intestinal damage induced by zinc deficiency in rats. *J Nutr* 1999; **129**: 792-798 [PMID: 10203552]

67 **Blanchard RK**, Cousins RJ. Upregulation of rat intestinal

uroguanylin mRNA by dietary zinc restriction. *Am J Physiol* 1997; **272**: G972-G978 [PMID: 9176203]
68 **Blanchard RK**, Cousins RJ. Regulation of intestinal gene expression by dietary zinc: induction of uroguanylin mRNA by zinc deficiency. *J Nutr* 2000; **130**: 1393S-1398S [PMID: 10801950]
69 **Koo SI**, Turk DE. Effect of zinc deficiency on intestinal transport triglyceride in the rat. *J Nutr* 1977; **107**: 909-919 [PMID: 192862]
70 **Moran JR**, Lyerly A. The effects of severe zinc deficiency on intestinal amino acid losses in the rat. *Life Sci* 1985; **36**: 2515-2521 [PMID: 4010465]
71 **Ying AJ**, Shu XL, Gu WZ, Huang XM, Shuai XH, Yang LR, Jiang MZ. [Effect of zinc deficiency on intestinal mucosal morphology and digestive enzyme activity in growing rat]. *Zhonghua Erke Zazhi* 2011; **49**: 249-254 [PMID: 21624198]
72 **Hambidge KM**. Zinc and diarrhea. *Acta Paediatr Suppl* 1992; **381**: 82-86 [PMID: 1421947]
73 **Dutta P**, Mitra U, Dutta S, Naik TN, Rajendran K, Chatterjee MK. Zinc, vitamin A, and micronutrient supplementation in children with diarrhea: a randomized controlled clinical trial of combination therapy versus monotherapy. *J Pediatr* 2011; **159**: 633-637 [PMID: 21592508 DOI: 10.1016/j.jpeds.2011.03.028]
74 **Sazawal S**, Jalla S, Mazumder S, Sinha A, Black RE, Bhan MK. Effect of zinc supplementation on cell-mediated immunity and lymphocyte subsets in preschool children. *Indian Pediatr* 1997; **34**: 589-597 [PMID: 9401251]
75 **Faruque AS**, Mahalanabis D, Haque SS, Fuchs GJ, Habte D. Double-blind, randomized, controlled trial of zinc or vitamin A supplementation in young children with acute diarrhoea. *Acta Paediatr* 1999; **88**: 154-160 [PMID: 10102147]
76 **Awasthi S**. Zinc supplementation in acute diarrhea is acceptable, does not interfere with oral rehydration, and reduces the use of other medications: a randomized trial in five countries. *J Pediatr Gastroenterol Nutr* 2006; **42**: 300-305 [PMID: 16540790 DOI: 10.1097/01.mpg.0000189340.00516.30]
77 **Fischer Walker CL**, Black RE. Micronutrients and diarrheal disease. *Clin Infect Dis* 2007; **45** Suppl 1: S73-S77 [PMID: 17582575]
78 **Unger CC**, Salam SS, Sarker MS, Black R, Cravioto A, El Arifeen S. Treating diarrhoeal disease in children under five: the global picture. *Arch Dis Child* 2014; **99**: 273-278 [PMID: 24197873]
79 **Oleske JM**, Westphal ML, Shore S, Gorden D, Bogden JD, Nahmias A. Zinc therapy of depressed cellular immunity in acrodermatitis enteropathica. Its correction. *Am J Dis Child* 1979; **133**: 915-918 [PMID: 112858 DOI: 10.1001/archpedi.1979.02130090043007]
80 **Zhang Y**, Lee B, Thompson M, Glass R, Cama RI, Figueroa D, Gilman R, Taylor D, Stephenson C. Lactulose-mannitol intestinal permeability test in children with diarrhea caused by rotavirus and cryptosporidium. Diarrhea Working Group, Peru. *J Pediatr Gastroenterol Nutr* 2000; **31**: 16-21 [PMID: 10896065]
81 **Alam AN**, Sarker SA, Wahed MA, Khatun M, Rahaman MM. Enteric protein loss and intestinal permeability changes in children during acute shigellosis and after recovery: effect of zinc supplementation. *Gut* 1994; **35**: 1707-1711 [PMID: 7829006 DOI: 10.1136/gut.35.12.1707]
82 **Sturniolo GC**, Fries W, Mazzon E, Di Leo V, Barollo M, D'inca R. Effect of zinc supplementation on intestinal permeability in experimental colitis. *J Lab Clin Med* 2002; **139**: 311-315 [PMID: 12032492]
83 **Roy SK**, Behrens RH, Haider R, Akramuzzaman SM, Mahalanabis D, Wahed MA, Tomkins AM. Impact of zinc supplementation on intestinal permeability in Bangladeshi children with acute diarrhoea and persistent diarrhoea syndrome. *J Pediatr Gastroenterol Nutr* 1992; **15**: 289-296 [PMID: 1432467]
84 **Roy SK**, Tomkins AM, Ara G, Jolly SP, Khatun W, Chowdhury R, Chakrabarty B. Impact of zinc deficiency on vibrio cholerae enterotoxin-stimulated water and electrolyte transport in animal model. *J Health Popul Nutr* 2006; **24**: 42-47 [PMID: 16796149]
85 **Qadir MI**, Arshad A, Ahmad B. Zinc: Role in the management of diarrhea and cholera. *World J Clin Cases* 2013; **1**: 140-142 [PMID: 24303485]
86 **Hoque KM**, Rajendran VM, Binder HJ. Zinc inhibits cAMP-stimulated Cl secretion via basolateral K-channel blockade in rat ileum. *Am J Physiol Gastrointest Liver Physiol* 2005; **288**: G956-G963 [PMID: 15618279 DOI: 10.1152/ajpgi.00441.2004]
87 **Bzik VA**, Medani M, Baird AW, Winter DC, Brayden DJ. Mechanisms of action of zinc on rat intestinal epithelial electrogenic ion secretion: insights into its antidiarrhoeal actions. *J Pharm Pharmacol* 2012; **64**: 644-653 [PMID: 22471360 DOI: 10.1111/j.2042-7158.2011.01441.x]
88 **Sargeant HR**, McDowall KJ, Miller HM, Shaw MA. Dietary zinc oxide affects the expression of genes associated with inflammation: Transcriptome analysis in piglets challenged with ETEC K88. *Vet Immunol Immunopathol* 2010; **137**: 120-129 [PMID: 20605641]
89 **Kwon CH**, Lee CY, Han SJ, Kim SJ, Park BC, Jang I, Han JH. Effects of dietary supplementation of lipid-encapsulated zinc oxide on colibacillosis, growth and intestinal morphology in weaned piglets challenged with enterotoxigenic Escherichia coli. *Anim Sci J* 2014; **85**: 805-813 [PMID: 24799095 DOI: 10.1111/asj.12215]
90 **Berni Canani R**, Secondo A, Passariello A, Buccigrossi V, Canzoniero LM, Ruotolo S, Puzone C, Porcaro F, Pensa M, Braucci A, Pedata M, Annunziato L, Guarino A. Zinc inhibits calcium-mediated and nitric oxide-mediated ion secretion in human enterocytes. *Eur J Pharmacol* 2010; **626**: 266-270 [PMID: 19819236 DOI: 10.1016/j.ejphar.2009.09.042]
91 **Surjawidjaja JE**, Hidayat A, Lesmana M. Growth inhibition of enteric pathogens by zinc sulfate: an in vitro study. *Med Princ Pract* 2010; **13**: 286-289 [PMID: 15316263]
92 **Sturniolo GC**, Molokhia MM, Shields R, Turnberg LA. Zinc absorption in Crohn's disease. *Gut* 1980; **21**: 387-391 [PMID: 7429301 DOI: 10.1136/gut.21.5.387]
93 **Nishida K**, Yao T, Tsuzuki O. [A study on a low serum zinc level in Crohn's disease]. *Nihon Shokakibyo Gakkai Zasshi* 1985; **82**: 424-433 [PMID: 4010010]
94 **Matsui T**. Zinc deficiency in Crohn's disease. *J Gastroenterol* 1998; **33**: 924-925 [PMID: 9853577]
95 **Hendricks KM**, Walker WA. Zinc deficiency in inflammatory bowel disease. *Nutr Rev* 1988; **46**: 401-408 [PMID: 3070446]
96 **McClain C**, Soutor C, Zieve L. Zinc deficiency: a complication of Crohn's disease. *Gastroenterology* 1980; **78**: 272-279 [PMID: 7350050]
97 **Vagianos K**, Bector S, McConnell J, Bernstein CN. Nutrition assessment of patients with inflammatory bowel disease. *JPEN J Parenter Enteral Nutr* 2007; **31**: 311-319 [PMID: 17595441 DOI: 10.1177/0148607107031004311]
98 **Filippi J**, Al-Jaouni R, Wiroth JB, Hébuterne X, Schneider SM. Nutritional deficiencies in patients with Crohn's disease in remission. *Inflamm Bowel Dis* 2006; **12**: 185-191 [PMID: 16534419 DOI: 10.1097/01.MIB.0000206541.15963.c3]
99 **Myung SJ**, Yang SK, Jung HY, Jung SA, Kang GH, Ha HK, Hong WS, Min YI. Zinc deficiency manifested by dermatitis and visual dysfunction in a patient with Crohn's disease. *J Gastroenterol* 1998; **33**: 876-879 [PMID: 9853564 DOI: 10.1007/s005350050192]
100 **Fauci AS**. Harrison's principles of internal medicine. New York, USA: McGraw-Hill Medical, 2008
101 **Ainley C**, Cason J, Slavin BM, Wolstencroft RA, Thompson RP. The influence of zinc status and malnutrition on immunological function in Crohn's disease. *Gastroenterology* 1991; **100**: 1616-1625 [PMID: 1902189]

102 **Haase H**, Rink L. Multiple impacts of zinc on immune function. *Metallomics* 2014; **6**: 1175-1180 [PMID: 24531756 DOI: 10.1039/c3mt00353a]

103 **Ukabam SO**, Clamp JR, Cooper BT. Abnormal small intestinal permeability to sugars in patients with Crohn's disease of the terminal ileum and colon. *Digestion* 1983; **27**: 70-74 [PMID: 6414866 DOI: 10.1159/000198932]

104 **Wyatt J**, Vogelsang H, Hübl W, Waldhöer T, Lochs H. Intestinal permeability and the prediction of relapse in Crohn's disease. *Lancet* 1993; **341**: 1437-1439 [PMID: 8099141]

105 **Ranaldi G**, Ferruzza S, Canali R, Leoni G, Zalewski PD, Sambuy Y, Perozzi G, Murgia C. Intracellular zinc is required for intestinal cell survival signals triggered by the inflammatory cytokine TNFα. *J Nutr Biochem* 2013; **24**: 967-976 [PMID: 22967671]

106 **Sturniolo GC**, Di Leo V, Ferronato A, D'Odorico A, D'Incà R. Zinc supplementation tightens "leaky gut" in Crohn's disease. *Inflamm Bowel Dis* 2001; **7**: 94-98 [PMID: 11383597 DOI: 10.1097/00054725-200105000-00003]

107 **El-Tawil AM**. Zinc supplementation tightens leaky gut in Crohn's disease. *Inflamm Bowel Dis* 2012; **18**: E399 [PMID: 21994075 DOI: 10.1002/ibd.21926]

108 **Pedersen J**, Coskun M, Soendergaard C, Salem M, Nielsen OH. Inflammatory pathways of importance for management of inflammatory bowel disease. *World J Gastroenterol* 2014; **20**: 64-77 [PMID: 24415859 DOI: 10.3748/wjg.v20.i1.64]

109 **Finamore A**, Massimi M, Conti Devirgiliis L, Mengheri E. Zinc deficiency induces membrane barrier damage and increases neutrophil transmigration in Caco-2 cells. *J Nutr* 2008; **138**: 1664-1670 [PMID: 18716167]

110 **Sandborn WJ**, Colombel JF, Enns R, Feagan BG, Hanauer SB, Lawrance IC, Panaccione R, Sanders M, Schreiber S, Targan S, van Deventer S, Goldblum R, Despain D, Hogge GS, Rutgeerts P. Natalizumab induction and maintenance therapy for Crohn's disease. *N Engl J Med* 2005; **353**: 1912-1925 [PMID: 16267322]

111 **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]

112 **Mulder TP**, van der Sluys Veer A, Verspaget HW, Griffioen G, Peña AS, Janssens AR, Lamers CB. Effect of oral zinc supplementation on metallothionein and superoxide dismutase concentrations in patients with inflammatory bowel disease. *J Gastroenterol Hepatol* 1994; **9**: 472-477 [PMID: 7827298]

113 **Brignola C**, Belloli C, De Simone G, Evangelisti A, Parente R, Mancini R, Iannone P, Mocheggiani E, Fabris N, Morini MC. Zinc supplementation restores plasma concentrations of zinc and thymulin in patients with Crohn's disease. *Aliment Pharmacol Ther* 1993; **7**: 275-280 [PMID: 8364132]

114 **Van de Wal Y**, Van der Sluys Veer A, Verspaget HW, Mulder TP, Griffioen G, Van Tol EA, Peña AS, Lamers CB. Effect of zinc therapy on natural killer cell activity in inflammatory bowel disease. *Aliment Pharmacol Ther* 1993; **7**: 281-286 [PMID: 8364133]

115 **Lee HH**, Prasad AS, Brewer GJ, Owyang C. Zinc absorption in human small intestine. *Am J Physiol* 1989; **256**: G87-G91 [PMID: 2912154]

116 **Sturniolo GC**, Mestriner C, Lecis PE, D'Odorico A, Venturi C, Irato P, Cecchetto A, Tropea A, Longo G, D'Inca R. Altered plasma and mucosal concentrations of trace elements and antioxidants in active ulcerative colitis. *Scand J Gastroenterol* 1998; **33**: 644-649 [PMID: 9669638]

117 **Ringstad J**, Kildebo S, Thomassen Y. Serum selenium, copper, and zinc concentrations in Crohn's disease and ulcerative colitis. *Scand J Gastroenterol* 1993; **28**: 605-608 [PMID: 8362213]

118 **Ojuawo A**, Keith L. The serum concentrations of zinc, copper and selenium in children with inflammatory bowel disease. *Cent Afr J Med* 2002; **48**: 116-119 [PMID: 14562534]

119 **Dalekos GN**, Ringstad J, Savaidis I, Seferiadis KI, Tsianos EV. Zinc, copper and immunological markers in the circulation of well nourished patients with ulcerative colitis. *Eur J Gastroenterol Hepatol* 1998; **10**: 331-337 [PMID: 9855050]

120 **Barahona-Garrido J**, Camacho-Escobedo J, García-Martínez CI, Tocay H, Cabiedes J, Yamamoto-Furusho JK. Antinuclear antibodies: a marker associated with steroid dependence in patients with ulcerative colitis. *Inflamm Bowel Dis* 2009; **15**: 1039-1043 [PMID: 19107779 DOI: 10.1002/ibd.20852]

121 **Hocke M**, Winnefeld K, Bosseckert H. Zinc concentration in serum and leucocytes in chronic inflammatory diseases. *J Trace Elem Med Biol* 1995; **9**: 112-116 [PMID: 8825984 DOI: 10.1016/S0946-672X(11)80019-2]

122 **Whineray E**, Inder WJ, Roche D, Dobbs BR, Frizelle FA. Comparison of micronutrients in patients having had panproctocolectomy and either ileal pouch anal anastomosis or Brooke ileostomy for chronic ulcerative colitis (UC). *Colorectal Dis* 2000; **2**: 351-354 [PMID: 23578154]

123 **Ma X**, Zhao K, Wei L, Song P, Liu G, Han H, Wang C. Altered plasma concentrations of trace elements in ulcerative colitis patients before and after surgery. *Biol Trace Elem Res* 2013; **153**: 100-104 [PMID: 23666686 DOI: 10.1007/s12011-013-9662-3]

124 **El Muhtaseb MS**, Duncan A, Talwar DK, O'Reilly DS, McKee RF, Anderson JH, Finlay IG. Assessment of dietary intake and trace element status in patients with ileal pouch-anal anastomosis. *Dis Colon Rectum* 2007; **50**: 1553-1557 [PMID: 17701072 DOI: 10.1007/s10350-007-9003-8]

125 **Geerling BJ**, Badart-Smook A, Stockbrügger RW, Brummer RJ. Comprehensive nutritional status in recently diagnosed patients with inflammatory bowel disease compared with population controls. *Eur J Clin Nutr* 2000; **54**: 514-521 [PMID: 10878655]

126 **Ainley CC**, Cason J, Carlsson LK, Slavin BM, Thompson RP. Zinc status in inflammatory bowel disease. *Clin Sci* (Lond) 1988; **75**: 277-283 [PMID: 3416562]

127 **Weiss SJ**. Tissue destruction by neutrophils. *N Engl J Med* 1989; **320**: 365-376 [PMID: 2536474 DOI: 10.1056/NEJM198902093200606]

128 **Machlin LJ**, Bendich A. Free radical tissue damage: protective role of antioxidant nutrients. *FASEB J* 1987; **1**: 441-445 [PMID: 3315807]

129 **Xia B**, Deng CS, Chen DJ, Zhou Y, Xiao JQ. Role of copper zinc superoxide dismutase in the short-term treatment of acetic acid-induced colitis in rats. *Acta Gastroenterol Latinoam* 1996; **26**: 227-230 [PMID: 9335927]

130 **Kruidenier L**, Kuiper I, van Duijn W, Marklund SL, van Hogezand RA, Lamers CB, Verspaget HW. Differential mucosal expression of three superoxide dismutase isoforms in inflammatory bowel disease. *J Pathol* 2003; **201**: 7-16 [PMID: 12950012 DOI: 10.1002/path.1407]

131 **Mulder TP**, Verspaget HW, Janssens AR, de Bruin PA, Peña AS, Lamers CB. Decrease in two intestinal copper/zinc containing proteins with antioxidant function in inflammatory bowel disease. *Gut* 1991; **32**: 1146-1150 [PMID: 1955169 DOI: 10.1136/gut.32.10.1146]

132 **Kruidenier L**, van Meeteren ME, Kuiper I, Jaarsma D, Lamers CB, Zijlstra FJ, Verspaget HW. Attenuated mild colonic inflammation and improved survival from severe DSS-colitis of transgenic Cu/Zn-SOD mice. *Free Radic Biol Med* 2003; **34**: 753-765 [PMID: 12633752 DOI: 10.1016/S0891-5849(02)01426-0]

133 **Lih-Brody L**, Powell SR, Collier KP, Reddy GM, Cerchia R, Kahn E, Weissman GS, Katz S, Floyd RA, McKinley MJ, Fisher SE, Mullin GE. Increased oxidative stress and decreased antioxidant defenses in mucosa of inflammatory bowel dis-

ease. *Dig Dis Sci* 1996; **41**: 2078-2086 [PMID: 8888724]
134 **Suwendi E**, Iwaya H, Lee JS, Hara H, Ishizuka S. Zinc deficiency induces dysregulation of cytokine productions in an experimental colitis of rats. *Biomed Res* 2012; **33**: 329-336 [PMID: 23268956]
135 **Bruewer M**, Schmid KW, Krieglstein CF, Senninger N, Schuermann G. Metallothionein: early marker in the carcinogenesis of ulcerative colitis-associated colorectal carcinoma. *World J Surg* 2002; **26**: 726-731 [PMID: 12053227 DOI: 10.1007/s00268-002-6266-z]
136 **Di Leo V**, D'Incà R, Barollo M, Tropea A, Fries W, Mazzon E, Irato P, Cecchetto A, Sturniolo GC. Effect of zinc supplementation on trace elements and intestinal metallothionein concentrations in experimental colitis in the rat. *Dig Liver Dis* 2001; **33**: 135-139 [PMID: 11346141 DOI: 10.1016/S1590-8658(01)80068-2]
137 **Tran CD**, Ball JM, Sundar S, Coyle P, Howarth GS. The role of zinc and metallothionein in the dextran sulfate sodium-induced colitis mouse model. *Dig Dis Sci* 2007; **52**: 2113-2121 [PMID: 17410436]
138 **Tran CD**, Butler RN, Philcox JC, Rofe AM, Howarth GS, Coyle P. Regional distribution of metallothionein and zinc in the mouse gut: comparison with metallothionien-null mice. *Biol Trace Elem Res* 1998; **63**: 239-251 [PMID: 9840820 DOI: 10.1007/BF02778942]
139 **Luk HH**, Ko JK, Fung HS, Cho CH. Delineation of the protective action of zinc sulfate on ulcerative colitis in rats. *Eur J Pharmacol* 2002; **443**: 197-204 [PMID: 12044810 DOI: 10.1016/S0014-2999(02)01592-3]
140 **Chen BW**, Wang HH, Liu JX, Liu XG. Zinc sulphate solution enema decreases inflammation in experimental colitis in rats. *J Gastroenterol Hepatol* 1999; **14**: 1088-1092 [PMID: 10574136 DOI: 10.1046/j.1440-1746.1999.02013.x]
141 **Yoshikawa T**, Yamaguchi T, Yoshida N, Yamamoto H, Kitazumi S, Takahashi S, Naito Y, Kondo M. Effect of Z-103 on TNB-induced colitis in rats. *Digestion* 1997; **58**: 464-468 [PMID: 9383638 DOI: 10.1159/000201484]
142 **Ohkawara T**, Takeda H, Kato K, Miyashita K, Kato M, Iwanaga T, Asaka M. Polaprezinc (N-(3-aminopropionyl)-L-histidinato zinc) ameliorates dextran sulfate sodium-induced colitis in mice. *Scand J Gastroenterol* 2005; **40**: 1321-1327 [PMID: 16334442 DOI: 10.1080/00365520510023530]
143 **Wierdsma NJ**, van Bokhorst-de van der Schueren MA, Berkenpas M, Mulder CJ, van Bodegraven AA. Vitamin and mineral deficiencies are highly prevalent in newly diagnosed celiac disease patients. *Nutrients* 2013; **5**: 3975-3992 [PMID: 24084055 DOI: 10.3390/nu5103975]
144 **Solomons NW**, Rosenberg IH, Sandstead HH. Zinc nutrition in celiac sprue. *Am J Clin Nutr* 1976; **29**: 371-375 [PMID: 1266787]
145 **Kuloğlu Z**, Kirsaçlioğlu CT, Kansu A, Ensari A, Girgin N. Celiac disease: presentation of 109 children. *Yonsei Med J* 2009; **50**: 617-623 [PMID: 19881963 DOI: 10.3349/ymj.2009.50.5.617]
146 **Henker J**, Gabsch HC. [Serum zinc levels in children with celiac disease]. *Helv Paediatr Acta* 1985; **40**: 47-53 [PMID: 3843236]
147 **Naveh Y**, Lightman A, Zinder O. A prospective study of serum zinc concentration in children with celiac disease. *J Pediatr* 1983; **102**: 734-736 [PMID: 6842333 DOI: 10.1016/S0022-3476(83)80248-0]
148 **Singhal N**, Alam S, Sherwani R, Musarrat J. Serum zinc levels in celiac disease. *Indian Pediatr* 2008; **45**: 319-321 [PMID: 18451454]
149 **Jameson S**. Zinc deficiency in malabsorption states: a cause of infertility? *Acta Med Scand Suppl* 1976; **593**: 38-49 [PMID: 1067747]
150 **Jameson S**. Trace element metabolism in coeliac disease. Trace elements in health and disease. In: Boström H, Ljungstedt N, editors. Stockholm: Almqvist and Wiksell International, 1985: 242-252
151 **Jameson S**. Villous atrophy and nutritional status in celiac disease. *Am J Clin Nutr* 1999; **69**: 573-575 [PMID: 10075349]
152 **Jameson S,** Björklund O, **Lööf** L, Ulfberg J. Zinc and folate malabsorption in coeliac disease. In: Howell JMC, Gawthorne JM, White CL, editors. Trace element metabolism in man and animals. Canberra, Australia: Springer-Verlag, 1981: 495-497
153 **Högberg L**, Danielsson L, Jarleman S, Sundqvist T, Stenhammar L. Serum zinc in small children with coeliac disease. *Acta Paediatr* 2009; **98**: 343-345 [PMID: 19006534 DOI: 10.1111/j.1651-2227.2008.01085.x]
154 **Rea F**, Polito C, Marotta A, Di Toro A, Iovene A, Collini R, Rea L, Sessa G. Restoration of body composition in celiac children after one year of gluten-free diet. *J Pediatr Gastroenterol Nutr* 1996; **23**: 408-412 [PMID: 8956176 DOI: 10.1097/00005176-199611000-00007]
155 **Jones PE**, Peters TJ. Oral zinc supplements in non-responsive coeliac syndrome: effect on jejunal morphology, enterocyte production, and brush border disaccharidase activities. *Gut* 1981; **22**: 194-198 [PMID: 7227852]
156 **Crofton RW**, Aggett PJ, Gvozdanovic S, Gvozdanovic D, Mowat NA, Brunt PW. Zinc metabolism in celiac disease. *Am J Clin Nutr* 1990; **52**: 379-382 [PMID: 2375305]
157 **Tran CD**, Katsikeros R, Manton N, Krebs NF, Hambidge KM, Butler RN, Davidson GP. Zinc homeostasis and gut function in children with celiac disease. *Am J Clin Nutr* 2011; **94**: 1026-1032 [PMID: 21865333]
158 **Krebs NF**, Miller LV, Naake VL, Lei S, Westcott JE, Fennessey PV, Michael Hambidge K. The use of stable isotope techniques to assess zinc metabolism. *J Nutr Biochem* 1995; **6**: 292-301 [DOI: 10.1016/0955-2863(95)00043-Y]
159 **Krebs NF**, Hambidge KM, Westcott JE, Miller LV, Sian L, Bell M, Grunwald G. Exchangeable zinc pool size in infants is related to key variables of zinc homeostasis. *J Nutr* 2003; **133**: 1498S-1501S [PMID: 12730452]
160 **Stenberg P**, Roth EB, Sjöberg K. Transglutaminase and the pathogenesis of coeliac disease. *Eur J Intern Med* 2008; **19**: 83-91 [PMID: 18249302 DOI: 10.1016/j.ejim.2007.05.012]
161 **Thomas KE**, Sapone A, Fasano A, Vogel SN. Gliadin stimulation of murine macrophage inflammatory gene expression and intestinal permeability are MyD88-dependent: role of the innate immune response in Celiac disease. *J Immunol* 2006; **176**: 2512-2521 [PMID: 16456012]
162 **Fasano A**. Regulation of intercellular tight junctions by zonula occludens toxin and its eukaryotic analogue zonulin. *Ann N Y Acad Sci* 2000; **915**: 214-222 [PMID: 11193578 DOI: 10.1111/j.1749-6632.2000.tb05244.x]
163 **Ciccocioppo R**, Finamore A, Ara C, Di Sabatino A, Mengheri E, Corazza GR. Altered expression, localization, and phosphorylation of epithelial junctional proteins in celiac disease. *Am J Clin Pathol* 2006; **125**: 502-511 [PMID: 16627260 DOI: 10.1309/DTYRA91G8R0KTM8M]
164 **Schulzke JD**, Bentzel CJ, Schulzke I, Riecken EO, Fromm M. Epithelial tight junction structure in the jejunum of children with acute and treated celiac sprue. *Pediatr Res* 1998; **43**: 435-441 [PMID: 9544995]
165 **Ventura A**, Magazzù G, Greco L. Duration of exposure to gluten and risk for autoimmune disorders in patients with celiac disease. SIGEP Study Group for Autoimmune Disorders in Celiac Disease. *Gastroenterology* 1999; **117**: 297-303 [PMID: 10419909]
166 **Wang X**, Valenzano MC, Mercado JM, Zurbach EP, Mullin JM. Zinc supplementation modifies tight junctions and alters barrier function of CACO-2 human intestinal epithelial layers. *Dig Dis Sci* 2013; **58**: 77-87 [PMID: 22903217]
167 **D'Souza RS**, Dhume VG. Gastric cytoprotection. *Indian J Physiol Pharmacol* 1991; **35**: 88-98 [PMID: 1791051]

168 **Bandyopadhyay B**, Bandyopadhyay SK. Protective effect of zinc gluconate on chemically induced gastric ulcer. *Indian J Med Res* 1997; **106**: 27-32 [PMID: 9248212]
169 **Escolar G**, Bulbena O. Zinc compounds, a new treatment in peptic ulcer. *Drugs Exp Clin Res* 1989; **15**: 83-89 [PMID: 2661183]
170 **Frommer DJ**. The healing of gastric ulcers by zinc sulphate. *Med J Aust* 1975; **2**: 793-796 [PMID: 1107772]
171 **Alcala-Santaella R**, Castellanos D, Velo JL, Gonzalez Lara V. Zinc acexamate in treatment of duocenal ulcer. *Lancet* 1985; **2**: 157
172 **Kirchhoff P**, Socrates T, Sidani S, Duffy A, Breidthardt T, Grob C, Viehl CT, Beglinger C, Oertli D, Geibel JP. Zinc salts provide a novel, prolonged and rapid inhibition of gastric acid secretion. *Am J Gastroenterol* 2011; **106**: 62-70 [PMID: 20736941 DOI: 10.1038/ajg.2010.327]
173 **Pfeiffer CJ**, Cho CH, Cheema A, Saltman D. Reserpine-induced gastric ulcers: protection by lysosomal stabilization due to zinc. *Eur J Pharmacol* 1980; **61**: 347-353 [PMID: 7371712 DOI: 10.1016/0014-2999(80)90073-4]
174 **Ozutemiz AO**, Aydin HH, Isler M, Celik HA, Batur Y. Effect of omeprazole on plasma zinc levels after oral zinc administration. *Indian J Gastroenterol* 1980; **21**: 216-218 [PMID: 12546170]
175 **Serfaty-Lacrosniere C**, Wood RJ, Voytko D, Saltzman JR, Pedrosa M, Sepe TE, Russell RR. Hypochlorhydria from short-term omeprazole treatment does not inhibit intestinal absorption of calcium, phosphorus, magnesium or zinc from food in humans. *J Am Coll Nutr* 1995; **14**: 364-368 [PMID: 8568113]
176 **Joshaghani H**, Amiriani T, Vaghari G, Besharat S, Molana A, Badeleh M, Roshandel G. Effects of omeprazole consumption on serum levels of trace elements. *J Trace Elem Med Biol* 2012; **26**: 234-237 [PMID: 22677542 DOI: 10.1016/j.jtemb.2012.02.002]
177 **Sturniolo GC**, Montino MC, Rossetto L, Martin A, D'Inca R, D'Odorico A, Naccarato R. Inhibition of gastric acid secretion reduces zinc absorption in man. *J Am Coll Nutr* 1991; **10**: 372-375 [PMID: 1894892]
178 **Nakamura T**, Arai Y, Tando Y, Terada A, Yamada N, Tsujino M, Imamura K, Machida K, Kikuchi H, Takebe K. Effect of omeprazole on changes in gastric and upper small intestine pH levels in patients with chronic pancreatitis. *Clin Ther* 1991; **17**: 448-459 [PMID: 7585849 DOI: 10.1016/0149-2918(95)80110-3]
179 **Hara H**, Konishi A, Kasai T. Contribution of the cecum and colon to zinc absorption in rats. *J Nutr* 2000; **130**: 83-89 [PMID: 10613771]
180 **Schulzke JD**, Fromm M. Tight junctions: molecular structure meets function. *Ann N Y Acad Sci* 2009; **1165**: 1-6 [PMID: 19538280 DOI: 10.1111/j.1749-6632.2009.04925.x]
181 **Walter JK**, Rueckert C, Voss M, Mueller SL, Piontek J, Gast K, Blasig IE. The oligomerization of the coiled coil-domain of occludin is redox sensitive. *Ann N Y Acad Sci* 2009; **1165**: 19-27 [PMID: 19538283 DOI: 10.1111/j.1749-6632.2009.04058.x]
182 **Tsukita S**, Katsuno T, Yamazaki Y, Umeda K, Tamura A, Tsukita S. Roles of ZO-1 and ZO-2 in establishment of the belt-like adherens and tight junctions with paracellular permselective barrier function. *Ann N Y Acad Sci* 2009; **1165**: 44-52 [PMID: 19538286 DOI: 10.1111/j.1749-6632.2009.04056.x]
183 **Ulluwishewa D**, Anderson RC, McNabb WC, Moughan PJ, Wells JM, Roy NC. Regulation of tight junction permeability by intestinal bacteria and dietary components. *J Nutr* 2011; **141**: 769-776 [PMID: 21430248 DOI: 10.3945/jn.110.135657]
184 **Krause G**, Winkler L, Piehl C, Blasig I, Piontek J, Müller SL. Structure and function of extracellular claudin domains. *Ann N Y Acad Sci* 2009; **1165**: 34-43 [PMID: 19538285 DOI: 10.1111/j.1749-6632.2009.04057.x]
185 **Yu AS**. Molecular basis for cation selectivity in claudin-2-based pores. *Ann N Y Acad Sci* 2009; **1165**: 53-57 [PMID: 19538287]
186 **Rao R**. Occludin phosphorylation in regulation of epithelial tight junctions. *Ann N Y Acad Sci* 2009; **1165**: 62-68 [PMID: 19538289 DOI: 10.1111/j.1749-6632.2009.04054.x]
187 **Stefanidou M**, Maravelias C, Dona A, Spiliopoulou C. Zinc: a multipurpose trace element. *Arch Toxicol* 2006; **80**: 1-9 [PMID: 16187101]
188 **Falchuk KH**. The molecular basis for the role of zinc in developmental biology. *Mol Cell Biochem* 1998; **188**: 41-48 [PMID: 9823009]
189 **Kondoh M**, Imada N, Kamada K, Tsukahara R, Higashimoto M, Takiguchi M, Watanabe Y, Sato M. Property of metallothionein as a Zn pool differs depending on the induced condition of metallothionein. *Toxicol Lett* 2003; **142**: 11-18 [PMID: 12765234 DOI: 10.1016/S0378-4274(02)00470-8]
190 **Seve M**, Chimienti F, Favier A. [Role of intracellular zinc in programmed cell death]. *Pathol Biol* (Paris) 2002; **50**: 212-221 [PMID: 11980336 DOI: 10.1016/S0369-8114(02)00290-0]
191 **Wellinghausen N**, Kirchner H, Rink L. The immunobiology of zinc. *Immunol Today* 1997; **18**: 519-521 [PMID: 9386346 DOI: 10.1016/S0167-5699(97)01146-8]
192 **Zhong W**, McClain CJ, Cave M, Kang YJ, Zhou Z. The role of zinc deficiency in alcohol-induced intestinal barrier dysfunction. *Am J Physiol Gastrointest Liver Physiol* 2010; **298**: G625-G633 [PMID: 20167873 DOI: 10.1152/ajpgi.00350.2009]
193 **Chang KL**, Hung TC, Hsieh BS, Chen YH, Chen TF, Cheng HL. Zinc at pharmacologic concentrations affects cytokine expression and induces apoptosis of human peripheral blood mononuclear cells. *Nutrition* 2006; **22**: 465-474 [PMID: 16472982]
194 **Weissgarten J**, Berman S, Modai D, Rosenberg R, Rapoport M, Cohen M, Averbukh Z. Zn metabolism affects apoptosis rate and proliferative responsiveness of PBMC from patients on chronic hemodialysis. *Metabolism* 2002; **51**: 1392-1396 [PMID: 12404186]
195 **Soler AP**, Laughlin KV, Mullin JM. Effects of epidermal growth factor versus phorbol ester on kidney epithelial (LLC-PK1) tight junction permeability and cell division. *Exp Cell Res* 1993; **207**: 398-406 [PMID: 7688317]
196 **Purohit V**, Bode JC, Bode C, Brenner DA, Choudhry MA, Hamilton F, Kang YJ, Keshavarzian A, Rao R, Sartor RB, Swanson C, Turner JR. Alcohol, intestinal bacterial growth, intestinal permeability to endotoxin, and medical consequences: summary of a symposium. *Alcohol* 2008; **42**: 349-361 [PMID: 18504085 DOI: 10.1016/j.alcohol.2008.03.131]
197 **Zhong W**, Zhao Y, McClain CJ, Kang YJ, Zhou Z. Inactivation of hepatocyte nuclear factor-4{alpha} mediates alcohol-induced downregulation of intestinal tight junction proteins. *Am J Physiol Gastrointest Liver Physiol* 2010; **299**: G643-G651 [PMID: 20576917 DOI: 10.1152/ajpgi.00515.2009]
198 **Lambert JC**, Zhou Z, Wang L, Song Z, McClain CJ, Kang YJ. Prevention of alterations in intestinal permeability is involved in zinc inhibition of acute ethanol-induced liver damage in mice. *J Pharmacol Exp Ther* 2003; **305**: 880-886 [PMID: 12626662 DOI: 10.1124/jpet.102.047852]
199 **Fernandez AL**, Koval M, Fan X, Guidot DM. Chronic alcohol ingestion alters claudin expression in the alveolar epithelium of rats. *Alcohol* 2007; **41**: 371-379 [PMID: 17889313 DOI: 10.1016/j.alcohol.2007.04.010]
200 **Coyne CB**, Gambling TM, Boucher RC, Carson JL, Johnson LG. Role of claudin interactions in airway tight junctional permeability. *Am J Physiol Lung Cell Mol Physiol* 2003; **285**: L1166-L1178 [PMID: 12909588 DOI: 10.1152/ajplung.00182.2003]
201 **Wang F**, Daugherty B, Keise LL, Wei Z, Foley JP, Savani RC, Koval M. Heterogeneity of claudin expression by alveolar epithelial cells. *Am J Respir Cell Mol Biol* 2003; **29**: 62-70 [PMID: 12600828 DOI: 10.1165/rcmb.2002-0180OC]
202 **Bao S**, Knoell DL. Zinc modulates cytokine-induced lung

epithelial cell barrier permeability. *Am J Physiol Lung Cell Mol Physiol* 2006; **291**: L1132-L1141 [PMID: 16844947 DOI: 10.1152/ajplung.00207.2006]
203 **Edens HA**, Levi BP, Jaye DL, Walsh S, Reaves TA, Turner JR, Nusrat A, Parkos CA. Neutrophil transepithelial migration: evidence for sequential, contact-dependent signaling events and enhanced paracellular permeability independent of transjunctional migration. *J Immunol* 2002; **169**: 476-486 [PMID: 12077279 DOI: 10.4049/jimmunol.169.1.476]
204 **Elliott SN**, Wallace JL. Neutrophil-mediated gastrointestinal injury. *Can J Gastroenterol* 2002; **12**: 559-568 [PMID: 9926266]
205 **Maes M**, Coucke F, Leunis JC. Normalization of the increased translocation of endotoxin from gram negative enterobacteria (leaky gut) is accompanied by a remission of chronic fatigue syndrome. *Neuro Endocrinol Lett* 2007; **28**: 739-744 [PMID: 18063928]
206 **Maes M**, Leunis JC. Normalization of leaky gut in chronic fatigue syndrome (CFS) is accompanied by a clinical improvement: effects of age, duration of illness and the translocation of LPS from gram-negative bacteria. *Neuro Endocrinol Lett* 2008; **29**: 902-910 [PMID: 19112401]
207 **Jacquillet G**, Barbier O, Cougnon M, Tauc M, Namorado MC, Martin D, Reyes JL, Poujeol P. Zinc protects renal function during cadmium intoxication in the rat. *Am J Physiol Renal Physiol* 2006; **290**: F127-F137 [PMID: 15942048 DOI: 10.1152/ajprenal.00366.2004]
208 **Prozialeck WC**, Lamar PC, Lynch SM. Cadmium alters the localization of N-cadherin, E-cadherin, and beta-catenin in the proximal tubule epithelium. *Toxicol Appl Pharmacol* 2003; **189**: 180-195 [PMID: 12791303 DOI: 10.1016/S0041-008X(03)00130-3]
209 **Santon A**, Formigari A, Irato P. The influence of metallothionein on exposure to metals: an in vitro study on cellular models. *Toxicol In Vitro* 2008; **22**: 980-987 [PMID: 18356017]
210 **Medici V**, Sturniolo GC, Santon A, D'Incà R, Bortolami M, Cardin R, Basso D, Albergoni V, Irato P. Efficacy of zinc supplementation in preventing acute hepatitis in Long-Evans Cinnamon rats. *Liver Int* 2005; **25**: 888-895 [PMID: 15998441 DOI: 10.1111/j.1478-3231.2005.01108.x]
211 **Formigari A**, Santon A, Irato P. Efficacy of zinc treatment against iron-induced toxicity in rat hepatoma cell line H4-II-E-C3. *Liver Int* 2007; **27**: 120-127 [PMID: 17241390 DOI: 10.1111/j.1478-3231.2006.01389.x]
212 **Rohweder J**, Runkel N, Fromm M, Schulzke JD, Buhr HJ. [Zinc acts as a protective agent on the mucosal barrier in experimental TNBS colitis]. *Langenbecks Arch Chir Suppl Kongressbd* 1998; **115**: 223-227 [PMID: 14518248]
213 **Tran CD**, Howarth GS, Coyle P, Philcox JC, Rofe AM, Butler RN. Dietary supplementation with zinc and a growth factor extract derived from bovine cheese whey improves methotrexate-damaged rat intestine. *Am J Clin Nutr* 2003; **77**: 1296-1303 [PMID: 12716685]
214 **Mahmood A**, FitzGerald AJ, Marchbank T, Ntatsaki E, Murray D, Ghosh S, Playford RJ. Zinc carnosine, a health food supplement that stabilises small bowel integrity and stimulates gut repair processes. *Gut* 2007; **56**: 168-175 [PMID: 16777920 DOI: 10.1136/gut.2006.099929]
215 **Roselli M**, Finamore A, Garaguso I, Britti MS, Mengheri E. Zinc oxide protects cultured enterocytes from the damage induced by Escherichia coli. *J Nutr* 2003; **133**: 4077-4082 [PMID: 14652351]
216 **Mullin JM**, Snock KV. Effect of tumor necrosis factor on epithelial tight junctions and transepithelial permeability. *Cancer Res* 1990; **50**: 2172-2176 [PMID: 2180562]
217 **Howe KL**, Reardon C, Wang A, Nazli A, McKay DM. Transforming growth factor-beta regulation of epithelial tight junction proteins enhances barrier function and blocks enterohemorrhagic Escherichia coli O157: H7-induced increased permeability. *Am J Pathol* 2005; **167**: 1587-1597 [PMID: 16314472 DOI: 10.1016/S0002-9440(10)61243-6]
218 **Agren MS**, Chvapil M, Franzén L. Enhancement of re-epithelialization with topical zinc oxide in porcine partial-thickness wounds. *J Surg Res* 1991; **50**: 101-105 [PMID: 1990212 DOI: 10.1016/0022-4804(91)90230-J]
219 **Lambert D**, Padfield PJ, McLaughlin J, Cannell S, O'Neill CA. Ochratoxin A displaces claudins from detergent resistant membrane microdomains. *Biochem Biophys Res Commun* 2007; **358**: 632-636 [PMID: 17499213 DOI: 10.1016/j.bbrc.2007.04.180]
220 **Ranaldi G**, Mancini E, Ferruzza S, Sambuy Y, Perozzi G. Effects of red wine on ochratoxin A toxicity in intestinal Caco-2/TC7 cells. *Toxicol In Vitro* 2007; **21**: 204-210 [PMID: 17107771 DOI: 10.1016/j.tiv.2006.09.005]
221 **Lambert GP**, Gisolfi CV, Berg DJ, Moseley PL, Oberley LW, Kregel KC. Selected contribution: Hyperthermia-induced intestinal permeability and the role of oxidative and nitrosative stress. *J Appl Physiol* (1985) 2002; **92**: 1750-1761; discussion 1749 [PMID: 11896046]
222 **Pearce SC**, Mani V, Boddicker RL, Johnson JS, Weber TE, Ross JW, Rhoads RP, Baumgard LH, Gabler NK. Heat stress reduces intestinal barrier integrity and favors intestinal glucose transport in growing pigs. *PLoS One* 2013; **8**: e70215 [PMID: 23936392 DOI: 10.1371/journal.pone.0070215]
223 **Dokladny K**, Moseley PL, Ma TY. Physiologically relevant increase in temperature causes an increase in intestinal epithelial tight junction permeability. *Am J Physiol Gastrointest Liver Physiol* 2006; **290**: G204-G212 [PMID: 16407590 DOI: 10.1152/ajpgi.00401.2005]
224 **Sanz Fernandez MV**, Pearce SC, Gabler NK, Patience JF, Wilson ME, Socha MT, Torrison JL, Rhoads RP, Baumgard LH. Effects of supplemental zinc amino acid complex on gut integrity in heat-stressed growing pigs. *Animal* 2014; **8**: 43-50 [PMID: 24229744 DOI: 10.1017/S1751731113001961]
225 **Fujimura T**, Matsui T, Funaba M. Regulatory responses to excess zinc ingestion in growing rats. *Br J Nutr* 2012; **107**: 1655-1663 [PMID: 21906406 DOI: 10.1017/S0007114511004867]
226 **Boudry G**, Péron V, Le Huërou-Luron I, Lallès JP, Sève B. Weaning induces both transient and long-lasting modifications of absorptive, secretory, and barrier properties of piglet intestine. *J Nutr* 2004; **134**: 2256-2262 [PMID: 15333713]
227 **Sun X**, Yang H, Nose K, Nose S, Haxhija EQ, Koga H, Feng Y, Teitelbaum DH. Decline in intestinal mucosal IL-10 expression and decreased intestinal barrier function in a mouse model of total parenteral nutrition. *Am J Physiol Gastrointest Liver Physiol* 2008; **294**: G139-G147 [PMID: 17991705]
228 **Zhang B**, Guo Y. Supplemental zinc reduced intestinal permeability by enhancing occludin and zonula occludens protein-1 (ZO-1) expression in weaning piglets. *Br J Nutr* 2009; **102**: 687-693 [PMID: 19267955 DOI: 10.1017/S0007114509289033]
229 **Hu C**, Song J, Li Y, Luan Z, Zhu K. Diosmectite-zinc oxide composite improves intestinal barrier function, modulates expression of pro-inflammatory cytokines and tight junction protein in early weaned pigs. *Br J Nutr* 2013; **110**: 681-688 [PMID: 23308387 DOI: 10.1017/S0007114512005508]
230 **Mercado J**, Valenzano MC, Jeffers C, Sedlak J, Cugliari MK, Papanikolaou E, Clouse J, Miao J, Wertan NE, Mullin JM. Enhancement of tight junctional barrier function by micronutrients: compound-specific effects on permeability and claudin composition. *PLoS One* 2013; **8**: e78775 [PMID: 24236048 DOI: 10.1371/journal.pone.0078775]
231 **Carr G**, Wright JA, Simmons NL. Epithelial barrier resistance is increased by the divalent cation zinc in cultured MDCKII epithelial monolayers. *J Membr Biol* 2010; **237**: 115-123 [PMID: 21057779 DOI: 10.1007/s00232-010-9312-z]
232 **Gitter AH**, Bertog M, Schulzke J, Fromm M. Measurement of paracellular epithelial conductivity by conductance scanning. *Pflugers Arch* 1997; **434**: 830-840 [PMID: 9306019]
233 **Furuse M**, Furuse K, Sasaki H, Tsukita S. Conversion of

zonulae occludentes from tight to leaky strand type by introducing claudin-2 into Madin-Darby canine kidney I cells. *J Cell Biol* 2001; **153**: 263-272 [PMID: 11309408 DOI: 10.1083/jcb.153.2.263]

234 **Hoque KM**, Sarker R, Guggino SE, Tse CM. A new insight into pathophysiological mechanisms of zinc in diarrhea. *Ann N Y Acad Sci* 2009; **1165**: 279-284 [PMID: 19538317 DOI: 10.1111/j.1749-6632.2009.04442.x]

235 **Moran JR**, Lewis JC. The effects of severe zinc deficiency on intestinal permeability: an ultrastructural study. *Pediatr Res* 1985; **19**: 968-973 [PMID: 4047766 DOI: 10.1203/00006450-198509000-00020]

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REVIEW

Alterations of the gut microbiome and metabolome in alcoholic liver disease

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Abstract

Alcohol consumption is one of the leading causes of liver diseases and liver-related death worldwide. The gut is a habitat for billions of microorganisms which promotes metabolism and digestion in their symbiotic relationship with the host. Alterations of gut microbiome by alcohol consumption are referred to bacterial overgrowth, release of bacteria-derived products, and/or changed microbiota equilibrium. Alcohol consumption also perturbs the function of gastrointestinal mucosa and elicits a pathophysiological condition. These adverse effects caused by alcohol may ultimately result in a broad change of gastrointestinal luminal metabolites such as bile acids, short chain fatty acids, and branched chain amino acids. Gut microbiota alterations, metabolic changes produced in a dysbiotic intestinal environment, and the host factors are all critical contributors to the development and progression of alcoholic liver disease. This review summarizes recent findings of how alcohol-induced alterations of gut microbiota and metabolome, and discusses the mechanistic link between gastrointestinal dyshomeostasis and alcoholic liver injury.

Key words: Alcoholic liver disease; Microbiome; Gut metabolome

Core tip: Excessive alcohol consumption causes alcoholic liver disease (ALD) with the mechanisms of pathogenesis largely unknown. Alterations of gut microbiota and metabolites are critical contributors to the development of ALD, which may lead to identification of therapeutic targets for ALD. This review summarizes recent findings of how alcohol-induced alterations of gut microbiota and metabolome, and discusses the mechanistic link between gastrointestinal dyshomeostasis and alcoholic liver injury.

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INTRODUCTION

Alcohol abuse is one of the leading causes of liver disease-related morbidity and mortality worldwide. Alcoholic liver disease (ALD) may progress from steatosis (fatty liver) to steatohepatitis, liver cirrhosis, and eventually hepatic carcinoma[1,2]. According to the National Institute on Alcoholic Abuse and Alcoholism, liver cirrhosis is the 12th leading cause of death in the United States, about 50% of which are alcohol related[3]. Even though enormous efforts have been made, the pathogenesis of ALD is still poorly understood, which makes the progress in finding proper treatments slow. In the last decade, the

role of the gastrointestinal tract (GI) in the pathogenesis and progression of ALD has drawn more and more attention. It is estimated that there are multiple times more microbial cells in the gut than the total number of cells in the human body[4]. The microbes contribute to a complex of biological processes such as digestion[5], synthesis of vitamins[6], and regulation of immunity[7]. Disruption of intestinal homeostasis and alterations in the gut microbiome and metabolome contribute to the pathogenesis of many disorders including ALD[4,8,9]. This review summarizes recent findings on how alcohol affects the composition of the gut microbiota and metabolites, and discusses the mechanistic link between GI dyshomeostasis and the pathogenesis of alcohol consumption-induced liver injury.

INTESTINAL MICROBIOME AND ALCOHOLIC LIVER DISEASE

Quantitative (bacterial overgrowth) and qualitative (dysbiosis) changes of the GI microbiome have long been associated with liver diseases including ALD[10]. Disturbed gut microbiota homeostasis results in dysfunction of the intestinal barrier and translocation of bacteria and/or bacterial products, which eventually contribute to the progression of ALD. Interventions focusing on gut bacteria and/or bacterial products in preventing ALD have drawn increasing attention in the last decade.

Intestinal bacterial overgrowth and translocation in the development of ALD

Alcohol consumption is well known to elicit bacterial overgrowth along the GI tract[11]. The number of both aerobic and anaerobic bacteria cultures of jejunal aspirates from alcoholic patients was distinctly higher than that from the control patients[12]. Similar trends were observed in patients with alcoholic cirrhosis[13]. Bacterial overgrowth has also been documented in experimental models of ALD[14,15]. Overgrowth of bacteria affects ethanol metabolism. Experimental induction of bacteria overgrowth resulted in enhanced endogenous and/or exogenous ethanol metabolism and high concentrations of acetaldehyde in both the intestinal lumen and the portal blood[16-18]. Oral administration of metronidazole, an antibiotic drug, led to a higher level of intracolonic acetaldehyde by increasing aerobic bacteria and reducing anaerobic bacteria in the intestine[19]. On the other hand, intracolonic acetaldehyde accumulation was prevented by antibiotic ciprofloxacin, which decreased colonic microbiota and fecal alcohol dehydrogenase activity[20].

Bacterial translocation is defined as the passage of viable bacteria from the GI tract to extraintestinal sites, such as the mesenteric lymph node, liver, kidney, and bloodstream. Experimental induction of small bowel bacterial overgrowth caused bacterial translocation in association with hepatic inflammation in rats[21]. The translocation of bacteria has been reported as early as 14 d after alcohol consumption in rats[22], while some studies did not show significant bacterial translocation after alcohol administration for 2 wk[23,24]. Moreover, Yan *et al*[14] reported that the bacterial translocation occurred prior to changes observed in the microbiome in a mouse model of continuous intragastric alcohol feeding for up to 3 wk. On the contrary, in a rat model of ALD combined with bacterial inoculation, rats chronically fed with alcohol presented markedly less bacterial translocation to the mesenteric lymph nodes and to the other organs examined compared to rats fed with an isocaloric liquid diet[25].

Bacterial products and gut permeability in the development of ALD

Bacteria, particularly the Gram-negative bacteria, produce endotoxins in the GI tract. Under physiological condition, endotoxin is excluded out of the body along with feces, and only trace amount of endotoxin can penetrate through the GI epithelium to the systemic circulation due to the gut barrier[26]. Alcohol consumption increases the serum endotoxin level, namely endotoxemia. The development of endotoxemia mainly results from bacterial overgrowth and/or increased gut permeability. Endotoxemia has been well documented in patients with ALD[26], and the blood endotoxin levels correlate well with tumor necrosis factor α (TNF-α) levels and the severity of ALD[27-29]. Elevated endotoxin in systemic circulation activates hepatic Kupffer cells *via* Toll-like receptor 4 to produce inflammatory cytokines and chemokines which, in turn, attract neutrophils and monocytes into the liver[30]. In addition to endotoxin, other bacterial products, such as bacterial DNA, peptidoglycan, and flagellin, could also translocate from the intestinal lumen to extraintestinal space and organs, and play a critical role in ALD progression. It was reported that bacterial DNA was elevated in the plasma of patients with alcoholic cirrhosis[31]. Bacterial DNA is recognized by TLR9 and sensitizes the liver to endotoxin-induced injury[32]. Alcohol exposure increased peptidoglycan levels and injected peptidoglycan deteriorated liver injury and inflammation in alcohol-fed mice[33,34].

Intestinal barrier dysfunction has been repeatedly reported in alcohol-induced endotoxemia and liver damage. Alcoholic patients showed increased gut permeability to a variety of macromolecules, such as polyethyleneglycol, lactulose/mannitol, or ^{51}CrEDTA[35-38]. In animal studies, gut permeability to macromolecules such horse radish peroxidase was also increased in association with alcohol-induced endotoxemia and liver damage[39-43]. Orally administrated lipopolysaccharide could be detected in the plasma of acute alcohol-intoxicated mice but not in the control mice[44]. Chronic alcohol exposure reduced the distribution of tight junction proteins, but did not significantly affect the intestinal histopathology[45]; and the gut leakiness only occurred in the ileum instead of in the duodenum or jejunum[45]. Taken together, intestinal barrier dysfunction enables bacteria and bacterial products to translocate from the intestinal lumen to the liver which, as a result, facilitates the development of ALD.

Intestinal dysbiosis in the progression of ALD

Alcohol consumption not only results in quantitative changes of the intestinal microbiota, but also leads to enteric dysbiosis. Enteric dysbiosis refers to an imbalance in the intestinal bacterial composition that participates in the normal activities of the GI tract. Clinical studies have shown that patients with alcoholic cirrhosis had a lower proportion of *Bacteroidetes* and higher ones of *Proteobacteria* in the colon as compared to alcoholic patients without liver cirrhosis[46]. In another study, patients with alcoholic liver cirrhosis showed higher amounts of *Prevotellaceae* in the feces compared to cirrhotic patients with hepatitis B or healthy controls[47]. Animal studies also demonstrated that alcohol consumption for 10 wk altered colonic mucosa-associated microbiota composition in rats[48]. The abundance of *Bacteroidetes* and *Verrucomicrobia* were elevated in the cecum of mice intragastrically fed alcohol for 3 wk, while *Firmicutes* bacteria (including *Lactobacillus*, *Pediococcus*, *Leuconostoc*, and *Lactococcus*) were predominant in the control mice[14]. A recent animal study showed that chronic alcohol feeding for 8 wk caused a decline in the abundance of both *Bacteroidetes* and *Firmicutes* phyla, with a proportional increase in the Gram-negative *Proteobacteria* and Gram-positive *Actinobacteria* phyla in mice feces[15].

The interactions between alcohol-induced liver injury and alterations in the amount/proportion of certain bacteria phylum remain largely unknown. The *Proteobacteria* phylum includes Gram-negative bacteria, most of which are regarded as pathogenic species. Alcohol exposure-induced *Proteobacteria* expansion in the GI tract strongly indicates a link between alcohol-induced alterations of gut microbiota and the elevated plasma endotoxin level as well as hepatic inflammation. Studies have described opportunistic infections of *Corynebacterium*, a member of the *Actinobacteria* phylum, in individuals with ALD[49,50]. As mentioned above, intestinal bacteria like *Escherichia coli* metabolize alcohol and increase luminal acetaldehyde levels through alcohol dehydrogenase-dependent[51] or catalase-dependent pathway[52]. Acetaldehyde is known to disrupt the intestinal barrier through disassembling tight junction proteins[53-56], which implicates another mechanism of how microbiota participate in the development of ALD. The relevance of the gut microbiota changes for ALD progression still requires further investigation.

Intervention for ALD via modulating intestinal microbiome

Efforts on exploring therapeutic strategies for treating ALD have been made for decades, and one of the major attempts was to ameliorate alcohol-induced endotoxemia. Indeed, animal studies demonstrated that abrogating endotoxin signal cascade in the liver by administration of antibiotics[57] or neutralization of circulating endotoxin[58], led to attenuation of alcohol-induced cytokine production and liver damage. Dietary supplementation of milk osteopontin prevented alcohol-induced liver injury through blocking enteric Gram-negative bacterial translocation and the endotoxin-mediated effects in the liver[59].

The effects of probiotics and prebiotics in modulating alcohol-induced liver injury in both patients with ALD and experimental models have been widely studied and the related references are summarized in Table 1. The first report was the study by Nanji *et al*[60], which showed that *Lactobacillus GG* treatment reduced endotoxemia and the severity of ALD. Treatment with *Lactobacillus GG* attenuated alcohol-induced intestinal barrier stress, gut leakiness, and liver injury in rats[40,61-64] and mice[65,66]. A short-term therapy with *Bifidobacterium bifidum* and *Lactobacillus plantarum 8PA3* to alcoholic patients lowered plasma alanine aminotransferase and aspartate aminotransferase levels, restored the gut microbiota, and improved ALD compared to patients treated with standard therapy (abstinence plus vitamins) alone[67]. Another human study showed that *Lactobacillus casei* Shirota administration for 4 wk restored neutrophil phagocytic capacity in alcoholic cirrhotic patients[68]. Notably, the beneficial effects of probiotics were achieved not only by live bacteria, but also by heat-inactivated bacteria[63,69] or bacteria culture supernatant[70].

Short-chain fructooligosaccharides and other prebiotics are used to stimulate the growth and activity of probiotics such as *Lactobacillii* and *Bifidobacteria*. Dietary supplementation of oats prevented alcohol-altered colonic musoca-associated microbiota composition in rats[48]. It was shown that administration of prebiotic (fructooligosaccharides) to alcohol-fed mice reduced bacterial overgrowth and ameliorated alcoholic steatohepatitis through partially restoring the host antimicrobioal protein Reg3g[14].

There are few reports addressing the impact of probiotic and/or prebiotic supplementation on gut microbiome during the development of ALD. To date the most comprehensive study employed 16S ribosome RNA sequencing to characterize gut microbiome changes in mice feces after chronic alcohol exposure and *Lactobacillus GG* supplementation[15]. *Lactobacillus GG* not only reduced bacterial overgrowth in alcohol-fed mice, but also prevented the alcohol-induced expansion of the *Proteobacteria* and *Actinobacteria* phyla.

INTESTINAL METABOLOME AND ALD

Research into alterations in gut metabolome in ALD is unfortunately not as advanced as that for alterations in gut microbiota. To the best of our knowledge, our group, for the first time, applied mass spectrometry-based high throughput technology for characterization of the metabolic alterations of the GI tract contents in a rat model of chronic alcohol consumption. First of all, we conducted a comprehensive metabolite profiling using a high performance liquid chromatography time-of-flight mass spectrometry (HPLC-TOF MS). Secondly, since the HPLC-TOF MS-based profiling approach may not be able to detect or generate accurate data of short chain amino acids (SCFAs) and branched chain

Table 1 Summary of references related to the protective effects of probiotic/prebiotic against alcoholic liver disease

Probiotic/prebiotic	Subjects	Duration of treatment	Outcome	Ref.	Year
Probiotics					
Lactobacillus rhamnosus GG	Male Wistar rats	1 mo	Probiotic feeding reduced alcohol-induced endotoxemia and liver injury	Nanji *et al*[60]	1994
A mixture containing 450 billion bacteria (VSL #3)	Alcoholic cirrhosis patients	3 mo	Treatment of probiotic lowered plasma levels of cytokines and oxidative stress parameters	Loguercio *et al*[103]	2005
L. casei Shirota	Alcoholic cirrhosis patients	4 wk	Probiotic supplementation restored neutrophil phagocytic capacity	Stadlbauer *et al*[68]	2008
Heat-killed *L. brevis* SBC8803	C57BL/6N mice	35 d	*L. brevis* SBC8803 ameliorated alcohol-induced liver injury and fatty liver	Segawa *et al*[69]	2008
Bifidobacterium bifidum and *L. plantarum* 8PA3	Male Russian adults	5 d	Patients treated with probiotics had significantly lower ALT and AST activity, and restored gut microbiota compared to patients treated with standard therapy alone	Kirpich *et al*[67]	2008
L. rhamnosus GG	Male Sprague-Dawley rats	10 wk	*L.* GG reduced alcohol-induced gut leakiness and blunted alcohol-induced oxidative stress and inflammation both in the intestine and liver	Forsyth *et al*[40]	2009
L. rhamnosus GG	Male C57BL/6N mice	Last 2 wk of the 8-wk feeding	*L.* GG supplementation reduced alcohol-induced endotoxemia and hepatic steatosis	Wang *et al*[65,66]	2011, 2013
L.paracasei	Male Fischer 344 rats	10 wk	*L. paracasei* altered the fatty acid composition of the plasma and liver	Komatsuzaki *et al*[61]	2012
L. rhamnosus GG culture supernatant	Male C57BL/6N mice	5 d	Bacteria-free *L.* GG culture supernatant ameliorated acute alcohol-induced gut leakiness and liver injury	Wang *et al*[70]	2012
Combined *Bifidobacterium*, *Lactobacillus*, *Enterococcus* and *Bacillus cereus* tablets	Male Sprague-Dawley rats	Up to 8 wk	Probiotic administration reduced plasma elevated-endotoxin levels caused by alcohol and altered gut microbiota	Zhang *et al*[62]	2012
Live or heat-killed VSL #3	Male rats	Up to 12 h	VSL #3 administration reduced plasma endotoxin level and cytokine production caused by acute alcohol exposure	Chang *et al*[63]	2013
Heat-killed *L. casei* MYL01	HepG2 cells	20 h	*L. casei* MYL01 modulated proinflammatory cytokine production	Chiu *et al*[104]	2014
Escherichia coli Nissle 1917 secreting pyrroloquinoline quinone	Male Foster rats	10 wk	Probiotic treatment ameliorated alcohol-induced oxidative damage and hyperlipidemia in rats	Singh *et al*[64]	2014
Prebiotics					
L. rhamnosus GG or oats	Male Sprague-Dawley rats	10 wk	Supplementation of *L. rhamnosus* GG or oats prevented alcohol-altered colonic musoca-associated microbiota composition in rats	Mutlu *et al*[48]	2009
Fructooligosaccharides	Male C57BL/6J mice	3 wk	Administration of fructooligosaccharides to alcohol-fed mice reduced bacterial overgrowth and ameliorated alcoholic steatohepatitis through partially restoring the host antimicrobial protein Reg3g	Yan *et al*[14]	2011

fatty acids (BCAAs) due to their volatile properties, a gas chromatography mass spectrometry (GC-MS) was used to quantitatively measure specific metabolic panels of SCFAs and BCAAs. The methods were described in more detail elsewhere[71,72]. Thirdly, a targeted quantitative metabolomics approach for a panel of 20-30 bile acids using ultraperformance liquid chromatography-triple-quadrupole mass spectrometry was utilized[73]. Alcohol consumption markedly altered bile acids[73], increased fatty acids and steroids, decreased carnitines, amino acids, branched chain amino acids, and all short chain fatty acids except for acetic acid[71] in the GI luminal contents of rats after 8-wk of alcohol exposure. Bile acids, SCFAs, and BCAAs were the top three categories among the significantly changed metabolites by alcohol consumption. Therefore, they were quantitatively measured in our study and the results will be discussed in more detail below.

Global profiling of metabolites in the GI tract

Chronic alcohol consumption resulted in a global metabolite alteration including amino acids, fatty acids, steroids, lipids, carnitine, SCFAs, BCAAs[71], and bile acids[73] along the GI tract of rats. Almost all amino acids detected were decreased in GI contents of alcohol-fed rats compared to the control. Notably, high abundances of alanine, arginine, glutamic acid, proline, and threoine were observed in all the intestinal segments (from duodenum to rectum) and they were dramatically decreased after alcohol exposure. Amino acids derived from dietary protein may serve as substrates for luminal conversion by the gut microbiota which, in turn, regulate the host homeostasis. For example, one constituent of the gut microbiome, *Lactobacillus reuteri*, is able to convert L-histidine into histamine, which is an immune-regulatory signal suppressing TNF-α production[74]. Intestinal bacteria also involve in converting glutamate to γ-amino butyric acid *via* gluta-

mate decarboxylase[75]. Taken together, it is possible that the reduced abundance of amino acids in alcohol-fed rats was resulted from a perturbed gut microbial-host co-metabolism under the enteric dysbiosis condition.

The levels of steroids and steroid derivatives were significantly increased after alcohol consumption in the stomach, duodenum, jejunum, and ileum. Carnitines and metabolites involved in lipid metabolism were decreased in alcohol-fed rats. Most of the fatty acids detected were at higher levels including 17-HDoHE and 19,20-DiH-DPA, the two metabolic products from docosahexaenoic acid (DHA), and DHA itself. The elevation of DHA and DHA metabolites in the intestinal lumen, especially the large intestine, indicates a disrupted absorption of this nutrient induced by alcohol exposure.

Bile acids

Alcohol consumption significantly perturbed all 21 bile acids detected along the GI tract with the ileum showed the most significant alteration[73]. The concentration of unconjugated bile acids in control rats was low in duodenum (0.04 nmol/mg wet weight), whereas it was increased in the alcohol group (1.30 nmol/mg wet weight). Taurine-conjugated bile acids are the most abundant bile acids in the small intestine and the liver of control rats[73,76,77]. Alcohol consumption led to lower levels of taurine-conjugated bile acids in the duodenum and ileum (0.15 and 0.02 nmol/mg wet weight) compared to control rats (2.39 and 5.66 nmol/mg wet weight, respectively), which made unconjugated bile acids accounted for the largest proportion of the total bile acids in the entire GI tract. Meanwhile, the amount and proportion of taurine-conjugated bile acids were decreased both in the liver and blood[73].

Bile acid metabolism is dependent on the biological activities of the gut microbiota and the host, and both bacterial and hepatic enzymes further modify bile acids during enterohepatic circulation[78,79]. Perturbed gut microbiome may result in a disturbance of bile acid metabolism and reabsorption, leading to altered bile acid profiles in the blood, liver, kidney, and heart[80]. Indeed, inhibiting intestinal microbiota with ampicillin increased the expression of the apical sodium-dependent bile acid transporter (ASBT/Slc10a2) in the brush-border membrane of the ileum, which in turn increased bile acid transport into portal blood[81]. Germ-free mice and rats have a higher proportion of taurine-conjugated bile acids in their livers and intestines[79,82], demonstrating a close association between gut microbiota and bile acid composition. It has been reported that the ratio of glycine-conjugated to taurine-conjugated bile acids is dependent on the hepatic taurine concentration[83]. In our study, we found that the hepatic bile salt taurine to glycine ratio was 30:1 in control rats, while the ratio was 1:1 in alcohol-treated rats. The majority of taurine is usually degraded by the gut microbiota to inorganic sulfate[84]. For this reason, an overgrowth of gut microbiota caused by alcohol exposure would be expected to decrease taurine bioavailability, which provides an explanation for alcohol-induced decrease in taurine-conjugated bile acids in our study. In addition, another investigation suggests that the reduction of taurine in the liver in alcohol-fed mice may be due to the formation and excretion of N-acetyltaurine, a novel metabolite synthesized from taurine and acetate[85].

SCFAs and BCAAs

Acetic acid, propionic acid, and butyric acid are the most predominant SCFAs within the intestine[86]. Our study revealed that the distal intestine (ileum to rectum, especially cecum) processed the majority of SCFAs, within which acetic acid, propionic acid, and butyric acid were predominant (85% in ileum, 94% in cecum, 97% in colon, and 93% in rectum)[71]. Alcohol consumption dramatically reduced all 9 SCFAs detected in the distal intestine except for acetic acid. SCFAs are mainly produced by microbial fermentation of indigestible dietary fibers in the gut[87]. The alteration of SCFAs in alcohol-fed rats may be a result from alcohol-perturbed gut microbiota. The elevated acetic acid levels after alcohol consumption may presumably be due to the oxidation of ethanol to acetaldehyde and subsequent oxidation to acetic acid[88]. Since bacterial aldehyde dehydrogenase activity is limited[18], gut microbiota may not be the major player for the elevated luminal acetic acid level. On the other hand, SCFAs may influence the gut microbiota through stimulating *Bifidibacteria* growth and inhibiting Gram-negative facultative and anaerobic bacteria[89]. SCFAs are known as energy sources to regulate the homeostasis of the intestine and other organs[86]. In a recent study, SCFAs were approved to be beneficial against alcohol-induced intestinal barrier dysfunction through activating AMP-activated protein kinase in Caco-2 cells[90].

BCAAs are essential nutrients obtained from food, as they cannot be synthesized *de novo* by mammals[91]. Gut microbiota, however, are capable to produce BCAAs efficiently[92]. BCAA supplementation has been widely used to improve energy metabolism[93,94], insulin resistance[95-97], and severity of liver disease[98]. Our study reported that all three BCAAs, valine, leucine, and isoleucine, in the GI lumen were predominant in the small intestine (duodenum, jejunum, and ileum) and to a lesser extent in the cecum in rats[71]. Alcohol consumption led to significantly lower levels of all three BCAAs in the GI contents[71]. Previous findings have shown that chronic alcohol consumption increased incorporation of leucine into hepatic proteins[99] and accelerated the absorption of leucine from the small intestine[100], which may explain the dramatic reduction of BCAAs in the gut lumen observed in our study. Notably, a low ratio of plasma BCAAs to aromatic amino acids is a hallmark of liver cirrhosis. Indeed, elevated leucine and isoleucine levels were reported in the plasma of non-alcholic steatotic and non-alcoholic steatohepatitic patients compared to healthy controls[101], which indicate the homeostasis of BCAAs may be involved in the pathogenesis of liver diseases. Moreover, branched chain SCFAs, 2-methylpropanoic acid, 2-methylbutyric acid, and 3-methylbutyric acid are derived from the catabolism of BCAAs[102]. The decreased enteric BCAA levels may

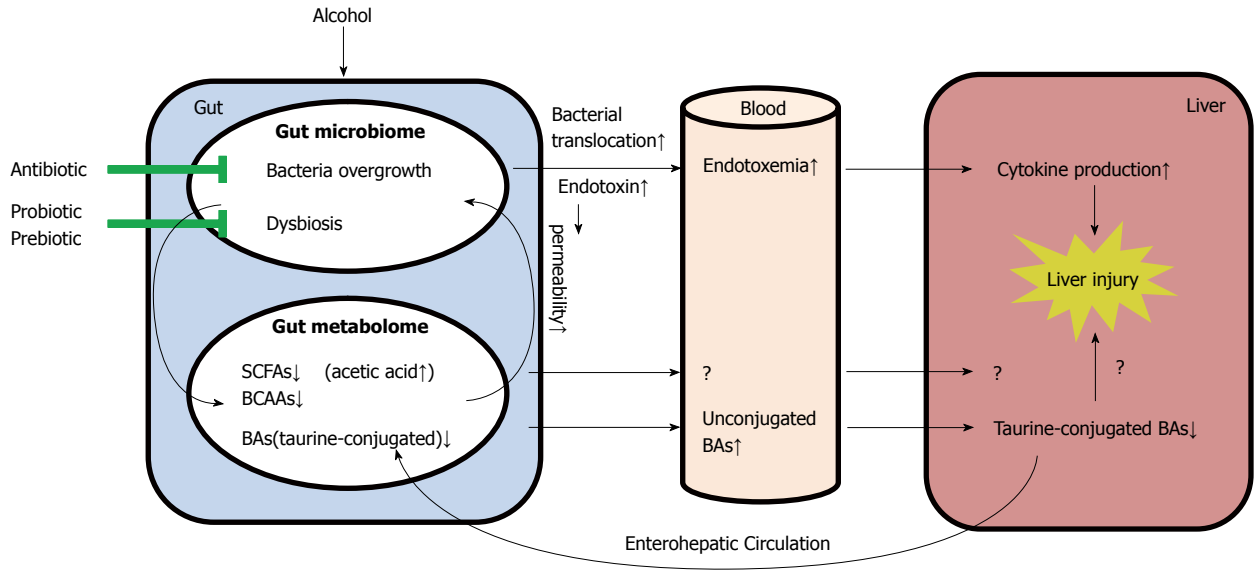

Figure 1 Schematic diagram of the impact of alcohol consumption on the gut microbiota and metabolome during the development and progression of alcoholic liver disease. BA: Bile acids; SCFA: Short chain fatty acid; BCAA: Branched chain amino acid.

further contribute to the decreased levels of branched chain SCFAs after alcohol consumption.

CONCLUSION

Alcoholic consumption is one of the leading causes of liver diseases and liver-related death worldwide. Of the major factors that contribute to the pathogenesis of ALD, the gut microbiota and metabolites have recently drawn more and more attention. Altered intestinal microbiota and gut-associated endotoxemia are recognized as pathophysiological factors in the development of ALD. Prebiotics and probiotics have been applied to prevent alcohol-induced disease development and progression. Taking the advantages of metabonomics approaches, detailed metabolic profiling provides novel information on alcohol-induced alterations in microbiota-host co-metabolism. The impact of alcohol consumption on the gut microbiome and metabolome during the development of ALD is summarized in Figure 1. Despite the recent progression in understanding the importance of the GI tract in the development of ALD, questions of how alcohol consumption results in gut microbiome and metabolome alterations and what are the consequences of such changes to the host have not been fully addressed. Future investigations on the cause-effect relationship between alterations of gut microbiome/metabolome and the liver pathophysiology will not only provide novel insights into the pathogenesis of ALD but also pave the way to the development of therapeutic interventions to cure ALD.

REFERENCES

1 **Miller AM**, Horiguchi N, Jeong WI, Radaeva S, Gao B. Molecular mechanisms of alcoholic liver disease: innate immunity and cytokines. *Alcohol Clin Exp Res* 2011; **35**: 787-793 [PMID: 21284667 DOI: 10.1111/j.1530-0277.2010.01399.x]

2 **Mandrekar P**. Epigenetic regulation in alcoholic liver disease. *World J Gastroenterol* 2011; **17**: 2456-2464 [PMID: 21633650 DOI: 10.3748/wjg.v17.i20.2456]

3 **Gao B**, Bataller R. Alcoholic liver disease: pathogenesis and new therapeutic targets. *Gastroenterology* 2011; **141**: 1572-1585 [PMID: 21920463 DOI: 10.1053/j.gastro.2011.09.002]

4 **Shoaie S**, Nielsen J. Elucidating the interactions between the human gut microbiota and its host through metabolic modeling. *Front Genet* 2014; **5**: 86 [PMID: 24795748 DOI: 10.3389/fgene.2014.00086]

5 **Breen DM**, Rasmussen BA, Côté CD, Jackson VM, Lam TK. Nutrient-sensing mechanisms in the gut as therapeutic targets for diabetes. *Diabetes* 2013; **62**: 3005-3013 [PMID: 23970519 DOI: 10.2337/db13-0523]

6 **LeBlanc JG**, Milani C, de Giori GS, Sesma F, van Sinderen D, Ventura M. Bacteria as vitamin suppliers to their host: a gut microbiota perspective. *Curr Opin Biotechnol* 2013; **24**: 160-168 [PMID: 22940212 DOI: 10.1016/j.copbio.2012.08.005]

7 **Chen VL**, Kasper DL. Interactions between the intestinal microbiota and innate lymphoid cells. *Gut Microbes* 2014; **5**: 129-140 [PMID: 24418741 DOI: 10.4161/gmic.27289]

8 **Parekh PJ**, Arusi E, Vinik AI, Johnson DA. The role and influence of gut microbiota in pathogenesis and management of obesity and metabolic syndrome. *Front Endocrinol* (Lausanne) 2014; **5**: 47 [PMID: 24778627 DOI: 10.3389/fendo.2014.00047]

9 **Kanauchi O**, Andoh A, Mitsuyama K. Effects of the modulation of microbiota on the gastrointestinal immune system and bowel function. *J Agric Food Chem* 2013; **61**: 9977-9983 [PMID: 24070265 DOI: 10.1021/jf402441f]

10 **Chen P**, Schnabl B. Host-microbiome interactions in alcoholic liver disease. *Gut Liver* 2014; **8**: 237-241 [PMID: 24827618 DOI: 10.5009/gnl.2014.8.3.237]

11 **Gabbard SL**, Lacy BE, Levine GM, Crowell MD. The impact of alcohol consumption and cholecystectomy on small intestinal bacterial overgrowth. *Dig Dis Sci* 2014; **59**: 638-644 [PMID: 24323179 DOI: 10.1007/s10620-013-2960-y]

12 **Bode JC**, Bode C, Heidelbach R, Dürr HK, Martini GA. Jejunal microflora in patients with chronic alcohol abuse. *Hepatogastroenterology* 1984; **31**: 30-34 [PMID: 6698486]

13 **Casafont Morencos F**, de las Heras Castaño G, Martín Ramos L, López Arias MJ, Ledesma F, Pons Romero F. Small bowel bacterial overgrowth in patients with alcoholic cirrhosis. *Dig Dis Sci* 1996; **41**: 552-556 [PMID: 8617135]

14 **Yan AW**, Fouts DE, Brandl J, Stärkel P, Torralba M, Schott E, Tsukamoto H, Nelson KE, Brenner DA, Schnabl B. Enteric dysbiosis associated with a mouse model of alcoholic liver disease. *Hepatology* 2011; **53**: 96-105 [PMID: 21254165 DOI: 10.1002/hep.24018]

15 **Bull-Otterson L**, Feng W, Kirpich I, Wang Y, Qin X, Liu Y, Gobejishvili L, Joshi-Barve S, Ayvaz T, Petrosino J, Kong M, Barker D, McClain C, Barve S. Metagenomic analyses of alcohol induced pathogenic alterations in the intestinal microbiome and the effect of Lactobacillus rhamnosus GG treatment. *PLoS One* 2013; **8**: e53028 [PMID: 23326376 DOI: 10.1371/journal.pone.0053028]

16 **Baraona E**, Julkunen R, Tannenbaum L, Lieber CS. Role of intestinal bacterial overgrowth in ethanol production and metabolism in rats. *Gastroenterology* 1986; **90**: 103-110 [PMID: 3940238]

17 **Väkeväinen S**, Tillonen J, Salaspuro M, Jousimies-Somer H, Nuutinen H, Färkkilä M. Hypochlorhydria induced by a proton pump inhibitor leads to intragastric microbial production of acetaldehyde from ethanol. *Aliment Pharmacol Ther* 2000; **14**: 1511-1518 [PMID: 11069323]

18 **Visapää JP**, Tillonen J, Salaspuro M. Microbes and mucosa in the regulation of intracolonic acetaldehyde concentration during ethanol challenge. *Alcohol Alcohol* 2002; **37**: 322-326 [PMID: 12107032]

19 **Tillonen J**, Väkeväinen S, Salaspuro V, Zhang Y, Rautio M, Jousimies-Somer H, Lindros K, Salaspuro M. Metronidazole increases intracolonic but not peripheral blood acetaldehyde in chronic ethanol-treated rats. *Alcohol Clin Exp Res* 2000; **24**: 570-575 [PMID: 10798595]

20 **Visapää JP**, Jokelainen K, Nosova T, Salaspuro M. Inhibition of intracolonic acetaldehyde production and alcoholic fermentation in rats by ciprofloxacin. *Alcohol Clin Exp Res* 1998; **22**: 1161-1164 [PMID: 9726290]

21 **Lichtman SN**, Sartor RB, Keku J, Schwab JH. Hepatic inflammation in rats with experimental small intestinal bacterial overgrowth. *Gastroenterology* 1990; **98**: 414-423 [PMID: 2295397]

22 **Napolitano LM**, Koruda MJ, Zimmerman K, McCowan K, Chang J, Meyer AA. Chronic ethanol intake and burn injury: evidence for synergistic alteration in gut and immune integrity. *J Trauma* 1995; **38**: 198-207 [PMID: 7869435]

23 **Mason CM**, Dobard E, Kolls J, Nelson S. Effect of alcohol on bacterial translocation in rats. *Alcohol Clin Exp Res* 1998; **22**: 1640-1645 [PMID: 9835276]

24 **Fleming S**, Toratani S, Shea-Donohue T, Kashiwabara Y, Vogel SN, Metcalf ES. Pro- and anti-inflammatory gene expression in the murine small intestine and liver after chronic exposure to alcohol. *Alcohol Clin Exp Res* 2001; **25**: 579-589 [PMID: 11329499]

25 **Braulio VB**, De Queiroz Côrtes M, Borges-Neto AA, Bastos MA, Cruz MS, Fracalanza SE. Inhibition of bacterial translocation by chronic ethanol consumption in the rat. *APMIS* 2001; **109**: 809-815 [PMID: 11846721]

26 **Rao RK**, Seth A, Sheth P. Recent Advances in Alcoholic Liver Disease I. Role of intestinal permeability and endotoxemia in alcoholic liver disease. *Am J Physiol Gastrointest Liver Physiol* 2004; **286**: G881-G884 [PMID: 15132946 DOI: 10.1152/ajpgi.00006.2004]

27 **Fujimoto M**, Uemura M, Nakatani Y, Tsujita S, Hoppo K, Tamagawa T, Kitano H, Kikukawa M, Ann T, Ishii Y, Kojima H, Sakurai S, Tanaka R, Namisaki T, Noguchi R, Higashino T, Kikuchi E, Nishimura K, Takaya A, Fukui H. Plasma endotoxin and serum cytokine levels in patients with alcoholic hepatitis: relation to severity of liver disturbance. *Alcohol Clin Exp Res* 2000; **24**: 48S-54S [PMID: 10803780]

28 **Hanck C**, Rossol S, Böcker U, Tokus M, Singer MV. Presence of plasma endotoxin is correlated with tumour necrosis factor receptor levels and disease activity in alcoholic cirrhosis. *Alcohol Alcohol* 1998; **33**: 606-608 [PMID: 9872348]

29 **Urbaschek R**, McCuskey RS, Rudi V, Becker KP, Stickel F, Urbaschek B, Seitz HK. Endotoxin, endotoxin-neutralizing-capacity, sCD14, sICAM-1, and cytokines in patients with various degrees of alcoholic liver disease. *Alcohol Clin Exp Res* 2001; **25**: 261-268 [PMID: 11236841]

30 **Szabo G**, Bala S. Alcoholic liver disease and the gut-liver axis. *World J Gastroenterol* 2010; **16**: 1321-1329 [PMID: 20238398]

31 **Francés R**, Benlloch S, Zapater P, González JM, Lozano B, Muñoz C, Pascual S, Casellas JA, Uceda F, Palazón JM, Carnicer F, Pérez-Mateo M, Such J. A sequential study of serum bacterial DNA in patients with advanced cirrhosis and ascites. *Hepatology* 2004; **39**: 484-491 [PMID: 14768002 DOI: 10.1002/hep.20055]

32 **Romics L**, Dolganiuc A, Kodys K, Drechsler Y, Oak S, Velayudham A, Mandrekar P, Szabo G. Selective priming to Toll-like receptor 4 (TLR4), not TLR2, ligands by P. acnes involves up-regulation of MD-2 in mice. *Hepatology* 2004; **40**: 555-564 [PMID: 15349893 DOI: 10.1002/hep.20350]

33 **Gustot T**, Lemmers A, Moreno C, Nagy N, Quertinmont E, Nicaise C, Franchimont D, Louis H, Devière J, Le Moine O. Differential liver sensitization to toll-like receptor pathways in mice with alcoholic fatty liver. *Hepatology* 2006; **43**: 989-1000 [PMID: 16628628 DOI: 10.1002/hep.21138]

34 **Purohit V**, Bode JC, Bode C, Brenner DA, Choudhry MA, Hamilton F, Kang YJ, Keshavarzian A, Rao R, Sartor RB, Swanson C, Turner JR. Alcohol, intestinal bacterial growth, intestinal permeability to endotoxin, and medical consequences: summary of a symposium. *Alcohol* 2008; **42**: 349-361 [PMID: 18504085 DOI: 10.1016/j.alcohol.2008.03.131]

35 **Bjarnason I**, Peters TJ, Wise RJ. The leaky gut of alcoholism: possible route of entry for toxic compounds. *Lancet* 1984; **1**: 179-182 [PMID: 6141332]

36 **Keshavarzian A**, Fields JZ, Vaeth J, Holmes EW. The differing effects of acute and chronic alcohol on gastric and intestinal permeability. *Am J Gastroenterol* 1994; **89**: 2205-2211 [PMID: 7977243]

37 **Keshavarzian A**, Holmes EW, Patel M, Iber F, Fields JZ, Pethkar S. Leaky gut in alcoholic cirrhosis: a possible mechanism for alcohol-induced liver damage. *Am J Gastroenterol* 1999; **94**: 200-207 [PMID: 9934756 DOI: 10.1111/j.1572-0241.1999.00797.x]

38 **Parlesak A**, Schäfer C, Schütz T, Bode JC, Bode C. Increased intestinal permeability to macromolecules and endotoxemia in patients with chronic alcohol abuse in different stages of alcohol-induced liver disease. *J Hepatol* 2000; **32**: 742-747 [PMID: 10845660]

39 **Enomoto N**, Takei Y, Hirose M, Ikejima K, Miwa H, Kitamura T, Sato N. Thalidomide prevents alcoholic liver injury in rats through suppression of Kupffer cell sensitization and TNF-alpha production. *Gastroenterology* 2002; **123**: 291-300 [PMID: 12105857]

40 **Forsyth CB**, Farhadi A, Jakate SM, Tang Y, Shaikh M, Keshavarzian A. Lactobacillus GG treatment ameliorates alcohol-induced intestinal oxidative stress, gut leakiness, and liver injury in a rat model of alcoholic steatohepatitis. *Alcohol* 2009; **43**: 163-172 [PMID: 19251117 DOI: 10.1016/j.alcohol.2008.12.009]

41 **Mathurin P**, Deng QG, Keshavarzian A, Choudhary S, Holmes EW, Tsukamoto H. Exacerbation of alcoholic liver injury by enteral endotoxin in rats. *Hepatology* 2000; **32**: 1008-1017 [PMID: 11050051 DOI: 10.1053/jhep.2000.19621]

42 **Keshavarzian A**, Choudhary S, Holmes EW, Yong S, Banan A, Jakate S, Fields JZ. Preventing gut leakiness by oats supplementation ameliorates alcohol-induced liver damage in rats. *J Pharmacol Exp Ther* 2001; **299**: 442-448 [PMID: 11602653]

43 **Keshavarzian A**, Farhadi A, Forsyth CB, Rangan J, Jakate S, Shaikh M, Banan A, Fields JZ. Evidence that chronic alcohol exposure promotes intestinal oxidative stress, intestinal hyperpermeability and endotoxemia prior to development of alcoholic steatohepatitis in rats. *J Hepatol* 2009; **50**: 538-547 [PMID: 19155080 DOI: 10.1016/j.jhep.2008.10.028]

44 **Lambert JC**, Zhou Z, Wang L, Song Z, McClain CJ, Kang YJ. Prevention of alterations in intestinal permeability is involved in zinc inhibition of acute ethanol-induced liver damage in mice. *J Pharmacol Exp Ther* 2003; **305**: 880-886 [PMID: 12626662 DOI: 10.1124/jpet.102.047852]

45 **Zhong W**, McClain CJ, Cave M, Kang YJ, Zhou Z. The role of zinc deficiency in alcohol-induced intestinal barrier dysfunction. *Am J Physiol Gastrointest Liver Physiol* 2010; **298**: G625-G633 [PMID: 20167873 DOI: 10.1152/ajpgi.00350.2009]

46 **Mutlu EA**, Gillevet PM, Rangwala H, Sikaroodi M, Naqvi A, Engen PA, Kwasny M, Lau CK, Keshavarzian A. Colonic microbiome is altered in alcoholism. *Am J Physiol Gastrointest Liver Physiol* 2012; **302**: G966-G978 [PMID: 22241860 DOI: 10.1152/ajpgi.00380.2011]

47 **Chen Y**, Yang F, Lu H, Wang B, Chen Y, Lei D, Wang Y, Zhu B, Li L. Characterization of fecal microbial communities in patients with liver cirrhosis. *Hepatology* 2011; **54**: 562-572 [PMID: 21574172 DOI: 10.1002/hep.24423]

48 **Mutlu E**, Keshavarzian A, Engen P, Forsyth CB, Sikaroodi M, Gillevet P. Intestinal dysbiosis: a possible mechanism of alcohol-induced endotoxemia and alcoholic steatohepatitis in rats. *Alcohol Clin Exp Res* 2009; **33**: 1836-1846 [PMID: 19645728]

49 **Harnisch JP**, Tronca E, Nolan CM, Turck M, Holmes KK. Diphtheria among alcoholic urban adults. A decade of experience in Seattle. *Ann Intern Med* 1989; **111**: 71-82 [PMID: 2472081]

50 **Cericco M**, Iglicki F, Guillaumont MP, Schmitt JL, Dupas JL, Capron JP. [Corynebacterium xerosis endocarditis associated with alcoholic cirrhosis]. *Gastroenterol Clin Biol* 1996; **20**: 514 [PMID: 8761156]

51 **Salaspuro V**, Nyfors S, Heine R, Siitonen A, Salaspuro M, Jousimies-Somer H. Ethanol oxidation and acetaldehyde production in vitro by human intestinal strains of Escherichia coli under aerobic, microaerobic, and anaerobic conditions. *Scand J Gastroenterol* 1999; **34**: 967-973 [PMID: 10563665]

52 **Tillonen J**, Kaihovaara P, Jousimies-Somer H, Heine R, Salaspuro M. Role of catalase in in vitro acetaldehyde formation by human colonic contents. *Alcohol Clin Exp Res* 1998; **22**: 1113-1119 [PMID: 9726283]

53 **Rao RK**. Acetaldehyde-induced increase in paracellular permeability in Caco-2 cell monolayer. *Alcohol Clin Exp Res* 1998; **22**: 1724-1730 [PMID: 9835287]

54 **Atkinson KJ**, Rao RK. Role of protein tyrosine phosphorylation in acetaldehyde-induced disruption of epithelial tight junctions. *Am J Physiol Gastrointest Liver Physiol* 2001; **280**: G1280-G1288 [PMID: 11352822]

55 **Basuroy S**, Sheth P, Mansbach CM, Rao RK. Acetaldehyde disrupts tight junctions and adherens junctions in human colonic mucosa: protection by EGF and L-glutamine. *Am J Physiol Gastrointest Liver Physiol* 2005; **289**: G367-G375 [PMID: 15718285 DOI: 10.1152/ajpgi.00464.2004]

56 **Dunagan M**, Chaudhry K, Samak G, Rao RK. Acetaldehyde disrupts tight junctions in Caco-2 cell monolayers by a protein phosphatase 2A-dependent mechanism. *Am J Physiol Gastrointest Liver Physiol* 2012; **303**: G1356-G1364 [PMID: 23064762 DOI: 10.1152/ajpgi.00526.2011]

57 **Adachi Y**, Moore LE, Bradford BU, Gao W, Thurman RG. Antibiotics prevent liver injury in rats following long-term exposure to ethanol. *Gastroenterology* 1995; **108**: 218-224 [PMID: 7806045]

58 **Zhou Z**, Wang L, Song Z, Lambert JC, McClain CJ, Kang YJ. A critical involvement of oxidative stress in acute alcohol-induced hepatic TNF-alpha production. *Am J Pathol* 2003; **163**: 1137-1146 [PMID: 12937155]

59 **Ge X**, Lu Y, Leung TM, Sørensen ES, Nieto N. Milk osteopontin, a nutritional approach to prevent alcohol-induced liver injury. *Am J Physiol Gastrointest Liver Physiol* 2013; **304**: G929-G939 [PMID: 23518682 DOI: 10.1152/ajpgi.00014.2013]

60 **Nanji AA**, Khettry U, Sadrzadeh SM. Lactobacillus feeding reduces endotoxemia and severity of experimental alcoholic liver (disease). *Proc Soc Exp Biol Med* 1994; **205**: 243-247 [PMID: 8171045]

61 **Komatsuzaki N**, Shima J. Effects of live Lactobacillus paracasei on plasma lipid concentration in rats fed an ethanol-containing diet. *Biosci Biotechnol Biochem* 2012; **76**: 232-237 [PMID: 22313752]

62 **Zhang B**, Lu XL, Song YH, Shi HT, Li J, Geng Y. [Changes in the intestinal microenvironment during development of alcoholic fatty liver disease and related effects of probiotic therapy]. *Zhonghua Ganzangbing Zazhi* 2012; **20**: 848-852 [PMID: 23206305 DOI: 10.3760/cma.j.issn.1007-3418.2012.11.010]

63 **Chang B**, Sang L, Wang Y, Tong J, Zhang D, Wang B. The protective effect of VSL#3 on intestinal permeability in a rat model of alcoholic intestinal injury. *BMC Gastroenterol* 2013; **13**: 151 [PMID: 24138544 DOI: 10.1186/1471-230X-13-151]

64 **Singh AK**, Pandey SK, Naresh Kumar G. Pyrroloquinoline quinone-secreting probiotic Escherichia coli Nissle 1917 ameliorates ethanol-induced oxidative damage and hyperlipidemia in rats. *Alcohol Clin Exp Res* 2014; **38**: 2127-2137 [PMID: 24930470 DOI: 10.1111/acer.12456]

65 **Wang Y**, Kirpich I, Liu Y, Ma Z, Barve S, McClain CJ, Feng W. Lactobacillus rhamnosus GG treatment potentiates intestinal hypoxia-inducible factor, promotes intestinal integrity and ameliorates alcohol-induced liver injury. *Am J Pathol* 2011; **179**: 2866-2875 [PMID: 22093263 DOI: 10.1016/j.ajpath.2011.08.039]

66 **Wang Y**, Liu Y, Kirpich I, Ma Z, Wang C, Zhang M, Suttles J, McClain C, Feng W. Lactobacillus rhamnosus GG reduces hepatic TNFα production and inflammation in chronic alcohol-induced liver injury. *J Nutr Biochem* 2013; **24**: 1609-1615 [PMID: 23618528 DOI: 10.1016/j.jnutbio.2013.02.001]

67 **Kirpich IA**, Solovieva NV, Leikhter SN, Shidakova NA, Lebedeva OV, Sidorov PI, Bazhukova TA, Soloviev AG, Barve SS, McClain CJ, Cave M. Probiotics restore bowel flora and improve liver enzymes in human alcohol-induced liver injury: a pilot study. *Alcohol* 2008; **42**: 675-682 [PMID: 19038698 DOI: 10.1016/j.alcohol.2008.08.006]

68 **Stadlbauer V**, Mookerjee RP, Hodges S, Wright GA, Davies NA, Jalan R. Effect of probiotic treatment on deranged neutrophil function and cytokine responses in patients with compensated alcoholic cirrhosis. *J Hepatol* 2008; **48**: 945-951 [PMID: 18433921 DOI: 10.1016/j.jhep.2008.02.015]

69 **Segawa S**, Wakita Y, Hirata H, Watari J. Oral administration of heat-killed Lactobacillus brevis SBC8803 ameliorates alcoholic liver disease in ethanol-containing diet-fed C57BL/6N mice. *Int J Food Microbiol* 2008; **128**: 371-377 [PMID: 18976829 DOI: 10.1016/j.ijfoodmicro.2008.09.023]

70 **Wang Y**, Liu Y, Sidhu A, Ma Z, McClain C, Feng W. Lactobacillus rhamnosus GG culture supernatant ameliorates acute alcohol-induced intestinal permeability and liver injury. *Am J Physiol Gastrointest Liver Physiol* 2012; **303**: G32-G41 [PMID: 22538402 DOI: 10.1152/ajpgi.00024.2012]

71 **Xie G**, Zhong W, Zheng X, Li Q, Qiu Y, Li H, Chen H, Zhou Z, Jia W. Chronic ethanol consumption alters mammalian gastrointestinal content metabolites. *J Proteome Res* 2013; **12**: 3297-3306 [PMID: 23763674 DOI: 10.1021/pr400362z]

72 **Zheng X**, Qiu Y, Zhong W, Baxter S, Su M, Li Q, Xie G, Ore BM, Qiao S, Spencer MD, Zeisel SH, Zhou Z, Zhao A, Jia W. A targeted metabolomic protocol for short-chain fatty acids and branched-chain amino acids. *Metabolomics* 2013; **9**: 818-827 [PMID: 23997757 DOI: 10.1007/s11306-013-0500-6]

73 **Xie G**, Zhong W, Li H, Li Q, Qiu Y, Zheng X, Chen H, Zhao X, Zhang S, Zhou Z, Zeisel SH, Jia W. Alteration of bile acid metabolism in the rat induced by chronic ethanol consumption. *FASEB J* 2013; **27**: 3583-3593 [PMID: 23709616 DOI: 10.1096/fj.13-231860]

74 **Thomas CM**, Hong T, van Pijkeren JP, Hemarajata P, Trinh DV, Hu W, Britton RA, Kalkum M, Versalovic J. Histamine derived from probiotic Lactobacillus reuteri suppresses TNF via modulation of PKA and ERK signaling. *PLoS One* 2012; **7**:

e31951 [PMID: 22384111 DOI: 10.1371/journal.pone.0031951]
75 **Kim JY**, Chung EJ, Kim JH, Jung KY, Lee WY. Response to steroid treatment in anti-glutamic acid decarboxylase antibody-associated cerebellar ataxia, stiff person syndrome and polyendocrinopathy. *Mov Disord* 2006; **21**: 2263-2264 [PMID: 17013903 DOI: 10.1002/mds.21041]
76 **Dupont J**, Oh SY, O'Deen LA, Geller S. Cholanoic (bile) acids in hepatic and nonhepatic tissues of miniature swine. *Lipids* 1974; **9**: 294-297 [PMID: 4833441]
77 **García-Cañaveras JC**, Donato MT, Castell JV, Lahoz A. Targeted profiling of circulating and hepatic bile acids in human, mouse, and rat using a UPLC-MRM-MS-validated method. *J Lipid Res* 2012; **53**: 2231-2241 [PMID: 22822028 DOI: 10.1194/jlr.D028803]
78 **Martin FP**, Dumas ME, Wang Y, Legido-Quigley C, Yap IK, Tang H, Zirah S, Murphy GM, Cloarec O, Lindon JC, Sprenger N, Fay LB, Kochhar S, van Bladeren P, Holmes E, Nicholson JK. A top-down systems biology view of microbiome-mammalian metabolic interactions in a mouse model. *Mol Syst Biol* 2007; **3**: 112 [PMID: 17515922 DOI: 10.1038/msb4100153]
79 **Claus SP**, Tsang TM, Wang Y, Cloarec O, Skordi E, Martin FP, Rezzi S, Ross A, Kochhar S, Holmes E, Nicholson JK. Systemic multicompartmental effects of the gut microbiome on mouse metabolic phenotypes. *Mol Syst Biol* 2008; **4**: 219 [PMID: 18854818 DOI: 10.1038/msb.2008.56]
80 **Swann JR**, Want EJ, Geier FM, Spagou K, Wilson ID, Sidaway JE, Nicholson JK, Holmes E. Systemic gut microbial modulation of bile acid metabolism in host tissue compartments. *Proc Natl Acad Sci USA* 2011; **108** Suppl 1: 4523-4530 [PMID: 20837534 DOI: 10.1073/pnas.1006734107]
81 **Miyata M**, Yamakawa H, Hamatsu M, Kuribayashi H, Takamatsu Y, Yamazoe Y. Enterobacteria modulate intestinal bile acid transport and homeostasis through apical sodium-dependent bile acid transporter (SLC10A2) expression. *J Pharmacol Exp Ther* 2011; **336**: 188-196 [PMID: 20884752 DOI: 10.1124/jpet.110.171736]
82 **Wostmann BS**. Intestinal bile acids and cholesterol absorption in the germfree rat. *J Nutr* 1973; **103**: 982-990 [PMID: 4716197]
83 **Hardison WG**, Proffitt JH. Influence of hepatic taurine concentration on bile acid conjugation with taurine. *Am J Physiol* 1977; **232**: E75-E79 [PMID: 835705]
84 **Hepner GW**, Sturman JA, Hofmann AF, Thomas PJ. Metabolism of steroid and amino acid moieties of conjugated bile acids in man. 3. Cholyltaurine (taurocholic acid). *J Clin Invest* 1973; **52**: 433-440 [PMID: 4683882 DOI: 10.1172/JCI107200]
85 **Shi X**, Yao D, Chen C. Identification of N-acetyltaurine as a novel metabolite of ethanol through metabolomics-guided biochemical analysis. *J Biol Chem* 2012; **287**: 6336-6349 [PMID: 22228769 DOI: 10.1074/jbc.M111.312199]
86 **Tan J**, McKenzie C, Potamitis M, Thorburn AN, Mackay CR, Macia L. The role of short-chain fatty acids in health and disease. *Adv Immunol* 2014; **121**: 91-119 [PMID: 24388214 DOI: 10.1016/B978-0-12-800100-4.00003-9]
87 **Cummings JH**. Short chain fatty acids in the human colon. *Gut* 1981; **22**: 763-779 [PMID: 7028579]
88 **Israel Y**, Orrego H, Carmichael FJ. Acetate-mediated effects of ethanol. *Alcohol Clin Exp Res* 1994; **18**: 144-148 [PMID: 8198211]
89 **Andoh A**, Tsujikawa T, Fujiyama Y. Role of dietary fiber and short-chain fatty acids in the colon. *Curr Pharm Des* 2003; **9**: 347-358 [PMID: 12570825]
90 **Elamin EE**, Masclee AA, Dekker J, Pieters HJ, Jonkers DM. Short-chain fatty acids activate AMP-activated protein kinase and ameliorate ethanol-induced intestinal barrier dysfunction in Caco-2 cell monolayers. *J Nutr* 2013; **143**: 1872-1881 [PMID: 24132573 DOI: 10.3945/jn.113.179549]
91 **Tom A**, Nair KS. Assessment of branched-chain amino Acid status and potential for biomarkers. *J Nutr* 2006; **136**: 324S-330S [PMID: 16365107]
92 **Park JH**, Lee SY. Fermentative production of branched chain amino acids: a focus on metabolic engineering. *Appl Microbiol Biotechnol* 2010; **85**: 491-506 [PMID: 19844702 DOI: 10.1007/s00253-009-2307-y]
93 **Higuchi N**, Kato M, Miyazaki M, Tanaka M, Kohjima M, Ito T, Nakamuta M, Enjoji M, Kotoh K, Takayanagi R. Potential role of branched-chain amino acids in glucose metabolism through the accelerated induction of the glucose-sensing apparatus in the liver. *J Cell Biochem* 2011; **112**: 30-38 [PMID: 20506195 DOI: 10.1002/jcb.22688]
94 **Kawaguchi T**, Izumi N, Charlton MR, Sata M. Branched-chain amino acids as pharmacological nutrients in chronic liver disease. *Hepatology* 2011; **54**: 1063-1070 [PMID: 21563202 DOI: 10.1002/hep.24412]
95 **She P**, Reid TM, Bronson SK, Vary TC, Hajnal A, Lynch CJ, Hutson SM. Disruption of BCATm in mice leads to increased energy expenditure associated with the activation of a futile protein turnover cycle. *Cell Metab* 2007; **6**: 181-194 [PMID: 17767905 DOI: 10.1016/j.cmet.2007.08.003]
96 **Zhang Y**, Guo K, LeBlanc RE, Loh D, Schwartz GJ, Yu YH. Increasing dietary leucine intake reduces diet-induced obesity and improves glucose and cholesterol metabolism in mice via multimechanisms. *Diabetes* 2007; **56**: 1647-1654 [PMID: 17360978 DOI: 10.2337/db07-0123]
97 **Ikehara O**, Kawasaki N, Maezono K, Komatsu M, Konishi A. Acute and chronic treatment of L-isoleucine ameliorates glucose metabolism in glucose-intolerant and diabetic mice. *Biol Pharm Bull* 2008; **31**: 469-472 [PMID: 18310912]
98 **Shirabe K**, Yoshimatsu M, Motomura T, Takeishi K, Toshima T, Muto J, Matono R, Taketomi A, Uchiyama H, Maehara Y. Beneficial effects of supplementation with branched-chain amino acids on postoperative bacteremia in living donor liver transplant recipients. *Liver Transpl* 2011; **17**: 1073-1080 [PMID: 21542128 DOI: 10.1002/lt.22324]
99 **Baraona E**, Leo MA, Borowsky SA, Lieber CS. Pathogenesis of alcohol-induced accumulation of protein in the liver. *J Clin Invest* 1977; **60**: 546-554 [PMID: 561118 DOI: 10.1172/JCI108806]
100 **Martines D**, Morris AI, Billington D. The effect of chronic ethanol intake on leucine absorption from the rat small intestine. *Alcohol Alcohol* 1989; **24**: 525-531 [PMID: 2627240]
101 **Kalhan SC**, Guo L, Edmison J, Dasarathy S, McCullough AJ, Hanson RW, Milburn M. Plasma metabolomic profile in nonalcoholic fatty liver disease. *Metabolism* 2011; **60**: 404-413 [PMID: 20423748 DOI: 10.1016/j.metabol.2010.03.006]
102 **Macfarlane S**, Macfarlane GT. Regulation of short-chain fatty acid production. *Proc Nutr Soc* 2003; **62**: 67-72 [PMID: 12740060 DOI: 10.1079/PNS2002207]
103 **Loguercio C**, Federico A, Tuccillo C, Terracciano F, D'Auria MV, De Simone C, Del Vecchio Blanco C. Beneficial effects of a probiotic VSL#3 on parameters of liver dysfunction in chronic liver diseases. *J Clin Gastroenterol* 2005; **39**: 540-543 [PMID: 15942443]
104 **Chiu YH**, Tsai JJ, Lin SL, Lin MY. Lactobacillus casei MYL01 modulates the proinflammatory state induced by ethanol in an in vitro model. *J Dairy Sci* 2014; **97**: 2009-2016 [PMID: 24485689 DOI: 10.3168/jds.2013-7514]

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REVIEW

Continuing challenges in the diagnosis and management of obscure gastrointestinal bleeding

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Abstract

The diagnosis and management of obscure gastrointestinal bleeding (OGIB) have changed dramatically since the introduction of video capsule endoscopy (VCE) followed by deep enteroscopy and other imaging technologies in the last decade. Significant advances have been made, yet there remains room for improvement in our diagnostic yield and treatment capabilities for recurrent OGIB. In this review, we will summarize the latest technologies for the diagnosis of OGIB, limitations of VCE, technological enhancement in VCE, and different management options for OGIB.

Key words: Obscure gastrointestinal bleeding; Video capsule endoscopy; Deep enteroscopy; Computed tomography enterography; Magnetic resonance enterography

Core tip: Since the advent of capsule endoscopy, significant advances have been made in the imaging of the small bowel that allow for the diagnosis and management of obscure gastrointestinal bleeding. With a decade of knowledge, it is now appropriate for us to look back, critically evaluate our achievements, improve on our current technologies and develop ideas to circumvent some of the shortcomings.

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INTRODUCTION

Remarkable progress has been made since 2001 in the development of technologies that are available to investigate disorders of the small intestine. The wireless video capsule endoscope (VCE) and double balloon enteroscope became available in 2001, followed by the development of computed tomography enterography (CTE) and magnetic resonance enterography (MRE). These tools and newer variations have allowed us to diagnose and manage small bowel lesions in ways that were previously unimaginable. After a 10 year period of remarkable progress it is appropriate to look back, critically evaluate where we are, and use that evaluation as a springboard to critique the technologies and circumvent some of their shortcomings.

Many studies have shown that the diagnostic yield of VCE and deep enteroscopy are similar for obscure gastrointestinal bleeding (OGIB) - approximately in the 40% to 60% range. The higher numbers are for overt obscure bleeding (40% to 92%), occult bleeding (40% to 60%), and iron deficiency (10% to 30%). Assuming indications for these studies are appropriate, a failure-to-diagnose rate of 50% with current techniques appears to be an inconvenient truth. Similarly, recurrent bleeding is distress-

Table 1 Specifications of available capsules

	MiroCam (IntroMedic)	PillCam SB/SB2/SB3 (Given Imaging)	EndoCapsule, EC-10 (Olympus)
Size (mm)	11 × 24	11 × 26	11 × 26
Weight (g)	3.4	3.45	-
Resolution (pixels)	320 × 320	256 × 256	-
Frames per second (fps)	3	2	2
Battery life (h)	11	8-12	8-12
Field of view (°)	150	140/156	145
Communication	Human body communication	Radiofrequency	Radiofrequency
Real time viewer	Yes	Yes	Yes

ingly common. A recent large study from South Korea involving 13 centers and 305 patients showed a detection rate of 51.5%. After VCE only 11.8% received interventional treatment. The overall re-bleeding rate was 19% during the mean of 30 mo[1]. Interestingly the re-bleeding rate was not different between those with positive capsule results and those that had therapy. These observations confirm that we still have a long way to go in terms of better diagnosis and therapy.

DIAGNOSIS OF OGIB

Technology

Various invasive and non-invasive modalities are available for the evaluation of OGIB. These include VCE, deep enteroscopy and a variety of radiological modalities such as CTE, MRE, and conventional and provocative angiography.

Video capsule endoscopy: Currently there are three FDA video capsule endoscopes available in the United States (Table 1). PillCam SB2 (now SB3; Given Imaging, Yoqneam, Israel), EndoCapsule (now EC-10; Olympus, Tokyo, Japan), and MiroCam (IntroMedic, Seoul, South Korea). These capsules are all approximately the same size (11 × 24-26 mm). Most are able to image for up to 12 h, thus reducing the occurrence of incomplete transit. The field of view for all is also similar (140°-156°).

CapsoCam (Capsovision, Saratoga CA, United States) which is in clinical trials in the United States has four cameras allowing for 360° imaging. Unlike traditional capsules that take pictures at a rate of 2-3 frames per second (fps), each of the CapsoCam camera images at the rate of 5 fps for the first two hours and thereafter at 3 fps resulting in 20 and 12 fps respectively. In a prospective comparative study by Pioche *et al*[2], both CapsoCam and PillCam SB2 were found to have similar diagnostic yield (81.8% and 84.8% respectively), however, CapsoCam detected significantly more lesions (108 lesions *vs* 85 lesions), but had a longer reading time (32.0 min *vs* 26.2 min)[2].

MiroCam uses Human Body Communication (HBC) for data transmission which is different from the radiofrequency telemetry of PillCam SB2, SB3 and EndoCapsule. HBC technology uses the human body as a conductive medium for data transmission which expends less electrical power, thereby conferring a longer operating time (12 h) and providing a higher resolution of images (320 × 320 pixels) compared to PillCam SB2 (256 × 256 pixels)[3]. Complete small bowel examination was achieved in 93.3% for MiroCam compared with 84.3% for PillCam SB2. When comparing both capsules for the evaluation of OGIB, the overall concordance was 78.65%, with 77.42% positive agreement and 79.31% negative agreement. MiroCam also has a 42% reduction in missed lesions compared with PillCam[4]. However, the longer recording time along with more image frames captured per second also translate to longer reading time, which may negate the 9% higher rate of small bowel completion and lower missed lesion rate with MiroCam.

Overall, the diagnostic yield of VCE is reported as 38%-92%[5-7]. With the use of VCE in the evaluation and management of OGIB, rebleeding rate was noted to be 15.6% within a 12 mo follow-up period[8]. VCE has the highest yield (92.3%) in those with active overt gastrointestinal (GI) bleeding, and the lowest diagnostic yield (12.9%) in those with a history of obscure GI bleeding[9,10].

Deep enteroscopy: Deep enteroscopy can be performed using double balloon enteroscopy (DBE), single balloon enteroscopy (SBE) or through spiral enteroscopy (SE). These techniques work by pleating the small bowel back over the overtube to minimize looping, thereby allowing the enteroscope to advance forward.

DBE involves two balloons, one each on the distal end of the enteroscope and the overtube. Studies have found the diagnostic yield of DBE is approximately 60%-80% for evaluating OGIB. Success rate of total enteroscopy, with the evaluation of the entire small bowel, either in the antegrade or antegrade plus retrograde fashion, was reported to be 16%-86%[11].

Kamalaporn *et al*[12] looked at detection rates of combined DBE following VCE in OGIB. Overall detection rates for both techniques were similar. Each technique detected lesions not seen by the other, and are complementary in the evaluation of OGIB. VCE is generally performed before DBE, as it can potentially localize the bleeding source and guide the direction of subsequent deep enteroscopy, either in the antegrade or retrograde fashion[13]. In a recent systematic review of over 12000 DBE over a 10 year period, the most common indication for performing DBE (62.5%) was for management of suspected bleeding in the small bowel. DBE was successful in detecting small bowel bleeding in 68.1%[14].

In contrast, SBE involves one balloon. With the use of an angulated enteroscopic tip that can hook onto the small bowel, this technique allows for the enteroscope to advance with a single balloon. Diagnostic yield and intervention rate of SBE were similar to that of DBE (57% *vs* 53% and 32% *vs* 26% respectively)[15], and the procedure

times were both the same at 60 min[16].

SE consists of the Endo-Ease Discovery SB which is a 118 cm long spiral-shaped overtube with spiral ribbing on its surface that is used for enteroscopy *via* the oral route. Diagnostic yield of SE was 57.1%, similar to that of SBE and DBE, and 60% of the angioectasias seen on VCE were detected during SE[17]. Recurrent overt bleeding after SE was 26% during a mean follow up of 2 years[18]. Compared with DBE, SE had both a shorter examination time, along with shorter time to reach the farthest point of examination (43 min *vs* 65 min and 24 min *vs* 43 min respectively). However, DBE allowed for deeper advancement of the enteroscope than SE (310 min *vs* 250 cm)[19].

A recent comparison review noted similar diagnostic yields for SBE, DBE, and SE (53.9%, 64.4%, and 47.0% respectively). Procedure time was fastest in the SE group (oral: 41.0 min, anal: 46 min) followed by SBE (oral: 59.8 min, anal: 68.8 min), and DBE (oral: 71.6 min, anal: 84.5 min). Therapeutic interventions were highest for DBE (40.1%), compared with SBE and SE (26.8% and 29.7%)[20].

CTE and MRE: Similar to VCE, CTE may be used in the evaluation of OGIB to provide a potential road map prior to performing the more invasive DBE. In comparing CTE and VCE, the diagnostic yield for all findings was 34% and 53% respectively. This yield was similar for the detection of neoplastic or mass lesions. However, VCE was superior over CTE in the detection of vascular or inflammatory mucosal lesions. In comparing CTE with DBE, the diagnostic yield was higher for DBE (78% *vs* 38%). Other studies comparing CTE with digital subtraction angiography showed similar yield of 64% and 60% respectively[21].

Agrawal *et al*[22] recently reported that in patients which VCE failed to localize bleeding, CTE may have a utility for the subsequent work up of overt, but not occult or OGIB. The authors found the diagnostic yield to be 50% in overt GI bleed, but 0% in OGIB.

MRE may be considered as an alternative for the initial examination in patients with clinical suspicion of small-bowel stenosis[23] and is also used as a complementary modality for evaluation of patients with small bowel tumors and Crohn's disease.

Angiography: Limited data exists on provocative angiography. In a single center series of patients with obscure and recurrent lower GI bleed, Kim *et al*[24] reported successful definitive treatment of recurrent hemorrhage in 11 of 36 studies (31%) that were performed on 34 patients, with only one complication of ischemic bowel perforation that necessitated bowel resection. There were otherwise no bleeding complications. Leung *et al*[25] compared VCE and angiography for acute overt OGIB and found that the diagnostic yield of VCE was significantly higher than that of angiography (53.3% *vs* 20.0%).

LIMITATIONS OF CAPSULE ENDOSCOPY

Why do we miss lesions?

It has been reported that VCE missed about 11% of all abnormalities in the small bowel. With single mass lesions, the miss rate can be up to 18.9%[26]. Multiple factors account for missed lesions in VCE, including rapid transit through the duodenum and proximal jejunum, unidirectional field of view (about 150°), coupled with a non-axial transit which does not permit the camera to capture the entirety of the mucosal surface. This latter issue explains the discordance of repeat capsule studies in the same patient. Furthermore, inadequate luminal distension and the presence of luminal contents and bubbles further impair complete visualization of the mucosa.

Despite the ability of deep enteroscopes to distend the intestine and wash the mucosa, the diagnostic yield is comparable to that of VCE in most patients when the first deep enteroscopy is attempted from only one direction[12]; achievement of pan enteroscopy is uncommon. The field of view for deep enteroscopes is comparable to VCE, thus it may be difficult to see lesions on the distal aspect of a fold. Anesthesia may also alter hemodynamics of intestinal blood flow in a manner quite different to that of video capsule, and active bleeding originating from a submucosal source is not commonly seen.

Preparation for capsule endoscopy

Capsule image quality plays an important role in the accuracy of capsule interpretation. Presence of food residue, bile, and air bubbles can obscure images. There are currently no guidelines regarding bowel preparation before VCE. Different centers and studies have used various regimens including overnight fasting only for 12 h, clear liquid diet of varying duration, and use of polyethylene glycol (PEG) and/or simethicone. Studies evaluating the role of purgatives/prokinetics as bowel preparation for VCE have been heterogeneous. In a meta-analysis by Song *et al*[27] and the Korean Gut Image Study Group, small bowel visualization quality was found to be enhanced fourfold with use of bowel prep with PEG solution. Two liter (2 L) of PEG solution was similarly effective as 4 L. Diagnostic yield was also slightly improved using PEG solution as compared with overnight fast or clear liquid diet. In another meta-analysis of eight randomized controlled trials comparing use of laxative bowel preparation with fasting alone, PEG based regimens were found to offer better visibility than fasting alone[28]. This is similar to the European Society of gastrointestinal Endoscopy guideline in 2009 recommending purgative bowel preparations that would enhance diagnostic yield of VCE[29]. Use of simethicone with or without bowel prep may also enhance image quality.

Capsule completion rate, however, was not affected with the use of bowel prep, simethicone, or prokinetics. Currently overnight fasting is the standard preparation for VCE in many centers. Alternatively the ASGE recommends 2 L of PEG the evening before the procedure.

Surrounding the controversy regarding use of bowel prep is also the subjective nature of assessing the cleanliness grading system. Current grading systems such as the 10-point quantitative index, or overall assessment of adequacy (adequate or inadequate) are either too cumbersome to calculate or too simplified to be of much utility. With the goal of having an objective assessment, Van Weyenberg *et al*[30] designed a scoring system to assess the quality of bowel preparation with a computed quantitative scale using the color intensities of the tissue color bar. This scoring system, known by the authors as Computed Assessment of Cleansing score is an objective measure, eliminating subjective interpretation by individual readers, and is potentially more reproducible and objective.

Reader error

Recently, a portrayal of physician performance and error in capsule reading was shown in a study by Zheng *et al*[31] to play a big role in missed lesions. In this study, 24 prepared clips of capsule images were read at different modes (single view, duo view, or quad view) and frame speed (15, 20, or 25) by 17 endoscopists, ranging from novice to experienced readers. The detection rates in this study were disappointingly low, ranging from 16% for the detection of blood to 69% for the detection of angioectasias. As expected, ulcers or erosions were more readily detected if they were larger, and masses or polyps were more distinguishable if their color and texture differ from the surrounding mucosa. Abnormal findings appearing in more frames were more likely to be picked up than those appearing in only 1-2 frames. The overall detection rate was also significantly higher when reading in the single view-15 and quad view-20 modes (45% and 47% respectively) compared with reading in single view-25 (26%). This may be explained by the longer dwell time on the screen for each image in quad compared with single view. In another study looking at inter-observer agreement in describing VCE findings, the best agreement was observed in identifying the presence of active bleeding, whereas the worst agreement was in describing size of lesion. Diagnostic concordance was better with angioectasias than for polyps or ulcers/erosions[32].

Missed lesions in the proximal small intestine

Several case reports have noted missed lesions on VCE that were subsequently detected on other imaging modalities such as DBE, CTE or MRE. These lesions were mostly in the proximal small bowel which can be poorly visualized on VCE. This has been evidenced by an earlier study demonstrating that the ampulla of Vater being missed in > 50% of capsule examinations[33]. In a study by Baichi *et al*[34], VCE was performed on 300 consecutive patients presenting with OGIB. Among those patients, 10 small bowel masses were identified, and of those lesions noted, three duodenal masses were missed on previous EGD, with one missed on VCE as well. Further evidence of VCE missing lesions in the proximal small intestine came from a study by Postgate *et al*[35] who reported five tumors missed on VCE for evaluation of OGIB. Three of these tumors were in the distal duodenum, one in the proximal jejunum, and the fifth was a large Peutz-Jegher's polyp in the proximal ileum.

In a study by Selby *et al*[36], capsule endoscopes with varying field of view were evaluated for the ability to identify the ampulla of Vater. The ampulla of Vater was seen in 18% of PillCam SB2 that has a wider field of view (156°) compared to 0% of PillCam (140°). The PillCam SB3 has a variable frame rate of 6 fps when moving fast. It remains to be seen how effective this enhancement will be in visualizing the ampulla of Vater and allowing for better identification of proximal small bowel lesions in the future.

Timing of VCE

The diagnostic yield of VCE for the evaluation of OGIB has been demonstrated to be higher if VCE is performed soon after the onset of bleeding. Of one hundred consecutive patients evaluated for obscure GI bleeding, Pennazio *et al*[9] reported 92.3% positive yield in patients with ongoing overt bleeding (n = 26), 12.9% yield in patients with previous overt bleeding (n = 31) and 44.2% in patients with guaiac positive stools and iron deficiency anemia (n = 43). Similarly, Bresci *et al*[37] reported a positive yield of 91% in patients who underwent VCE within 15 d of obscure GI bleed event versus a yield of 34% in patients who underwent VCE placement after 15 d. Goenka *et al*[38] reported that out of 385 patients investigated for obscure GI bleed, patients with VCE placement within 48 h of overt GI bleed had the highest diagnostic yield (87%). This was significantly greater ($P < 0.05$) compared to patients who had VCE placed after 48 h (68%) of overt GI bleed, as well as those with occult obscure GI bleed (59%).

Recently our group reported that early use of VCE (*i.e.,* within 3 d of hospital admission) led to improved diagnostic yield, higher rate of therapeutic interventions, along with decreased hospital length of stay[39]. In the early deployment group, VCE findings of active bleeding or vascular angioectasia were significantly higher than in the group where VCE was deployed late (after more than 3 d of admission), or in the group where VCE was performed as an outpatient (44.4% *vs* 27.8% *vs* 25.8%). Therapeutic intervention was also carried out more often in the early deployment group compared to the other groups (18.9% *vs* 7.4% for the late group *vs* 10.3% for the outpatient group). Hospital length of stay was also shorter at 6.1 d in the early deployment group, compared with 10.3 d in the late deployment group.

Second look video capsule endoscopy

Several studies have shown that VCE can detect a bleeding site in 45%-66% patients with OGIB, and it is often felt that patients with OGIB and a negative VCE had a low rate of re-bleeding. However, in a study looking at the outcome of 35 patients who had negative VCE for

the evaluation of OGIB, the overall re-bleeding rate was 23% (8 patients) at a median of 15.9 mo of follow up. Four of these patients underwent repeat endoscopy after negative VCE and found previously missed lesions with potential as a bleeding source in the stomach. Overall 13 patients (37%) with or without re-bleeding underwent repeat endoscopy after a negative VCE, which lead to a definitive diagnosis in nine patients (69% who underwent repeat endoscopy). Lesions were located in the stomach and colon in eight of these nine patients[40]. In another study, Vlachogiannakos *et al*[41] noted that in 317 VCE performed for the evaluation of OGIB with negative prior upper endoscopy and colonoscopy, a bleeding source was found on VCE to be outside the small bowel in 3.5% cases, typically duodenum or cecum that was missed by conventional upper endoscopy or colonoscopy. Min *et al*[42] found a higher diagnostic yield for back-to-back VCE and showed that for a single VCE, yield was 37.5%, which increased to 43.8% with a second VCE, and up to 62.5% with back-to-back VCE. Therefore repeat VCE and/or endoscopic evaluations are recommended in cases of severe anemia, or persistent obscure/overt GIB. Timing of VCE is also important as discussed above.

TECHNOLOGICAL ENHANCEMENTS IN CAPSULE ENDOSCOPY

Technological enhancements

Abnormal findings may only be present in a few image frames, and the usefulness of VCE relies on accurate detection of these fleeting images. Several features have been built-in to capsule endoscopy software with the goal of improving detection, such as Suspected Blood Indicator (SBI) and flexible spectral imaging color enhancement (FICE). Different software programs are also equipped with special viewing modes to decrease reading time, such as with QuickView (Given Imaging) and "auto-speed-adjusted" and "express-selected" playback modes (Olympus).

Suspected blood indicator: SBI automatically highlights frames containing several red pixels in an attempt to help capsule reader localize bleeding source. However, use of the SBI is identifying clinically significant lesions is limited by its low sensitivity 56.4% and specificity 33.5%. SBI only has a 24.0% positive predictive value and 67.3% negative predictive value[43]. Intra-luminal bubbles created many of the false positive results. Anecdotally, SBI does have value in overt bleeding where it becomes a solid red bar, the proximal end of which nearly always marks the site of bleeding.

Flexible spectral imaging color enhancement: FICE is an image enhancement system that can obtain bright and high-contrast images. In a study looking at the ability of FICE to detect angioectasia as compared with conventional images, the sensitivity and specificity of detecting angioectasia with FICE images were 91% and 86%, compared with 80% and 100% with conventional mode[44]. FICE reading resulted in more false positive lesions, which can be correctly identified by converting the images to conventional mode.

Quickview system: The QuickView system scans each frame and analyzes patterns/colors to select significant images to create a short video that can then be a quick preview of the entire capsule. Even though QuickView mode may reduce reading time, prospective trials showed a high 8%-12% miss rate[45-47], and it is not recommended as a substitute to reading the entire capsule study.

Auto-speed-adjusted and express-selected playback modes: Olympus capsule endoscopy software systems have equipped an "auto-speed-adjusted" and "express-selected"playback modes. There is also an overview feature which is a one page summary of selected still images which provides the reader with a quick glance of characteristic frames from the capsule study. In the "auto-speed-adjusted" mode, the software speeds up the fps of the video to a maximum of 25 fps when the software detects repeated images similar to the previous frames, thereby potentially reducing the reading time. In the "express-selected" viewing mode, the software skips similar images, and produces a running video stream of only dissimilar images for viewing by the reader. Those skipped images are then gathered into the "expressed-skipped" mode for subsequent viewing if necessary. In a retrospective study of 70 patients to evaluate the clinical efficacy of these functions, Subramanian *et al*[48] noted that the capsule reading time using "express-selected" mode with the overview feature was much lower (19 ± 5 min) than using "auto-speed-adjusted" mode with the overview feature (34 ± 10 min). The missed rate was 8% when the overview function was used alone, but decreased to 0.03% when the overview function was combined with either "express-selected" or "auto-speed-adjusted" playback functions. Though this appears to be promising, further prospective evaluation in a large multicenter trial is needed before this could be recommended for widespread use in clinical practice.

Localization

The clinical problem: While video capsule endoscopy (VCE) is the gold standard for diagnosis of small bowel bleeding, endoscopists are still faced with the clinical challenge of localizing bleeding sources identified by VCE[49,50]. There are many issues at play here. First, the small intestine is a featureless tube which offers only two reliable landmarks for endoscopists - the pylorus and the cecum. Second, the small bowel is stacked upon itself in the peritoneal cavity, meaning that the capsule will traverse through multiple planes as it relates to a single point on the abdominal wall.

Because capsule transit time from the pylorus to the cecum is consistently about four hours, current clinical

practice involves identifying time points associated with the pylorus and cecum and then noting the time point associated with visualization of the bleeding source. Endoscopists can then approximate the distance of the lesion between these two landmarks. One issue with this technique is that if the capsule is not able to visualize the cecum (*e.g.,* because of slow transit time), localization solely based on knowing the time point associated with the pylorus becomes very inaccurate[51,52].

Since the advent of the video capsule, multiple studies have been undertaken to provide a more definitive system of localization. The two major techniques being studied are magnetic field and radiofrequency (RF) localization.

Magnetic field localization: Unlike radiofrequencies, magnetic fields are unaffected by human tissue, allowing for more accurate localization. Magnetic field-based systems also allow the opportunity to control the movement of the capsule while it travels the small bowel using one system[53,54]. Using one system for both capsule control and localization, however, will produce interference that can obstruct both of these functions. Given the accuracy required to control the movements of a capsule through the bowel, this system may not be appropriate. A second issue with magnet-based localization is its application in the clinical setting. Specifically, an examination performed using this system would require a space containing no ferromagnetic materials. Also, magnet-based systems are very complex and would be difficult to utilize in clinics[55].

Radiofrequency localization: An issue surrounding RF-based systems is that radiofrequencies do not easily travel through human tissue. In an effort to better understand the behavior of radiofrequency signals within the human body, a series of multidisciplinary conferences have been convened to address the problems associated with what is called body area networking[56,57]. What is clear is that RF within the body is influenced by multiple factors including tissue densities, juxtaposition of different organs, and other anatomic considerations. Despite this, one advantage of RF-based systems is the ease of utilization in the clinical setting. The first commercially available localization system was the RF-based system attached to the M2A capsule developed by Given Imaging[58]. This localization system has been discontinued due to its inaccuracy. In addition, this system only produces localization data in two dimensions and, therefore, its clinical utility is decreased since capsules travel in multiple planes. Marya *et al*[59] recently reported on a new RF-based localization system developed by Olympus Medical Systems for the new EC-10 capsule. This system has similar accuracy to the Given system while providing three-dimensional localization instead of only two dimensions. The clinical utility of this system cannot be properly assessed until a prospective trial is performed.

Future considerations: Although progress is being made in the development of new localization systems, there are still issues to be addressed. Research needs to be focused on developing a localization system that provides information related to the distance the capsule travels from the pylorus to the suspected bleeding lesion. It is this distance, not simply the three-dimensional location of a capsule within the abdominal cavity, which has the greatest clinical utility to an endoscopist or surgeon in the management of obscure GI bleeding.

MANAGEMENT OF OGIB

Therapeutic options

While progress is being made in the diagnosis of lesions contributing to obscure GI bleeding, the clinical challenge of treating the suspected lesions persists. Traditionally, analysis of particular therapeutic options has been limited to case series or small clinical trials. The decision to choose a particular option is based on several factors. Specifically, clinicians consider where the suspected lesions are within the GI tract, the number of suspected lesions at risk of further bleeding, the degree of bleeding and anemia, co-morbidities and the severity of symptoms experienced by the patient before deciding on a particular therapeutic intervention.

Endoscopic therapy

Once an obscure lesion is localized through endoscopy, the endoscopist has several options available for treatment. Treatments include APC and endoscopic band ligation (EBL).

Argon plasma coagulation: APC therapy is the gold standard therapy for gastric antral vascular ectasias (GAVE) and is widely used to treat angioectasia throughout the GI tract[60-62]. A prospective study by Kwan *et al*[63] provided definitive evidence of the usefulness of APC therapy in 100 patients with both angioectasia and GAVE. In their study the authors found that in a previously transfusion-dependent subset of subjects, over half did not require further transfusions post-APC. In a smaller study population, Herrera *et al*[64] demonstrated a 90% success rate for APC therapy in patients with focal vascular ectasias. In that study, APC was not associated with any adverse effects. Other studies have reported a 2.5% rate of adverse events[65].

Endoscopic band ligation: Historically, EBL has been a treatment for esophageal varices, but its usefulness as a treatment for GAVE and other angioectasia throughout the GI tract is now being realized[66]. In a study of 22 patients, Wells *et al*[67] demonstrated the benefits of EBL, as a subgroup receiving the therapy required fewer treatment sessions and had better-controlled bleeding than those receiving thermal therapy. Earlier studies have shown an equal efficacy and safety profile for EBL in the treatment of Dieulafoy's lesions compared to hemostatic clips or injection therapy[68].

Future directions: Despite important advances in treatments, a large prospective study comparing endoscopic therapies is lacking. As diagnostic measures improve, such a study could prove vital in allowing endoscopists more opportunities to treat obscure GI bleeds.

Pharmacologic therapy

If multiple lesions are suspected to be throughout the GI tract, or if a patient is found to have persistent bleeding despite repeated endoscopic or surgical interventions, pharmacologic therapy should be considered. Pharmacologic therapy may also be pursued for patients with multiple medical co-morbidities that may make them poor candidates for repeated endoscopy or surgical interventions.

Hormonal therapy: The utilization of hormonal therapy (*i.e.,* estrogen plus progesterone) for the treatment of suspected vascular malformations in the GI tract originated from the treatment of hereditary hemorrhagic telangiectasia (HHT, also known as Rendu-Osler-Weber disease). Much of the support for this therapy, however, came only from case reports or from studies with very small sample sizes.

van Cutsem *et al*[69] demonstrated a significant benefit of hormonal therapy compared to placebo (failure rate of therapy 29% compared to 100% for placebo). Despite impressive results, there are many significant issues with this study. Specifically, the sample size of patients was small and the study population included several individuals with HHT who represent a small sub-population of patients with OGIB[69]. These results have been countered by other studies including a larger more recent study from Junquera *et al*[70], which demonstrated no definitive benefit from hormonal therapy compared to the placebo. As other studies suggest, the pathogenesis of vascular malformations in the GI tract is quite different from the process associated with HHT[71,72]. Currently, there is no definitive evidence for the efficacy of hormonal therapy in GI bleeding that is unrelated to HHT.

Anti-angiogenics: The pathogenesis of vascular malformations related to GI bleeding provides multiple options for therapy. In particular, much focus has been placed on the role of vascular endothelial growth factors (VEGF) in the development of these lesions. Junquera *et al*[73] demonstrated that patients with recurrent bleeding secondary to intestinal angiodysplasias (AD) have accumulations of VEGF along the endothelial lining of colonic resection specimens. Research suggests that VEGF becomes overexpressed in oxygen-depleted mucosa, contributing to the formation of AD in older tissues[74,75]. This role of VEGF in the development of AD has created a niche for anti-angiogenic therapy in AD-associated bleeding.

Thalidomide (which has been used in Crohn's disease patients due to its anti-TNF effects) is now being studied in patients with AD-related bleeding. Recently, Ge *et al*[76] performed a randomized controlled trial demonstrating the efficacy of thalidomide in treating AD, 71.4% of patients responded, compared to 3.7% in the control group. Although these results are promising there are significant adverse events associated with thalidomide therapy including leukopenia, deep vein thrombosis, and peripheral neuropathy[77]. Bevacizumab is another anti-angiogenic medication recently studied as a VEGF-inhibitor. Studies have demonstrated its usefulness as an antiangiogenic medication for colon and renal cancer, but there have been no formal studies performed in patients with recurrent GI bleeding from suspected vascular malformations[78].

Somatostatin analogues: The most well-studied somatostatin analogue for the treatment of OGIB is octreotide. The suspected mechanism of action is the ability to inhibit the production of intestinal enzymes (*e.g.,* cholecystokinin, gastrin, and vasointestinal peptide), decrease splanchnic blood flow, decrease platelet aggregation, and decrease angiogenesis. One case series by Nardone *et al*[79], demonstrated that octreotide treatment stopped bleeding in 10 of 17 patients. The authors noted that patients receiving octreotide therapy experienced few side effects. The most common side effects of octreotide therapy are abdominal discomfort and diarrhea, considered to be relatively mild compared to side effects of some of the other therapies listed here[80].

Future directions: While the pathogenesis of AD-related bleeding offers multiple promising opportunities for intervention, a focus on developing randomized-controlled trials to better assess these therapies is needed to define their clinical utility and potential side effects.

Surgical therapy

With advances in endoscopic techniques that allow for visualization and treatment of lesions responsible for OGIB, surgery has become less of a necessity. Now, surgery may be pursued in patients who have failed medical and endoscopic therapy, as well as patients who present with an acute hemorrhage. But in all cases a target lesion needs to be defined preoperatively to avoid the high likelihood of a negative exploration. Research in this field has focused on the localization of lesions to allow surgeons the opportunity to make a curative resection. Studies have demonstrated the benefits of injecting methylene blue dye as an intraoperative technique to allow surgeons to identify the areas of the bowel affected by AD[81,82]. Although this technique has been used since 1978, recent studies have suggested adaptations to make the process easier. For example, D'Mello *et al*[83] presented a case report using digital subtraction angiography to reveal a vascular malformation which they then accessed easily using a microcatheter. Further investigation into capsule endoscopy localization and intraoperative localization of lesions will allow surgeons to make more definitive resections while decreasing the length of bowel needed to be removed.

RECURRENCE OF OGIB

One of the biggest challenges associated with OGIB is that of recurrence. The rate of re-bleeding varies in literature depending on center, duration of follow up and cause of bleeding. Studies have demonstrated the re-bleeding rate to be in the range of 40%-60% when associated with a finding of angiodysplasia on VCE[84,85]. Endo *et al*[86] studied the rate of re-bleeding after intervention for lesions detected on VCE, and found 50% re-bleeding rate in patients with angiodysplasia despite endoscopic intervention. The re-bleeding rate was also higher for patients with clinically insignificant lesions, regardless of whether endoscopic intervention was performed[86]. Patients requiring multiple transfusions for recurrent bleeding typically have multiple co-morbidities, such as chronic renal failure or use of anticoagulation, that are also independent risks for re-bleeding. Patients with recurrent bleeding require multiple endoscopic procedures and are thus at increased risk of complications from these procedures.

Another challenge associated with recurrent OGIB is negative endoscopic findings on VCE or deep enteroscopy. Evidence is conflicting in this area with some studies showing a higher rate of bleeding with normal mucosa or insignificant lesions on endoscopy[84], whereas others show higher rate of recurrent bleeding associated with positive findings on VCE[85]. Studies have reported re-bleeding rate as low as 5.6% and 11% in patients with negative VCE[85,87]. Koh *et al*[88] investigated long-term outcome in OGIB after negative VCE, and found that the overall re-bleeding rate was 28.4%. The re-bleeding rate was higher in patients with positive VCE (36.8%) than in those with negative findings (22.8%)[88]. It is also reported that in VCE-directed interventions, 50%-66% of patients remain transfusion-free without recurrent bleeding[89,90].

FUTURE IN THE DIAGNOSIS AND MANAGEMENT OF OGIB

Advances in capsule endoscopy have included longer battery life, higher image capture frame rate, wider angle of view, improved image resolution, along with enhanced software features to assist in reading. Studies suggest that there is room for further education in reading VCE videos. The more widespread use of early capsule deployment in overt OGIB should enhance diagnostic yields and increase therapeutic intervention rates. The ability to define distance travelled by the capsule would be very helpful in lesion localization. The new 3-D localization software is a step in the right direction. Difficult to detect sources of bleeding may be better controlled by medical means and should stimulate drug development and clinical trials of such agents. The tools for deep enteroscopy are likely to evolve shortly. However, cost constraints for these procedures would preclude their primary deployment in most parts of the world. Thus VCE and DE are likely to remain complimentary procedures for the foreseeable future.

REFERENCES

1 **Min YW**, Kim JS, Jeon SW, Jeen YT, Im JP, Cheung DY, Choi MG, Kim JO, Lee KJ, Ye BD, Shim KN, Moon JS, Kim JH, Hong SP, Chang DK. Long-term outcome of capsule endoscopy in obscure gastrointestinal bleeding: a nationwide analysis. *Endoscopy* 2014; **46**: 59-65 [PMID: 24254387 DOI: 10.1055/s-0033-1358803]

2 **Pioche M**, Vanbiervliet G, Jacob P, Duburque C, Gincul R, Filoche B, Daudet J, Filippi J, Saurin JC. Prospective randomized comparison between axial- and lateral-viewing capsule endoscopy systems in patients with obscure digestive bleeding. *Endoscopy* 2014; **46**: 479-484 [PMID: 24285122 DOI: 10.1055/s-0033-1358832]

3 **Bang S**, Park JY, Jeong S, Kim YH, Shim HB, Kim TS, Lee DH, Song SY. First clinical trial of the "MiRo" capsule endoscope by using a novel transmission technology: electric-field propagation. *Gastrointest Endosc* 2009; **69**: 253-259 [PMID: 18640676 DOI: 10.1016/j.gie.2008.04.033]

4 **Choi EH**, Mergener K, Semrad C, Fisher L, Cave DR, Dodig M, Burke C, Leighton JA, Kastenberg D, Simpson P, Sul J, Bhattacharya K, Charles R, Gerson L, Weber L, Eisen G, Reidel W, Vargo JJ, Wakim-Fleming J, Lo SK. A multicenter, prospective, randomized comparison of a novel signal transmission capsule endoscope to an existing capsule endoscope. *Gastrointest Endosc* 2013; **78**: 325-332 [PMID: 23664161 DOI: 10.1016/j.gie.2013.02.039]

5 **Fireman Z**, Friedman S. Diagnostic yield of capsule endoscopy in obscure gastrointestinal bleeding. *Digestion* 2004; **70**: 201-206 [PMID: 15627766 DOI: 10.1159/000082834]

6 **Saurin JC**, Delvaux M, Gaudin JL, Fassler I, Villarejo J, Vahedi K, Bitoun A, Canard JM, Souquet JC, Ponchon T, Florent C, Gay G. Diagnostic value of endoscopic capsule in patients with obscure digestive bleeding: blinded comparison with video push-enteroscopy. *Endoscopy* 2003; **35**: 576-584 [PMID: 12822092 DOI: 10.1055/s-2003-40244]

7 **Rondonotti E**, Villa F, Mulder CJ, Jacobs MA, de Franchis R. Small bowel capsule endoscopy in 2007: indications, risks and limitations. *World J Gastroenterol* 2007; **13**: 6140-6149 [PMID: 18069752 DOI: 10.3748/wjg.13.6140]

8 **Apostolopoulos P**, Liatsos C, Gralnek IM, Kalantzis C, Giannakoulopoulou E, Alexandrakis G, Tsibouris P, Kalafatis E, Kalantzis N. Evaluation of capsule endoscopy in active, mild-to-moderate, overt, obscure GI bleeding. *Gastrointest Endosc* 2007; **66**: 1174-1181 [PMID: 18061718 DOI: 10.1016/j.gie.2007.06.058]

9 **Pennazio M**, Santucci R, Rondonotti E, Abbiati C, Beccari G, Rossini FP, De Franchis R. Outcome of patients with obscure gastrointestinal bleeding after capsule endoscopy: report of 100 consecutive cases. *Gastroenterology* 2004; **126**: 643-653 [PMID: 14988816 DOI: 10.1053/j.gastro.2003.11.057]

10 **Singh A**, Baptista V, Stoicov C, Cave DR. Evaluation of small bowel bleeding. *Curr Opin Gastroenterol* 2013; **29**: 119-124 [PMID: 23168835 DOI: 10.1097/MOG.0b013e32835bdc1a]

11 **Leighton JA**. The role of endoscopic imaging of the small bowel in clinical practice. *Am J Gastroenterol* 2011; **106**: 27-36; quiz 37 [PMID: 20978483 DOI: 10.1038/ajg.2010.410]

12 **Kamalaporn P**, Cho S, Basset N, Cirocco M, May G, Kortan P, Kandel G, Marcon N. Double-balloon enteroscopy following capsule endoscopy in the management of obscure gastrointestinal bleeding: outcome of a combined approach. *Can J Gastroenterol* 2008; **22**: 491-495 [PMID: 18478135]

13 **Shishido T**, Oka S, Tanaka S, Aoyama T, Watari I, Imagawa H, Yoshida S, Chayama K. Diagnostic yield of capsule endoscopy vs. double-balloon endoscopy for patients who have undergone total enteroscopy with obscure gastrointes-

tinal bleeding. *Hepatogastroenterology* 2012; **59**: 955-959 [PMID: 22580642 DOI: 10.5754/hge12242]
14 **Xin L**, Liao Z, Jiang YP, Li ZS. Indications, detectability, positive findings, total enteroscopy, and complications of diagnostic double-balloon endoscopy: a systematic review of data over the first decade of use. *Gastrointest Endosc* 2011; **74**: 563-570 [PMID: 21620401 DOI: 10.1016/j.gie.2011.03.1239]
15 **Teshima CW**. Small bowel endoscopy for obscure GI bleeding. *Best Pract Res Clin Gastroenterol* 2012; **26**: 247-261 [PMID: 22704568 DOI: 10.1016/j.bpg.2012.01.020]
16 **Efthymiou M**, Desmond PV, Brown G, La Nauze R, Kaffes A, Chua TJ, Taylor AC. SINGLE-01: a randomized, controlled trial comparing the efficacy and depth of insertion of single- and double-balloon enteroscopy by using a novel method to determine insertion depth. *Gastrointest Endosc* 2012; **76**: 972-980 [PMID: 22980289 DOI: 10.1016/j.gie.2012.06.033]
17 **Buscaglia JM**, Richards R, Wilkinson MN, Judah JR, Lam Y, Nagula S, Draganov PV. Diagnostic yield of spiral enteroscopy when performed for the evaluation of abnormal capsule endoscopy findings. *J Clin Gastroenterol* 2011; **45**: 342-346 [PMID: 20861800 DOI: 10.1097/MCG.0b013e3181eeb74b]
18 **Williamson JB**, Judah JR, Gaidos JK, Collins DP, Wagh MS, Chauhan SS, Zoeb S, Buscaglia JM, Yan H, Hou W, Draganov PV. Prospective evaluation of the long-term outcomes after deep small-bowel spiral enteroscopy in patients with obscure GI bleeding. *Gastrointest Endosc* 2012; **76**: 771-778 [PMID: 22771101 DOI: 10.1016/j.gie.2012.05.025]
19 **May A**, Manner H, Aschmoneit I, Ell C. Prospective, crossover, single-center trial comparing oral double-balloon enteroscopy and oral spiral enteroscopy in patients with suspected small-bowel vascular malformations. *Endoscopy* 2011; **43**: 477-483 [PMID: 21437852 DOI: 10.1055/s-0030-1256340]
20 **Lenz P**, Domagk D. Double- vs. single-balloon vs. spiral enteroscopy. *Best Pract Res Clin Gastroenterol* 2012; **26**: 303-313 [PMID: 22704572 DOI: 10.1016/j.bpg.2012.01.021]
21 **Wang Z**, Chen JQ, Liu JL, Qin XG, Huang Y. CT enterography in obscure gastrointestinal bleeding: a systematic review and meta-analysis. *J Med Imaging Radiat Oncol* 2013; **57**: 263-273 [PMID: 23721134 DOI: 10.1111/1754-9485.12035]
22 **Agrawal JR**, Travis AC, Mortele KJ, Silverman SG, Maurer R, Reddy SI, Saltzman JR. Diagnostic yield of dual-phase computed tomography enterography in patients with obscure gastrointestinal bleeding and a non-diagnostic capsule endoscopy. *J Gastroenterol Hepatol* 2012; **27**: 751-759 [PMID: 22098076 DOI: 10.1111/j.1440-1746.2011.06959.x]
23 **Wiarda BM**, Heine DG, Mensink P, Stolk M, Dees J, Hazenberg HJ, Stoker J, Kuipers EJ. Comparison of magnetic resonance enteroclysis and capsule endoscopy with balloon-assisted enteroscopy in patients with obscure gastrointestinal bleeding. *Endoscopy* 2012; **44**: 668-673 [PMID: 22528671]
24 **Kim CY**, Suhocki PV, Miller MJ, Khan M, Janus G, Smith TP. Provocative mesenteric angiography for lower gastrointestinal hemorrhage: results from a single-institution study. *J Vasc Interv Radiol* 2010; **21**: 477-483 [PMID: 20171902 DOI: 10.1055/s-0032-1309386]
25 **Leung WK**, Ho SS, Suen BY, Lai LH, Yu S, Ng EK, Ng SS, Chiu PW, Sung JJ, Chan FK, Lau JY. Capsule endoscopy or angiography in patients with acute overt obscure gastrointestinal bleeding: a prospective randomized study with long-term follow-up. *Am J Gastroenterol* 2012; **107**: 1370-1376 [PMID: 22825363 DOI: 10.1038/ajg.2012.212]
26 **Lewis BS**, Eisen GM, Friedman S. A pooled analysis to evaluate results of capsule endoscopy trials. *Endoscopy* 2005; **37**: 960-965 [PMID: 16189768 DOI: 10.1055/s-2005-870353]
27 **Song HJ**, Moon JS, Do JH, Cha IH, Yang CH, Choi MG, Jeen YT, Kim HJ. Guidelines for Bowel Preparation before Video Capsule Endoscopy. *Clin Endosc* 2013; **46**: 147-154 [PMID: 23614124 DOI: 10.5946/ce.2013.46.2.147]
28 **Belsey J**, Crosta C, Epstein O, Fischbach W, Layer P, Parente F, Halphen M. Meta-analysis: efficacy of small bowel preparation for small bowel video capsule endoscopy. *Curr Med Res Opin* 2012; **28**: 1883-1890 [PMID: 23136911 DOI: 10.1185/03007995.2012.747953]
29 **Ladas SD**, Triantafyllou K, Spada C, Riccioni ME, Rey JF, Niv Y, Delvaux M, de Franchis R, Costamagna G. European Society of Gastrointestinal Endoscopy (ESGE): recommendations (2009) on clinical use of video capsule endoscopy to investigate small-bowel, esophageal and colonic diseases. *Endoscopy* 2010; **42**: 220-227 [PMID: 20195992 DOI: 10.1055/s-0029-1243968]
30 **Van Weyenberg SJ**, De Leest HT, Mulder CJ. Description of a novel grading system to assess the quality of bowel preparation in video capsule endoscopy. *Endoscopy* 2011; **43**: 406-411 [PMID: 21425039]
31 **Zheng Y**, Hawkins L, Wolff J, Goloubeva O, Goldberg E. Detection of lesions during capsule endoscopy: physician performance is disappointing. *Am J Gastroenterol* 2012; **107**: 554-560 [PMID: 22233695 DOI: 10.1055/s-0030-1256228]
32 **Pezzoli A**, Cannizzaro R, Pennazio M, Rondonotti E, Zancanella L, Fusetti N, Simoni M, Cantoni F, Melina R, Alberani A, Caravelli G, Villa F, Chilovi F, Casetti T, Iaquinto G, D'imperio N, Gullini S. Interobserver agreement in describing video capsule endoscopy findings: a multicentre prospective study. *Dig Liver Dis* 2011; **43**: 126-131 [PMID: 20817579 DOI: 10.1016/j.dld.2010.07.007]
33 **Kong H**, Kim YS, Hyun JJ, Cho YJ, Keum B, Jeen YT, Lee HS, Chun HJ, Um SH, Lee SW, Choi JH, Kim CD, Ryu HS, Hyun JH. Limited ability of capsule endoscopy to detect normally positioned duodenal papilla. *Gastrointest Endosc* 2006; **64**: 538-541 [PMID: 16996345 DOI: 10.1016/j.gie.2006.02.028]
34 **Baichi MM**, Arifuddin RM, Mantry PS. Small-bowel masses found and missed on capsule endoscopy for obscure bleeding. *Scand J Gastroenterol* 2007; **42**: 1127-1132 [PMID: 17710681 DOI: 10.1080/00365520701266898]
35 **Postgate A**, Despott E, Burling D, Gupta A, Phillips R, O'Beirne J, Patch D, Fraser C. Significant small-bowel lesions detected by alternative diagnostic modalities after negative capsule endoscopy. *Gastrointest Endosc* 2008; **68**: 1209-1214 [PMID: 19028234 DOI: 10.1016/j.gie.2008.06.035]
36 **Selby WS**, Prakoso E. The inability to visualize the ampulla of Vater is an inherent limitation of capsule endoscopy. *Eur J Gastroenterol Hepatol* 2011; **23**: 101-103 [PMID: 21030868 DOI: 10.1097/MEG.0b013e3283410210]
37 **Bresci G**, Parisi G, Bertoni M, Tumino E, Capria A. The role of video capsule endoscopy for evaluating obscure gastrointestinal bleeding: usefulness of early use. *J Gastroenterol* 2005; **40**: 256-259 [PMID: 15830284 DOI: 10.1007/s00535-004-1532-5]
38 **Goenka MK**, Majumder S, Kumar S, Sethy PK, Goenka U. Single center experience of capsule endoscopy in patients with obscure gastrointestinal bleeding. *World J Gastroenterol* 2011; **17**: 774-778 [PMID: 21390148 DOI: 10.3748/wjg.v17.i6.774]
39 **Singh A**, Marshall C, Chaudhuri B, Okoli C, Foley A, Person SD, Bhattacharya K, Cave DR. Timing of video capsule endoscopy relative to overt obscure GI bleeding: implications from a retrospective study. *Gastrointest Endosc* 2013; **77**: 761-766 [PMID: 23375526 DOI: 10.1016/j.gie.2012.11.041]
40 **Lorenceau-Savale C**, Ben-Soussan E, Ramirez S, Antonietti M, Lerebours E, Ducrotté P. Outcome of patients with obscure gastrointestinal bleeding after negative capsule endoscopy: results of a one-year follow-up study. *Gastroenterol Clin Biol* 2010; **34**: 606-611 [PMID: 20822872]
41 **Vlachogiannakos J**, Papaxoinis K, Viazis N, Kegioglou A, Binas I, Karamanolis D, Ladas SD. Bleeding lesions within reach of conventional endoscopy in capsule endoscopy examinations for obscure gastrointestinal bleeding: is repeating endoscopy economically feasible? *Dig Dis Sci* 2011; **56**: 1763-1768 [PMID: 21302137 DOI: 10.1016/j.gcb.2010.06.009]
42 **Min BH**, Chang DK, Kim BJ, Lee IS, Choi MG. Does Back-

To-Back Capsule Endoscopy Increase the Diagnostic Yield over a Single Examination in Patients with Obscure Gastrointestinal Bleeding? *Gut Liver* 2010; **4**: 54-59 [PMID: 20479913 DOI: 10.5009/gnl.2010.4.1.54]

43 **D'Halluin PN**, Delvaux M, Lapalus MG, Sacher-Huvelin S, Ben Soussan E, Heyries L, Filoche B, Saurin JC, Gay G, Heresbach D. Does the "Suspected Blood Indicator" improve the detection of bleeding lesions by capsule endoscopy? *Gastrointest Endosc* 2005; **61**: 243-249 [PMID: 15729233 DOI: 10.1016/S0016-5107(04)02587-8]

44 **Nakamura M**, Ohmiya N, Miyahara R, Ando T, Watanabe O, Kawashima H, Itoh A, Hirooka Y, Goto H. Usefulness of flexible spectral imaging color enhancement (FICE) for the detection of angiodysplasia in the preview of capsule endoscopy. *Hepatogastroenterology* 2012; **59**: 1474-1477 [PMID: 22683964 DOI: 10.5754/hge10747]

45 **Shiotani A**, Honda K, Kawakami M, Murao T, Matsumoto H, Tarumi K, Kusunoki H, Hata J, Haruma K. Evaluation of RAPID(®) 5 Access software for examination of capsule endoscopies and reading of the capsule by an endoscopy nurse. *J Gastroenterol* 2011; **46**: 138-142 [PMID: 20821237 DOI: 10.1007/s00535-010-0312-7]

46 **Westerhof J**, Koornstra JJ, Weersma RK. Can we reduce capsule endoscopy reading times? *Gastrointest Endosc* 2009; **69**: 497-502 [PMID: 19012887 DOI: 10.1016/j.gie.2008.05.070]

47 **Kyriakos N**, Karagiannis S, Galanis P, Liatsos C, Zouboulis-Vafiadis I, Georgiou E, Mavrogiannis C. Evaluation of four time-saving methods of reading capsule endoscopy videos. *Eur J Gastroenterol Hepatol* 2012; **24**: 1276-1280 [PMID: 22825645 DOI: 10.1097/MEG.0b013e32835718d2]

48 **Subramanian V**, Mannath J, Telakis E, Ragunath K, Hawkey CJ. Efficacy of new playback functions at reducing small-bowel wireless capsule endoscopy reading times. *Dig Dis Sci* 2012; **57**: 1624-1628 [PMID: 22327240 DOI: 10.1007/s10620-012-2074-y]

49 **Ginsberg GG**, Barkun AN, Bosco JJ, Isenberg GA, Nguyen CC, Petersen BT, Silverman WB, Slivka A, Taitelbaum G. Wireless capsule endoscopy: August 2002. *Gastrointest Endosc* 2002; **56**: 621-624 [PMID: 12397265]

50 **Iddan G**, Meron G, Glukhovsky A, Swain P. Wireless capsule endoscopy. *Nature* 2000; **405**: 417 [PMID: 10839527 DOI: 10.1038/35013140]

51 **Moglia A**, Menciassi A, Dario P, Cuschieri A. Capsule endoscopy: progress update and challenges ahead. *Nat Rev Gastroenterol Hepatol* 2009; **6**: 353-362 [PMID: 19434097 DOI: 10.1038/nrgastro.2009.69]

52 **Wang X**, Meng M. Perspective of active capsule endoscope: actuation and localization. *Int J Mechatron Autom* 2011; **1**: 38-45 [DOI: 10.1504/IJMA.2011.039154]

53 **Hu C**, -H Meng M, Mandal M. Efficient linear algorithm for magnetic localization and orientation in capsule endoscopy. *Conf Proc IEEE Eng Med Biol Soc* 2005; **7**: 7143-7146 [PMID: 17281923 DOI: 10.1109/IEMBS.2005.1616154]

54 **Wang X**, Meng MQ, Hu C. A localization method using 3-axis magnetoresistive sensors for tracking of capsule endoscope. *Conf Proc IEEE Eng Med Biol Soc* 2006; **1**: 2522-2525 [PMID: 17946518 DOI: 10.1109/IEMBS.2006.260711]

55 **Than TD**, Alici G, Zhou H, Li W. A review of localization systems for robotic endoscopic capsules. *IEEE Trans Biomed Eng* 2012; **59**: 2387-2399 [PMID: 22736628 DOI: 10.1109/TBME.2012.2201715]

56 **Pahlavan K**, Rao G, Ye Y, Makarov S, Khan U, Swar P, Cave D, Karellas A, Krishnamurthy P, Sayrafian K. RF Localization for Wireless Capsule Endoscopy. *Int J Wirel Inf Netw* 2012; **19**: 326-340 [DOI: 10.1007/s10776-012-0195-z]

57 **Pahlavan K**, Ye Y, Fu R, Khan U. Challenges in channel measurement and modeling for RF localization inside the human body. *Int J Embed Real-time Commun Syst* 2012; **3**: 18-37 [DOI: 10.4018/jertcs.2012070102]

58 **Fischer D**, Schreiber R, Levi D, Eliakim R. Capsule endoscopy: the localization system. *Gastrointest Endosc Clin N Am* 2004; **14**: 25-31 [PMID: 15062377 DOI: 10.1016/j.giec.2003.10.020]

59 **Marya N**, Karellas A, Foley A, Roychowdhury A, Cave D. Computerized 3-dimensional localization of a video capsule in the abdominal cavity: validation by digital radiography. *Gastrointest Endosc* 2014; **79**: 669-674 [PMID: 24424401 DOI: 10.1016/j.gie.2013.11.022]

60 **Fuccio L**, Zagari RM, Serrani M, Eusebi LH, Grilli D, Cennamo V, Laterza L, Asioli S, Ceroni L, Bazzoli F. Endoscopic argon plasma coagulation for the treatment of gastric antral vascular ectasia-related bleeding in patients with liver cirrhosis. *Digestion* 2009; **79**: 143-150 [PMID: 19329853 DOI: 10.1159/000210087]

61 **Lecleire S**, Ben-Soussan E, Antonietti M, Goria O, Riachi G, Lerebours E, Ducrotté P. Bleeding gastric vascular ectasia treated by argon plasma coagulation: a comparison between patients with and without cirrhosis. *Gastrointest Endosc* 2008; **67**: 219-225 [PMID: 18226684 DOI: 10.1016/j.gie.2007]

62 **Sebastian S**, McLoughlin R, Qasim A, O'Morain CA, Buckley MJ. Endoscopic argon plasma coagulation for the treatment of gastric antral vascular ectasia (watermelon stomach): long-term results. *Dig Liver Dis* 2004; **36**: 212-217 [PMID: 15046192 DOI: 10.1016/j.dld.2003.11.028]

63 **Kwan V**, Bourke MJ, Williams SJ, Gillespie PE, Murray MA, Kaffes AJ, Henriquez MS, Chan RO. Argon plasma coagulation in the management of symptomatic gastrointestinal vascular lesions: experience in 100 consecutive patients with long-term follow-up. *Am J Gastroenterol* 2006; **101**: 58-63 [PMID: 16405534 DOI: 10.1111/j.1572-0241.2006.00370.x]

64 **Herrera S**, Bordas JM, Llach J, Ginès A, Pellisé M, Fernández-Esparrach G, Mondelo F, Mata A, Cárdenas A, Castells A. The beneficial effects of argon plasma coagulation in the management of different types of gastric vascular ectasia lesions in patients admitted for GI hemorrhage. *Gastrointest Endosc* 2008; **68**: 440-446 [PMID: 18423466 DOI: 10.1016/j.gie.2008.02.009]

65 **Canard JM**, Védrenne B. Clinical application of argon plasma coagulation in gastrointestinal endoscopy: has the time come to replace the laser? *Endoscopy* 2001; **33**: 353-357 [PMID: 11315899 DOI: 10.1055/s-2001-13686]

66 **Park CH**, Joo YE, Kim HS, Choi SK, Rew JS, Kim SJ. A prospective, randomized trial of endoscopic band ligation versus endoscopic hemoclip placement for bleeding gastric Dieulafoy's lesions. *Endoscopy* 2004; **36**: 677-681 [PMID: 15280971 DOI: 10.1055/s-2004-825661]

67 **Wells CD**, Harrison ME, Gurudu SR, Crowell MD, Byrne TJ, Depetris G, Sharma VK. Treatment of gastric antral vascular ectasia (watermelon stomach) with endoscopic band ligation. *Gastrointest Endosc* 2008; **68**: 231-236 [PMID: 18533150 DOI: 10.1016/j.gie.2008.02.021]

68 **Mumtaz R**, Shaukat M, Ramirez FC. Outcomes of endoscopic treatment of gastroduodenal Dieulafoy's lesion with rubber band ligation and thermal/injection therapy. *J Clin Gastroenterol* 2003; **36**: 310-314 [PMID: 12642736 DOI: 10.1097/00004836-200304000-00006]

69 **van Cutsem E**, Rutgeerts P, Vantrappen G. Treatment of bleeding gastrointestinal vascular malformations with oestrogen-progesterone. *Lancet* 1990; **335**: 953-955 [PMID: 1970032 DOI: 10.1016/0140-6736(90)91010-8]

70 **Junquera F**, Feu F, Papo M, Videla S, Armengol JR, Bordas JM, Saperas E, Piqué JM, Malagelada JR. A multicenter, randomized, clinical trial of hormonal therapy in the prevention of rebleeding from gastrointestinal angiodysplasia. *Gastroenterology* 2001; **121**: 1073-1079 [PMID: 11677198 DOI: 10.1053/gast.2001.28650]

71 **McAllister KA**, Grogg KM, Johnson DW, Gallione CJ, Baldwin MA, Jackson CE, Helmbold EA, Markel DS, McKinnon WC, Murrell J. Endoglin, a TGF-beta binding protein of endothelial cells, is the gene for hereditary haemorrhagic tel-

angiectasia type 1. *Nat Genet* 1994; **8**: 345-351 [PMID: 7894484 DOI: 10.1038/ng1294-345]
72 **Sami SS**, Al-Araji SA, Ragunath K. Review article: gastrointestinal angiodysplasia - pathogenesis, diagnosis and management. *Aliment Pharmacol Ther* 2014; **39**: 15-34 [PMID: 24138285 DOI: 10.1111/apt.12527]
73 **Junquera F**, Saperas E, de Torres I, Vidal MT, Malagelada JR. Increased expression of angiogenic factors in human colonic angiodysplasia. *Am J Gastroenterol* 1999; **94**: 1070-1076 [PMID: 10201485 DOI: 10.1111/j.1572-0241.1999.01017.x]
74 **Shweiki D**, Itin A, Soffer D, Keshet E. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature* 1992; **359**: 843-845 [PMID: 1279431 DOI: 10.1038/359843a0]
75 **Stavri GT**, Zachary IC, Baskerville PA, Martin JF, Erusalimsky JD. Basic fibroblast growth factor upregulates the expression of vascular endothelial growth factor in vascular smooth muscle cells. Synergistic interaction with hypoxia. *Circulation* 1995; **92**: 11-14 [PMID: 7788904 DOI: 10.1161/01.CIR.92.1.11]
76 **Ge ZZ**, Chen HM, Gao YJ, Liu WZ, Xu CH, Tan HH, Chen HY, Wei W, Fang JY, Xiao SD. Efficacy of thalidomide for refractory gastrointestinal bleeding from vascular malformation. *Gastroenterology* 2011; **141**: 1629-1637.e1-4 [PMID: 21784047 DOI: 10.1053/j.gastro.2011.07.018]
77 **Szilagyi A**, Ghali MP. Pharmacological therapy of vascular malformations of the gastrointestinal tract. *Can J Gastroenterol* 2006; **20**: 171-178 [PMID: 16550261]
78 **Bauditz J**, Lochs H. Angiogenesis and vascular malformations: antiangiogenic drugs for treatment of gastrointestinal bleeding. *World J Gastroenterol* 2007; **13**: 5979-5984 [PMID: 18023086 DOI: 10.3748/wjg.13.5979]
79 **Nardone G**, Rocco A, Balzano T, Budillon G. The efficacy of octreotide therapy in chronic bleeding due to vascular abnormalities of the gastrointestinal tract. *Aliment Pharmacol Ther* 1999; **13**: 1429-1436 [PMID: 10571598 DOI: 10.1046/j.1365-2036.1999.00647.x]
80 **Brown C**, Subramanian V, Wilcox CM, Peter S. Somatostatin analogues in the treatment of recurrent bleeding from gastrointestinal vascular malformations: an overview and systematic review of prospective observational studies. *Dig Dis Sci* 2010; **55**: 2129-2134 [PMID: 20393879 DOI: 10.1007/s10620-010-1193-6]
81 **Schechter S**, Deorchis D, Bass J, Marcoux DA. Angiographic selective methylene blue staining of an occult small-bowel arteriovenous malformation. *Am Surg* 2002; **68**: 15-17 [PMID: 12467310]
82 **Schrodt JF**, Bradford WR. Presurgical angiographic localization of small bowel bleeding site with methylene blue injection. *J Ky Med Assoc* 1996; **94**: 192-195 [PMID: 8935401]
83 **D'Mello A**, Sawant AD, Kulkarni A, Joshi A, Shah SR. Methylene blue dye injection for identification of occult gastrointestinal bleeding. *Indian J Gastroenterol* 2014; **33**: 85-86 [PMID: 23990392 DOI: 10.1007/s12664-013-0380-8]
84 **Gerson LB**, Batenic MA, Newsom SL, Ross A, Semrad CE. Long-term outcomes after double-balloon enteroscopy for obscure gastrointestinal bleeding. *Clin Gastroenterol Hepatol* 2009; **7**: 664-669 [PMID: 19514115 DOI: 10.1016/j.cgh.2009.01.021]
85 **Lai LH**, Wong GL, Chow DK, Lau JY, Sung JJ, Leung WK. Long-term follow-up of patients with obscure gastrointestinal bleeding after negative capsule endoscopy. *Am J Gastroenterol* 2006; **101**: 1224-1228 [PMID: 16771942 DOI: 10.1111/j.1572-0241.2006.00565.x]
86 **Endo H**, Matsuhashi N, Inamori M, Akimoto K, Ohya T, Yanagawa T, Asayama M, Hisatomi K, Teratani T, Fujita K, Yoneda M, Nakajima A. Rebleeding rate after interventional therapy directed by capsule endoscopy in patients with obscure gastrointestinal bleeding. *BMC Gastroenterol* 2008; **8**: 12 [PMID: 18430253 DOI: 10.1186/1471-230X-8-12]
87 **Macdonald J**, Porter V, McNamara D. Negative capsule endoscopy in patients with obscure GI bleeding predicts low rebleeding rates. *Gastrointest Endosc* 2008; **68**: 1122-1127 [PMID: 19028220 DOI: 10.1016/j.gie.2008.06.054]
88 **Koh SJ**, Im JP, Kim JW, Kim BG, Lee KL, Kim SG, Kim JS, Jung HC. Long-term outcome in patients with obscure gastrointestinal bleeding after negative capsule endoscopy. *World J Gastroenterol* 2013; **19**: 1632-1638 [PMID: 23539070 DOI: 10.3748/wjg.v19.i10.1632]
89 **Delvaux M**, Fassler I, Gay G. Clinical usefulness of the endoscopic video capsule as the initial intestinal investigation in patients with obscure digestive bleeding: validation of a diagnostic strategy based on the patient outcome after 12 months. *Endoscopy* 2004; **36**: 1067-1073 [PMID: 15578296 DOI: 10.1055/s-2004-826034]
90 **Estévez E**, González-Conde B, Vázquez-Iglesias JL, de Los Angeles Vázquez-Millán M, Pértega S, Alonso PA, Clofent J, Santos E, Ulla JL, Sánchez E. Diagnostic yield and clinical outcomes after capsule endoscopy in 100 consecutive patients with obscure gastrointestinal bleeding. *Eur J Gastroenterol Hepatol* 2006; **18**: 881-888 [PMID: 16825907]

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REVIEW

Pathophysiological mechanisms linking obesity and esophageal adenocarcinoma

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Abstract

In recent decades there has been a dramatic rise in the incidence of esophageal adenocarcinoma (EAC) in the developed world. Over approximately the same period there has also been an increase in the prevalence of obesity. Obesity, especially visceral obesity, is an important independent risk factor for the development of gastro-esophageal reflux disease, Barrett's esophagus and EAC. Although the simplest explanation is that this mediated by the mechanical effects of abdominal obesity promoting gastro-esophageal reflux, the epidemiological data suggest that the EAC-promoting effects are independent of reflux. Several, not mutually exclusive, mechanisms have been implicated, which may have different effects at various points along the reflux-Barrett's-cancer pathway. These mechanisms include a reduction in the prevalence of *Helicobacter pylori* infection enhancing gastric acidity and possibly appetite by increasing gastric ghrelin secretion, induction of both low-grade systemic inflammation by factors secreted by adipose tissue and the metabolic syndrome with insulin-resistance. Obesity is associated with enhanced secretion of leptin and decreased secretion of adiponectin from adipose tissue and both increased leptin and decreased adiponectin have been shown to be independent risk factors for progression to EAC. Leptin and adiponectin have a set of mutually antagonistic actions on Barrett's cells which appear to influence the progression of malignant behaviour. At present no drugs are of proven benefit to prevent obesity associated EAC. Roux-en-Y reconstruction is the preferred bariatric surgical option for weight loss in patients with reflux. Statins and aspirin may have chemopreventative effects and are indicated for their circulatory benefits.

Key words: Adipose; Body mass index; Reflux; Barrett's esophagus

Core tip: Excess adipose tissue, particularly visceral obesity, is an important risk factor for esophageal adenocarcinoma (EAC). The mechanisms involve both the promotion of gastro-esophageal reflux and reflux-independent mechanisms. Abnormal secretion of the adipokines leptin and adiponectin from adipose tissue in obesity may promote the development of EAC. Increased leptin levels are an independent risk factor for EAC and leptin enhances proliferation and invasion and inhibits apoptosis in Barrett's cell lines. Relative adiponectin deficiency is an independent risk factor for EAC and adiponectin blocks the cancer promoting effects of leptin in experimental models. Obesity may influence EAC development *via* adipokine secretion.

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INTRODUCTION

Esophageal adenocarcinoma is a health problem of increasing global health significance. The overall prognosis of esophageal adenocarcinoma (EAC), the most prevalent form of esophageal cancer in the developed world, is dismal, with a 5-year survival of 15%-20% at best[1]. At the same time the incidence of this cancer has increased dramatically, by approximately 600% in the last 30 years, leading some commentators to call this an epidemic[2]. A detailed understanding of the pathogenic mechanisms leading to this malignancy is required to enable the development of strategies for both prevention and treatment. Over a similar period the prevalence of obesity has increased in the developed world and this increase in obesity has been linked with increased risks of several cancers, including oesophageal adenocarcinoma (OAC)[3,4]. Over the last 30 years rates of obesity have been increasing steadily, most obviously in the United States and Western Europe[5] but also in lower and middle income countries[6]. Estimates from The World Health Organisation suggest that 12% of the world's population aged over 20 years old is now obese which equates to approximately 500 million adults. It is estimated that 10% of men and 14% of women are obese by standard criteria. This has doubled from the 1980s[6].

Well-designed epidemiological investigations have been instrumental in detecting and defining the association between obesity and EAC. Relevant features of the association have been explored in detail to determine features of causality and to help clarify the potential implicated mechanisms through which obesity may act. This review summarises relevant recent observational data on the association between measures of obesity and risk of EAC, gastro-esophageal reflux and Barrett's esophagus; and experimental data of plausible mechanisms contributing to carcinogenesis and explores where and how interventions may reduce the burden of this disease.

CLINICAL MEASURES OF ADIPOSITY AND METHODOLOGICAL CONSIDERATIONS IN RELEVANT OBSERVATIONAL RESEARCH

The World Health Organization definition for overweight and obesity is abnormal or excessive fat accumulation that may impair health. Adiposity has been quantified in observational research relevant to this topic using a number of measures including anthropometric measurements and imaging. Overall adiposity is commonly measured using body mass index (BMI) [weight (kg) per height squared (m^2)], and central adiposity (synonymous to visceral and abdominal adiposity) is measured using waist-to-hip ratio (WTHR), waist circumference and anterior-posterior abdominal diameter (cm); and imaging such as visceral adipose tissue area (m^2) (VATA) or volume (m^3) as determined by computerised tomography or magnetic resonance imaging. While BMI is a simple measure of overall adiposity that is practical for large epidemiological studies, it is crude and does not necessarily reflect the varying proportions of fat and lean (non-fat) mass or body fat distribution. Measures of central obesity have been demonstrated to vary substantially within a narrow range of BMI, whilst central obesity itself is a combination of subcutaneous obesity around the abdomen and mesenteric adipose tissue. Furthermore, as body composition changes with age, and height decreases, for example through kyphosis and loss of vertebral height, BMI may be overestimated in older participants[7]. Imaging modalities used to quantify fat distribution, can precisely estimate the size of body fat compartments. However, they are less suitable for large scale prospective studies: they are often performed at diagnosis rather than for preceding time points and could therefore underestimate associations depending on weight loss associated with the diagnosis; and their use requires convenience sampling for controls (*e.g.*, patients undergoing investigation for other reasons), and therefore may be less representative of the population under study. These are important considerations when appraising the evidence on the risk of reflux, Barrett's esophagus and EAC with adiposity.

OBESITY AND RISK OF ESOPHAGEAL ADENOCARCINOMA

The association between measures of obesity and risk of OAC has been extensively examined in epidemiological investigations. The most striking evidence for a potential causal association between adiposity and risk of this cancer is the wealth of consistent data to suggest the association is among the strongest than for any other malignancy with evidence of a biological gradient[4]. A systematic review of prospective studies from Europe, Australia and the Asia-Pacific region, that measured BMI at baseline and followed participants until the development of incident cancer (hence supporting a temporal relationship), included 1315 male cases of OAC and 735 female cases, demonstrated the magnitude of the association in men was stronger than for any other malignancy, from 16 sites; and in women was only second to endometrial from 19 sites. The strength of the associations (per increase in BMI by 5 kg/m^2) was almost the same in both genders (RR = 1.52, 95%CI: 1.33-1.74 for men; RR = 1.51, 95%CI: 1.31-1.74 for women) with minimal heterogeneity. This implies the association between BMI and risk of EAC is consistent between well-designed prospective studies, further supporting the causality, and that sex-specific differences in the incidence of EAC are likely unrelated to adiposity as measured by BMI. Interestingly,

for squamous cell carcinoma, the most common histological type of esophageal cancer worldwide, in both men and women, risk was significantly reduced with increased BMI, more strongly than for any of the included malignancies[4]. A recent meta-analysis of five observational studies, including four prospective studies[8-11] and one case-control study[12], reported a significant association between abdominal obesity (as a composite measure of VATA, WHR, AC and abdominal diameter) and risk of EAC (adjusted OR = 2.51; 95%CI: 1.56-4.04)[13]. Indeed, the effect of abdominal obesity, as measured by WHR or AD, on risk of OAC has been reported to be independent of BMI[8,10]. This implies a role of abdominal obesity in the pathogenesis EAC over and above general obesity. Furthermore, the association between general or central adiposity and risk of EAC has been demonstrated to persist despite inclusion of plausible confounders in multivariable analyses, including: symptomatic reflux, physical activity, smoking, and intakes of total energy, red meat, fruit and vegetables[10].

Although the available cohort studies have not clearly shown that visceral adiposity is associated with an increased risk of invasive neoplasia in patients with Barrett' s esophagus, two recent studies have suggested that increased visceral fat tissue[14] or total abdominal obesity[15] are associated with the progression to high-grade dysplasia. Equally a recent meta-analysis including measures of central adiposity at least 5 years before the diagnosis of EAC showed a significant increased risk of cancer with central obesity[13]. Given our understanding of the biology of Barrett's esophagus, these data do suggest that central obesity promotes cancers in patients with Barrett's esophagus.

OBESITY AND RISK OF GASTRO-ESOPHAGEAL REFLUX

A commonly proposed mechanical explanation for the associations between obesity and EAC is through the following sequence: increased abdominal adiposity leading to increased intra-abdominal pressure, then consequent reflux predisposing to Barrett's esophagus and then EAC[16]. While either abdominal or central adiposity has been associated with each of these "steps"[7,17-19] it has not been possible so far to empirically demonstrate this whole sequence to be causal[17]. Measures of central obesity appear strongly associated with symptomatic reflux, independent of BMI, in a dose-dependent manner[20]. However, in patients with reflux, for each kg/m^2 increase in BMI, while both intra-gastric pressure and gastro-esophageal pressure gradient (GEPG) rise[18]; increments in GEPG are not associated with acid exposure as determined by 24-h pH monitoring. Therefore, obesity does not appear to promote reflux through a purely mechanical means, which suggests alternative obesity-induced mechanisms of esophageal dysfunction are operating. While increased reflux could feasibly contribute to the increased risk of EAC observed in obese persons, other mechanisms are likely at play as obesity is strongly associated with risk of EAC, independent of symptomatic reflux. A recent well-conceived Swedish population-based case-control study, which included 189 incident cases of EAC and 816 population-based controls, demonstrated the effect sizes for overweight BMI categories (> 25 kg/m^2) verses underweight BMI category (< 25 kg/m^2) on risk of EAC were not significantly different in adjusted or unadjusted models for severity, frequency or duration of reflux[21]. This study demonstrated significant synergy between BMI and reflux, most strikingly for frequency (for more than 3 times per week), but also for severity and duration of reflux, on risk of EAC. Other epidemiological studies have also demonstrated the reflux-independent effects of BMI[8,22-24] and abdominal obesity[8] on the risk of EAC, however, It should be noted these studies rely on the reporting of symptomatic reflux and do not necessarily reflect the actual amount of acid reflux.

It might be the location of the fat rather than pure BMI that is important. Abdominal obesity, rather than excess weight has been suggested as the true association of the increase in GORD. The association between BMI and GORD was attenuated when adjusted for waist circumference suggesting BMI has its affect by increasing abdominal obesity[25].

Although one large cross-sectional study found no association between GERD and waist circumference or waist: hip ratio[26], a considerable body of research suggests that an enlarged waist circumference increases the risk of erosive oesophagitis[27-29]. A Korean study of 5329 subjects reported an association between abdominal visceral adipose tissue volume, but not BMI or waist circumference, and erosive esophagitis[30]. Visceral adipose tissue has been assessed by CT scan and high levels of visceral adipose tissue were significantly associated with the duration of GERD symptoms[31]. The association is most obvious in the white population, which could help explain the high levels in the developed world. It is not associated with black or Asian ethnicities[20].

ADIPOSITY AND RISK OF BARRETT'S ESOPHAGUS

In a recent pooled analysis from the BEACON consortium, including 1102 cases of long segment BE (> 3 cm) and 1400 population-based controls from four case-control studies, increasing waist circumference was significantly associated with risk of BE, independent of BMI (OR = 1.87, 95%CI: 1.22-1.32) for the highest *vs* lowest quartile), with evidence of a significant biological gradient (OR = 1.16, 95%CI: 1.02-1.32, per 5 cm increase in waist circumference)[19]. The effect sizes for the association between waist circumference and risk of BE were similar in both men and women and were almost unchanged after adjustment for symptomatic reflux.

A meta-analysis reported that BMI per se was not associated with BE[32] but that increased waist circumference that confers a two-fold risk for BE[33]. Further

studies have reported similar findings as to the effect of visceral obesity on the risk of BE but have also shown an inverse relationship with glutofemoral obesity[34,35]. This could be due to the less metabolically active nature of glutofemoral adipose tissue which further supports the theory it is distribution of adipose tissue, not just overall increase in weight or the excess fat tissue that is a risk for BE. Recent studies have shown that even abdominal obesity is too crude a tool: it appears to be the visceral (mesenteric) component rather than the subcutaneous component that is the most important risk factor for BE[31,36].

Furthermore, the preponderance of BE in men is not explained by differential risk in men and women according to BMI alone. Although waist circumference increased the risk of BE in male and females, the association in females but not males is attenuated when adjusted for GORD symptoms[37]. It is possible that non-reflux related mechanisms contribute more to development of BE in males and these extra mechanisms could explain the higher male prevalence of BE. Abdominal subcutaneous fat was not associated with the development of BE, whereas visceral adiposity was[14].

The pathogeneses of GERD, BE and cancer are complex and multifactorial[38]. It is important to note that symptoms of GERD are fairly uniformly distributed globally (albeit generally less prevalent in Eastern countries compared to Western or Middle Eastern[38]) but the burden of erosive esophagitis, Barrett's esophagus and adenocarcinoma becomes increasingly concentrated in white males in the Western world[38]. Whilst this has an important correlation with exactly the group with the greatest increase in visceral obesity, it does limit the global generalizability of the data; whilst the links between obesity and GERD are generally consistent worldwide, the majority of the epidemiological data related to obesity, Barrett's and cancer, are from this most prevalent group and it is possible, although unproven that other factors may be more important in other racial groups or geographical areas.

WEIGHT LOSS AND RISK OF EAC

Unsurprisingly, to date there are is no significant body of literature on the effect of interventions to promote weight loss as a means to reduce the risk of BE or EAC. At a general population level the age-standardized incidence of EAC is relatively low (approximately 12 per 100000 per year in the United Kingdom)[2] and therefore a randomised controlled trial would require an unfeasibly large sample size to empirically demonstrate this. A clinical trial to determine whether or not a weight loss programme could reduce the risk of EAC in a group at higher risk of progression, such as those with known BE, may be more feasible. However, such a clinical trial would be problematic in interpretation as causality could not be attributed to obesity (or lack of) *per se*, but ascribed to the intervention designed to promote weight loss, which may plausibly act through a number of pathways (*e.g.*, exercise and diet). There is evidence that weight loss secondary to lifestyle, dietary changes or surgery is associated with a reduction in symptomatic reflux[39]. Ascribing causality to obesity on the risk of EAC can therefore only be determined through comprehension of the available epidemiological data on the key features of the association, which are consistent with a causal relationship, and an appraisal of laboratory data.

MOLECULAR MECHANISMS

Whilst the pathogenesis of EAC is not fully defined, increasingly the molecular changes are being understood[40]. Detailed discussion of the cellular and molecular changes leading to the development and persistence of the clone(s) of cells which give rise to initially Barrett's Esophagus and the later progression in some cases to adenocarcinoma are outside the scope of this review[41,42] but it seems clear that reflux of gastro-duodenal contents is involved in the initiation, perpetuation and progression of the esophageal changes. However there must be other factors also driving these changes. As reviewed above, there are considerable epidemiological data linking various markers of obesity with the development of EAC and several, not mutually exclusive, biologically plausible mechanisms will be explored (Figure 1). However exactly how these mechanisms associated with obesity interact to promote EAC remains unclear, but exploration of these mechanisms is likely to be fruitful in order to explore new treatment and preventative therapies.

POSSIBLE MECHANISMS LINKING OBESITY WITH ESOPHAGEAL ADENOCARCINOMA

The association is by chance

It is first necessary to consider that the association is merely chance and that obesity does not directly contribute to the pathogenesis of EAC. There have generally been parallel increases in obesity and EAC in the last few decades, and as discussed previously, obesity (especially abdominal visceral obesity) is clearly a risk factor for Barrett's esophagus and EAC[43]. Some inconsistencies in the data deserve further comment: there have been dramatic rises in the incidence of EAC incidence Australia and Denmark but with much more modest changes in obesity. The epidemic of EAC in the United Kingdom appeared to start about 10 years before that in the United States, yet the United Kingdom was about 10 years behind the United States in the increase in obesity rates[2]. Despite these uncertainties, the vast majority of the epidemiology showing obesity as a risk factor of EAC is compelling and obesity is also associated with the risk of many other cancers. There is biological plausibility and the relative risk of EAC with obesity is higher than other cancers, all suggesting that the association is real even if

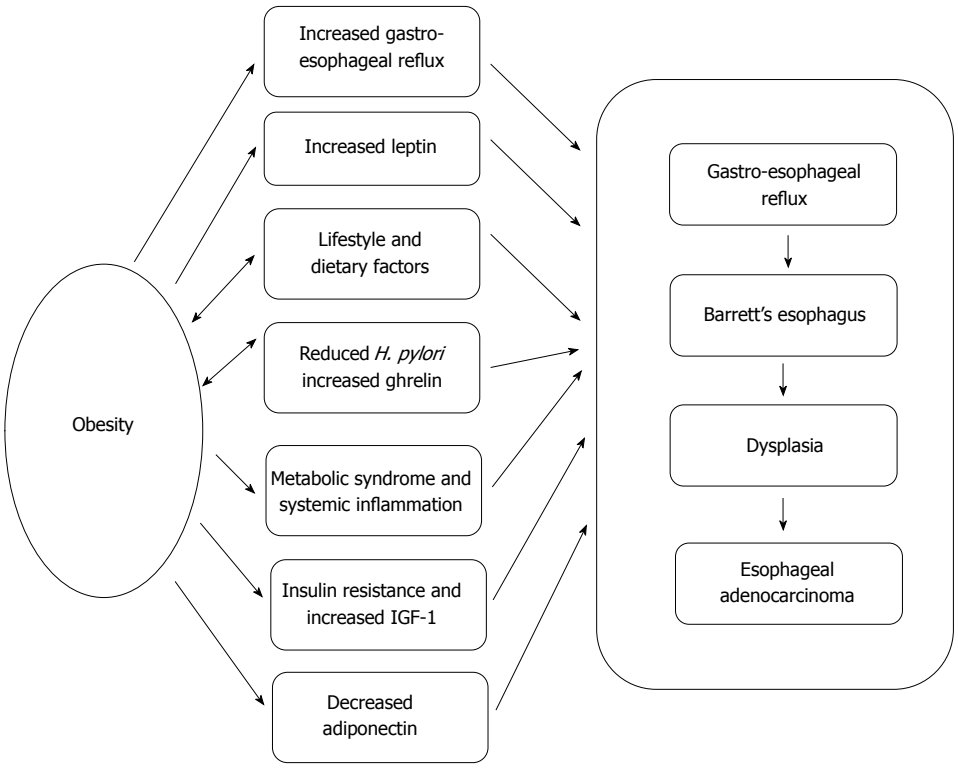

Figure 1 Possible mechanisms linking obesity with the development of esophageal adenocarcinoma. There are several potential and not mutually exclusive mechanisms that could link obesity and esophageal adenocarcinoma. Adipose tissue can exert both mechanical and endocrine effects that could enhance gastro-esophageal reflux and progression to adenocarcinoma. Decreased *H. pylori* could promote both gastro-esophageal reflux by increasing gastric acidity and increase body mass by enhancing production of the gastric appetite-stimulating peptide ghrelin.

obesity is not the sole driver of EAC[3].

Lifestyle or dietary factors associated with obesity increase the risk of EAC

It is possible that specific dietary or lifestyle factors associated with obesity promote EAC development. There are many potential individual variables in but no data specifically implicating any one factor. Smoking, for instance, is a risk factor for both Barrett's esophagus and progression to EAC[44,45] and lifestyle choices associated with significant obesity may be associated with greater proclivity to smoking but smoking is associated with a lower body mass, including patients with BE[46]. There is a complex and not completely understood inter-relationship between smoking, obesity, Barrett's esophagus and cancer. Whilst smoking does seem to be a consistent risk factor for progression to EAC[47], the effects on the development of BE are rather more variable; positive[46] and negative[48,49] associations have been reported and a meta-analysis concluded that being an "ever-smoker" was associated with an increased risk of BE when compared to population-based (OR = 1.42, 95%CI: 1.15-1.76) or non-GERD-controls (OR = 1.44, 95%CI: 1.20-1.74) but not GERD-controls (OR = (1.18, 95%CI: 0.75-1.86)[50]. In one study the positive association between EAC and smoking was removed after adjusting co-variables[51]. There are very limited data examining the combination of measures of obesity and smoking of the risks of BE and EAC. Hardikar *et al*[15] reported that the increased risk of progression to EAC associated with a high WTHR was only seen in male "never smokers" and not in male regular smokers. In a case-control study of endoscopy patients smoking was a risk factor for the development of BE: there was a suggestion that the risk associated with smoking was higher in the more obese (in those with BMI > 30, OR = 5.6, 95%CI: 1.7-18.3) than those of lower body weight (BMI < 30, OR = 3.0, 95%CI: 1.5-6.1)[46], but these were not statistically significant differences. Other studies have failed to show any interaction[23,51] or have not specifically explored any possible interaction[48,49,52]. The decline in the prevalence of smoking has occurred over the same period as EAC has increased and smoking would not explain the racial differences in EAC incidence. Thus although cigarette smoking itself seems to be a risk factor for BE and progression to EAC, there are insufficient data to implicate smoking as direct line between markers of obesity and development of EAC.

Although moderate-severe exercise acutely can precipitate gastro-esophageal reflux, regular exercise is associated with a lower rate of erosive oesophagitis and also protects against obesity[53]. It is possible certain dietary substances may promote both obesity and relaxation of the lower esophageal sphincter (LOS) so promoting reflux disease and EAC. Although there are few convincing data implicating any specific dietary constituents, several possibilities exist: it seems a high calorie content of meals

independently of fat content is most likely to provoke reflux[54] and chocolate promotes LOS-relaxation[53]. EAC is also associated with increased meat intake and reduced fruit and vegetable intake[55] and there are many other putative dietary components that could directly or indirectly promote EAC development in obese patients.

Increased gastro-esophageal reflux as the link between obesity and EAC

The link between EAC and fat tissue is much stronger for visceral obesity than overall obesity[43]. Perhaps the most obvious pathogenic link is that the visceral fat tissue exerts mechanical effects on the upper GI tract to promote gastro-esophageal reflux directly and hence the Barrett's-cancer sequence indirectly. There are considerable experimental data showing that acid and/or bile exert effects on the esophageal epithelium that would be expected to promote cancer (including stimulation of proliferation, inhibition of apoptosis, generation of free radicals[56-58], hence factors that provoke reflux would be expected to enhance the development and progression of Barrett's esophagus. There are some data to support this hypothesis: obesity is indeed associated with an increased prevalence and severity of reflux[59-61] and also with size of hiatus hernia[62], greater esophageal acid exposure[25], and increased transient lower esophageal relaxations[63]. However it seems likely that visceral fat tissue exerts both direct and indirect effects on the promotion of esophageal carcinogenesis, the majority of the data show that obesity is associated with Barrett's oesophagus and/or EAC independently of measures of reflux[21,31,64,65].

A separate factor or factors have increased EAC and obesity

One alternative hypothesis is that a separate factor or mechanism has promoted both obesity and EAC independently of each other. There are some data implicating *Helicobacter pylori* (*H. pylori*) infection in this situation. Infection of the stomach with *H. pylori*, particularly the CagA positive strains that provoke more intense gastric mucosal inflammation is inversely associated with both erosive esophagitis and BE[66]. The most plausible explanation for this is that infection and the resulting inflammation of the gastric body leads to a reduction in gastric acid secretion due to either local cytokine production[67] or more irreversible process due to the subsequent development of gastric atrophy[68]. Thus *H. pylori* infection would be associated with less reflux severe reflux disease due to relatively decreased gastric acid secretion. The prevalence of *H. pylori* infection has fallen, whilst the incidence of EAC has increased[69] over the last century. Weight gain is common after *H. pylori* eradication and hence a reduced prevalence of *H. pylori* could directly provoke more severe reflux disease and an overall increase in body mass. In a recent meta-analysis infection with CagA positive *H. pylori* was associated with a significantly lower risk of esophageal adenocarcinoma 0.74 (95%CI: 0.57-0.97), although no significant relationship was seen with CagA negative strains or between *H. pylori* and esophageal squamous cancer[70].

Changes in dyspeptic symptoms could underlie the weight gain but a more direct link between these two has been postulated *via* the role of gastric ghrelin. Ghrelin is a peptide hormone produced in, and secreted from, the P/D1 cells in the gastric body. Ghrelin stimulates appetite. *H. pylori* infection is associated with lower levels of gastric mucosa ghrelin and these mucosal levels increase after H. pylori eradication[71,72]. Hence it possible that lower levels of *H. pylori* infection are directly linked to obesity by increasing appetite. Whilst this is an attractive hypothesis and serum ghrelin levels have been shown increase after successful *H. pylori* eradication[73], this link between *H. pylori* status and circulating ghrelin has not be found consistently[74,75] to reliably increase after H. pylori eradication and higher plasma ghrelin levels have themselves been associated with both a lower incidence of erosive oesophagitis (possibly by enhancing gastric emptying[76] and also protection against esophageal adenocarcinoma[77]. However, more in keeping with this hypothesis high plasma ghrelin levels have been shown to be positively associated with the development of Barrett's esophagus[76]. The time course of *H. pylori* prevalence is not completely consistent with the changing epidemiology of EAC. The prevalence of H. pylori had been falling steadily throughout the 20th century well before the upsurge in EAC[69] and the increase in EAC incidence in Sweden seemed to begin in the early 1990s, well after the discovery and active treatment of H. pylori. The beginning of the upsurge in EAC in the United Kingdom began in the 1960s, well before the discovery of *H. pylori*. Hypothesizing that decreased gastric *H. pylori* infection as a direct cause of both obesity and EAC is also unable to explain the clear gender and racial differences in EAC[2] Therefore *H. pylori* infection and gastric ghrelin seem not be major contributors to the link between obesity and EAC but these potential mechanisms do outline the potential importance of factors influencing both appetite and mucosal biology.

Meta-inflammation and the metabolic syndrome

Adipose tissue is now recognised as a complex metabolically-active tissue, which secretes a variety of mediators that can have effects throughout the body. These mediators can conveniently, if rather simplistically be grouped into two: those relatively specific for adipose tissue, generally called adipokines or adipocytokines which include several important mediators including leptin, adiponectin, resistin and omentin, these are generally primarily involved in energy balance homeostasis and a second group of systemic cytokines that can be produced by a variety of tissues not limited to fat cells[3,78,79]. Most commentators now accept that obesity is a state of chronic low-grade, systemic inflammation, also termed "meta-inflammation". This is predominantly caused by the secretion of a variety of pro-inflammatory mediators by the fat tissue. These include tumour necrosis factor-α

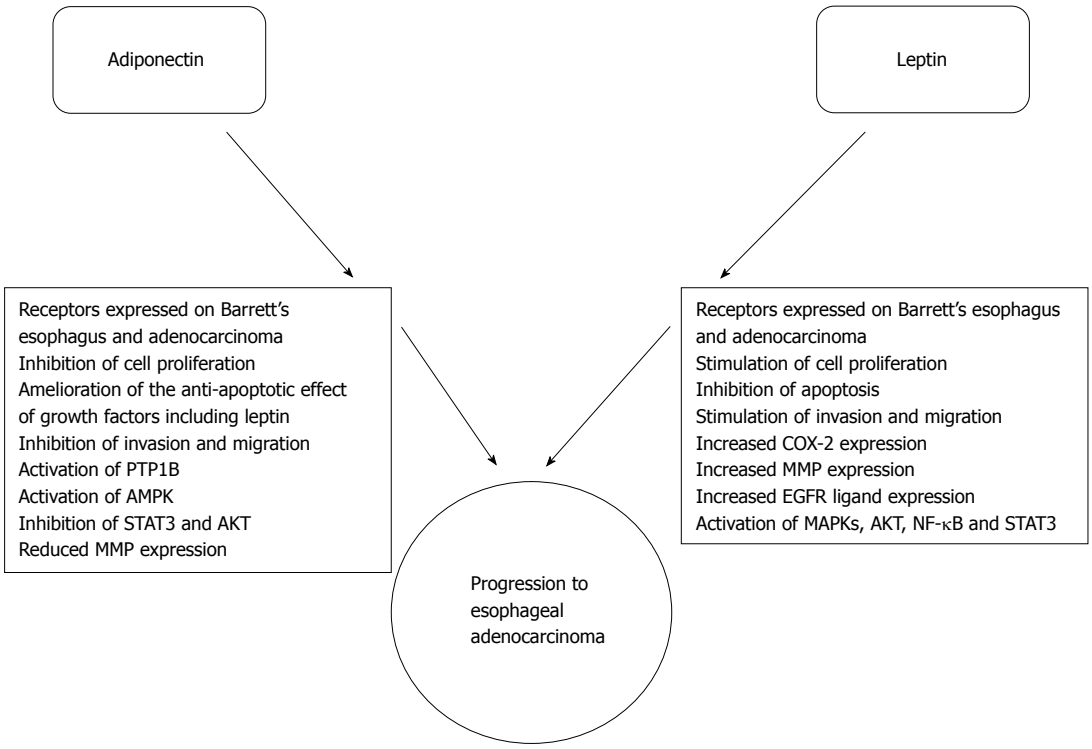

Figure 2 Effects of the adipokines leptin and adiponectin on Barrett's esophagus and esophageal adenocarcinoma. Obesity, more specifically visceral obesity, is associated with increased serum leptin and decreased serum adiponectin levels. Leptin and adiponectin have a set of antagonistic pathophysiological actions on Barrett's esophageal and adenocarcinoma cells.

(TNF-α), IL-1, IL-6, IL-8, interferon-β, MCP-1, VEGF and it is believed these mediators contribute not only to the development of the metabolic syndrome, with resulting insulin resistance and the related complications but also the increased risk of many cancers associated with obesity[79]. Systemic inflammation is recognised as a classical precursor to cancer, it is not completely understood how this systematic inflammatory state promotes cancer although, simplistically, many of these mediators promote cell proliferation, inhibit apoptosis and stimulate angiogenesis, all of which would be expected to promote cancer.

Faecal calprotectin, which is a marker of luminal inflammation, is increased in obesity[80]. There is an increased incidence of most gastrointestinal cancers associated with this obesity-induced inflammatory state[3], but the relative risk of EAC is higher than other cancers. Exactly how this meta-inflammation promotes EAC in the face of what would seem to be more severe and prolonged esophageal inflammation driven separately by acid and bile reflux is uncertain, although it again underlines the potential effects of circulating fat-derived mediators.

The meta-inflammation associated with obesity is associated with insulin resistance and increased circulating concentrations of both insulin and insulin growth factor-1 (IGF-1). This increase in insulin-related factors is at least partly driven by the secretions from metabolically-active visceral adipose tissue. As discussed above, a feature of obesity, and more specifically visceral obesity, is increased levels of inflammatory cytokine and mediators, including free fatty acids, TNF-α, leptin and resistin[81,82] and reduced secretion of adiponectin[83]. Insulin stimulates the production of IGF-1 and decreases production of the major serum proteins which bind insulin and IGF-1, insulin growth factor binding proteins 1 (IGFBP-1) and 3 (IGFBP-3)[84]. The overall effect is to increase the bioavailable levels of IGF-1. Both insulin and IGF-1 can bind to the insulin growth factor receptor complex, stimulating pathways that promote cellular proliferation. In a Barrett's cohort this insulin resistance has been associated with progression to adenocarcinoma[43]. Insulin and IGF-1 are mitogenic for many tissues, including Barrett's esophageal cells, which express IGF-1 receptors[85]. IGF-1 receptor expression is increased in EAC specimens resected from viscerally obese patients[86]. However the available data are conflicting on the role of IGF-1 and insulin as risk factors for malignant progression in BE. An increase in risk of cancer or BE have been reported[84,86], but other studies have failed to demonstrate any association between the metabolic syndrome and the risk of EAC [43] or between serum IGF-1 or IGFBP3 (the predominant serum binding protein) levels and progression of Barrett's[87].

Adipokines as effectors of the esophageal mucosal changes

These general inflammatory changes may be important in the development of EAC, but the specific role of adipokines is attracting considerable attention. Leptin and adiponectin have been examined in some detail and

it is possible they are a direct mechanistic link between obesity and progression to EAC (Figure 2). There are other adipokines such as resistin and omentin[88]: there no cell line or in vitro mechanistic studies examining the effects of these on esophageal tissues and there is a single epidemiological study showing that circulating resistin levels were higher in those with gastro-esophageal reflux disease than either Barrett's esophagus or controls[83] but further studies are required.

LEPTIN

Leptin is the archetypal adipokine. It is secreted as a 16 kDa protein from fat cells and serum levels are proportionate to body fat mass, as might be expected from it playing an important role as a regulator of appetite, energy metabolism and body weight. In the vast majority of obese subject, serum levels are significantly elevated and leptin deficiency is very rare cause of human obesity, in contrast to the gross obesity of the naturally occurring ob/ob leptin-deficient mouse[89]. It is thought that a degree of hypothalamic hyposensitivity to leptin is a more important cause of clinical obesity[90]. Many studies have reported that increased leptin levels are an independent risk factor for many cancers including breast, colorectal, prostate, ovarian, lung and endometrial[91]. Leptin levels have be an shown to be an independent risk factor for the development of Barrett's oesophagus[65,76,83], one study showed this effect was seen in male and not females[92] but another study confirmed this association in females[93]. Increased leptin levels have been shown to be an independent risk factor for progression to cancer in a cohort of patients with Barrett's esophagus[43]. Consistent with these data suggesting that leptin could directly affect the esophagus, leptin receptor expression has been detected in non-dysplastic Barrett's cell lines, esophageal adenocarcinoma cell lines, Barrett's esophagus and EAC[94-97]. One study has reported an association between increased leptin-receptor expression and more advanced stage in EAC[96].

Leptin promotes malignant behaviour in experimental esophageal cell line models. Leptin signals *via* the leptin receptor and increases proliferation, inhibits apoptosis, and stimulates migration and invasion. This is accompanied by the production of the matrix metalloproteinases (MMPs) MMP-2 and MMP-9 which are involved in invasion[94,98]. In a separate study, conditioned media from visceral adipocytes stimulated production of MMP-9 from esophageal adenocarcinoma cell lines and there is a clear association between *in vivo* MMP-9 production by EAC tissues and visceral obesity[99], suggesting that fat-derived mediators can influence esophageal epithelial behaviours, although the latter study did not confirm a specific role of leptin.

The cell signalling pathways involved in these leptin-induced effects have been well described[94,98]. Binding of leptin to the full-length receptor stimulates phosphorylation of the receptor-associated JAK2 tyrosine kinase which subsequently leads to activation of the protein kinase B/Akt and extra-cellular signal related kinase (ERK) cascades. The p38 MAP kinase pathway is also activated in a JAK2-independent manner downstream of the leptin receptor. The NF-κB pathway is activated, predominantly *via* upstream Akt activation. Inhibitor studies have shown that the ERK, Akt, NF-κB and p38 pathways are all essential to the proliferative and anti-apoptotic effects of leptin. Co-ordinated activation of these pathways leads to enhanced expression of the cyclo-oxygenase-2 (*COX-2*) gene. This in turn enhances prostaglandin E2 (PGE2) production. PGE2 leads to by transactivation of the epidermal growth factor receptor (EGFR) and subsequent EGFR-dependant activation of the mitogen activated protein kinase cascades and late activation of c-Jun N-terminal kinase. As well as stimulating the initial steps in the pathway, leptin increases mRNA expression of the EGFR ligands and heparin binding EGF (HB-EGF) in EAC cells and immunoneutralisation of these growth factors blocks the proliferative effects of leptin, confirming their role in the pathway[95].

A separate JAK2-dependant pathway leading activation of signal transducer and activator of transcription 3 (STAT3) is also stimulated by leptin in EAC cells. Activated STAT3 also essential to the proliferation, anti-apoptotic and pro-invasive effects of leptin[98].

The experimental data show that leptin is able to stimulate malignant behaviour in Barrett's cells bearing the leptin receptor and thus may be a direct link between obesity and progression to EAC. As discussed previously, obesity seems to promote the development of Barrett's oesophagus and EAC through both reflux-dependent and independent mechanisms. Epidemiologically this combination of obesity and reflux is associated with a cancer risk significantly greater than either alone or when summated[21,31,43,64,65]. There are interesting parallels in the experimental cell models. The combination of exposure to leptin (as a model for obesity) and transient acid exposure (as a model of transient acid reflux) produced significantly greater (and synergistic) cell proliferation and reduction in apoptosis in EAC cell lines[56]. This acid-leptin combination resulted in synergistic activation of the Akt and ERK signalling cascades, without any further increases in leptin-receptor expression, COX-2 expression, PGE2 production and phosphorylation of either p38 MAP kinase or the EGFR[56]. *In vivo*, esophageal acid exposure enhances MAP kinase activation and mucosal proliferation[100] and increased AKT activation is associated with decreased apoptosis and progression to high grade dysplasia and cancer[101] Therefore it is possible that the continued exposure to the high levels of serum leptin seen in obese subjects enhances the response of Barrett's mucosa to even physiological acid reflux and promotes malignant change.

In addition to adipocytes, leptin is also synthesised and secreted by chief cells in the gastric body and can be detected in gastric juice. The function of this luminally-secreted leptin is unclear but it is possible it is a physiological regulator of mucosal integrity or nutrient absorption. Therefore esophageal mucosa is potentially exposed

to both circulating leptin and that in gastric refluxate, again suggesting some point of convergence between reflux-dependant and -independent mechanisms. Further studies are required into the possible role of gastric leptin however the presence of Barrett's oesophagus has been associated with increased levels of gastric fundic leptin[102].

ADIPONECTIN

Adiponectin, a 30 kDa protein, is the predominant protein secreted by adipocytes. Unlike leptin, adiponectin secretion falls as obesity increases and so obesity is characterised by relative adiponectin deficiency. The exact mechanisms causing this inverse relationship between fat mass and adiponectin secretion are unclear[103]. As might be expected, in general the effects of adiponectin are to oppose those of leptin and relative adiponectin deficiency has been implicated in the pathogenesis of the metabolic syndrome and its complications, including systemic inflammation. In general low systemic adiponectin levels have been associated with an increased risk of many cancers (including breast, colorectal, prostate, endometrial and gastric)[104]. Comparison between studies is complicated by the various circulating forms of adiponectin, which may have different biological actions and are detected in different assays[105]. Adiponectin is secreted as a full-length monomer (f-adiponectin) than then aggregates into both low- molecular and high-molecular weight oligomers. A truncated form (globular adiponectin (g-adiponectin)) is also found, this is at least partly formed by breakdown of full-length adiponectin by enzymes released in inflammation and circulating levels may not accurately reflect tissue levels of g-adiponectin[106]. There at two specific cell surface adiponectin receptors: AdipoR1 which appears to be relatively globular adiponectin specific and AdipoR2 which has equal affinity for globular and full length adiponectin[107]. Adiponectin may also be able to exert cellular effects by binding to, and inhibiting the action of, HB-EGF[108].

Data are available to support relative adiponectin deficiency in the promotion of EAC (Figure 2). AdipoR1 and AdipoR2 are expressed on both non-dysplastic and neoplastic Barrett's epithelium[97,109,110]. Circulating adiponectin levels have been shown to be inversely associated with the risk of both Barrett's oesophagus[83,93,111], and erosive oesophagitis[112]. Increased levels of low-molecular weight adiponectin and a high low-molecular weight/total adiponectin ratio have been shown to be independently associated with a reduced risk of developing Barrett's esophagus in patients with gastro-esophageal reflux disease[113].This relationship has not been seen in all studies[92]. Perhaps more convincingly low serum levels of adiponectin have been reported to be an independent risk factor for neoplastic progression in a cohort of Barrett's patients[43].

In a variety of experimental studies adiponectin has been shown to exert anti-cancer effects in Barrett's cancer cell lines. Adiponectin inhibits leptin-induced proliferation, inhibits leptin-induced invasion and migration and ameliorates the anti-apoptotic effect of leptin. Inhibition of AdipoR1 with RNA interference prevented these effects. Downstream of AdipoR1, these effects are mediated by 5'-AMP activated kinase (AMPK), which ultimately leads to blunting of leptin signalling *via* inhibition of the Akt pathway[110]. Further detailed studies have shown these inhibitory effects are mediated by the activation of the relatively non-specific protein tyrosine phosphatase PTP1B. Adiponectin leads to increases in both PTP1B mRNA and protein expression and also a separate activation of PTP1B enzyme activity. Activation of this tyrosine phosphatase inhibits signalling *via* the leptin receptor. These experimental models provide a basis to explain how leptin, adiponectin and acid may interact at the cellular level to promote either the promotion or persistence of Barrett's epithelium or malignant behaviour in cancer cells and how obesity can remotely influence the risk so EAC[98]. Although speculative at this stage, this potential mechanism of adiponectin *via* PTP1B could have wider importance. PTP1B is a relatively non-specific phosphatase and would also be expected to inhibit signalling *via* other pathways that are believed to be important in driving malignant behaviour in Barrett's epithelium, such as EGFR ligands, IL-6 and bile acids or even those pathways leading to cdx2 expression, which are believed to be central to the development of the Barrett's phenotype[41,114,115]. Hence relative adiponectin deficiency in obesity could contribute to the development and progression of Barrett's esophagus at many steps.

The different types of adipose tissue have different hormonal effects. As discussed previously EAC and Barrett's are most clearly associated with abdominal rather than general obesity[31]. Even within this abdominal obesity there are variable contributions from the separate visceral and subcutaneous fat tissues. More specifically, excess visceral fat being specifically associated with Barrett's esophagus. Gluteofemoral fat ("hips") (which is subcutaneous fat) does not seem to be a specific risk factor of BE and may even be protective[34] It is thought gluteofemoral and subcutaneous fat is even less metabolically active and has less effect on progression of Barrett's oesophagus. In light of this, it is believed that visceral, rather than subcutaneous, fat is usually the predominant source of circulating adiponectin[116-118] and this might explain how reduction in adiponectin secretion from visceral fat probably specifically contributes to the Barrett's-carcinoma sequence.

IMPLICATIONS FOR THERAPY

The fact that obesity is a risk factor for both BE and EAC is established. This is already being translated into the clinical arena: for example the British Society of Gastroenterology guidelines now suggest that screening and case finding for Barrett's esophagus be considered

in males with reflux symptoms and at least two other risk factors (Caucasian, obesity, smoker), this has the advantage of detecting premalignant cases of Barrett' s esophagus that may be amenable to surveillance and endoscopic therapies if required[119]. A broader question is how may our understanding of the pathophysiological links between obesity and EAC be translated into useful therapeutic gains for prevention or treatment?

The mechanisms linking obesity and esophageal are undoubtedly complex and likely multifactorial and are likely to differ depending on the histological stage of the esophageal mucosa. Experimental and epidemiological studies support a role of the adipokines leptin and adiponectin in the progression to EAC but further mechanistic and clinical studies are still required. At the present time, these pathophysiological insights have suggested several new areas of therapy.

Although it is accepted that gastro-esophageal reflux plays a central role in the pathogenesis of BE and EAC and appears to accentuate the risks associated with obesity, profound acid suppression with either proton pump inhibitors or anti-reflux surgery have not conclusively been shown to have chemopreventative effects. The large United Kingdom AspECT trial comparing placebo, aspirin and standard- and very high dose-esomeprazole in a randomized trial may provide clarity on this issue when data become available[120].

At both a population and individual level weight loss with dietary and behavioural modifications remains the first line approach for obese patients. Gastric bands tend to accentuate reflux and for those patients with reflux symptoms and significant obesity[121], a Roux-en-Y gastric bypass appears to be the preferable procedure, although it cannot be advocated purely to prevent esophageal cancer. Interestingly, as well as a reduction in body mass and visceral fat, and reducing symptoms from gastro-esophageal reflux, this procedure is associated with potentially beneficial metabolic effects including higher serum adiponectin[39,122].

There may yet be some developments in therapies aimed to improve the metabolic/endocrine profile of adipose tissue that may translate into useful clinical interventions. Antagonists of CB1 receptors, such as rimonabant reduce visceral fat[123] and the PPAR-α agonists such as rosiglitazone enhance adiponectin release from visceral fat[118]. Unfortunately at the present time the adverse effects; psychiatric problems with rimonabant and bladder cancer and the increased cardiovascular mortality with PPAR-α agonists preclude their wider use. A variety of other agents have been shown to usefully increase serum adiponectin levels: these include PPAR-α agonists, inhibitors of the renin-angiotensin system, calcium channel modulators and some beta-receptor antagonists[124] and various phyochemical such as catechin[125]. All these deserve further study, although at this time, data are limited and these drugs and their effect on adipokine profiles have not been investigated in the context of esophageal disease[124]. Preclinical development of adiponectin-analogues[126] and leptin-receptor antagonists[127] is continuing but these are some way off clinical use.

Metformin seems to have some potential as a chemopreventative agent in the context of obesity-associated EAC. Metformin is a direct activator of AMPK kinase and exerts potentially useful anti-cancer effects[128]. In Barrett's cell line studies, the inhibitory effects of adiponectin are also mediated *via* activation of AMPK[110]. In case-control studies, metformin use is associated with a reduced incidence of many cancers including esophageal cancer[129]. Metformin is inexpensive, has a low incidence of side effects and could be promising chemopreventative agent, although more studies specifically in EAC are needed.

There appear to be more data to recommend aspirin and statins (HMG-CoA reductase inhibitors) as the most appropriate potential chemopreventative agents to reduce the incidence of EAC associated with, or indeed without obesity. Several lines of experimental data show that cyclooxygenase inhibitors, such as aspirin, reduce malignant behaviours such as proliferation and apoptosis-inhibition in EAC and non-neoplastic Barrett's cells lines. Non-specific and COX-2 selective inhibitors block the effects of leptin in cell line models[130-132]. Definitive conclusions on the preventative effects of aspirin may have to wait until the UK AspECT trial has reported[120]. Observational studies and meta-analyses show that aspirin use is associated with a reduced incidence of both Barrett's esophagus and EAC[133,134]. Statins exert potent anti-cancer effects in EAC cells line models. By inhibiting the post-translational modification (prenylation) of small signalling G protein of the Ras/Rho family and so ameliorating pro-carcinogenic signalling from growth factor receptors, statins inhibit cell growth and induce apoptosis[135]. Experimentally the effect of inhibition of the COX-2/PGE2 pathway, by using a variety of small molecule COX-inhibitors, inhibition of microsomal PGES-1 or RNA interference, and the effect statins were additive[131,135]. A similar magnitude of reduced risk has been reported in two separate meta-analyses of Barrett's cohorts, where the combination of COX-inhibitor and statin was associated with a 85% reduction in EAC incidence[133,136]. Statins may also have beneficial effects by increasing increase serum adiponectin levels[124]. It is probably premature to advocate aspirin and statin therapy as primary preventative therapy for all. It is essential to consider that cardiovascular disease and not EAC is predominant cause of death in Barrett's cohorts and hence that statins and aspirin should be utilised for the beneficial effects on circulatory diseases pending further clarification of the chemopreventative actions[131].

CONCLUSION

Overall a large amount of epidemiological data shows that obesity is likely to be causally associated with esophageal adenocarcinoma. This cancer is strongly associated with an increase in BMI, in fact more so than for other cancers. There are also strong associations between mea-

sures of adiposity and gastro-esophageal reflux including the more serious sequelae, reflux esophagitis and Barrett's esophagus. Abdominal, and in particular visceral, obesity is likely to play a key role in its pathogenesis though both reflux-dependent and -independent mechanisms. Leptin and adiponectin are adipokines secreted by visceral fat cells and both an increased serum leptin decreased serum adiponectin have been reported to be risk factors for progression to EAC. Experimentally, leptin enhances, and adiponectin inhibits malignant behaviour in Barrett's cell lines, consistent with these mediators having a direct role in the pathogenesis of EAC. No specific chemopreventative strategies are of proven benefit, but appropriate weight loss in overweight subjects seems appropriate. Aspirin and statins seem to have the most potential as chemopreventative actions and should be utilized in patients with Barrett's esophagus according to the cardiovascular risk profile.

REFERENCES

1 **Jemal A**, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin* 2010; **60**: 277-300 [PMID: 20610543 DOI: 10.3322/caac.20073]

2 **Edgren G**, Adami HO, Weiderpass E, Nyrén O. A global assessment of the oesophageal adenocarcinoma epidemic. *Gut* 2013; **62**: 1406-1414 [PMID: 22917659 DOI: 10.1136/gutjnl-2012-302412]

3 **Alemán JO**, Eusebi LH, Ricciardiello L, Patidar K, Sanyal AJ, Holt PR. Mechanisms of obesity-induced gastrointestinal neoplasia. *Gastroenterology* 2014; **146**: 357-373 [PMID: 24315827 DOI: 10.1053/j.gastro.2013.11.051]

4 **Renehan AG**, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet* 2008; **371**: 569-578 [PMID: 18280327 DOI: 10.1016/S0140-6736(08)60269-X]

5 **Nguyen DM**, El-Serag HB. The epidemiology of obesity. *Gastroenterol Clin North Am* 2010; **39**: 1-7 [PMID: 20202574 DOI: 10.1016/j.gtc.2009.12.014]

6 **Popkin BM**, Adair LS, Ng SW. Global nutrition transition and the pandemic of obesity in developing countries. *Nutr Rev* 2012; **70**: 3-21 [PMID: 22221213 DOI: 10.1111/j.1753-4887.2011.00456.x]

7 **Snijder MB**, van Dam RM, Visser M, Seidell JC. What aspects of body fat are particularly hazardous and how do we measure them? *Int J Epidemiol* 2006; **35**: 83-92 [PMID: 16339600 DOI: 10.1093/ije/dyi253]

8 **Corley DA**, Kubo A, Zhao W. Abdominal obesity and the risk of esophageal and gastric cardia carcinomas. *Cancer Epidemiol Biomarkers Prev* 2008; **17**: 352-358 [PMID: 18268119 DOI: 10.1158/1055-9965.EPI-07-0748]

9 **MacInnis RJ**, English DR, Hopper JL, Giles GG. Body size and composition and the risk of gastric and oesophageal adenocarcinoma. *Int J Cancer* 2006; **118**: 2628-2631 [PMID: 16353151 DOI: 10.1002/ijc.21638]

10 **O'Doherty MG**, Freedman ND, Hollenbeck AR, Schatzkin A, Abnet CC. A prospective cohort study of obesity and risk of oesophageal and gastric adenocarcinoma in the NIH-AARP Diet and Health Study. *Gut* 2012; **61**: 1261-1268 [PMID: 22174193 DOI: 10.1136/gutjnl-2011-300551]

11 **Steffen A**, Schulze MB, Pischon T, Dietrich T, Molina E, Chirlaque MD, Barricarte A, Amiano P, Quirós JR, Tumino R, Mattiello A, Palli D, Vineis P, Agnoli C, Misirli G, Boffetta P, Kaaks R, Rohrmann S, Bueno-de-Mesquita HB, Peeters PH, May AM, Spencer EA, Allen NE, Bingham S, Tjønneland A, Halkjaer J, Overvad K, Stegger J, Manjer J, Lindkvist B, Hallmanns G, Stenling R, Lund E, Riboli E, Gonzalez CA, Boeing H. Anthropometry and esophageal cancer risk in the European prospective investigation into cancer and nutrition. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 2079-2089 [PMID: 19567501 DOI: 10.1158/1055-9965.EPI-09-0265]

12 **Beddy P**, Howard J, McMahon C, Knox M, de Blacam C, Ravi N, Reynolds JV, Keogan MT. Association of visceral adiposity with oesophageal and junctional adenocarcinomas. *Br J Surg* 2010; **97**: 1028-1034 [PMID: 20632268 DOI: 10.1002/bjs.7100]

13 **Singh S**, Sharma AN, Murad MH, Buttar NS, El-Serag HB, Katzka DA, Iyer PG. Central adiposity is associated with increased risk of esophageal inflammation, metaplasia, and adenocarcinoma: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2013; **11**: 1399-1412.e7 [PMID: 23707461 DOI: 10.1016/j.cgh.2013.05.009]

14 **Nelsen EM**, Kirihara Y, Takahashi N, Shi Q, Lewis JT, Namasivayam V, Buttar NS, Dunagan KT, Prasad GA. Distribution of body fat and its influence on esophageal inflammation and dysplasia in patients with Barrett's esophagus. *Clin Gastroenterol Hepatol* 2012; **10**: 728-734; quiz e61-62 [PMID: 22433923 DOI: 10.1016/j.cgh.2012.03.007]

15 **Hardikar S**, Onstad L, Blount PL, Odze RD, Reid BJ, Vaughan TL. The role of tobacco, alcohol, and obesity in neoplastic progression to esophageal adenocarcinoma: a prospective study of Barrett's esophagus. *PLoS One* 2013; **8**: e52192 [PMID: 23300966 DOI: 10.1371/journal.pone.0052192]

16 **Lagergren J**. Influence of obesity on the risk of esophageal disorders. *Nat Rev Gastroenterol Hepatol* 2011; **8**: 340-347 [PMID: 21643038 DOI: 10.1038/nrgastro.2011.73]

17 **Anggiansah R**, Sweis R, Anggiansah A, Wong T, Cooper D, Fox M. The effects of obesity on oesophageal function, acid exposure and the symptoms of gastro-oesophageal reflux disease. *Aliment Pharmacol Ther* 2013; **37**: 555-563 [PMID: 23305085 DOI: 10.1111/apt.12208]

18 **de Vries DR**, van Herwaarden MA, Smout AJ, Samsom M. Gastroesophageal pressure gradients in gastroesophageal reflux disease: relations with hiatal hernia, body mass index, and esophageal acid exposure. *Am J Gastroenterol* 2008; **103**: 1349-1354 [PMID: 18510603 DOI: 10.1111/j.1572-0241.2008.01909.x]

19 **Kubo A**, Cook MB, Shaheen NJ, Vaughan TL, Whiteman DC, Murray L, Corley DA. Sex-specific associations between body mass index, waist circumference and the risk of Barrett's oesophagus: a pooled analysis from the international BEACON consortium. *Gut* 2013; **62**: 1684-1691 [PMID: 23355549 DOI: 10.1136/gutjnl-2012-303753]

20 **Corley DA**, Kubo A, Zhao W. Abdominal obesity, ethnicity and gastro-oesophageal reflux symptoms. *Gut* 2007; **56**: 756-762 [PMID: 17047097 DOI: 10.1136/gut.2006.109413]

21 **Lagergren J**, Mattsson F, Nyrén O. Gastroesophageal reflux does not alter effects of body mass index on risk of esophageal adenocarcinoma. *Clin Gastroenterol Hepatol* 2014; **12**: 45-51 [PMID: 23920035 DOI: 10.1016/j.cgh.2013.07.027]

22 **Chow WH**, Blot WJ, Vaughan TL, Risch HA, Gammon MD, Stanford JL, Dubrow R, Schoenberg JB, Mayne ST, Farrow DC, Ahsan H, West AB, Rotterdam H, Niwa S, Fraumeni JF. Body mass index and risk of adenocarcinomas of the esophagus and gastric cardia. *J Natl Cancer Inst* 1998; **90**: 150-155 [PMID: 9450576 DOI: 10.1093/jnci/90.2.150]

23 **Whiteman DC**, Sadeghi S, Pandeya N, Smithers BM, Gotley DC, Bain CJ, Webb PM, Green AC. Combined effects of obesity, acid reflux and smoking on the risk of adenocarcinomas of the oesophagus. *Gut* 2008; **57**: 173-180 [PMID: 17932103 DOI: 10.1136/gut.2007.131375]

24 **Lindblad M**, Rodríguez LA, Lagergren J. Body mass, tobacco and alcohol and risk of esophageal, gastric cardia, and

gastric non-cardia adenocarcinoma among men and women in a nested case-control study. *Cancer Causes Control* 2005; **16**: 285-294 [PMID: 15947880]

25 **El-Serag HB**, Ergun GA, Pandolfino J, Fitzgerald S, Tran T, Kramer JR. Obesity increases oesophageal acid exposure. *Gut* 2007; **56**: 749-755 [PMID: 17127706 DOI: 10.1136/gut.2006.100263]

26 **Chen T**, Lu M, Wang X, Yang Y, Zhang J, Jin L, Ye W. Prevalence and risk factors of gastroesophageal reflux symptoms in a Chinese retiree cohort. *BMC Gastroenterol* 2012; **12**: 161 [PMID: 23153099 DOI: 10.1186/1471-230X-12-161]

27 **Tai CM**, Lee YC, Tu HP, Huang CK, Wu MT, Chang CY, Lee CT, Wu MS, Lin JT, Wang WM. The relationship between visceral adiposity and the risk of erosive esophagitis in severely obese Chinese patients. *Obesity* (Silver Spring) 2010; **18**: 2165-2169 [PMID: 20559298 DOI: 10.1038/oby.2010.143]

28 **Ha NR**, Lee HL, Lee OY, Yoon BC, Choi HS, Hahm JS, Ahn YH, Koh DH. Differences in clinical characteristics between patients with non-erosive reflux disease and erosive esophagitis in Korea. *J Korean Med Sci* 2010; **25**: 1318-1322 [PMID: 20808675 DOI: 10.3346/jkms.2010.25.9.1318]

29 **Kang MS**, Park DI, Oh SY, Yoo TW, Ryu SH, Park JH, Kim HJ, Cho YK, Sohn CI, Jeon WK, Kim BI. Abdominal obesity is an independent risk factor for erosive esophagitis in a Korean population. *J Gastroenterol Hepatol* 2007; **22**: 1656-1661 [PMID: 17845694 DOI: 10.1111/j.1440-1746.2006.04518.x]

30 **Nam SY**, Choi IJ, Ryu KH, Park BJ, Kim HB, Nam BH. Abdominal visceral adipose tissue volume is associated with increased risk of erosive esophagitis in men and women. *Gastroenterology* 2010; **139**: 1902-1911.e2 [PMID: 20727886 DOI: 10.1053/j.gastro.2010.08.019]

31 **El-Serag HB**, Hashmi A, Garcia J, Richardson P, Alsarraj A, Fitzgerald S, Vela M, Shaib Y, Abraham NS, Velez M, Cole R, Rodriguez MB, Anand B, Graham DY, Kramer JR. Visceral abdominal obesity measured by CT scan is associated with an increased risk of Barrett's oesophagus: a case-control study. *Gut* 2014; **63**: 220-229 [PMID: 23408348 DOI: 10.1136/gutjnl-2012-304189]

32 **Cook MB**, Greenwood DC, Hardie LJ, Wild CP, Forman D. A systematic review and meta-analysis of the risk of increasing adiposity on Barrett's esophagus. *Am J Gastroenterol* 2008; **103**: 292-300 [PMID: 17986313 DOI: 10.1111/j.1572-0241.2007.01621.x]

33 **Kramer JR**, Fischbach LA, Richardson P, Alsarraj A, Fitzgerald S, Shaib Y, Abraham NS, Velez M, Cole R, Anand B, Verstovsek G, Rugge M, Parente P, Graham DY, El-Serag HB. Waist-to-hip ratio, but not body mass index, is associated with an increased risk of Barrett's esophagus in white men. *Clin Gastroenterol Hepatol* 2013; **11**: 373-381.e1 [PMID: 23220167 DOI: 10.1016/j.cgh.2012.11.028]

34 **Rubenstein JH**, Morgenstern H, Chey WD, Murray J, Scheiman JM, Schoenfeld P, Appelman HD, McMahon L, Metko V, Kellenberg J, Kalish T, Baker J, Inadomi JM. Protective role of gluteofemoral obesity in erosive oesophagitis and Barrett's oesophagus. *Gut* 2014; **63**: 230-235 [PMID: 23461896 DOI: 10.1136/gutjnl-2012-304103]

35 **Edelstein ZR**, Farrow DC, Bronner MP, Rosen SN, Vaughan TL. Central adiposity and risk of Barrett's esophagus. *Gastroenterology* 2007; **133**: 403-411 [PMID: 17681161 DOI: 10.1053/j.gastro.2007.05.026]

36 **Akiyama T**, Yoneda M, Inamori M, Iida H, Endo H, Hosono K, Yoneda K, Fujita K, Koide T, Tokoro C, Takahashi H, Goto A, Abe Y, Kirikoshi H, Kobayashi N, Kubota K, Saito S, Nakajima A. Visceral obesity and the risk of Barrett's esophagus in Japanese patients with non-alcoholic fatty liver disease. *BMC Gastroenterol* 2009; **9**: 56 [PMID: 19622165 DOI: 10.1186/1471-230X-9-56]

37 **Kendall BJ**, Macdonald GA, Hayward NK, Prins JB, O' Brien S, Whiteman DC. The risk of Barrett's esophagus associated with abdominal obesity in males and females. *Int J Cancer* 2013; **132**: 2192-2199 [PMID: 23034724 DOI: 10.1002/ijc.27887]

38 **Boeckxstaens G**, El-Serag HB, Smout AJ, Kahrilas PJ. Symptomatic reflux disease: the present, the past and the future. *Gut* 2014; **63**: 1185-1193 [PMID: 24607936 DOI: 10.1136/gutjnl-2013-306393]

39 **De Groot NL**, Burgerhart JS, Van De Meeberg PC, de Vries DR, Smout AJ, Siersema PD. Systematic review: the effects of conservative and surgical treatment for obesity on gastro-oesophageal reflux disease. *Aliment Pharmacol Ther* 2009; **30**: 1091-1102 [PMID: 19758397 DOI: 10.1111/j.1365-2036.2009.04146.x]

40 **Gibson MK**, Dhaliwal AS, Clemons NJ, Phillips WA, Dvorak K, Tong D, Law S, Pirchi ED, Räsänen J, Krasna MJ, Parikh K, Krishnadath KK, Chen Y, Griffiths L, Colleypriest BJ, Farrant JM, Tosh D, Das KM, Bajpai M. Barrett's esophagus: cancer and molecular biology. *Ann N Y Acad Sci* 2013; **1300**: 296-314 [PMID: 24117650]

41 **Dvorak K**, Goldman A, Kong J, Lynch JP, Hutchinson L, Houghton JM, Chen H, Chen X, Krishnadath KK, Westra WM. Molecular mechanisms of Barrett's esophagus and adenocarcinoma. *Ann N Y Acad Sci* 2011; **1232**: 381-391 [PMID: 21950830 DOI: 10.1111/j.1749-6632.2011.06062.x]

42 **Fang Y**, Chen X, Bajpai M, Verma A, Das KM, Souza RF, Garman KS, Donohoe CL, O'Farrell NJ, Reynolds JV, Dvorak K. Cellular origins and molecular mechanisms of Barrett's esophagus and esophageal adenocarcinoma. *Ann N Y Acad Sci* 2013; **1300**: 187-199 [PMID: 24117642 DOI: 10.1111/nyas.12249]

43 **Duggan C**, Onstad L, Hardikar S, Blount PL, Reid BJ, Vaughan TL. Association between markers of obesity and progression from Barrett's esophagus to esophageal adenocarcinoma. *Clin Gastroenterol Hepatol* 2013; **11**: 934-943 [PMID: 23466711 DOI: 10.1016/j.cgh.2013.02.017]

44 **Cook MB**, Shaheen NJ, Anderson LA, Giffen C, Chow WH, Vaughan TL, Whiteman DC, Corley DA. Cigarette smoking increases risk of Barrett's esophagus: an analysis of the Barrett's and Esophageal Adenocarcinoma Consortium. *Gastroenterology* 2012; **142**: 744-753 [PMID: 22245667 DOI: 10.1053/j.gastro.2011.12.049]

45 **Pohl H**, Wrobel K, Bojarski C, Voderholzer W, Sonnenberg A, Rösch T, Baumgart DC. Risk factors in the development of esophageal adenocarcinoma. *Am J Gastroenterol* 2013; **108**: 200-207 [PMID: 23247577 DOI: 10.1038/ajg.2012.387]

46 **Balasubramanian G**, Gupta N, Giacchino M, Singh M, Kanakadandi V, Gaddam S, Wani SB, Higbee AD, Rastogi A, Bansal A, Sharma P. Cigarette smoking is a modifiable risk factor for Barrett's oesophagus. *United European Gastroenterol J* 2013; **1**: 430-437 [PMID: 24917994 DOI: 10.1177/2050640613504917]

47 **Coleman HG**, Bhat S, Johnston BT, McManus D, Gavin AT, Murray LJ. Tobacco smoking increases the risk of high-grade dysplasia and cancer among patients with Barrett's esophagus. *Gastroenterology* 2012; **142**: 233-240 [PMID: 22062359 DOI: 10.1053/j.gastro.2011.10.034]

48 **Yates M**, Cheong E, Luben R, Igali L, Fitzgerald R, Khaw KT, Hart A. Body mass index, smoking, and alcohol and risks of Barrett's esophagus and esophageal adenocarcinoma: a UK prospective cohort study. *Dig Dis Sci* 2014; **59**: 1552-1559 [PMID: 24500448 DOI: 10.1007/s10620-013-3024-z]

49 **Thrift AP**, Kramer JR, Richardson PA, El-Serag HB. No significant effects of smoking or alcohol consumption on risk of Barrett's esophagus. *Dig Dis Sci* 2014; **59**: 108-116 [PMID: 24114046 DOI: 10.1007/s10620-013-2892-6]

50 **Andrici J**, Cox MR, Eslick GD. Cigarette smoking and the risk of Barrett's esophagus: a systematic review and meta-analysis. *J Gastroenterol Hepatol* 2013; **28**: 1258-1273 [PMID: 23611750 DOI: 10.1111/jgh.12230]

51 **Cooper S**, Menon S, Nightingale P, Trudgill Nj. Risk factors for the development of oesophageal adenocarcinoma in Barrett's oesophagus: a UK primary care retrospective nested case-control study. *United European Gastroenterol J* 2014; **2**: 91-98 [PMID: 24918013 DOI: 10.1177/2050640614523596]

52 **Steevens J**, Schouten LJ, Driessen AL, Huysentruyt CJ, Keulemans YC, Goldbohm RA, van den Brandt PA. A prospective cohort study on overweight, smoking, alcohol consumption, and risk of Barrett's esophagus. *Cancer Epidemiol Biomarkers Prev* 2011; **20**: 345-358 [PMID: 21173169 DOI: 10.1158/1055-9965.EPI-10-0636]

53 **Falk GW**, Jacobson BC, Riddell RH, Rubenstein JH, El-Zimaity H, Drewes AM, Roark KS, Sontag SJ, Schnell TG, Leya J, Chejfec G, Richter JE, Jenkins G, Goldman A, Dvorak K, Nardone G. Barrett's esophagus: prevalence-incidence and etiology-origins. *Ann N Y Acad Sci* 2011; **1232**: 1-17 [PMID: 21950804 DOI: 10.1111/j.1749-6632.2011.06042.x]

54 **Fox M**, Barr C, Nolan S, Lomer M, Anggiansah A, Wong T. The effects of dietary fat and calorie density on esophageal acid exposure and reflux symptoms. *Clin Gastroenterol Hepatol* 2007; **5**: 439-444 [PMID: 17363334 DOI: 10.1016/j.cgh.2006.12.013]

55 **Navarro Silvera SA**, Mayne ST, Risch HA, Gammon MD, Vaughan T, Chow WH, Dubin JA, Dubrow R, Schoenberg J, Stanford JL, West AB, Rotterdam H, Blot WJ. Principal component analysis of dietary and lifestyle patterns in relation to risk of subtypes of esophageal and gastric cancer. *Ann Epidemiol* 2011; **21**: 543-550 [PMID: 21435900 DOI: 10.1016/j.annepidem.2010.11.019]

56 **Beales IL**, Ogunwobi OO. Leptin synergistically enhances the anti-apoptotic and growth-promoting effects of acid in OE33 oesophageal adenocarcinoma cells in culture. *Mol Cell Endocrinol* 2007; **274**: 60-68 [PMID: 17618045 DOI: 10.1016/j.mce.2007.05.017]

57 **Dvorak K**, Payne CM, Chavarria M, Ramsey L, Dvorakova B, Bernstein H, Holubec H, Sampliner RE, Guy N, Condon A, Bernstein C, Green SB, Prasad A, Garewal HS. Bile acids in combination with low pH induce oxidative stress and oxidative DNA damage: relevance to the pathogenesis of Barrett's oesophagus. *Gut* 2007; **56**: 763-771 [PMID: 17145738 DOI: 10.1136/gut.2006.103697]

58 **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]

59 **Jacobson BC**, Somers SC, Fuchs CS, Kelly CP, Camargo CA. Body-mass index and symptoms of gastroesophageal reflux in women. *N Engl J Med* 2006; **354**: 2340-2348 [PMID: 16738270 DOI: 10.1056/NEJMoa054391]

60 **Peura DA**, Pilmer B, Hunt B, Mody R, Perez MC. The effects of increasing body mass index on heartburn severity, frequency and response to treatment with dexlansoprazole or lansoprazole. *Aliment Pharmacol Ther* 2013; **37**: 810-818 [PMID: 23451835 DOI: 10.1111/apt.12270]

61 **Nocon M**, Labenz J, Jaspersen D, Meyer-Sabellek W, Stolte M, Lind T, Malfertheiner P, Willich SN. Association of body mass index with heartburn, regurgitation and esophagitis: results of the Progression of Gastroesophageal Reflux Disease study. *J Gastroenterol Hepatol* 2007; **22**: 1728-1731 [PMID: 17914941 DOI: 10.1111/j.1440-1746.2006.04549.x]

62 **Pandolfino JE**, El-Serag HB, Zhang Q, Shah N, Ghosh SK, Kahrilas PJ. Obesity: a challenge to esophagogastric junction integrity. *Gastroenterology* 2006; **130**: 639-649 [PMID: 16530504 DOI: 10.1053/j.gastro.2005.12.016]

63 **Wu JC**, Mui LM, Cheung CM, Chan Y, Sung JJ. Obesity is associated with increased transient lower esophageal sphincter relaxation. *Gastroenterology* 2007; **132**: 883-889 [PMID: 17324403 DOI: 10.1053/j.gastro.2006.12.032]

64 **Lagergren J**, Bergström R, Nyrén O. Association between body mass and adenocarcinoma of the esophagus and gastric cardia. *Ann Intern Med* 1999; **130**: 883-890 [PMID: 10375336 DOI: 10.7326/0003-4819-130-11-199906010-00003]

65 **Garcia JM**, Splenser AE, Kramer J, Alsarraj A, Fitzgerald S, Ramsey D, El-Serag HB. Circulating inflammatory cytokines and adipokines are associated with increased risk of Barrett's esophagus: a case-control study. *Clin Gastroenterol Hepatol* 2014; **12**: 229-238.e3 [PMID: 23954649 DOI: 10.1016/j.cgh.2013.07.038]

66 **Rubenstein JH**, Inadomi JM, Scheiman J, Schoenfeld P, Appelman H, Zhang M, Metko V, Kao JY. Association between Helicobacter pylori and Barrett's esophagus, erosive esophagitis, and gastroesophageal reflux symptoms. *Clin Gastroenterol Hepatol* 2014; **12**: 239-245 [PMID: 23988686 DOI: 10.1016/j.cgh.2013.08.029]

67 **Beales IL**, Calam J. Interleukin 1 beta and tumour necrosis factor alpha inhibit acid secretion in cultured rabbit parietal cells by multiple pathways. *Gut* 1998; **42**: 227-234 [PMID: 9536948 DOI: 10.1136/gut.42.2.227]

68 **El-Omar EM**, Oien K, El-Nujumi A, Gillen D, Wirz A, Dahill S, Williams C, Ardill JE, McColl KE. Helicobacter pylori infection and chronic gastric acid hyposecretion. *Gastroenterology* 1997; **113**: 15-24 [PMID: 9207257 DOI: 10.1016/S0016-5085(97)70075-1]

69 **Banatvala N**, Mayo K, Megraud F, Jennings R, Deeks JJ, Feldman RA. The cohort effect and Helicobacter pylori. *J Infect Dis* 1993; **168**: 219-221 [PMID: 8515114 DOI: 10.1093/infdis/168.1.219]

70 **Nie S**, Chen T, Yang X, Huai P, Lu M. Association of Helicobacter pylori infection with esophageal adenocarcinoma and squamous cell carcinoma: a meta-analysis. *Dis Esophagus* 2014; **27**: 645-653 [PMID: 24635571 DOI: 10.1111/dote.12194]

71 **Osawa H**. Ghrelin and Helicobacter pylori infection. *World J Gastroenterol* 2008; **14**: 6327-6333 [PMID: 19009647 DOI: 10.3748/wjg.14.6327]

72 **Boltin D**, Niv Y. Ghrelin, Helicobacter pylori and body mass: is there an association? *Isr Med Assoc J* 2012; **14**: 130-132 [PMID: 22693798]

73 **Nwokolo CU**, Freshwater DA, O'Hare P, Randeva HS. Plasma ghrelin following cure of Helicobacter pylori. *Gut* 2003; **52**: 637-640 [PMID: 12692045 DOI: 10.1136/gut.52.5.637]

74 **Gokcel A**, Gumurdulu Y, Kayaselcuk F, Serin E, Ozer B, Ozsahin AK, Guvener N. Helicobacter pylori has no effect on plasma ghrelin levels. *Eur J Endocrinol* 2003; **148**: 423-426 [PMID: 12656662 DOI: 10.1530/eje.0.1480423]

75 **Isomoto H**, Ueno H, Nishi Y, Wen CY, Nakazato M, Kohno S. Impact of Helicobacter pylori infection on ghrelin and various neuroendocrine hormones in plasma. *World J Gastroenterol* 2005; **11**: 1644-1648 [PMID: 15786542]

76 **Rubenstein JH**, Morgenstern H, McConell D, Scheiman JM, Schoenfeld P, Appelman H, McMahon LF, Kao JY, Metko V, Zhang M, Inadomi JM. Associations of diabetes mellitus, insulin, leptin, and ghrelin with gastroesophageal reflux and Barrett's esophagus. *Gastroenterology* 2013; **145**: 1237-1244.e1-5 [PMID: 23999171 DOI: 10.1053/j.gastro.2013.08.052]

77 **de Martel C**, Haggerty TD, Corley DA, Vogelman JH, Orentreich N, Parsonnet J. Serum ghrelin levels and risk of subsequent adenocarcinoma of the esophagus. *Am J Gastroenterol* 2007; **102**: 1166-1172 [PMID: 17378911 DOI: 10.1111/j.1572-0241.2007.01116.x]

78 **Donohoe CL**, O'Farrell NJ, Doyle SL, Reynolds JV. The role of obesity in gastrointestinal cancer: evidence and opinion. *Therap Adv Gastroenterol* 2014; **7**: 38-50 [PMID: 24381646 DOI: 10.1177/1756283X13501786]

79 **Yeung CY**, Tso AW, Xu A, Wang Y, Woo YC, Lam TH, Lo SV, Fong CH, Wat NM, Woo J, Cheung BM, Lam KS. Pro-

inflammatory adipokines as predictors of incident cancers in a Chinese cohort of low obesity prevalence in Hong Kong. *PLoS One* 2013; **8**: e78594 [PMID: 24205276 DOI: 10.1371/journal.pone.0078594]

80 **Kant P**, Fazakerley R, Hull MA. Faecal calprotectin levels before and after weight loss in obese and overweight subjects. *Int J Obes* (Lond) 2013; **37**: 317-319 [PMID: 22410961 DOI: 10.1038/ijo.2012.38]

81 **Eksteen JA**, Scott PA, Perry I, Jankowski JA. Inflammation promotes Barrett's metaplasia and cancer: a unique role for TNFalpha. *Eur J Cancer Prev* 2001; **10**: 163-166 [PMID: 11330458]

82 **Ryan AM**, Healy LA, Power DG, Byrne M, Murphy S, Byrne PJ, Kelleher D, Reynolds JV. Barrett esophagus: prevalence of central adiposity, metabolic syndrome, and a proinflammatory state. *Ann Surg* 2008; **247**: 909-915 [PMID: 18520215 DOI: 10.1097/SLA.0b013e3181612cac]

83 **Mokrowiecka A**, Daniel P, Jasinska A, Pietruczuk M, Pawlowski M, Szczesniak P, Orszulak-Michalak D, Malecka-Panas E. Serum adiponectin, resistin, leptin concentration and central adiposity parameters in Barrett's esophagus patients with and without intestinal metaplasia in comparison to healthy controls and patients with GERD. *Hepatogastroenterology* 2012; **59**: 2395-2399 [PMID: 22944288]

84 **Greer KB**, Thompson CL, Brenner L, Bednarchik B, Dawson D, Willis J, Grady WM, Falk GW, Cooper GS, Li L, Chak A. Association of insulin and insulin-like growth factors with Barrett's oesophagus. *Gut* 2012; **61**: 665-672 [PMID: 21930730 DOI: 10.1136/gutjnl-2011-300641]

85 **Doyle SL**, Donohoe CL, Finn SP, Howard JM, Lithander FE, Reynolds JV, Pidgeon GP, Lysaght J. IGF-1 and its receptor in esophageal cancer: association with adenocarcinoma and visceral obesity. *Am J Gastroenterol* 2012; **107**: 196-204 [PMID: 22146489 DOI: 10.1038/ajg.2011.417]

86 **Donohoe CL**, Doyle SL, McGarrigle S, Cathcart MC, Daly E, O'Grady A, Lysaght J, Pidgeon GP, Reynolds JV. Role of the insulin-like growth factor 1 axis and visceral adiposity in oesophageal adenocarcinoma. *Br J Surg* 2012; **99**: 387-396 [PMID: 22241325 DOI: 10.1002/bjs.8658]

87 **Siahpush SH**, Vaughan TL, Lampe JN, Freeman R, Lewis S, Odze RD, Blount PL, Ayub K, Rabinovitch PS, Reid BJ, Chen C. Longitudinal study of insulin-like growth factor, insulin-like growth factor binding protein-3, and their polymorphisms: risk of neoplastic progression in Barrett's esophagus. *Cancer Epidemiol Biomarkers Prev* 2007; **16**: 2387-2395 [PMID: 18006928 DOI: 10.1158/1055-9965.EPI-06-0986]

88 **Ohashi K**, Shibata R, Murohara T, Ouchi N. Role of anti-inflammatory adipokines in obesity-related diseases. *Trends Endocrinol Metab* 2014; **25**: 348-355 [PMID: 24746980 DOI: 10.1016/j.tem.2014.03.009]

89 **Zhang Y**, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994; **372**: 425-432 [PMID: 7984236 DOI: 10.1038/372425a0]

90 **Vansaun MN**. Molecular pathways: adiponectin and leptin signaling in cancer. *Clin Cancer Res* 2013; **19**: 1926-1932 [PMID: 23355630 DOI: 10.1158/1078-0432.CCR-12-0930]

91 **Garofalo C**, Surmacz E. Leptin and cancer. *J Cell Physiol* 2006; **207**: 12-22 [PMID: 16110483 DOI: 10.1002/jcp.20472]

92 **Kendall BJ**, Macdonald GA, Hayward NK, Prins JB, Brown I, Walker N, Pandeya N, Green AC, Webb PM, Whiteman DC. Leptin and the risk of Barrett's oesophagus. *Gut* 2008; **57**: 448-454 [PMID: 18178609 DOI: 10.1136/gut.2007.131243]

93 **Thompson OM**, Beresford SA, Kirk EA, Bronner MP, Vaughan TL. Serum leptin and adiponectin levels and risk of Barrett's esophagus and intestinal metaplasia of the gastroesophageal junction. *Obesity* (Silver Spring) 2010; **18**: 2204-2211 [PMID: 20111023 DOI: 10.1038/oby.2009.508]

94 **Ogunwobi O**, Mutungi G, Beales IL. Leptin stimulates proliferation and inhibits apoptosis in Barrett's esophageal adenocarcinoma cells by cyclooxygenase-2-dependent, prostaglandin-E2-mediated transactivation of the epidermal growth factor receptor and c-Jun NH2-terminal kinase activation. *Endocrinology* 2006; **147**: 4505-4516 [PMID: 16740977 DOI: 10.1210/en.2006-0224]

95 **Ogunwobi OO**, Beales IL. Leptin stimulates the proliferation of human oesophageal adenocarcinoma cells via HB-EGF and Tgfalpha mediated transactivation of the epidermal growth factor receptor. *Br J Biomed Sci* 2008; **65**: 121-127 [PMID: 18986098]

96 **Howard JM**, Beddy P, Ennis D, Keogan M, Pidgeon GP, Reynolds JV. Associations between leptin and adiponectin receptor upregulation, visceral obesity and tumour stage in oesophageal and junctional adenocarcinoma. *Br J Surg* 2010; **97**: 1020-1027 [PMID: 20632267 DOI: 10.1002/bjs.7072]

97 **Mokrowiecka A**, Sokolowska M, Luczak E, Dudojc M, Wieczfinska J, Kacprzak D, Wierzchniewska-Lawska A, Pawliczak R, Malecka-Panas E. Adiponectin and leptin receptors expression in Barrett's esophagus and normal squamous epithelium in relation to central obesity status. *J Physiol Pharmacol* 2013; **64**: 193-199 [PMID: 23756394]

98 **Beales IL**, Garcia-Morales C, Ogunwobi OO, Mutungi G. Adiponectin inhibits leptin-induced oncogenic signalling in oesophageal cancer cells by activation of PTP1B. *Mol Cell Endocrinol* 2014; **382**: 150-158 [PMID: 23994026 DOI: 10.1016/j.mce.2013.08.013]

99 **Allott EH**, Lysaght J, Cathcart MC, Donohoe CL, Cummins R, McGarrigle SA, Kay E, Reynolds JV, Pidgeon GP. MMP9 expression in oesophageal adenocarcinoma is upregulated with visceral obesity and is associated with poor tumour differentiation. *Mol Carcinog* 2013; **52**: 144-154 [PMID: 22121096 DOI: 10.1002/mc.21840]

100 **Souza RF**, Shewmake K, Terada LS, Spechler SJ. Acid exposure activates the mitogen-activated protein kinase pathways in Barrett's esophagus. *Gastroenterology* 2002; **122**: 299-307 [PMID: 11832445 DOI: 10.1053/gast.2002.30993]

101 **Beales IL**, Ogunwobi O, Cameron E, El-Amin K, Mutungi G, Wilkinson M. Activation of Akt is increased in the dysplasia-carcinoma sequence in Barrett's oesophagus and contributes to increased proliferation and inhibition of apoptosis: a histopathological and functional study. *BMC Cancer* 2007; **7**: 97 [PMID: 17559672 DOI: 10.1186/1471-2407-7-97]

102 **Francois F**, Roper J, Goodman AJ, Pei Z, Ghumman M, Mourad M, de Perez AZ, Perez-Perez GI, Tseng CH, Blaser MJ. The association of gastric leptin with oesophageal inflammation and metaplasia. *Gut* 2008; **57**: 16-24 [PMID: 17761783 DOI: 10.1136/gut.2007.131672]

103 **Li FY**, Lam KS, Xu A. Therapeutic perspectives for adiponectin: an update. *Curr Med Chem* 2012; **19**: 5513-5523 [PMID: 22876919 DOI: 10.2174/092986712803833173]

104 **Kelesidis I**, Kelesidis T, Mantzoros CS. Adiponectin and cancer: a systematic review. *Br J Cancer* 2006; **94**: 1221-1225 [PMID: 16570048 DOI: 10.1038/sj.bjc.6603051]

105 **Dalamaga M**, Diakopoulos KN, Mantzoros CS. The role of adiponectin in cancer: a review of current evidence. *Endocr Rev* 2012; **33**: 547-594 [PMID: 22547160 DOI: 10.1210/er.2011-1015]

106 **Waki H**, Yamauchi T, Kamon J, Kita S, Ito Y, Hada Y, Uchida S, Tsuchida A, Takekawa S, Kadowaki T. Generation of globular fragment of adiponectin by leukocyte elastase secreted by monocytic cell line THP-1. *Endocrinology* 2005; **146**: 790-796 [PMID: 15528304 DOI: 10.1210/en.2004-1096]

107 **Yamauchi T**, Iwabu M, Okada-Iwabu M, Kadowaki T. Adiponectin receptors: a review of their structure, function and how they work. *Best Pract Res Clin Endocrinol Metab* 2014; **28**: 15-23 [PMID: 24417942 DOI: 10.1016/j.beem.2013.09.003]

108 **Wang Y**, Lam KS, Xu JY, Lu G, Xu LY, Cooper GJ, Xu A. Adiponectin inhibits cell proliferation by interacting with

several growth factors in an oligomerization-dependent manner. *J Biol Chem* 2005; **280**: 18341-18347 [PMID: 15734737 DOI: 10.1074/jbc.M501149200]

109 **Konturek PC**, Burnat G, Rau T, Hahn EG, Konturek S. Effect of adiponectin and ghrelin on apoptosis of Barrett adenocarcinoma cell line. *Dig Dis Sci* 2008; **53**: 597-605 [PMID: 17763959]

110 **Ogunwobi OO**, Beales IL. Globular adiponectin, acting via adiponectin receptor-1, inhibits leptin-stimulated oesophageal adenocarcinoma cell proliferation. *Mol Cell Endocrinol* 2008; **285**: 43-50 [PMID: 18313838 DOI: 10.1016/j.mce.2008.01.023]

111 **Rubenstein JH**, Dahlkemper A, Kao JY, Zhang M, Morgenstern H, McMahon L, Inadomi JM. A pilot study of the association of low plasma adiponectin and Barrett's esophagus. *Am J Gastroenterol* 2008; **103**: 1358-1364 [PMID: 18510610 DOI: 10.1111/j.1572-0241.2008.01823.x]

112 **Kato M**, Watabe K, Hamasaki T, Umeda M, Furubayashi A, Kinoshita K, Kishida O, Fujimoto T, Yamada A, Tsukamoto Y, Yamamoto S, Kamada Y, Yoshida Y, Kiso S, Tsutsui S, Kihara S, Hayashi N, Matsuzawa Y. Association of low serum adiponectin levels with erosive esophagitis in men: an analysis of 2405 subjects undergoing physical check-ups. *J Gastroenterol* 2011; **46**: 1361-1367 [PMID: 21845377 DOI: 10.1007/s00535-011-0453-3]

113 **Rubenstein JH**, Kao JY, Madanick RD, Zhang M, Wang M, Spacek MB, Donovan JL, Bright SD, Shaheen NJ. Association of adiponectin multimers with Barrett's oesophagus. *Gut* 2009; **58**: 1583-1589 [PMID: 19570765 DOI: 10.1136/gut.2008.171553]

114 **Zhang HY**, Zhang Q, Zhang X, Yu C, Huo X, Cheng E, Wang DH, Spechler SJ, Souza RF. Cancer-related inflammation and Barrett's carcinogenesis: interleukin-6 and STAT3 mediate apoptotic resistance in transformed Barrett's cells. *Am J Physiol Gastrointest Liver Physiol* 2011; **300**: G454-G460 [PMID: 21148399 DOI: 10.1152/ajpgi.00458.2010]

115 **Beales IL**, Ogunwobi OO. Glycine-extended gastrin inhibits apoptosis in Barrett's oesophageal and oesophageal adenocarcinoma cells through JAK2/STAT3 activation. *J Mol Endocrinol* 2009; **42**: 305-318 [PMID: 19153190 DOI: 10.1677/JME-08-0096]

116 **Snijder MB**, Dekker JM, Visser M, Bouter LM, Stehouwer CD, Yudkin JS, Heine RJ, Nijpels G, Seidell JC. Trunk fat and leg fat have independent and opposite associations with fasting and postload glucose levels: the Hoorn study. *Diabetes Care* 2004; **27**: 372-377 [PMID: 14747216 DOI: 10.2337/diacare.27.2.372]

117 **Lysaght J**, van der Stok EP, Allott EH, Casey R, Donohoe CL, Howard JM, McGarrigle SA, Ravi N, Reynolds JV, Pidgeon GP. Pro-inflammatory and tumour proliferative properties of excess visceral adipose tissue. *Cancer Lett* 2011; **312**: 62-72 [PMID: 21890265 DOI: 10.1016/j.canlet.2011.07.034]

118 **Motoshima H**, Wu X, Sinha MK, Hardy VE, Rosato EL, Barbot DJ, Rosato FE, Goldstein BJ. Differential regulation of adiponectin secretion from cultured human omental and subcutaneous adipocytes: effects of insulin and rosiglitazone. *J Clin Endocrinol Metab* 2002; **87**: 5662-5667 [PMID: 12466369 DOI: 10.1210/jc.2002-020635]

119 **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]

120 **Das D**, Chilton AP, Jankowski JA. Chemoprevention of oesophageal cancer and the AspECT trial. *Recent Results Cancer Res* 2009; **181**: 161-169 [PMID: 19213566]

121 **Cunneen SA**, Brathwaite CE, Joyce C, Gersin K, Kim K, Schram JL, Wilson EB, Schwiers M, Gutierrez M. Clinical outcomes of the Realize Adjustable Gastric Band-C at 2 years in a United States population. *Surg Obes Relat Dis* 2013; **9**: 885-893 [PMID: 23642493 DOI: 10.1016/j.soard.2013.02.009]

122 **Umeda LM**, Pereira AZ, Carneiro G, Arasaki CH, Zanella MT. Postprandial adiponectin levels are associated with improvements in postprandial triglycerides after Roux-en-Y gastric bypass in type 2 diabetic patients. *Metab Syndr Relat Disord* 2013; **11**: 343-348 [PMID: 23745620 DOI: 10.1089/met.2012.0042]

123 **Després JP**, Ross R, Boka G, Alméras N, Lemieux I. Effect of rimonabant on the high-triglyceride/ low-HDL-cholesterol dyslipidemia, intraabdominal adiposity, and liver fat: the ADAGIO-Lipids trial. *Arterioscler Thromb Vasc Biol* 2009; **29**: 416-423 [PMID: 19112166 DOI: 10.1161/ATVBAHA.108.176362]

124 **Lim S**, Quon MJ, Koh KK. Modulation of adiponectin as a potential therapeutic strategy. *Atherosclerosis* 2014; **233**: 721-728 [PMID: 24603219 DOI: 10.1016/j.atherosclerosis.2014.01.051]

125 **Cho SY**, Park PJ, Shin HJ, Kim YK, Shin DW, Shin ES, Lee HH, Lee BG, Baik JH, Lee TR. (-)-Catechin suppresses expression of Kruppel-like factor 7 and increases expression and secretion of adiponectin protein in 3T3-L1 cells. *Am J Physiol Endocrinol Metab* 2007; **292**: E1166-E1172 [PMID: 17164435 DOI: 10.1152/ajpendo.00436.2006]

126 **Otvos L**, Haspinger E, La Russa F, Maspero F, Graziano P, Kovalszky I, Lovas S, Nama K, Hoffmann R, Knappe D, Cassone M, Wade J, Surmacz E. Design and development of a peptide-based adiponectin receptor agonist for cancer treatment. *BMC Biotechnol* 2011; **11**: 90 [PMID: 21974986 DOI: 10.1186/1472-6750-11-90]

127 **Rene Gonzalez R**, Watters A, Xu Y, Singh UP, Mann DR, Rueda BR, Penichet ML. Leptin-signaling inhibition results in efficient anti-tumor activity in estrogen receptor positive or negative breast cancer. *Breast Cancer Res* 2009; **11**: R36 [PMID: 19531256 DOI: 10.1186/bcr2321]

128 **Martin-Castillo B**, Vazquez-Martin A, Oliveras-Ferraros C, Menendez JA. Metformin and cancer: doses, mechanisms and the dandelion and hormetic phenomena. *Cell Cycle* 2010; **9**: 1057-1064 [PMID: 20305377 DOI: 10.4161/cc.9.6.10994]

129 **Franciosi M**, Lucisano G, Lapice E, Strippoli GF, Pellegrini F, Nicolucci A. Metformin therapy and risk of cancer in patients with type 2 diabetes: systematic review. *PLoS One* 2013; **8**: e71583 [PMID: 23936520 DOI: 10.1371/journal.pone.0071583]

130 **Beales IL**, Ogunwobi OO. Microsomal prostaglandin E synthase-1 inhibition blocks proliferation and enhances apoptosis in oesophageal adenocarcinoma cells without affecting endothelial prostacyclin production. *Int J Cancer* 2010; **126**: 2247-2255 [PMID: 19739114 DOI: 10.1002/ijc.24875]

131 **Fang D**, Das KM, Cao W, Malhotra U, Triadafilopoulos G, Najarian RM, Hardie LJ, Lightdale CJ, Beales IL, Felix VN, Schneider PM, Bellizzi AM. Barrett's esophagus: progression to adenocarcinoma and markers. *Ann N Y Acad Sci* 2011; **1232**: 210-229 [PMID: 21950815 DOI: 10.1111/j.1749-6632.2011.06053.x]

132 **Ogunwobi OO**, Beales IL. Glycine-extended gastrin stimulates proliferation via JAK2- and Akt-dependent NF-kappaB activation in Barrett's oesophageal adenocarcinoma cells. *Mol Cell Endocrinol* 2008; **296**: 94-102 [PMID: 18771702 DOI: 10.1016/j.mce.2008.08.004]

133 **Beales IL**, Hensley A, Loke Y. Reduced esophageal cancer incidence in statin users, particularly with cyclo-oxygenase inhibition. *World J Gastrointest Pharmacol Ther* 2013; **4**: 69-79 [PMID: 23919219 DOI: 10.4292/wjgpt.v4.i3.69]

134 **Omer ZB**, Ananthakrishnan AN, Nattinger KJ, Cole EB, Lin JJ, Kong CY, Hur C. Aspirin protects against Barrett's esophagus in a multivariate logistic regression analysis. *Clin Gastroenterol Hepatol* 2012; **10**: 722-727 [PMID: 22426086 DOI:

10.1016/j.cgh.2012.02.031]
135 **Ogunwobi OO**, Beales IL. Statins inhibit proliferation and induce apoptosis in Barrett's esophageal adenocarcinoma cells. *Am J Gastroenterol* 2008; **103**: 825-837 [PMID: 18371146 DOI: 10.1111/j.1572-0241.2007.01773.x]
136 **Singh S**, Singh AG, Singh PP, Murad MH, Iyer PG. Statins are associated with reduced risk of esophageal cancer, particularly in patients with Barrett's esophagus: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2013; **11**: 620-629 [PMID: 23357487 DOI: 10.1016/j.cgh.2012.12.036]

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REVIEW

Intestinal microbiota: The explosive mixture at the origin of inflammatory bowel disease?

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Abstract

Inflammatory bowel diseases (IBDs), namely Crohn's disease and ulcerative colitis, are lifelong chronic disorders arising from interactions among genetic, immunological and environmental factors. Although the origin of IBDs is closely linked to immune response alterations, which governs most medical decision-making, recent findings suggest that gut microbiota may be involved in IBD pathogenesis. Epidemiologic evidence and several studies have shown that a dysregulation of gut microbiota (*i.e.*, dysbiosis) may trigger the onset of intestinal disorders such as IBDs. Animal and human investigations focusing on the microbiota-IBD relationship have suggested an altered balance of the intestinal microbial population in the active phase of IBD. Rigorous microbiota typing could, therefore, soon become part of a complete phenotypic analysis of IBD patients. Moreover, individual susceptibility and environmental triggers such as nutrition, medications, age or smoking could modify bacterial strains in the bowel habitat. Pharmacological manipulation of bowel microbiota is somewhat controversial. The employment of antibiotics, probiotics, prebiotics and synbiotics has been widely addressed in the literature worldwide, with the aim of obtaining positive results in a number of IBD patient settings, and determining the appropriate timing and modality of this intervention. Recently, novel treatments for IBDs, such as fecal microbiota transplantation, when accepted by patients, have shown promising results. Controlled studies are being designed. In the near future, new therapeutic strategies can be expected, with non-pathogenic or modified food organisms that can be genetically modified to exert anti-inflammatory properties.

Key words: Intestinal microbiota; Inflammatory bowel diseases; Probiotics; Prebiotics; Symbiotics

Core tip: This paper focuses on the scientific scenario regarding the potential function of gut microbiota in inflammatory bowel diseases (IBDs). Epidemiologic findings suggest that the heterogeneity and disruption of gut microbiota can be significant in modulating and addressing the immune reactions underlying IBD pathogenesis. Traditional or innovative manipulation strategies of gut microbiota may be possible future treatment options for the management of these disorders.

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INTRODUCTION

Inflammatory bowel diseases (IBDs) are lifelong chronic disorders arising from interactions among genetic, immunological and environmental factors[1].

Technological advances have allowed novel predictive factors to be assessed, that can identify the disease at an early stage and provide an accurate diagnosis long before the onset of clinical manifestations[2]. Recent findings suggest that, in addition to genetic and environmental factors, interactions with the gut microbiota may play a relevant role in a "perfect storm" driving the pathogenesis of IBDs[3].

MICROBIOTA AND IBD

The human intestinal tract includes several multifaceted microbial populations with an essential function in general health. The human gut contains, in the assortment of 1000 bacterial species, 100-fold more genes than the human genome. The new high throughput sequencing technologies, the presence of 16S rRNA genes in the gut bacterial composition, as well as recent non genomic techniques, have well defined the function of gut microbiota in some human diseases[4,5].

Although the microbiota of the colon is apparently similar in different people, there are marked variations between individuals in bacterial populations of a single species. It has been demonstrated that an increase in biodiversity requires a different metabolic homeostasis and structural stability, while a reduction, due to age, illness or antibiotics, reduce the capacity of the intestinal environment to fight infecting pathogens[6,7]. In fact, epidemiological evidence and experimental studies have suggested that alterations in the gut microbiota (*i.e.*, dysbiosis) can be relevant in intestinal conditions such as chronic IBD[8].

Clinical evidence confirms the role of microbiota in IBD, and an abnormal microbial composition in IBD has been amply demonstrated. The most common site of IBD is the colon, where the highest intestinal bacterial concentrations are found. Additionally, fecal stream diversion can prevent and treat Crohn's disease (CD) and pouchitis. Finally, many studies have shown that antibiotics and probiotics improve the histological, endoscopic and clinical picture[9]. Despite this evidence-based findings, there are still some major unexplained points such as the IBD response to immunosuppressive therapy or the protective role of poor hygienic conditions, which do not appear likely to be related to the microbial state[10].

Animal studies

It is known that the non-pathogenic microbiota controls bowel immunity, but interactions in the gut with host microbes can be bidirectional. The mucosal immune system can be affected by the pro-inflammatory potential of abnormal growth of microbiota elements, which ultimately determine or influence an inflammatory reaction and induce the possibility of development of illness. Several animal studies have shown that this interaction is possible and can induce colitis.

Studies in germ-free interleukin 10-deficient (IL-10$^{-/-}$) mice, that fail to acquire spontaneous colitis and immune activation, support this hypothesis[11]. Indeed, some studies show that, regardless of the background strain of these animals, the onset and degree of spontaneous colitis depends on the composition of the enteric gut microbiota[11,12]. Penetrance of colitis increases to nearly 100% when the immune system response is characterized by a T-helper 1 (Th1) interferon (IFN)-γ reaction[12].

Therefore, in this model of colitis, it has been demonstrated that the disease may show different characteristics and distribution based on the intestinal bacteria present. Furthermore, in IL-10-/- germ-free mice, bacterial colonization of non-pathogenic bacteria such as *Escherichia coli* (*E. coli*) or *Bilophila wadsworthia* provokes different types of colitis[13]. In particular, *Bilophila wadsworthia* produces a low grade colitis involving the distal colon, associated with an exclusively Th1-mediated immune response. In contrast, *E. coli* leads to an early (3-wk) development of mild-to-moderate inflammation that is more severe in the cecum. In the same study, *Bacteroides vulgatus*, but not *E. coli*, provokes mucosal inflammation of the colon in HLA-B27 transgenic mice without bone marrow involvement as in transplanted CD3 transgenic mice[13].

Finally, novel experimental evidence demonstrated that *Klebsiella* may provoke moderate pancolitis while *Bifidobacterium animalis* could cause a mild degree of inflammation in the distal colon and duodenum[14,15].

Human studies

A few studies in humans have suggested that IBD patients have an altered balance of intestinal microbiota in the active phase. Bacterial 16S rRNA gene examination did not show relevant differences in bacterial constitution in the intestinal mucosa of CD and ulcerative colitis (UC) patients. Moreover, in UC patients, a decrease in bacterial load was observed even if it was not significant when compared to that of CD patients[16-18].

Another interesting finding, a thinner and less sulphated mucus in patients affected by UC, has been demonstrated and may account for an increased number of bacteria colonizing the mucosa[19,20]. Indeed, a poor mucus layer with a microbiota overgrowth could enhance the presentation of bacterial antigens to the immune system of the gut mucosa. In UC patients, the colonic surface and inflamed areas are colonized by a broad variety of bacteria. For example, in UC specimens *Clostridium histolyticum* and *lituseburense* accounted for 21% of the microbiota. *Enterobacteriaceae* such as *Escherichia* and *Klebsiella* have also been considered to be implicated in the pathogenetic mechanism of UC. Indeed, their aptitude to adhere to enterocytes, allowing them to penetrate the mucosal layer and deliver enterotoxins, might account for this hypothesis[21,22].

Genetics in IBD pathogenesis

The interaction between genetic factors, and a dysregulated response of the immune system to bacterial antigens are still strongly supported hypotheses in the pathogenesis of IBD. Indeed, genome-wide association studies (GWAS) showed that several genes were associated with

IBD susceptibility[23]. These genes, risk factors for CD and UC, encode for proteins that may regulate the microbiota (NOD2/CARD15) or may control host responses (IL-12-IL23R pathway or autophagy)[24,25], and constitute a barrier function notably for UC[26].

One of these proteins, NOD2, may be crucial for distinguishing between non-pathogenic and pathogenic organisms; indeed, it initiates signal transduction thus promoting NFκB translocation into the nucleus, where the expression of specific genes determinates the response of primary and adaptive immune mechanisms[27-29].

The multifunctional genetic linkage of NOD2/CARD15 is demonstrated by the protein's ability to identify bacterial muramyl-dipeptide and by its impact on the homeostasis of non-pathogenic bacteria, regulatory T cells (Tregs), and viral identification by immune system[24]. Although NOD2 homozygosity may carry a 20-fold increased risk for CD, notably in the ileal location, less than 20% of patients affected by CD are homozygous for NOD2[30,31]. So, while these studies and GWAS have provided important details about IBD pathogenesis, investigations on the distribution of genetic variants in different populations poorly explain the large discrepancies in IBD prevalence between different geographic areas as well as the increasing incidence of IBDs in Western countries over the past 5 decades[2].

The evidence strongly supports that IBDs are polygenetic disorders and their heterogeneity relates to the complexity of their genetic background as well as to different lifestyle and environmental factors, including variations in microbiota composition.

Environmental triggers

It is known that nutrition, medications (NSAIDs) and smoking affect the composition of the gut microbiota and it is known that changes in this multifaceted structure are contributing factors in the origin of some disorders, including IBDs.

Smoking is a relevant risk factor in CD pathogenesis[32-36]. Indeed, it may alter the intestinal microbiota and its cessation may further modify intestinal microbial composition. Indeed, simultaneous increased *Firmicutes* and *Actinobacteria* and decreased *Proteobacteria* and *Bacteroidetes* characterize smoking cessation; in contrast, the composition of the flora in continuous smokers and non-smokers remains stable[37].

Many studies have reported a modification of the gut microbiota composition in populations migrating from developing to developed countries[38]. In these subjects, diet, family size, antibiotic consumption, urbanization, reduced parasitism, and a reduction in exposure to childhood infections, such as hepatitis A and *Helicobacter pylori,* are associated with changes in the microbiota.

Neonates show a sterile or, at least, a very low microbial load in the intestine[39]. Bacterial strains colonize the infant bowel after delivery according to various factors, such as method of delivery, breast- or bottle-feeding, and antibiotic administration[40]. There is early colonization of *Lactobacillus* and *Prevotella* after vaginal delivery and greater colonization of *Firmicutes* in neonates delivered by cesarean section, that predisposes to a greater susceptibility to some pathogens and a higher risk of atopic disease[41,42]. Therefore, growth from newborn to early childhood and finally adulthood is associated with changes in the gut microbiota, featuring a reduction in *Lactobacillus* and *Bifidobacteria* and an increase in *Firmicutes, Clostridia* and *Bacteroides* species, that may lead to a high risk of allergic and immunological diseases[43]. This raises the hypothesis that a decreased biodiversity within non-pathogenic microbiota, with an altered immunity maturation, could negatively influence the immune recognition and activation, and thereby confer a risk for developing IBD in adulthood[38].

Regarding the impact of a high-fat dietary intake on the non-pathogenic microbiota, it has been demonstrated that it can radically remodel the intestinal microbiota[44,45]. Moreover, there is evidence that non-absorbed carbohydrates (inulin and fructooligosaccharides) promote the growth of beneficial species, supplying a substrate for the production of short-chain fatty acids (SCFAs)[46].

Recently, novel studies have focused on the role of NSAIDs in inducing and maintaining mucosal damage, thus contributing to the genesis of IBD. In particular, several studies demonstrated that NSAIDs were able to cause injury by means of microbiota modulation[47]. NSAIDs, indeed, can promote the overexpression of proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and IFN-γ through changes in the microbiota[48], and further allow bacterial translocation through the intestinal barrier. This hypothesis is confirmed by evidence that the levels of such proinflammatory cytokines are significantly increased in IBD patients.

Microbiota and IBDs: Comments on the literature

There can be no doubt, in view of all the experimental data, that the microbiota can be considered a key factor in the origin of IBDs and not a bystander. Studies performed on animal models provide strong evidence for a primary role played by microbiota in IBDs but human studies do not fully support this pathogenic hypothesis owing to the lack of sufficient scientific proof. For instance, it is well-known that, in CD, the entire alimentary tract from the oral cavity to the anus may be involved, but no data from human studies are available on this topic. Conversely, animal studies have demonstrated that the microbiota composition may influence the onset of IBDs in a selected part of the digestive system. El Aidy *et al*[49] investigated the responses of the jejunal mucosa to bacterial colonization in germ-free mice, showing a consequent shift to anaerobic metabolism, a condition that may strongly influence mucosal oxygenation in IBD. Moreover, in an experimental model of small bowel CD, a single strain of *E. coli* (LF82) has been demonstrated to stimulate the production of proinflammatory cytokines, an effect that was counteracted by lactoferrin, another microbial product[50].

There has been much discussion as to whether infec-

Table 1 Antibiotic therapy in inflammatory bowel diseases

Ref.	Year	Antibiotics	Duration	Result
Crohn's disease-primary therapy				
Ursing *et al*[53]	1982	Metronidazole 800 mg/d	16 wk	No difference from sulfasalazine
Sutherland *et al*[54]	1991	Metronidazole 10 or 20 mg/kg	16 wk	Superior to placebo (↓ CDAI), no difference in remission
Colombel *et al*[55]	1999	Ciprofloxacin 500 mg 2 × d	6 wk	No difference from mesalamine
Arnold *et al*[56]	2002	Ciprofloxacin 500 mg 2 × d	6 mo	Superior to placebo (CDAI)
Prantera *et al*[57]	1996	Ciprofloxacin 500 mg 2 × d + metronidazole 250 mg 4 × d	12 wk	No difference from prednisolone
Greenbloom *et al*[58]	1998	Ciprofloxacin 500 mg 2 × d + metronidazole 250 mg 3 × d	10 wk	Uncontrolled, 68% remission
Leiper *et al*[59]	2000	Clarithromycin 250 mg 2 × d	4 wk	Uncontrolled, 64% response, 48% remission
Steinhart *et al*[60]	2002	Ciprofloxacin 500 mg 2 × d + metronidazole 250 mg 3 × d	8 wk	No improvement over budesonide alone (33% *vs* 38% remission)
Crohn's disease-prevention of postsurgical relapse				
Rutgeerts *et al*[61]	1995	Metronidazole 20 mg/kg	12 wk	↓ clinical relapse 1 yr *vs* placebo
Rutgeerts *et al*[62]	2005	Ornidazole 1 g/d	52 wk	↓ severe endoscopic relapse *vs* placebo
Ulcerative colitis-primary therapy				
Turunen *et al*[63]	1999	Cipro 500 mg 2 × d	6 mo	Superior to placebo
Mantzaris *et al*[64]	1997	Cipro 500 mg 2 × d	6 mo	No benefit *vs* placebo
Casellas *et al*[65]	1998	Amoxicillin 1 g/ Clavulanic acid 250 mg	5 d	↓ mucosal IL-8 and eicosanoids *vs* placebo
Turner *et al*[66]	2014	metronidazole, amoxicillin, doxycycline (Paediatrics)		Remission (46.6%)
Pouchitis				
Shen *et al*[67]	2001	Metronidazole 20 mg/kg or Cipro 500 mg 2 × d	6 mo	Both effective, Cipro > metronidazole
Gionchetti *et al*[68]	2000	Cipro 500 mg 2 × d and Rifaximin 1 g 2 × d	5 d	89% response, 33% remission, uncontrolled

tious factors could be a trigger for IBD. No evidence is available from human studies, but animal models offer interesting insights. Couturier-Maillard *et al*[51] demonstrated that microbiota transplantation from healthy wild-type mice may reduce the IBD risk in Nod2-deficient mice and lead to long-term alterations in the gut microbiota. On the other hand, disease risk was promoted in wild-type mice that were recolonized with dysbiotic fecal microbiota from NOD2-deficient mice. In conclusion, animal models must be seen only as a starting point for microbiota investigation in humans, and the main lesson that we can deduce is that an imbalance of bacterial species is one of the main reasons that can explain the different types of colitis induced by the effect of different bacteria.

PHARMACOLOGICAL MANIPULATION OF MICROBIOTA IN IBDs

Antibiotics

Antibiotics are known to have an important role in the management of septic complications of IBD, *e.g.*, intra-abdominal and perianal abscesses and fistulae of CD, superinfections, and post-surgical wound infections. Nonetheless, treatment with antibiotics for active luminal CD and UC is not widely accepted as a first-line choice. Although the use of antibiotics against pathogenic bacteria is proven and based on reliable evidence of experimental enterocolitis and IBD, there are some clinical trials that do not sufficiently support the efficacy of these drugs in patients affected by IBD[52].

The most representative published studies are summarized in Table 1[53-68]. Metronidazole, ciprofloxacin, or the contemporary use of these agents are useful in Crohn's colitis, ileocolitis and pouchitis, but not in disease confined to the ileum. They are recommended for pouchitis in the European Crohn's and Colitis Organisation statements, which also indicate that ciprofloxacin appears to have fewer adverse effects (statements 8C, 8D)[69].

Probiotics

Probiotics are viable microorganisms that have been cultured from foods, in particular milk. Various species and bacterial strains that have been used in IBD clinical trials, are believed to have a potential beneficial role. The most evaluated probiotics are *E. coli Nissle*[70], *VSL#3* mixture (four strains of *lactobacilli*, three strains of *bifidobacteria*, and one strain of *Streptococcus salivarius thermophilus*)[68,71-73], *BIO-THREE* mixture (*S. faecalis, C. butyricum,* and *Bacillus mesentericus*)[74], a mixture of *L. rhamnosus* and *L. reuteri*[75], *L. rhamnosus GG*[76], Yakult strains of *Bifidobacterium brevis, Bifidobacterium bifidum and L. acidophilus*[77]. Recently, advanced genetic engineering has produced modified species that are able to produce immunosuppressive molecules such as IL-10[78].

These studies have shown that probiotic supplementation can re-establish bacterial homeostasis in the intestine and downregulate gut inflammation that is characteristic of IBD patients, thus modulating the inflammatory/anti-inflammatory balance. A reduction in the number of microbiome elements was also found. Indeed, the administration of probiotics can normalize

Table 2 Probiotic therapy in inflammatory bowel diseases

Model	Probiotic	Effect
Trinitrobenzene sulphonic acid or dinitrobenzene sulphonic acid	*Bi. infantis*	No effect
	L. acidophilus, L. casei and *Bi. animalis*	Reduced inflammation
	VSL#3	No effect
	Lactobacillus GG	No effect
	L. plantarum 299	No effect
	VSL#3 (DNA, subcutaneously)	Reduced inflammation
Iodoacetamide	VSL#3	Reduced inflammation
	Lactobacillus GG	Reduced inflammation
Acetic acid	*L. rhamnosus GG*	No effect
	L. reuteri R2LC	Reduced inflammation
	L. reuteri R2LC	Reduced inflammation
Dextran sodium sulphate	VSL#3 (irradiated and DNA*)	Reduced inflammation
IL-10 knockout mice	*L. salivarius* 118 (subcutaneously)	Reduced inflammation
	L. salivarius	Reduced inflammation
	Bi. infantis	Reduced inflammation
	L. plantarum 299V	Reduced inflammation
	VSL#3	Reduced inflammation
	L. salivarius	Reduced inflammation
	L. reuteri	Reduced inflammation
	VSL#3 (DNA, subcutaneously)	Reduced inflammation
E. coli-induced colitis in IL-2 knockout mice	*B. vulgatus*	Reduced inflammation
B. vulgatus-induced colitis	*Lactobacillus GG*	Prevented recurrent colitis
	L. plantarum 299*V*	No prevention of recurrent colitis

altered intestinal microbiota in IBD patients, and increase protective species by reducing the pathogen load, positively affecting intestinal permeability, balancing local immune response, producing beneficial substances, and disintegrating pathogenic antigens in the intestinal lumen[79].

In animal models (Table 2), *Lactobacilli* and *Bifidobacteria* reduced the severity of experimental colitis in IL-10 knockout mice[80,81]. In another study *L. plantarum* prevented colitis onset in HLA B27 transgenic rats. This and other reports confirm the protective effects of several probiotics in selected hosts and special inflammatory conditions. Therefore, in experimental colitis induced in B27 transgenic rats, which had remission with broad-spectrum antibiotics, probiotics prevented recurrence of the colitis. However, probiotic treatment alone was unable to produce remission of the induced disease[82].

The beneficial effect of probiotics was demonstrated in rats with colitis induced by instillation of 4% acetic acid, which causes altered intestinal permeability. In particular, after 4 d of acetic acid treatment the activity of myeloperoxidase (MPO) showed a 3-fold increase, in parallel with a 6-fold increase in mucosal permeability in the colonic samples. The use of *L. reuteri R2LC,* after acetic acid administration, reduced the morphological score, MPO activity, mucosal permeability, and prevented the onset of colitis[83].

In human studies, 9-mo daily use of a probiotic formula, *i.e.*, *VSL#3,* was effective in preventing the relapse of chronic pouchitis after remission induced by antibiotics[68]. Another investigation replicated the same results, and, in addition, showed a decreased frequency of pouchitis when *VSL#3* was given after pouch closure[84].

In cases of mild-to-moderate active UC treated with probiotics, there was an improvement in clinical severity, a reduction in relapses, and induction of remission. Moreover, these findings were accompanied by high histological scores and increased levels of fecal butyrate and other SCFAs[73-77].

Studies in UC patients found that the prevention of flare-ups by probiotics was associated with inactivation of NF-κB, downregulation of TNF-α and IL-1β, and a simultaneous increase in anti-inflammatory cytokines, such as IL-10[85]. Few data are available about the mechanism by which probiotics could modify the composition of the resident microbiota, even though it has been hypothesized that they might increase the load of *Lactobacilli* and/or *Bifidobacteria*[74,85].

On the other hand, clinical trials with the use of probiotics in CD, are less concordant than in UC. Malchow[86] found that *E. coli Nissle* was more effective than placebo in preventing relapse of CD in the remission phase induced by conventional therapy, but supplementation of probiotics was found to be ineffective in prolonging remission after the administration of *L. johnsonii LA1* following surgical resection[87,88]. Similarly, a study of Prantera *et al*[80] did not demonstrate any benefit by 1 year-long *Lactobacillus GG* consumption in the prevention of post-surgical clinical or endoscopic relapses in the neo-terminal ileum.

As reported above, Butterworth *et al*[89] evaluated 12 potentially relevant studies of the efficacy of probiotics in CD, even though 11 did not fulfill the inclusion criteria. In the only study satisfying the stated criteria, patients with moderately active CD received *L. rhamnosus* GG for 6 mo without obtaining an improvement.

Prebiotics

Prebiotics are dietary supplementations, usually non-digestible glycides, which are energy substrates for protective intestinal organisms. Lactosucrose, fructo-oligosaccharides, inulin, bran, psyllium, and germinated barley extracts promote *Lactobacilli and Bifidobacteria* growth, thus inducing SCFA production, in particular butyrate[90-92]. Therefore, these substances are able to re-establish the optimal beneficial/pathogen bacteria ratio in IBD patients. These physiological dietary supplements increase the protective lactic acid bacilli load, with a consequent inhibition of harmful species by decreasing the luminal pH, reducing epithelial adhesion, and producing bactericidal molecules. Animal studies showed a protective effect in rat colitis models (Table 3)[93,94]. Several small controlled studies but only a few randomized controlled

Table 3 Inflammatory bowel diseases prebiotic therapy

Model	Prebiotic	Effect
Trinitrobenzene sulphonic acid	Fructo-oligosaccharide	Reduced inflammation
	Galacto-oligosaccharide	No effect on inflammation
Dextran sodium sulphate	Fructo-oligosaccharide	No effect on inflammation
	Resistant starch	Reduced inflammation
	Germinated barley foodstuff	Reduced inflammation
	Germinated barley foodstuff	Reduced inflammation
	Inulin	Reduced inflammation
	Germinated barley foodstuff	Reduced inflammation
IL-10 knockout mice	Lactulose	Reduced inflammation

trials (RCT) in IBD patients have been performed, fewer than the studies with probiotics.

Interestingly, Welters *et al*[95] carried out a clinical trial in 20 patients with an ileal pouch-anal anastomosis who consumed 24 g of inulin or placebo daily for 3 wk. The pH, short chain fatty acids, microflora, and bile acids were determined in the stools, while the inflammatory status was evaluated by clinical, endoscopic and histological parameters. It was proven that the treatment enhanced butyrate levels, reduced pH, and reduced the number of *Bacteroides fragilis* as well as fecal concentrations of secondary bile acids. These findings were accompanied by a reduction in inflammation in the ileal reservoir mucosa.

In another open-label study, 10 patients with active ileocolonic CD were enrolled to receive a daily 15 g dose of fructo-oligosaccharides (FOS) for 3 wk. The Harvey-Bradshaw index was chosen to assess the disease activity, and fluorescence *in situ* hybridization was used to measure *Bifidobacteria* in stools; flow cytometry of dissociated rectal biopsies evaluated mucosal dendritic cell, IL-10 and TLR expression. The results of this study were promising: the use of FOS resulted in a significant reduction in the Harvey Bradshaw index, and a significant increase in fecal *Bifidobacteria* concentrations. The percentage of IL-10 positive dendritic cells was increased from 30% to 53%. Moreover, an increase in the percentage of dendritic cells expressing TLR2 and TLR4 was found (from 1.7% to 36.8% and from 3.6% to 75.4%, respectively)[96].

Symbiotics

Probiotic therapy can potentially be improved by simultaneous administering of prebiotics (non-digestible and non-absorbable carbohydrates) that enhance probiotic proliferation in the gut. This mixture is referred as a symbiotic. The main benefit of symbiotic formulation is that a prebiotic constituent could positively modulate the increase in local microbiota, which is further regulated by the probiotic component of the synbiotic formulation. In animal models, Schultz *et al*[97] evaluated the effect of a symbiotic preparation composed of a probiotic combination of *Lactobacilli, Bifidobacteria* and inulin (SIM) in HLA-B27-beta(2)-microglobulin transgenic rats affected by severe colitis. After 4 mo of SIM treatment, the colonic disease showed histological improvement and, furthermore, there was an alteration in the microflora profile, with an increased variety, and specifically, increased growth of *Bifidobacterium animalis* compared with untreated rats.

A few well conducted studies have supported the usefulness of symbiotic supplementation. Furrie *et al*[98], in a double-blinded RCT, developed a symbiotic called Synergy 1, made up of a combination of a probiotic (*Bifidobacterium longum*) and a prebiotic (inulin-oligofructose), which provided a metabolic substrate for the *Bifidobacterium* strain, and obtained promising results in UC patients.

Fecal transplantation

A novel treatment for IBD is fecal microbiota transplantation (FMT). FMT consists of extracting gastrointestinal microbiota from a healthy donor, which is then instilled *via* an enema through a liquid stool suspension. FMT has recently gained ground as a therapy for refractory and/or recurrent *C. difficile* infection[99-102].

In a recent systematic review conducted by Anderson *et al*[103], following Cochrane and PRISMA recommendations, 5320 articles on FMT in patients with IBD were identified. Seventeen articles were selected, including reports on FMT given in single cases to treat IBD, and in the management of infectious diarrhea in IBD. The 17 trials encluded 41 subjects followed up for 2 wk-13 years. FMT was able to produce a reduction in symptoms in most of the IBD patients, allow an interruption in IBD medication, and result in disease remission. In those patients who experienced a simultaneous *C. difficile* infection, complete eradication of the bacterium was achieved. Even though this procedure may face difficult acceptance by patients, the review describes promising results.

Despite insufficient data on FMT in IBD, this procedure is potentially an effective and safe treatment; it may be suggested for subjects who failed conventional treatments. It is necessary to perform new well-designed and randomized trials to enrich the data about FMT in IBD to: (1) evaluate safety and success rate; and (2) to standardize protocols. Without these considerations, FMT could not become a standard part of clinical therapy[103].

CONCLUSION

Patients affected by IBDs, either UC or CD, suffer from a heterogeneic entity whose pathogenic etiology must be explored in the context of a "multihit" phenomenon that precipitates the disease through a multifactorial platform resulting from interactions among genetic, immunological and environmental triggers. Although the microbiota may well play a crucial role in the origin of IBD, up-to-date therapeutic strategies have a primary purpose of suppressing the host response, and so a significant fraction of patients fail to accomplish sustained remission.

While novel techniques in molecular biology and engineering have enabled further discoveries about the gut microbiota, the relationship between intestinal microbiota

and IBD has not yet been completely clarified. A better understanding of the role that some bacterial species play in IBD pathogenesis is essential in order to develop appropriate management strategies.

The possibility of modulating our gut flora by interventions on microbial composition and the correct timing of this operation have important implications on efforts to improve gastrointestinal health. Nevertheless, microbiology should support, but not replace, the genetics of IBD, and meticulous typing of the intestinal microflora should soon take a decisive place in its complete characterization in order to explore the relationship between genes and the environment in health and disease. Finally, future research in microbial intervention needs to be directed towards two areas: (1) improvements in strain selection with the goal of realizing new screening procedures for a better understanding of the mechanisms of action, and ensuring adequate efficacy; (2) a new therapeutic strategy with non-pathogenic organisms of alimentary origin that can be genetically modified with the aim of producing anti-inflammatory substances.

REFERENCES

1 **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]

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 **Leone V**, Chang EB, Devkota S. Diet, microbes, and host genetics: the perfect storm in inflammatory bowel diseases. *J Gastroenterol* 2013; **48**: 315-321 [PMID: 23475322 DOI: 10.1007/s00535-013-0777-2]

4 **Ley RE**, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell* 2006; **124**: 837-848 [PMID: 16497592 DOI: 10.1016/j.cell.2006.02.017]

5 **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]

6 **Macfarlane S**, Macfarlane GT. Bacterial diversity in the human gut. *Adv Appl Microbiol* 2004; **54**: 261-289 [PMID: 15251284 DOI: 10.1016/S0065-2164(04)54010-8]

7 **Bartosch S**, Fite A, Macfarlane GT, McMurdo ME. Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by using real-time PCR and effects of antibiotic treatment on the fecal microbiota. *Appl Environ Microbiol* 2004; **70**: 3575-3581 [PMID: 15184159 DOI: 10.1128/AEM.70.6.3575-3581.2004]

8 **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 DOI: 10.1073/pnas.0706625104]

9 **Sartor RB**. Microbial influences in inflammatory bowel diseases. *Gastroenterology* 2008; **134**: 577-594 [PMID: 18242222 DOI: 10.1053/j.gastro.2007.11.059]

10 **Round JL**, Lee SM, Li J, Tran G, Jabri B, Chatila TA, Mazmanian SK. The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science* 2011; **332**: 974-977 [PMID: 21512004 DOI: 10.1126/science.1206095]

11 **Sellon RK**, Tonkonogy S, Schultz M, Dieleman LA, Grenther W, Balish E, Rennick DM, Sartor RB. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. *Infect Immun* 1998; **66**: 5224-5231 [PMID: 9784526]

12 **Kullberg MC**, Ward JM, Gorelick PL, Caspar P, Hieny S, Cheever A, Jankovic D, Sher A. Helicobacter hepaticus triggers colitis in specific-pathogen-free interleukin-10 (IL-10)-deficient mice through an IL-12- and gamma interferon-dependent mechanism. *Infect Immun* 1998; **66**: 5157-5166 [PMID: 9784517]

13 **Kim SC**, Tonkonogy SL, Albright CA, Tsang J, Balish EJ, Braun J, Huycke MM, Sartor RB. Variable phenotypes of enterocolitis in interleukin 10-deficient mice monoassociated with two different commensal bacteria. *Gastroenterology* 2005; **128**: 891-906 [PMID: 15825073 DOI: 10.1053/j.gastro.2005.02.009]

14 **Sartor RB**. Microbial influences in inflammatory bowel disease: role in pathogenesis and clinical implications. In: Sartor RB, Sandborn WJ, editors. Kirsner's inflammatory bowel diseases. Philadelphia: Elsevier, 2004: 138-162

15 **Moran JP**, Walter J, Tannock GW, Tonkonogy SL, Sartor RB. Bifidobacterium animalis causes extensive duodenitis and mild colonic inflammation in monoassociated interleukin-10-deficient mice. *Inflamm Bowel Dis* 2009; **15**: 1022-1031 [PMID: 19235917 DOI: 10.1002/ibd.20900]

16 **Kornbluth A**, Sachar DB. Ulcerative colitis practice guidelines in adults (update): American College of Gastroenterology, Practice Parameters Committee. *Am J Gastroenterol* 2004; **99**: 1371-1385 [PMID: 15233681 DOI: 10.1111/j.1572-0241.2004.40036.x]

17 **Ott SJ**, Musfeldt M, Wenderoth DF, Hampe J, Brant O, Fölsch UR, Timmis KN, Schreiber S. Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. *Gut* 2004; **53**: 685-693 [PMID: 15082587 DOI: 10.1136/gut.2003.025403]

18 **Swidsinski A**, Ladhoff A, Pernthaler A, Swidsinski S, Loening-Baucke V, Ortner M, Weber J, Hoffmann U, Schreiber S, Dietel M, Lochs H. Mucosal flora in inflammatory bowel disease. *Gastroenterology* 2002; **122**: 44-54 [PMID: 11781279 DOI: 10.1053/gast.2002.30294]

19 **Pullan RD**, Thomas GA, Rhodes M, Newcombe RG, Williams GT, Allen A, Rhodes J. Thickness of adherent mucus gel on colonic mucosa in humans and its relevance to colitis. *Gut* 1994; **35**: 353-359 [PMID: 8150346 DOI: 10.1136/gut.35.3.353]

20 **Corfield AP**, Myerscough N, Bradfield N, Corfield Cdo A, Gough M, Clamp JR, Durdey P, Warren BF, Bartolo DC, King KR, Williams JM. Colonic mucins in ulcerative colitis: evidence for loss of sulfation. *Glycoconj J* 1996; **13**: 809-822 [PMID: 8910008 DOI: 10.1007/BF00702345]

21 **Kleessen B**, Kroesen AJ, Buhr HJ, Blaut M. Mucosal and invading bacteria in patients with inflammatory bowel disease compared with controls. *Scand J Gastroenterol* 2002; **37**: 1034-1041 [PMID: 12374228 DOI: 10.1080/00365520232037822 0]

22 **Kotlowski R**, Bernstein CN, Sepehri S, Krause DO. High prevalence of Escherichia coli belonging to the B2+D phylogenetic group in inflammatory bowel disease. *Gut* 2007; **56**: 669-675 [PMID: 17028128 DOI: 10.1136/gut.2006.099796]

23 **Montgomery SM**, Ekbom A. Epidemiology of inflammatory bowel disease. *Curr Opin Gastroenterol* 2002; **18**: 416-420 [PMID: 17033315 DOI: 10.1097/00001574-200207000-00004]

24 **Henderson P**, Satsangi J. Genes in inflammatory bowel disease: lessons from complex diseases. *Clin Med* 2011; **11**: 8-10 [PMID: 21404774 DOI: 10.7861/clinmedicine.11-1-8]

25 **Stappenbeck TS**, Rioux JD, Mizoguchi A, Saitoh T, Huett A, Darfeuille-Michaud A, Wileman T, Mizushima N, Carding S, Akira S, Parkes M, Xavier RJ. Crohn disease: a current perspective on genetics, autophagy and immunity. *Autophagy* 2011; **7**: 355-374 [PMID: 20729636 DOI: 10.4161/auto.7.2.13074]

26 **Barrett JC**, Lee JC, Lees CW, Prescott NJ, Anderson CA, Phillips A, Wesley E, Parnell K, Zhang H, Drummond H, Nimmo ER, Massey D, Blaszczyk K, Elliott T, Cotterill L, Dallal H, Lobo AJ, Mowat C, Sanderson JD, Jewell DP, Newman WG, Edwards C, Ahmad T, Mansfield JC, Satsangi J, Parkes M, Mathew CG, Donnelly P, Peltonen L, Blackwell JM, Bramon E, Brown MA, Casas JP, Corvin A, Craddock N, Deloukas P, Duncanson A, Jankowski J, Markus HS, Mathew CG, McCarthy MI, Palmer CN, Plomin R, Rautanen A, Sawcer SJ, Samani N, Trembath RC, Viswanathan AC, Wood N, Spencer CC, Barrett JC, Bellenguez C, Davison D, Freeman C, Strange A, Donnelly P, Langford C, Hunt SE, Edkins S, Gwilliam R, Blackburn H, Bumpstead SJ, Dronov S, Gillman M, Gray E, Hammond N, Jayakumar A, McCann OT, Liddle J, Perez ML, Potter SC, Ravindrarajah R, Ricketts M, Waller M, Weston P, Widaa S, Whittaker P, Deloukas P, Peltonen L, Mathew CG, Blackwell JM, Brown MA, Corvin A, McCarthy MI, Spencer CC, Attwood AP, Stephens J, Sambrook J, Ouwehand WH, McArdle WL, Ring SM, Strachan DP. Genome-wide association study of ulcerative colitis identifies three new susceptibility loci, including the HNF4A region. *Nat Genet* 2009; **41**: 1330-1334 [PMID: 19915572 DOI: 10.1038/ng.483]

27 **Maeda S**, Hsu LC, Liu H, Bankston LA, Iimura M, Kagnoff MF, Eckmann L, Karin M. Nod2 mutation in Crohn's disease potentiates NF-kappaB activity and IL-1beta processing. *Science* 2005; **307**: 734-738 [PMID: 15692052 DOI: 10.1126/science.1103685]

28 **Cooney R**, Baker J, Brain O, Danis B, Pichulik T, Allan P, Ferguson DJ, Campbell BJ, Jewell D, Simmons A. NOD2 stimulation induces autophagy in dendritic cells influencing bacterial handling and antigen presentation. *Nat Med* 2010; **16**: 90-97 [PMID: 19966812 DOI: 10.1038/nm.2069]

29 **Travassos LH**, Carneiro LA, Ramjeet M, Hussey S, Kim YG, Magalhães JG, Yuan L, Soares F, Chea E, Le Bourhis L, Boneca IG, Allaoui A, Jones NL, Nuñez G, Girardin SE, Philpott DJ. Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. *Nat Immunol* 2010; **11**: 55-62 [PMID: 19898471 DOI: 10.1038/ni.1823]

30 **Gearry RB**, Roberts RL, Burt MJ, Frampton CM, Chapman BA, Collett JA, Shirley P, Allington MD, Kennedy MA, Barclay ML. Effect of inflammatory bowel disease classification changes on NOD2 genotype-phenotype associations in a population-based cohort. *Inflamm Bowel Dis* 2007; **13**: 1220-1227 [PMID: 17538984 DOI: 10.1002/ibd.20190]

31 **Lesage S**, Zouali H, Cézard JP, Colombel JF, Belaiche J, Almer S, Tysk C, O'Morain C, Gassull M, Binder V, Finkel Y, Modigliani R, Gower-Rousseau C, Macry J, Merlin F, Chamaillard M, Jannot AS, Thomas G, Hugot JP. CARD15/NOD2 mutational analysis and genotype-phenotype correlation in 612 patients with inflammatory bowel disease. *Am J Hum Genet* 2002; **70**: 845-857 [PMID: 11875755 DOI: 10.1086/339432]

32 **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]

33 **Cosnes J**, Carbonnel F, Beaugerie L, Le Quintrec Y, Gendre JP. Effects of cigarette smoking on the long-term course of Crohn's disease. *Gastroenterology* 1996; **110**: 424-431 [PMID: 8566589 DOI: 10.1053/gast.1996.v110.pm8566589]

34 **Lindberg E**, Järnerot G, Huitfeldt B. Smoking in Crohn's disease: effect on localisation and clinical course. *Gut* 1992; **33**: 779-782 [PMID: 1624159 DOI: 10.1136/gut.33.6.779]

35 **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]

36 **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]

37 **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]

38 **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]

39 **Jiménez E**, Marín ML, Martín R, Odriozola JM, Olivares M, Xaus J, Fernández L, Rodríguez JM. Is meconium from healthy newborns actually sterile? *Res Microbiol* 2008; **159**: 187-193 [PMID: 18281199 DOI: 10.1016/j.resmic.2007.12.007]

40 **Fouhy F**, Ross RP, Fitzgerald GF, Stanton C, Cotter PD. Composition of the early intestinal microbiota: knowledge, knowledge gaps and the use of high-throughput sequencing to address these gaps. *Gut Microbes* 2012; **3**: 203-220 [PMID: 22572829 DOI: 10.4161/gmic.20169]

41 **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]

42 **Palmer C**, Bik EM, DiGiulio DB, Relman DA, Brown PO. Development of the human infant intestinal microbiota. *PLoS Biol* 2007; **5**: e177 [PMID: 17594176 DOI: 10.1371/journal.pbio.0050177]

43 **Blaser MJ**, Falkow S. What are the consequences of the disappearing human microbiota? *Nat Rev Microbiol* 2009; **7**: 887-894 [PMID: 19898491 DOI: 10.1038/nrmicro2245]

44 **Hildebrandt MA**, Hoffmann C, Sherrill-Mix SA, Keilbaugh SA, Hamady M, Chen YY, Knight R, Ahima RS, Bushman F, Wu GD. High-fat diet determines the composition of the murine gut microbiome independently of obesity. *Gastroenterology* 2009; **137**: 1716-1724.e1-2 [PMID: 19706296 DOI: 10.1053/j.gastro.2009.08.042]

45 **Murphy EF**, Cotter PD, Healy S, Marques TM, O'Sullivan O, Fouhy F, Clarke SF, O'Toole PW, Quigley EM, Stanton C, Ross PR, O'Doherty RM, Shanahan F. Composition and energy harvesting capacity of the gut microbiota: relationship to diet, obesity and time in mouse models. *Gut* 2010; **59**: 1635-1642 [PMID: 20926643 DOI: 10.1136/gut.2010.215665]

46 **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]

47 **Montenegro L**, Losurdo G, Licinio R, Zamparella M, Giorgio F, Ierardi E, Leo AD, Principi M. Non steroidal anti-inflammatory drug induced damage on lower gastro-intestinal tract: is there an involvement of microbiota? *Curr Drug Saf* 2014; **9**: 196-204 [PMID: 24809527]

48 **Hayashi Y**, Aoyagi K, Morita I, Yamamoto C, Sakisaka S. Oral administration of mesalazine protects against mucosal injury and permeation in dextran sulfate sodium-induced colitis in rats. *Scand J Gastroenterol* 2009; **44**: 1323-1331 [PMID: 19891583 DOI: 10.3109/00365520903262414]

49 **El Aidy S**, Merrifield CA, Derrien M, van Baarlen P, Hoo-

iveld G, Levenez F, Doré J, Dekker J, Holmes E, Claus SP, Reijngoud DJ, Kleerebezem M. The gut microbiota elicits a profound metabolic reorientation in the mouse jejunal mucosa during conventionalisation. *Gut* 2013; **62**: 1306-1314 [PMID: 22722618 DOI: 10.1136/gutjnl-2011-301955]

50 **Bertuccini L**, Costanzo M, Iosi F, Tinari A, Terruzzi F, Stronati L, Aloi M, Cucchiara S, Superti F. Lactoferrin prevents invasion and inflammatory response following E. coli strain LF82 infection in experimental model of Crohn's disease. *Dig Liver Dis* 2014; **46**: 496-504 [PMID: 24631031 DOI: 10.1016/j.dld.2014.02.009]

51 **Couturier-Maillard A**, Secher T, Rehman A, Normand S, De Arcangelis A, Haesler R, Huot L, Grandjean T, Bressenot A, Delanoye-Crespin A, Gaillot O, Schreiber S, Lemoine Y, Ryffel B, Hot D, Nùñez G, Chen G, Rosenstiel P, Chamaillard M. NOD2-mediated dysbiosis predisposes mice to transmissible colitis and colorectal cancer. *J Clin Invest* 2013; **123**: 700-711 [PMID: 23281400 DOI: 10.1172/JCI62236]

52 **Sartor RB**. Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: antibiotics, probiotics, and prebiotics. *Gastroenterology* 2004; **126**: 1620-1633 [PMID: 15168372 DOI: 10.1053/j.gastro.2004.03.024]

53 **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]

54 **Sutherland L**, Singleton J, Sessions J, Hanauer S, Krawitt E, Rankin G, Summers R, Mekhjian H, Greenberger N, Kelly M. Double blind, placebo controlled trial of metronidazole in Crohn's disease. *Gut* 1991; **32**: 1071-1075 [PMID: 1916494]

55 **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]

56 **Arnold GL**, Beaves MR, Pryjdun VO, Mook WJ. Preliminary study of ciprofloxacin in active Crohn's disease. *Inflamm Bowel Dis* 2002; **8**: 10-15 [PMID: 11837933]

57 **Prantera C**, Zannoni F, Scribano ML, Berto E, Andreoli A, Kohn A, Luzi C. An antibiotic regimen for the treatment of active Crohn's disease: a randomized, controlled clinical trial of metronidazole plus ciprofloxacin. *Am J Gastroenterol* 1996; **91**: 328-332 [PMID: 8607501]

58 **Greenbloom SL**, Steinhart AH, Greenberg GR. Combination ciprofloxacin and metronidazole for active Crohn's disease. *Can J Gastroenterol* 1998; **12**: 53-56 [PMID: 9544412]

59 **Leiper K**, Morris AI, Rhodes JM. Open label trial of oral clarithromycin in active Crohn's disease. *Aliment Pharmacol Ther* 2000; **14**: 801-806 [PMID: 10848665]

60 **Steinhart AH**, Feagan BG, Wong CJ, Vandervoort M, Mikolainis S, Croitoru K, Seidman E, Leddin DJ, Bitton A, Drouin E, Cohen A, Greenberg GR. Combined budesonide and antibiotic therapy for active Crohn's disease: a randomized controlled trial. *Gastroenterology* 2002; **123**: 33-40 [PMID: 12105831]

61 **Rutgeerts P**, Hiele M, Geboes K, Peeters M, Penninckx F, Aerts R, Kerremans R. Controlled trial of metronidazole treatment for prevention of Crohn's recurrence after ileal resection. *Gastroenterology* 1995; **108**: 1617-1621 [PMID: 7768364]

62 **Rutgeerts P**, Van Assche G, Vermeire S, D'Haens G, Baert F, Noman M, Aerden I, De Hertogh G, Geboes K, Hiele M, D'Hoore A, Penninckx F. Ornidazole for prophylaxis of postoperative Crohn's disease recurrence: a randomized, double-blind, placebo-controlled trial. *Gastroenterology* 2005; **128**: 856-861 [PMID: 15825069]

63 **Turunen U**, Färkkilä V. Long-term treatment of ulcerative colitis with ciprofloxacin. *Gastroenterology* 1999; **117**: 282-283 [PMID: 10428613]

64 **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]

65 **Casellas F**, Borruel N, Papo M, Guarner F, Antolín M, Videla S, Malagelada JR. Antiinflammatory effects of enterically coated amoxicillin-clavulanic acid in active ulcerative colitis. *Inflamm Bowel Dis* 1998; **4**: 1-5 [PMID: 9552221]

66 **Turner D**, Levine A, Kolho KL, Shaoul R, Ledder O. Combination of oral antibiotics may be effective in severe pediatric ulcerative colitis: A preliminary report. *J Crohns Colitis* 2014; Epub ahead of print [PMID: 24958064 DOI: 10.1016/j.crohns.2014.05.010]

67 **Shen B**, Achkar JP, Lashner BA, Ormsby AH, Remzi FH, Brzezinski A, Bevins CL, Bambrick ML, Seidner DL, Fazio VW. A randomized clinical trial of ciprofloxacin and metronidazole to treat acute pouchitis. *Inflamm Bowel Dis* 2001; **7**: 301-305 [PMID: 11720319]

68 **Gionchetti P**, Rizzello F, Venturi A, Brigidi P, Matteuzzi D, Bazzocchi G, Poggioli G, Miglioli M, Campieri M. Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. *Gastroenterology* 2000; **119**: 305-309 [PMID: 10930365 DOI: 10.1053/gast.2000.9370]

69 **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]

70 **Matthes H**, Krummenerl T, Giensch M, Wolff C, Schulze J. Clinical trial: probiotic treatment of acute distal ulcerative colitis with rectally administered Escherichia coli Nissle 1917 (EcN). *BMC Complement Altern Med* 2010; **10**: 13 [PMID: 20398311 DOI: 10.1186/1472-6882-10-13]

71 **Sood A**, Midha V, Makharia GK, Ahuja V, Singal D, Goswami P, Tandon RK. The probiotic preparation, VSL#3 induces remission in patients with mild-to-moderately active ulcerative colitis. *Clin Gastroenterol Hepatol* 2009; **7**: 1202-1209, 1209.e1 [PMID: 19631292 DOI: 10.1016/j.cgh.2009.07.016]

72 **Pronio A**, Montesani C, Butteroni C, Vecchione S, Mumolo G, Vestri A, Vitolo D, Boirivant M. Probiotic administration in patients with ileal pouch-anal anastomosis for ulcerative colitis is associated with expansion of mucosal regulatory cells. *Inflamm Bowel Dis* 2008; **14**: 662-668 [PMID: 18240282 DOI: 10.1002/ibd.20369]

73 **Bibiloni R**, Fedorak RN, Tannock GW, Madsen KL, Gionchetti P, Campieri M, De Simone C, Sartor RB. VSL#3 probiotic-mixture induces remission in patients with active ulcerative colitis. *Am J Gastroenterol* 2005; **100**: 1539-1546 [PMID: 15984978 DOI: 10.1111/j.1572-0241.2005.41794.x]

74 **Tsuda Y**, Yoshimatsu Y, Aoki H, Nakamura K, Irie M, Fukuda K, Hosoe N, Takada N, Shirai K, Suzuki Y. Clinical effectiveness of probiotics therapy (BIO-THREE) in patients with ulcerative colitis refractory to conventional therapy. *Scand J Gastroenterol* 2007; **42**: 1306-1311 [PMID: 17852859 DOI: 10.1080/00365520701396091]

75 **Lorea Baroja M**, Kirjavainen PV, Hekmat S, Reid G. Anti-inflammatory effects of probiotic yogurt in inflammatory bowel disease patients. *Clin Exp Immunol* 2007; **149**: 470-479 [PMID: 17590176 DOI: 10.1111/j.1365-2249.2007.03434.x]

76 **Zocco MA**, dal Verme LZ, Cremonini F, Piscaglia AC, Nista EC, Candelli M, Novi M, Rigante D, Cazzato IA, Ojetti V, Armuzzi A, Gasbarrini G, Gasbarrini A. Efficacy of Lactobacillus GG in maintaining remission of ulcerative colitis. *Aliment Pharmacol Ther* 2006; **23**: 1567-1574 [PMID: 16696804 DOI: 10.1111/j.1365-2036.2006.02927.x]

77 **Kato K**, Mizuno S, Umesaki Y, Ishii Y, Sugitani M, Imaoka A,

Otsuka M, Hasunuma O, Kurihara R, Iwasaki A, Arakawa Y. Randomized placebo-controlled trial assessing the effect of bifidobacteria-fermented milk on active ulcerative colitis. *Aliment Pharmacol Ther* 2004; **20**: 1133-1141 [PMID: 15569116 DOI: 10.1111/j.1365-2036.2004.02268.x]

78 **Braat H**, Rottiers P, Hommes DW, Huyghebaert N, Remaut E, Remon JP, van Deventer SJ, Neirynck S, Peppelenbosch MP, Steidler L. A phase I trial with transgenic bacteria expressing interleukin-10 in Crohn's disease. *Clin Gastroenterol Hepatol* 2006; **4**: 754-759 [PMID: 16716759 DOI: 10.1016/j.cgh.2006.03.028]

79 **Bai AP**, Ouyang Q. Probiotics and inflammatory bowel diseases. *Postgrad Med J* 2006; **82**: 376-382 [PMID: 16754706 DOI: 10.1136/pgmj.2005.040899]

80 **Prantera C**, Scribano ML, Falasco G, Andreoli A, Luzi C. Ineffectiveness of probiotics in preventing recurrence after curative resection for Crohn's disease: a randomised controlled trial with Lactobacillus GG. *Gut* 2002; **51**: 405-409 [PMID: 12171964 DOI: 10.1136/gut.51.3.405]

81 **Schultz M**, Veltkamp C, Dieleman LA, Grenther WB, Wyrick PB, Tonkonogy SL, Sartor RB. Lactobacillus plantarum 299V in the treatment and prevention of spontaneous colitis in interleukin-10-deficient mice. *Inflamm Bowel Dis* 2002; **8**: 71-80 [PMID: 11854603 DOI: 10.1097/00054725-200203000-00001]

82 **Dieleman LA**, Goerres MS, Arends A, Sprengers D, Torrice C, Hoentjen F, Grenther WB, Sartor RB. Lactobacillus GG prevents recurrence of colitis in HLA-B27 transgenic rats after antibiotic treatment. *Gut* 2003; **52**: 370-376 [PMID: 12584218 DOI: 10.1136/gut.52.3.370]

83 **Fabia R**, Ar'Rajab A, Johansson ML, Willén R, Andersson R, Molin G, Bengmark S. The effect of exogenous administration of Lactobacillus reuteri R2LC and oat fiber on acetic acid-induced colitis in the rat. *Scand J Gastroenterol* 1993; **28**: 155-162 [PMID: 8382837 DOI: 10.3109/00365529309096063]

84 **Gionchetti P**, Rizzello F, Helwig U, Venturi A, Lammers KM, Brigidi P, Vitali B, Poggioli G, Miglioli M, Campieri M. Prophylaxis of pouchitis onset with probiotic therapy: a double-blind, placebo-controlled trial. *Gastroenterology* 2003; **124**: 1202-1209 [PMID: 12730861 DOI: 10.1016/S0016-5085(03)00171-9]

85 **Cui HH**, Chen CL, Wang JD, Yang YJ, Cun Y, Wu JB, Liu YH, Dan HL, Jian YT, Chen XQ. Effects of probiotic on intestinal mucosa of patients with ulcerative colitis. *World J Gastroenterol* 2004; **10**: 1521-1525 [PMID: 15133865]

86 **Malchow HA**. Crohn's disease and Escherichia coli. A new approach in therapy to maintain remission of colonic Crohn's disease? *J Clin Gastroenterol* 1997; **25**: 653-658 [PMID: 9451682 DOI: 10.1097/00004836-199712000-00021]

87 **Marteau P**, Lémann M, Seksik P, Laharie D, Colombel JF, Bouhnik Y, Cadiot G, Soulé JC, Bourreille A, Metman E, Lerebours E, Carbonnel F, Dupas JL, Veyrac M, Coffin B, Moreau J, Abitbol V, Blum-Sperisen S, Mary JY. Ineffectiveness of Lactobacillus johnsonii LA1 for prophylaxis of postoperative recurrence in Crohn's disease: a randomised, double blind, placebo controlled GETAID trial. *Gut* 2006; **55**: 842-847 [PMID: 16377775 DOI: 10.1136/gut.2005.076604]

88 **Van Gossum A**, Dewit O, Louis E, de Hertogh G, Baert F, Fontaine F, DeVos M, Enslen M, Paintin M, Franchimont D. Multicenter randomized-controlled clinical trial of probiotics (Lactobacillus johnsonii, LA1) on early endoscopic recurrence of Crohn's disease after lleo-caecal resection. *Inflamm Bowel Dis* 2007; **13**: 135-142 [PMID: 17206696 DOI: 10.1002/ibd.20063]

89 **Butterworth AD**, Thomas AG, Akobeng AK. Probiotics for induction of remission in Crohn's disease. *Cochrane Database Syst Rev* 2008; **(3)**: CD006634 [PMID: 18646162 DOI: 10.1002/14651858]

90 **Cummings JH**. Dietary carbohydrates and the colonic microflora. *Curr Opin Clin Nutr Metab Care* 1998; **1**: 409-414 [PMID: 10565385 DOI: 10.1097/00075197-199809000-00007]

91 **Bird AR**, Brown IL, Topping DL. Starches, resistant starches, the gut microflora and human health. *Curr Issues Intest Microbiol* 2000; **1**: 25-37 [PMID: 11709851]

92 **Jacobasch G**, Schmiedl D, Kruschewski M, Schmehl K. Dietary resistant starch and chronic inflammatory bowel diseases. *Int J Colorectal Dis* 1999; **14**: 201-211 [PMID: 10647628 DOI: 10.1007/s003840050212]

93 **Videla S**, Vilaseca J, Antolín M, García-Lafuente A, Guarner F, Crespo E, Casalots J, Salas A, Malagelada JR. Dietary inulin improves distal colitis induced by dextran sodium sulfate in the rat. *Am J Gastroenterol* 2001; **96**: 1486-1493 [PMID: 11374687 DOI: 10.1111/j.1572-0241.2001.03802.x]

94 **Camuesco D**, Peran L, Comalada M, Nieto A, Di Stasi LC, Rodriguez-Cabezas ME, Concha A, Zarzuelo A, Galvez J. Preventative effects of lactulose in the trinitrobenzenesulphonic acid model of rat colitis. *Inflamm Bowel Dis* 2005; **11**: 265-271 [PMID: 15735433 DOI: 10.1097/01.MIB.0000160808.30988.d9]

95 **Welters CF**, Heineman E, Thunnissen FB, van den Bogaard AE, Soeters PB, Baeten CG. Effect of dietary inulin supplementation on inflammation of pouch mucosa in patients with an ileal pouch-anal anastomosis. *Dis Colon Rectum* 2002; **45**: 621-627 [PMID: 12004211 DOI: 10.1007/s10350-004-6257-2]

96 **Lindsay JO**, Whelan K, Stagg AJ, Gobin P, Al-Hassi HO, Rayment N, Kamm MA, Knight SC, Forbes A. Clinical, microbiological, and immunological effects of fructo-oligosaccharide in patients with Crohn's disease. *Gut* 2006; **55**: 348-355 [PMID: 16162680 DOI: 10.1136/gut.2005.074971]

97 **Schultz M**, Munro K, Tannock GW, Melchner I, Göttl C, Schwietz H, Schölmerich J, Rath HC. Effects of feeding a probiotic preparation (SIM) containing inulin on the severity of colitis and on the composition of the intestinal microflora in HLA-B27 transgenic rats. *Clin Diagn Lab Immunol* 2004; **11**: 581-587 [PMID: 15138186]

98 **Furrie E**, Macfarlane S, Kennedy A, Cummings JH, Walsh SV, O'neil DA, Macfarlane GT. Synbiotic therapy (Bifidobacterium longum/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. *Gut* 2005; **54**: 242-249 [PMID: 15647189 DOI: 10.1136/gut.2004.044834]

99 **Gough E**, Shaikh H, Manges AR. Systematic review of intestinal microbiota transplantation (fecal bacteriotherapy) for recurrent Clostridium difficile infection. *Clin Infect Dis* 2011; **53**: 994-1002 [PMID: 22002980 DOI: 10.1093/cid/cir632]

100 **Borody TJ**, Khoruts A. Fecal microbiota transplantation and emerging applications. *Nat Rev Gastroenterol Hepatol* 2012; **9**: 88-96 [PMID: 22183182 DOI: 10.1038/nrgastro.2011.244]

101 **Landy J**, Al-Hassi HO, McLaughlin SD, Walker AW, Ciclitira PJ, Nicholls RJ, Clark SK, Hart AL. Review article: faecal transplantation therapy for gastrointestinal disease. *Aliment Pharmacol Ther* 2011; **34**: 409-415 [PMID: 21682755 DOI: 10.1111/j.1365-2036.2011.04737.x]

102 **Eiseman B**, Silen W, Bascom GS, Kauvar AJ. Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. *Surgery* 1958; **44**: 854-859 [PMID: 13592638]

103 **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]

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REVIEW

Patterns of airway involvement in inflammatory bowel diseases

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Abstract

Extraintestinal manifestations occur commonly in inflammatory bowel diseases (IBD). Pulmonary manifestations (PM) of IBD may be divided in airway disorders, interstitial lung disorders, serositis, pulmonary vasculitis, necrobiotic nodules, drug-induced lung disease, thromboembolic lung disease and enteropulmonary fistulas. Pulmonary involvement may often be asymptomatic and detected solely on the basis of abnormal screening tests. The common embryonic origin of the intestine and the lungs from the primitive foregut, the co-existence of mucosa associated lymphoid tissue in both organs, autoimmunity, smoking and bacterial translocation from the colon to the lungs may all be involved in the pathogenesis of PM in IBD. PM are mainly detected by pulmonary function tests and high-resolution computed tomography. This review will focus on the involvement of the airways in the context of IBD, especially stenoses of the large airways, tracheobronchitis, bronchiectasis, bronchitis, mucoid impaction, bronchial granulomas, bronchiolitis, bronchiolitis obliterans syndrome and the co-existence of IBD with asthma, chronic obstructive pulmonary disease, sarcoidosis and a1-antitrypsin deficiency.

Key words: Inflammatory bowel diseases; Airways; Bronchiolitis

Core tip: The lung is commonly involved in inflammatory bowel diseases; however, airway involvement is often overlooked. This review will help gastroenterologists recognize the involvement of the airways in the context of inflammatory bowel diseases (IBD), especially stenoses of the large airways, tracheobronchitis, bronchiectasis, bronchitis, mucoid impaction, bronchial granulomas, bronchiolitis, bronchiolitis obliterans syndrome and the co-existence of IBD with asthma, chronic obstructive pulmonary disease, sarcoidosis and a1-antitrypsin deficiency, and appropriately manage their patients.

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INTRODUCTION

Extraintestinal manifestations (EIM) commonly occur in inflammatory bowel diseases (IBD), with a prevalence rate between 21%-41% reported in various series. Crohn's disease (CD) presents with EIM more frequently than ulcerative colitis (UC)[1]. The most common EIM are erythema nodosum, pyoderma gangrenosum, arthritis, uveitis,

episcleritis, mouth ulcers, renal stones, thromboembolic disease and primary sclerosing cholangitis. Pulmonary involvement complicating IBD was originally considered rare (with a frequency rate < 1%), but the first case series published in 1976 assisted in better recognition, evaluation and description of IBD related respiratory disease[2].

Pulmonary manifestations (PM) of IBD have been studied in the literature by small in size case-control studies, case reports and epidemiological population-based studies. Depending on the anatomic site involved, PM in IBD may be divided in to airway disorders, interstitial lung disorders, serositis, pulmonary vasculitis, necrobiotic nodules, drug-induced lung disease, thromboembolic lung disease and enteropulmonary fistulas. Concomitant occurrence of IBD with specific respiratory diseases [granulomatosis with polyangiitis (GPA), asthma, chronic obstructive pulmonary disease (COPD), alpha 1 antitrypsin deficiency and sarcoidosis] is not uncommon. Pulmonary involvement is often asymptomatic and may be detected solely on the basis of abnormal screening tests. This review will focus on the involvement of the airways in the context of IBD.

EPIDEMIOLOGY

The exact incidence and prevalence of PM in IBD is not known; however, airway involvement constitutes a large proportion, responsible for 40%-63% of overall respiratory incidents[3,4]. PM in total and specifically airway involvement seem to occur more commonly in UC than in CD.

Although IBD related PM were originally considered rare, certain population-based studies have revealed significant interrelationships between the lungs and IBD. Bernstein and colleagues in a large population-based study in North America in 2005 reported that airway disorders in general (including asthma and bronchitis) were the most common extraintestinal manifestation in subjects with CD and the second most common in subjects with UC, with the prevalence of asthma in this population between 7%-8%[5]. In another retrospective study from the opposite view, Birring reported that IBD was 4 times more prevalent among patients with airway diseases, particularly non-asthmatic patients with productive cough, than in the general population[6].

These epidemiological studies along with the observation of concordant EIM among siblings and first degree relatives suffering from IBD led to genome-wide studies that showed certain genetic predispositions for various EIM. Hence, in CD, HLA-A2 and HLA-DR1 and in UC, haplotypes HLA-B27, HLA-B58 and HLA-B8/DR3 are all linked to IBD related skin, joint and eye disorders[7,8]. To our knowledge, such a genetic predisposition for respiratory involvement in IBD has not been demonstrated yet.

PATHOGENESIS

Current theory for the pathogenesis of IBD postulates that in genetically susceptible individuals, environmental triggering factors cause a local immunologically aberrant intestinal injury and repair as a response to commensal bacteria. Environmental factors implicated are smoking (for CD), stress, infection, drugs and diet. Polymorphisms in NOD2-CARD15 (caspase recruitment domain family member 15) are found in 25%-35% of patients of European descent with CD; haplotype HLA DRB1*0103 within major histocompatibility complex (MHC) is associated with susceptibility to and extensive UC. Various genetic mutations (ATG16L1, IL-23 receptor, TNF polymorphisms) confer to result in disease progression by loosening of the intestinal epithelial barrier, decreasing of microbial clearance, loss of tolerance of the mucosa to enteric microflora (*autophagy*) and immunological dysregulation. Innate and adaptive immunity recognize microbial antigens and through pattern recognition receptors [toll-like receptors (TLR), nucleotide binding domain like receptors (NLR)] initiate an abnormal T-cell expansion, in the form of Th-1 and Th-17 pathways in CD and mainly Th-2 pathway in UC, that lead to chronic mucosal inflammation and disease[9,10].

Although significant progression is noted in the understanding of the pathogenesis of IBD, the exact mechanisms responsible for the cross-talk between bowel disease and airway disorders are not clearly elucidated. This association is important since clinical observations have repeatedly pointed out the phenomenon of a respiratory exacerbation occurring suddenly in patients with IBD after a therapeutic intervention (enterectomy) for their colon disease, with respiratory disease being completely unresponsive to this intervention[11]. However, the common embryonic origin of the intestine and the lungs from the primitive foregut should be considered the basis for this association. Their common origin reflects their common structural features: an extensive luminal surface area, protected by a tight epithelial barrier that covers a submucosal layer of goblet cells, glands and, most importantly, lymphoid tissue responsible for homing of lymphocytes, as well as innate and adaptive immunity[12].

Bronchus associated lymphoid tissue (BALT) and gut associated lymphoid tissue (GALT) are both parts of the mucosa associated lymphoid tissue (MALT). Lymphocytes become activated according to the inflammatory stimuli they receive and mis-homing of lymphocytes may provide an explanation for the migration of inflammation[13,14]. Furthermore, as we described earlier, apart from immune-mediated phenomena, autoimmunity [pANCA, anti-Saccharomyces cerevisiae antibodies (ASCA) and antibodies against intestinal epithelial antigens] also contributes to the pathogenesis of IBD[15]. It is therefore likely that based on common structures and the affinities of the lymphoid tissue, circulating immune complexes, stimuli and autoantibodies migrate from the intestine to bronchial and possibly alveolar epithelium, leading to airway inflammation and disease. This shift of inflammation may become more dramatic when the colon is removed after colectomy. In line with this, Adenis and colleagues in a scintigraphy study demonstrated increased pulmo-

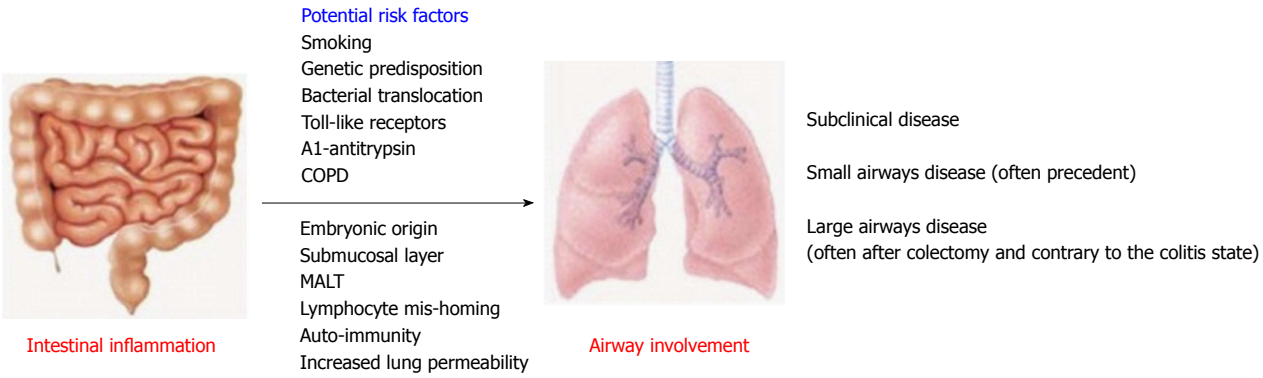

Figure 1 Possible pathogenesis of airway involvement and disease in the event of inflammatory bowel diseases. COPD: Chronic obstructive pulmonary disease; MALT: Mucosa associated lymphoid tissue.

nary permeability in patients with CD[16].

Certain contributing factors to such presumed pathogenesis may be proposed. Smoking is a well-known risk factor both for airway diseases and CD[17]. Bacterial translocation occurring in IBD may well affect the lung microbiome and confer to result in airway disorders since we already know that abnormal microbiome of the lungs carries implications in the pathogenesis of COPD[18]. Common TLR molecules (TLRs 2 and 4) participate in the pathogenesis of both COPD and IBD[19,20]. Lastly, matrix metalloproteinases (MMP) and anti-proteases like alpha-1 antitrypsin, well known as a cause of pulmonary emphysema, have been increasingly studied in the pathogenesis of IBD where their expression and balance seems to be disrupted, offering another potential link between the two systems[21]. A proposed schema for the pathogenesis of IBD related airway diseases is shown in Figure 1.

PATHOLOGY

As described earlier in this article, in the context of IBD, pathology examination may reveal abnormalities in different lung compartments, namely the airways, the interstitial tissue, the pleura, the parenchyma and the vessels. Interstitial pneumonitis and drug related eosinophilic pneumonia have been described; however, most intriguing for the pathologist is the differentiation between CD related pulmonary involvement and GPA in the case of pulmonary nodules, particularly since CD and GPA may coincide, as shown in several reports[22]. In this review, we shall focus on subclinical disease and airway pathology findings.

Bronchoalveolar lavage (BAL) studies have shown that chronic inflammation is common in the bronchi and alveoli of patients with CD. Wallaert *et al*[23] reported that 61% of asymptomatic patients with CD exhibit BAL features of an overt lymphocytic alveolitis. The clinical significance of this phenomenon, which provides further evidence for a systemic immunological manifestation of IBD to the lungs, is unknown. In our opinion, this alveolitis will not necessarily progress to clinical stage disease.

Small case series have described all types of biopsy proven bronchiolitis, documented with wedge and transbronchial biopsies mostly but also with open lung biopsies. Granulomatous bronchiolitis is more common; acute bronchiolitis with peribronchiolar inflammation, concentric small airways fibrosis, constrictive bronchiolitis and diffuse panbronchiolitis are also reported[4]. It should be highlighted that in the larger series by Casey and colleagues, bronchiolitides usually present before or concurrently with bowel disease, unlike other respiratory disorders that commonly follow bowel disease by a considerable time[24].

SCREENING FOR AIRWAY DISEASES

Airway disease, either latent and subclinical or clinically active, should be recognized for several reasons: (1) airway disease may complicate and follow a course independent of the course of the primary IBD; (2) IBD related airway diseases necessitate appropriate treatment and follow-up; (3) certain pulmonary function tests (PFT) may have a role as potential markers of disease activity; and (4) screening for respiratory disease may add to the recognition of concurrent diseases such as asthma and sarcoidosis. Screening for airway disease in the context of IBD may include medical history and clinical examination, PFT and radiological examinations.

Symptoms - clinical examination

Patients examined in an IBD clinic should be regularly asked about respiratory symptoms as nearly half of them report at least one symptom which they may attribute to anything but their primary disease. The most common symptoms reported are cough, sputum production, breathlessness mainly while exercising and wheezing. Stridor and hoarseness may develop in cases of upper airway narrowing.

Camus and colleagues reported that respiratory symptoms follow IBD presentation by months or even decades in nearly 85% of patients. In 10%-15%, respiratory symptoms precede and rarely (5%-10%) coincide with inflammatory bowel disease[3]. Respiratory symptom prevalence ranges from 25.6% in a recent study of 30 UC and 9 CD patients to 50% in an older study of 11

CD and 19 UC patients[25,26]. In a case control study of 64 IBD patients compared to 1346 controls, after adjustment for age, sex and smoking status, IBD patients were more likely to report shortness of breath and sputum production and to a lesser degree, cough [odds ratio (OR) respectively 3.4, 2.5, 1.8], highlighting the importance of relative clinical awareness[27].

Moreover, Higenbottam *et al*[28] described a respiratory exacerbation with cough and shortness of breath several years after coloproctectomy with the primary IBD in remission. This finding has been consistently described by other authors and several case reports. Thus, respiratory symptoms may occur independently of the course and activity of the bowel disease and are not responsive in parallel to its surgical treatment; on the contrary, pulmonary disease may be exacerbated. Most of these cases are reported with ulcerative colitis.

When respiratory symptoms occur acutely, the differential diagnosis includes pulmonary embolism, infectious pneumonia and drug toxicity. When respiratory disease follows an indolent course, airway disorders are more likely. Prompt evaluation in the latter scenario should include detailed medical history (with an emphasis on smoking habit, history of asthma and medication used), pulmonary function tests and radiological exams, including high resolution computed tomography scan (HRCT) of the chest with expiratory maneuvers. Bronchoscopy, as we will discuss further, is mandatory when there is evidence of upper airway involvement.

PFT

Symptomatic IBD patients may have normal PFT; this is apparently due to the anatomic site and the recruiting capacity of the airway system (bigger for small airways, smaller for large airways). On the other hand, up to two thirds of asymptomatic IBD patients have been found to demonstrate PFT abnormalities in recent studies, a surprising finding in light of the past reports of infrequent pulmonary involvement in IBD.

A prospective study of 40 IBD patients reported a 55% frequency of abnormal PFT in active IBD, equally distributed between UC and CD. This finding fell to 17.5% when IBD went into remission[29]. Herrlinger *et al*[30] reported 39% frequency of abnormal PFT in CD patients and 45% in UC, all asymptomatic, with values more affected during IBD activity but persisting in remission. Yilmaz *et al*[25] found abnormal PFT in 56% of IBD patients, with results directly affected by disease activity in UC cases. Another case-control study of 23 UC and 13 CD patients demonstrated abnormal PFT in 58% [75% of total events included low diffusing capacity for carbon monoxide (DLCO)], 81% of these patients with active IBD[31]. Lastly, in the larger study of 83 UC and 12 CD patients, Desai *et al*[32] reported abnormal PFT in 28.5% and low DLCO in 18%. Most of these studies included a mixed CD and UC population. UC patients, however, usually outnumbered CD patients, perhaps making PFT abnormalities seem more frequent in UC.

Decreased forced expiratory volume in the 1st second (FEV1), decreased FEV1/forced vital capacity (FVC) ratio, increased residual volume (RV)/total lung capacity (TLC) ratio, low forced expiratory flow (FEF_{25-75}) and, more importantly, decreased DLCO are the parameters noted to be abnormal in IBD patients in the existing literature. While FEV1 and FEV1/FVC ratio have been found to be normal in certain older studies, other studies and recent data suggest mild functional compromise[33,34]. Results should be interpreted with caution since different criteria have been used to define abnormal and certain studies included smoking patients or an inappropriate control population. In current studies, when an obstructive pattern has been noted, it demonstrates only partial reversibility which helps to differentiate it from asthma. RV/TLC ratio elevation has been demonstrated to correlate with bowel disease activity in several studies[35]. Tzanakis *et al*[36] have thoroughly described small airways disease, particularly in the event of active UC or CD.

The most consistent finding is a reduction in DLCO that commonly correlates with disease activity. In a large study of 47 CD and 85 UC patients, decreased DLCO was found in 19% and 17.6% respectively, with values being worse when the primary disease was active[37]. In another major study, DLCO was abnormal in 53% of inactive UC and in 81% of active UC patients; DLCO also correlated with pathological intestinal disease activity grading[38]. In children with IBD where PFT abnormalities are rather rare, reduced DLCO was the only finding in 53% of 26 children with active CD[39]. In conclusion, PFT and DLCO abnormalities in asymptomatic IBD are frequent but particularly so in active UC. It is believed that, as a systemic inflammatory disease, IBD affects the lung, creating a mild pulmonary inflammation corresponding to bowel inflammation. It is currently unknown, however, whether DLCO could serve as a disease activity marker.

Bronchial hyperresponsiveness (BHR) seems to occur more frequently in IBD than in a control population. This was demonstrated in 71% of children with CD and in 41% non-atopic IBD patients[40,41]. In line with this, airway eosinophilia and deranged induced sputum have also been reported in IBD[42]. These features may be associated with concomitant asthma or be subclinical and attributed solely to the underlying IBD. Thus, the etiology of BHR IBD may be two-fold: atopic and secondary to intestinal mucosal inflammation via the increased lung permeability observed in IBD[16].

It is currently unclear if asymptomatic patients with PFT abnormalities will progress to clinical respiratory disease and if so what defines this progression. Until more knowledge is acquired, PFT abnormalities should dictate closer follow-up of these patients.

Radiology - high resolution computed tomography

Studies show that HRCT in patients with IBD often demonstrates abnormal findings. The most common findings include bronchial wall thickening and bronchi-

Table 1 Classification of airway diseases secondary to inflammatory bowel diseases[1,3,4]

Site of involvement	Manifestations	Percent of total PM
Upper extrathoracic and intrathoracic airways (larynx/glottis, trachea, mainstem bronchi)	Stenoses, tracheobronchitis, acute respiratory failure	7%-8%
Large airways	Bronchiectasis	23%-26%
	Simple chronic bronchitis without suppuration	10%-20%
	Mucoid impaction	
	Bronchial granulomas	
	Suppurative bronchitis	3%-8%
Small airways	Granulomatous bronchiolitis	3%-10%
	Acute bronchiolitis	
	Diffuse panbronchiolitis	
	Bronchiolitis obliterans syndrome	
Concomitant diseases involving the airways	Asthma	
	Chronic obstructive pulmonary disease	
	Sarcoidosis	
	A1 antitrypsin deficiency	

PM: Pulmonary manifestations.

ectasis, cysts, emphysema, ground glass opacities and reticulonodular opacities. Centrilobular nodules, "tree-in-bud" opacities, air trapping and a mosaic pattern in expiratory scans all constitute a bronchiolitis radiological appearance. The prevalence ranges from 22% to 89% and radiological findings may be independent of symptoms, PFT results and primary disease intestinal activity[32]. This is because these findings may not be solely attributed to primary IBD related pulmonary disease. Alternative diagnoses include COPD, smoking related bronchiolitis, smoking related interstitial lung abnormalities, drug toxicities, thromboembolic disease and infections.

AIRWAY DISEASES IN IBD

Airway inflammation and disease are the most prevalent and distinctive pattern of respiratory involvement in IBD and accounts for 40%-63% of the total of clinically significant pulmonary complaints. Airway diseases (AD) usually follow IBD presentation by many years or even decades and the opposite presentation is the exception; when AD occur, IBD is rather inactive. If left untreated, AD can lead to irreversible stenoses in the airway. The clinical manifestations depend on the exact anatomic site involved. A classification for AD is shown in Table 1.

Upper airways

Upper airway disease (UAD) in IBD is a rare entity that may involve pharynx, larynx, trachea and mainstem bronchi. A total of 24 cases have been reported in the literature. Exudative lesions affect the bronchial mucosa and may cause subglottic stenosis and tracheobronchitis. UAD has been described in both UC and CD, with UC being predominant. Usually, it occurs years after diagnosis of the bowel disease, with IBD stable or in remission. UAD can occur after colectomy, with the time interval being as short as 30 d.

UAD may present with hoarseness, stridor and severe respiratory distress or just with cough, phlegm and shortness of breath[43,44]. Physical examination may reveal wheezing during inspiration, expiration or both. Because the clinical presentation may mimic infection or asthma, a certain degree of clinical suspicion is required to suspect an insult to the upper airway as a consequence of IBD. PFT and radiology are helpful in the diagnosis. A flow-volume loop demonstrates variable extrathoracic obstruction or fixed upper airway obstruction with plateau at both phases of respiration. While chest radiography has subtle findings, a CT scan of the chest may show circumferential or nodular narrowing of the trachea and bronchi. Bronchoscopy is the diagnostic procedure of choice[45]. Mucosal inflammation with exuberant pseudotumoral lesions, deformities, whitish lesions and narrowing of the lumen have all been described. Histology shows neutrophilic inflammation, granulation tissue, ulcerations, squamous cell metaplasia and plasma cell submucosal infiltrates. Noncaseating bronchial granulomas have also been reported in CD.

The differential diagnosis of IBD related UAD includes sarcoidosis, tuberculosis, amyloidosis and GPA[46]. The clinical presentation may be confusing if there is no bowel disease activity or other extraintestinal manifestations of IBD or if there is anti-neutrophil cytoplasmic antibodies (ANCA) positivity. ANCA are found to be positive in 50%-90% of UC and 10%-20% of CD patients but usually are neither cytoplasmic nor perinuclear in location[47]. The ANCA type specificity and histology of the airway lesions may help differentiate IBD-related UAD from GPA. Interestingly, isolated ANCA positivity without vasculitis has been associated with isolated subglottic stenosis in one study[48].

Empirical data suggest initial treatment with systemically administered high doses of corticosteroids (prednisone 1 mg/kg of lean body weight administered orally or methylprednisolone 60-80 mg intravenously per day); these suggestions are drawn from case reports, however, and not from randomized controlled trials, and should therefore be critically viewed[3]. In refractory cases, rigid bronchoscopy and interventional bronchoscopy procedures (laser beam, balloon dilation, stent placement) may be required in order to maintain an adequate airway[49,50].

Large airways

Large airways include the bronchi from the level of lobar bronchi to the level of terminal bronchioles. Large airways are the most common anatomic site of respiratory involvement in IBD, accounting for about 50% of total PM. Bronchial disease is more common in non-smoking females in their 5th decade of life in UC, particularly when other extraintestinal manifestations are present. Bronchial disease occurs many years after IBD in 8%-85% of pa-

tients, precedes IBD in younger patients (10%-15%) and less often coincides with active IBD (5%-10%). In 79% of cases, IBD is inactive and 50% of reported bronchial disease has followed colectomy[3,4].

The main large airway disorders are bronchiectasis (BE), chronic bronchitis (CB), suppurative bronchitis and mucoid impaction (Table 1). The classification depends on the patient's symptoms (the presence or absence of copious purulent sputum in the absence of BE classifies patient as suppurative bronchitis or CB, respectively) and HRCT features. Such features include luminal dilatation with bronchial wall thickening (definition of BE), bronchial wall thickening alone (CB) and mucoid impaction.

PFT usually reveal an obstructive pattern non-reversible to bronchodilators, or occasionally a mixed obstructive and restrictive pattern. CB diagnosis may be difficult to establish, particularly in long standing respiratory illnesses or in the presence of smoking. However, it should be noted that in most studies the reported patients were never smokers. Moreover, UC is epidemiologically connected to non-smoking individuals; in this setting, diagnosis of CB becomes more straightforward.

BE is the most important IBD related lung disorder. Typically, UC patients appear to present more commonly than CD. The bowel disease is often inactive and patients present with subacute symptoms of sputum production, cough and shortness of breath. In half of IBD-BE cases, a curative surgery (colectomy) has preceded the diagnosis of BE. In this setting, a close temporal relationship of weeks to months is well documented[11]. Spira and colleagues reported 6 UC and 1 CD patient with BE and CB in half of them manifesting after colectomy[51]. In another study of 14 UC and 3 CD patients, 76% developed BE, 41% shortly after enterectomy; such features are verified by other studies as well[3,52]. The most popular explanation for this sequence implicates a shift of mediators from the resected bowel to the lung, based on their common embryonic origin. In addition, withdrawal of immune-modulatory medication like corticosteroids after IBD remission may play a role in the flare up of pulmonary disease. Autoimmunity, as postulated by other authors, may also play a role as antinuclear antibodies have been discovered in some cases[53].

Importantly, bronchial disease is treated separately and independently from the bowel disease. As such, colectomy would aggravate rather than palliate bronchial disease. Treatment is the same as with any other cause of non-cystic fibrosis bronchiectasis. Antibiotics, bronchial toilet and bronchodilators should be offered as usual in all BE. Historically, certain authors advocate the use of corticosteroids in IBD-BE, based on case series and personal experience. These authors suggest inhaled corticosteroids should be used initially and if response is poor suggest prednisone administered orally at a dose of 0.5 mg/kg. Methylprednisolone has been also lavaged through the bronchoscope directly into the airways. Since no hard evidence supports the use of corticosteroids in IBD-BE or in bronchiectasis in general, and because of the concern for long term treatment with corticosteroids (CS), it is the authors' opinion that CS should not be administered in primary IBD related airway diseases[3,46,54].

Small airways

Small airways refer to the transitional airway zone from terminal bronchioles to alveolar ducts. Although larger case series attribute bronchiolitides as only 3%-10% of total IBD related pulmonary manifestations, their true involvement may be greater[4]. Kelly and colleagues evaluated 10 patients with IBD and bronchiectasis and found that 70% of them had abnormal FEF_{25-75}, suggesting that subclinical small airways disease is more frequent[55].

Bronchiolitides, the main form of small airway disorders, share a different clinical presentation than the clinical characteristics of upper and large airways involvement. They occur at a younger age, earlier in the disease course and in 1/3 of cases, pulmonary disease precedes intestinal disease[4]. As such, patients with bronchiolitis who have yet to be diagnosed with IBD often undergo invasive diagnostic procedures (bronchoscopy, open lung biopsy). In contrast to other pulmonary manifestations of IBD, the bronchiolitides are equally relevant in Crohn's disease and ulcerative colitis, as opposed to rest of airway disorders that are more prevalent in UC.

Pulmonary pathology in small airway involvement has been described earlier in this article. Granulomatous bronchiolitis is the most common finding, accounting for 59% of cases, and relates to CD as a systemic granulomatous disease[24]. PFT commonly demonstrates an obstructive pattern that may derange FEV1 or only FEF_{25-75}. DLCO is often abnormal as well[36]. HRCT features are the same as for all bronchiolitides (as already described).

This secondary bronchiolitis may be acute or, more commonly, chronic. Chronic bronchiolitis that persists untreated significantly worsens prognosis since it may eventually progress to diffuse airway narrowing and bronchiolitis obliterans syndrome (BOS). It may also lead to the progressive formation of bronchiolectasis and bronchiectasis[56]. This development is important because it explains physiologically the coexistence of small airways disease with larger airway involvement observed in patients with IBD. Since CS have a modest effect on small airways disease, we suggest the use of macrolides in the setting of IBD related bronchiolitis. Macrolides have shown clinical benefit in diffuse panbronchiolitis and BOS; azithromycin is shown to inhibit epithelial to mesenchymal transition and fibrosis to the small airways[57]. Nevertheless, in cases of BOS and despite therapy, transplantation may eventually be needed.

Concomitant diseases involving the airways

Asthma: After arthritis, asthma is the most common comorbidity in both UC and CD. A large population-based epidemiological study at the University of Manitoba compared 3873 UC cases with 38674 matched controls and found a 7.88% incidence of asthma in UC (especially in males); similarly, they studied 4187 CD cases with

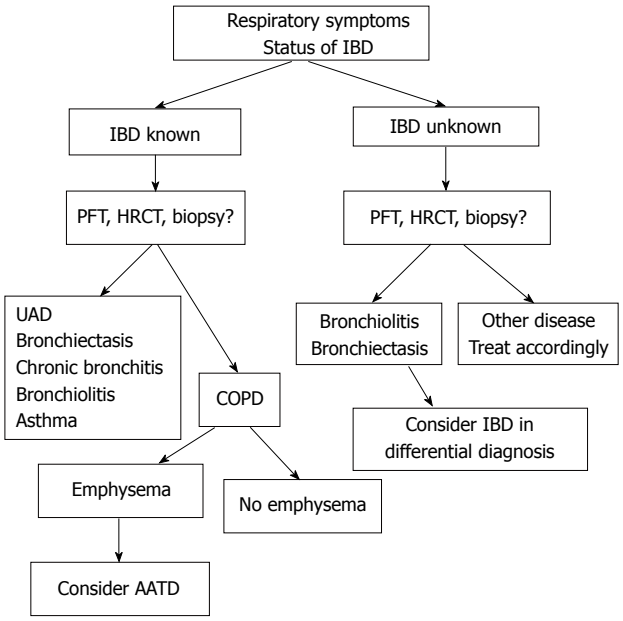

Figure 2 Proposed diagnostic algorithm for the evaluation of airway disease in inflammatory bowel diseases. IBD: Inflammatory bowel disease; PFT: Pulmonary function tests; HRCT: High resolution computed tomography; UAD: Upper airway disease; COPD: Chronic obstructive pulmonary disease; AATD: A1 antitrypsin deficiency.

41815 matched controls and found a 7.09% prevalence ratio of asthma in CD (especially in females)[5].

We have already discussed the increased prevalence of BHR and atopy in IBD. It should be noted that when a clinical phenotype of asthma is established in a patient with IBD, appropriate treatment is mandatory since there is epidemiological evidence of increased mortality in the asthma-UC population and laboratory evidence of more severe BHR in asthma-UC patients[58,59].

COPD: Cigarette smoke is known to protect against UC but promotes CD progression. Another population-based study investigated the relationship between COPD and IBD. Investigators found that COPD cases had a 1.83 hazard ratio (HR) for UC and a 2.72 HR for CD; this hazard extended to first degree relatives. As such, an inflammatory vulnerability in COPD patients has been postulated[60]. A very recent study evaluating the intestinal function of COPD patients demonstrated that COPD, regarded as a systemic inflammatory disease, causes intestinal hyperpermeability and enterocyte damage leading to intestinal compromise. The latter potentially provides an explanation for this coincidence from an etiological and environmental point of view[61].

COPD in IBD patients should be investigated, recognized and treated appropriately. COPD increases all-cause mortality and specific cause mortality in patients with CD. A meta-analysis by Duricova and colleagues reported a standardized mortality rate of 2.55 for CD-COPD subjects[62].

Sarcoidosis: Sarcoidosis shares many common characteristics with IBD, especially CD, as they both are granulomatous diseases. Sarcoidosis and IBD may coincide, as shown by case reports and population-based studies. They both share multi-organ manifestations (joints, eye, skin). Sarcoidosis and IBD seem to share a genetic overlap regarding cytoplasmic nucleotide oligomerization domains 1 and 2 and certain polymorphisms in chromosomes 1 (loci *IL-23R* and *1q.24.3*) and 15 (locus *HERC2*)[63].

IBD, as discussed earlier in this article, may exhibit granulomatous lung disease, mainly bronchiolitis. If infections have been ruled out, it is intriguing to differentiate between an IBD pulmonary localization and concomitant sarcoidosis. Histopathological features pointing to sarcoidosis are a lymphangitic distribution of granulomas and the absence of interstitial pneumonia and chronic bronchiolitis[24]. A clinical approach, however, is mandatory as the diagnosis of sarcoidosis apart from granuloma pathology demands compatible clinical and radiological findings.

A1 antitrypsin deficiency: Heterozygosity for the PiZ allele of a1 antitrypsin (AAT) deficiency (AATD) has been found to be more prevalent in patients with UC than in the general Swedish population (8.5% *vs* 4.7%)[64]. More importantly, a recent study from the United Kingdom confirmed a higher prevalence of UC among subjects suffering from emphysema due to homozygous PiZZ allele and AATD in comparison to the general population (1.5% *vs* 0.4%)[65]. Consequently, a blood test for AATD should be ordered when emphysema and IBD coincide in a young patient. It is unknown if AAT supplementation could be of use in the therapy of IBD.

Table 2 Key messages

In a patient with IBD and respiratory symptoms, symptoms should be initially attributed to the primary disease because of significant lung-intestine interference
IBD, asthma and COPD often coincide
IBD should be always remembered in the differential diagnosis of bronchiectasis and bronchiolitis
PFT and HRCT are necessary to evaluate a symptomatic patient
IBD related airway disease does not necessarily follow the course of colitis

IBD: Inflammatory bowel disease; COPD: Chronic obstructive pulmonary disease; PFT: Pulmonary function tests; HRCT: High resolution computed tomography.

CONCLUSION

Patients with IBD may present sometime during the course of their disease with various pulmonary incidents. The clinical approach relies upon the doctor's knowledge and judgment to attribute the patient's symptoms to the primary disease, comorbidities or to other complications after a thorough investigation. A proposed diagnostic algorithm for the evaluation of respiratory disease in IBD is shown in Figure 2. Table 2 contains the key messages

that, in our opinion, summarize the pulmonary-intestinal interrelationships in inflammatory bowel diseases.

REFERENCES

1 **Hoffmann RM**, Kruis W. Rare extraintestinal manifestations of inflammatory bowel disease. *Inflamm Bowel Dis* 2004; **10**: 140-147 [PMID: 15168815 DOI: 10.1097/00054725-200403000-00013]

2 **Kraft SC**, Earle RH, Roesler M, Esterly JR. Unexplained bronchopulmonary disease with inflammatory bowel disease. *Arch Intern Med* 1976; **136**: 454-459 [PMID: 1267553 DOI: 10.1001/archinte.1976.03630040056012]

3 **Camus P**, Piard F, Ashcroft T, Gal AA, Colby TV. The lung in inflammatory bowel disease. *Medicine* (Baltimore) 1993; **72**: 151-183 [PMID: 8502168 DOI: 10.1097/00005792-199372030-00003]

4 **Black H**, Mendoza M, Murin S. Thoracic manifestations of inflammatory bowel disease. *Chest* 2007; **131**: 524-532 [PMID: 17296657 DOI: 10.1378/chest.06-1074]

5 **Bernstein CN**, Wajda A, Blanchard JF. The clustering of other chronic inflammatory diseases in inflammatory bowel disease: a population-based study. *Gastroenterology* 2005; **129**: 827-836 [PMID: 16143122 DOI: 10.1053/j.gastro.2005.06.021]

6 **Raj AA**, Birring SS, Green R, Grant A, de Caestecker J, Pavord ID. Prevalence of inflammatory bowel disease in patients with airways disease. *Respir Med* 2008; **102**: 780-785 [PMID: 18321696 DOI: 10.1016/j.rmed.2007.08.014]

7 **Roussomoustakaki M**, Satsangi J, Welsh K, Louis E, Fanning G, Targan S, Landers C, Jewell DP. Genetic markers may predict disease behavior in patients with ulcerative colitis. *Gastroenterology* 1997; **112**: 1845-1853 [PMID: 9178675 DOI: 10.1053/gast.1997.v112.pm9178675]

8 **Orchard TR**, Chua CN, Ahmad T, Cheng H, Welsh KI, Jewell DP. Uveitis and erythema nodosum in inflammatory bowel disease: clinical features and the role of HLA genes. *Gastroenterology* 2002; **123**: 714-718 [PMID: 12198697 DOI: 10.1053/gast.2002.35396]

9 **Baumgart DC**, Sandborn WJ. Crohn's disease. *Lancet* 2012; **380**: 1590-1605 [PMID: 22914295 DOI: 10.1016/S0140-6736(12)60026-9]

10 **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]

11 **Eaton TE**, Lambie N, Wells AU. Bronchiectasis following colectomy for Crohn's disease. *Thorax* 1998; **53**: 529-531 [PMID: 9713458 DOI: 10.1136/thx.53.6.529]

12 **Mestecky J**. The common mucosal immune system and current strategies for induction of immune responses in external secretions. *J Clin Immunol* 1987; **7**: 265-276 [PMID: 3301884 DOI: 10.1007/BF00915547]

13 **Holt PG**. Development of bronchus associated lymphoid tissue (BALT) in human lung disease: a normal host defence mechanism awaiting therapeutic exploitation? *Thorax* 1993; **48**: 1097-1098 [PMID: 8296250 DOI: 10.1136/thx.48.11.1097]

14 **Keely S**, Talley NJ, Hansbro PM. Pulmonary-intestinal cross-talk in mucosal inflammatory disease. *Mucosal Immunol* 2012; **5**: 7-18 [PMID: 22089028 DOI: 10.1038/mi.2011.55]

15 **Wen Z**, Fiocchi C. Inflammatory bowel disease: autoimmune or immune-mediated pathogenesis? *Clin Dev Immunol* 2004; **11**: 195-204 [PMID: 15559364 DOI: 10.1080/17402520400004201]

16 **Adenis A**, Colombel JF, Lecouffe P, Wallaert B, Hecquet B, Marchandise X, Cortot A. Increased pulmonary and intestinal permeability in Crohn's disease. *Gut* 1992; **33**: 678-682 [PMID: 1612487 DOI: 10.1136/gut.33.5.678]

17 **Birrenbach T**, Böcker U. Inflammatory bowel disease and smoking: a review of epidemiology, pathophysiology, and therapeutic implications. *Inflamm Bowel Dis* 2004; **10**: 848-859 [PMID: 15626903 DOI: 10.1097/00054725-200411000-00019]

18 **Sethi S**. Chronic obstructive pulmonary disease and infection. Disruption of the microbiome? *Ann Am Thorac Soc* 2014; **11** Suppl 1: S43-S47 [PMID: 24437405 DOI: 10.1513/AnnalsATS.201307-212MG]

19 **Berenson CS**, Kruzel RL, Eberhardt E, Dolnick R, Minderman H, Wallace PK, Sethi S. Impaired innate immune alveolar macrophage response and the predilection for COPD exacerbations. *Thorax* 2014; **69**: 811-818 [PMID: 24686454]

20 **Bank S**, Andersen PS, Burisch J, Pedersen N, Roug S, Galsgaard J, Turino SY, Brodersen JB, Rashid S, Rasmussen BK, Avlund S, Olesen TB, Hoffmann HJ, Thomsen MK, Thomsen VO, Frydenberg M, Nexø BA, Sode J, Vogel U, Andersen V. Associations between functional polymorphisms in the NFκB signaling pathway and response to anti-TNF treatment in Danish patients with inflammatory bowel disease. *Pharmacogenomics J* 2014; Epub ahead of print [PMID: 24776844]

21 **Matusiewicz M**, Neubauer K, Mierzchala-Pasierb M, Gamian A, Krzystek-Korpacka M. Matrix metalloproteinase-9: its interplay with angiogenic factors in inflammatory bowel diseases. *Dis Markers* 2014; **2014**: 643645 [PMID: 24803722 DOI: 10.1155/2014/643645]

22 **Vaszar LT**, Orzechowski NM, Specks U, Ytterberg SR, Loftus EV, Mark EJ, Tazelaar HD. Coexistent pulmonary granulomatosis with polyangiitis (Wegener granulomatosis) and Crohn disease. *Am J Surg Pathol* 2014; **38**: 354-359 [PMID: 24525506 DOI: 10.1097/PAS.0000000000000135]

23 **Wallaert B**, Dugas M, Dansin E, Perez T, Marquette CH, Ramon P, Tonnel AB, Voisin C. Subclinical alveolitis in immunological systemic disorders. Transition between health and disease? *Eur Respir J* 1990; **3**: 1206-1216 [PMID: 2090485]

24 **Casey MB**, Tazelaar HD, Myers JL, Hunninghake GW, Kakar S, Kalra SX, Ashton R, Colby TV. Noninfectious lung pathology in patients with Crohn's disease. *Am J Surg Pathol* 2003; **27**: 213-219 [PMID: 12548168 DOI: 10.1097/00000478-200302000-00010]

25 **Yilmaz A**, Yilmaz Demirci N, Hoşgün D, Uner E, Erdoğan Y, Gökçek A, Cağlar A. Pulmonary involvement in inflammatory bowel disease. *World J Gastroenterol* 2010; **16**: 4952-4957 [PMID: 20954282 DOI: 10.3748/wjg.v16.i39.4952]

26 **Ceyhan BB**, Karakurt S, Cevik H, Sungur M. Bronchial hyperreactivity and allergic status in inflammatory bowel disease. *Respiration* 2003; **70**: 60-66 [PMID: 12584393 DOI: 10.1159/000068407]

27 **Birring SS**, Morgan AJ, Prudon B, McKeever TM, Lewis SA, Falconer Smith JF, Robinson RJ, Britton JR, Pavord ID. Respiratory symptoms in patients with treated hypothyroidism and inflammatory bowel disease. *Thorax* 2003; **58**: 533-536 [PMID: 12775870 DOI: 10.1136/thorax.58.6.533]

28 **Higenbottam T**, Cochrane GM, Clark TJ, Turner D, Millis R, Seymour W. Bronchial disease in ulcerative colitis. *Thorax* 1980; **35**: 581-585 [PMID: 7444824 DOI: 10.1136/thx.35.8.581]

29 **Ateş F**, Karincaoğlu M, Hacievlıyagıl SS, Yalniz M, Seçkın Y. Alterations in the pulmonary function tests of inflammatory bowel diseases. *Turk J Gastroenterol* 2011; **22**: 293-299 [PMID: 21805420]

30 **Herrlinger KR**, Noftz MK, Dalhoff K, Ludwig D, Stange EF, Fellermann K. Alterations in pulmonary function in inflammatory bowel disease are frequent and persist during remission. *Am J Gastroenterol* 2002; **97**: 377-381 [PMID: 11866276 DOI: 10.1111/j.1572-0241.2002.05473.x]

31 **Songür N**, Songür Y, Tüzün M, Doğan I, Tüzün D, Ensari A, Hekimoglu B. Pulmonary function tests and high-resolution CT in the detection of pulmonary involvement in inflammatory bowel disease. *J Clin Gastroenterol* 2003; **37**: 292-298 [PMID: 14506385 DOI: 10.1097/00004836-200310000-00006]

32 **Desai D**, Patil S, Udwadia Z, Maheshwari S, Abraham P, Joshi A. Pulmonary manifestations in inflammatory bowel disease: a prospective study. *Indian J Gastroenterol* 2011; **30**: 225-228 [PMID: 21935713 DOI: 10.1007/s12664-011-0129-1]

33 **Godet PG**, Cowie R, Woodman RC, Sutherland LR. Pulmonary function abnormalities in patients with ulcerative colitis. *Am J Gastroenterol* 1997; **92**: 1154-1156 [PMID: 9219789]

34 **Johnson NM**, Mee AS, Jewell DP, Clarke SW. Pulmonary function in inflammatory bowel disease. *Digestion* 1978; **18**: 416-418 [PMID: 35436 DOI: 10.1159/000198228]

35 **Neilly JB**, Main AN, McSharry C, Murray J, Russell RI, Moran F. Pulmonary abnormalities in Crohn's disease. *Respir Med* 1989; **83**: 487-491 [PMID: 2623217 DOI: 10.1016/S0954-6111(89)80132-5]

36 **Tzanakis N**, Samiou M, Bouros D, Mouzas J, Kouroumalis E, Siafakas NM. Small airways function in patients with inflammatory bowel disease. *Am J Respir Crit Care Med* 1998; **157**: 382-386 [PMID: 9476847 DOI: 10.1164/ajrccm.157.2.97-04075]

37 **Tzanakis N**, Bouros D, Samiou M, Panagou P, Mouzas J, Manousos O, Siafakas N. Lung function in patients with inflammatory bowel disease. *Respir Med* 1998; **92**: 516-522 [PMID: 9692115 DOI: 10.1016/S0954-6111(98)90301-8]

38 **Marvisi M**, Borrello PD, Brianti M, Fornarsari G, Marani G, Guariglia A. Changes in the carbon monoxide diffusing capacity of the lung in ulcerative colitis. *Eur Respir J* 2000; **16**: 965-968 [PMID: 11153600 DOI: 10.1183/09031936.00.16596500]

39 **Munck A**, Murciano D, Pariente R, Cezard JP, Navarro J. Latent pulmonary function abnormalities in children with Crohn's disease. *Eur Respir J* 1995; **8**: 377-380 [PMID: 7789480 DOI: 10.1183/09031936.95.08030377]

40 **Mansi A**, Cucchiara S, Greco L, Sarnelli P, Pisanti C, Franco MT, Santamaria F. Bronchial hyperresponsiveness in children and adolescents with Crohn's disease. *Am J Respir Crit Care Med* 2000; **161**: 1051-1054 [PMID: 10712362 DOI: 10.1164/ajrccm.161.3.9906013]

41 **Louis E**, Louis R, Drion V, Bonnet V, Lamproye A, Radermecker M, Belaiche J. Increased frequency of bronchial hyperresponsiveness in patients with inflammatory bowel disease. *Allergy* 1995; **50**: 729-733 [PMID: 8546267 DOI: 10.1111/j.1398-9995.1995.tb01214.x]

42 **Fireman Z**, Osipov A, Kivity S, Kopelman Y, Sternberg A, Lazarov E, Fireman E. The use of induced sputum in the assessment of pulmonary involvement in Crohn's disease. *Am J Gastroenterol* 2000; **95**: 730-734 [PMID: 10710066 DOI: 10.1111/j.1572-0241.2000.01843.x]

43 **Kuźniar T**, Sleiman C, Brugière O, Groussard O, Mal H, Mellot F, Pariente R, Malolepszy J, Fournier M. Severe tracheobronchial stenosis in a patient with Crohn's disease. *Eur Respir J* 2000; **15**: 209-212 [PMID: 10678648]

44 **Lamblin C**, Copin MC, Billaut C, Marti R, Tacq V, Riviere O, Wallaert B. Acute respiratory failure due to tracheobronchial involvement in Crohn's disease. *Eur Respir J* 1996; **9**: 2176-2178 [PMID: 8902486 DOI: 10.1183/09031936.96.09102176]

45 **Bayraktaroglu S**, Basoglu O, Ceylan N, Aydın A, Tuncel S, Savas R. A rare extraintestinal manifestation of ulcerative colitis: tracheobronchitis associated with ulcerative colitis. *J Crohns Colitis* 2010; **4**: 679-682 [PMID: 21122581 DOI: 10.1016/j.crohns.2010.06.003]

46 **Camus P**, Colby TV. The lung in inflammatory bowel disease. *Eur Respir J* 2000; **15**: 5-10 [PMID: 10678613 DOI: 10.1183/09031936.00.15100500]

47 **Roozendaal C**, Kallenberg CG. Are anti-neutrophil cytoplasmic antibodies (ANCA) clinically useful in inflammatory bowel disease (IBD)? *Clin Exp Immunol* 1999; **116**: 206-213 [PMID: 10337008 DOI: 10.1046/j.1365-2249.1999.00905.x]

48 **de Vries N**, Gans RO, Donker AJ, Goldschmeding R, Hoorntje SJ, Snow GB. Autoantibodies against constituents of neutrophils in the diagnosis and treatment of (isolated) subglottic stenosis. *Arch Otolaryngol Head Neck Surg* 1992; **118**: 1120-1123 [PMID: 1389063 DOI: 10.1001/archotol.1992.01880100112021]

49 **Wilcox P**, Miller R, Miller G, Heath J, Nelems B, Muller N, Ostrow D. Airway involvement in ulcerative colitis. *Chest* 1987; **92**: 18-22 [PMID: 3595232 DOI: 10.1378/chest.92.1.18]

50 **Plataki M**, Tzortzaki E, Lambiri I, Giannikaki E, Ernst A, Siafakas NM. Severe airway stenosis associated with Crohn's disease: case report. *BMC Pulm Med* 2006; **6**: 7 [PMID: 16603056 DOI: 10.1186/1471-2466-6-7]

51 **Spira A**, Grossman R, Balter M. Large airway disease associated with inflammatory bowel disease. *Chest* 1998; **113**: 1723-1726 [PMID: 9631822 DOI: 10.1378/chest.113.6.1723]

52 **Mahadeva R**, Walsh G, Flower CD, Shneerson JM. Clinical and radiological characteristics of lung disease in inflammatory bowel disease. *Eur Respir J* 2000; **15**: 41-48 [PMID: 10678619 DOI: 10.1183/09031936.00.15104100]

53 **Butland RJ**, Cole P, Citron KM, Turner-Warwick M. Chronic bronchial suppuration and inflammatory bowel disease. *Q J Med* 1981; **50**: 63-75 [PMID: 7267968]

54 **Pasteur MC**, Bilton D, Hill AT. British Thoracic Society guideline for non-CF bronchiectasis. *Thorax* 2010; **65** Suppl 1: i1-58 [PMID: 20627931 DOI: 10.1136/thx.2010.136119]

55 **Kelly MG**, Frizelle FA, Thornley PT, Beckert L, Epton M, Lynch AC. Inflammatory bowel disease and the lung: is there a link between surgery and bronchiectasis? *Int J Colorectal Dis* 2006; **21**: 754-757 [PMID: 16625374 DOI: 10.1007/s00384-006-0094-9]

56 **Papiris SA**, Malagari K, Manali ED, Kolilekas L, Triantafillidou C, Baou K, Rontogianni D, Bouros D, Kagouridis K. Bronchiolitis: adopting a unifying definition and a comprehensive etiological classification. *Expert Rev Respir Med* 2013; **7**: 289-306 [PMID: 23734650 DOI: 10.1586/ers.13.21]

57 **Banerjee B**, Musk M, Sutanto EN, Yerkovich ST, Hopkins P, Knight DA, Lindsey-Temple S, Stick SM, Kicic A, Chambers DC. Regional differences in susceptibiity of bronchial epithelium to mesenchymal transition and inhibition by the macrolide antibiotic azithromycin. *PLoS One* 2012; **7**: e52309 [PMID: 23284981 DOI: 10.1371/journal.pone.0052309]

58 **Persson PG**, Bernell O, Leijonmarck CE, Farahmand BY, Hellers G, Ahlbom A. Survival and cause-specific mortality in inflammatory bowel disease: a population-based cohort study. *Gastroenterology* 1996; **110**: 1339-1345 [PMID: 8613037 DOI: 10.1053/gast.1996.v110.pm8613037]

59 **Kanazawa H**, Yoshikawa J. A case-control study of bronchial asthma associated with ulcerative colitis: role of airway microvascular permeability. *Clin Exp Allergy* 2005; **35**: 1432-1436 [PMID: 16297138 DOI: 10.1111/j.1365-2222.2005.02358.x]

60 **Ekbom A**, Brandt L, Granath F, Löfdahl CG, Egesten A. Increased risk of both ulcerative colitis and Crohn's disease in a population suffering from COPD. *Lung* 2008; **186**: 167-172 [PMID: 18330638 DOI: 10.1007/s00408-008-9080-z]

61 **Rutten EP**, Lenaerts K, Buurman WA, Wouters EF. Disturbed intestinal integrity in patients with COPD: effects of activities of daily living. *Chest* 2014; **145**: 245-252 [PMID: 23928850 DOI: 10.1378/chest.13-0584]

62 **Duricova D**, Pedersen N, Elkjaer M, Gamborg M, Munkholm P, Jess T. Overall and cause-specific mortality in Crohn's disease: a meta-analysis of population-based studies. *Inflamm Bowel Dis* 2010; **16**: 347-353 [PMID: 19572377 DOI: 10.1002/ibd.21007]

63 **Fischer A**, Nothnagel M, Franke A, Jacobs G, Saadati HR, Gaede KI, Rosenstiel P, Schürmann M, Müller-Quernheim J, Schreiber S, Hofmann S. Association of inflammatory bowel disease risk loci with sarcoidosis, and its acute and chronic subphenotypes. *Eur Respir J* 2011; **37**: 610-616 [PMID: 20650992 DOI: 10.1183/09031936.00049410]

64 **Elzouki AN**, Eriksson S, Löfberg R, Nässberger L, Wi-

eslander J, Lindgren S. The prevalence and clinical significance of alpha 1-antitrypsin deficiency (PiZ) and ANCA specificities (proteinase 3, BPI) in patients with ulcerative colitis. *Inflamm Bowel Dis* 1999; **5**: 246-252 [PMID: 10579117 DOI: 10.1097/00054725-199911000-00002]

65 **Stone H**, Pye A, Stockley RA. Disease associations in alpha-1-antitrypsin deficiency. *Respir Med* 2014; **108**: 338-343 [PMID: 24176989 DOI: 10.1016/j.rmed.2013.10.006]

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MINIREVIEWS

Genetic and environmental determinants of risk for cholangiocarcinoma in Thailand

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Abstract

Cholangiocarcinoma (CCA) is a difficult cancer to diagnose in the early stage and to treat by curative resection. The incidence of CCA in the northeast of Thailand is the highest in the world. To make progress in detecting a high risk group and in the prevention and detection of CCA, we have been analyzing the risk factors for CCA. Although liver fluke infection is known to be a risk factor, there are patients who are not infected with the liver fluke and not all people infected with the liver fluke will suffer from the disease. Therefore, it is of the utmost importance to analyze the risk factors and the mechanism to prevent the disease and also to detect the disease in its early stage to save patients' lives. Through collaboration among Thai and Japanese researchers, we analyzed the genetic and environmental determinants of risks for CCA. Also, we have been trying to develop methods to detect the disease in a non-invasive way. Without repeating findings reported in various reviews on CCA, we will first discuss the environmental and genetic determinants of the risks for CCA. Second, we will discuss the properties of CCA, including the etiological agents and the mechanism of cholangiocarcinogenesis, and finally, we will discuss future approaches to prevent and cure CCA from the standpoint of evidence-based medicine. We will discuss these points by including the data from our laboratories. We would like to emphasize the importance of the genetic data, especially whole genome approaches, to understand the properties of CCA, to find a high risk population for CCA and to develop effective preventative methods to stop the carcinogenic steps toward CCA in the near future. In addition, it is of the upmost importance to develop a non-invasive, specific and sensitive method to detect CCA in its early stage for the application of modern medical approaches to help patients with CCA.

Key words: Alcohol drinking; Cholangiocarcinoma; DNA polymorphism; Glutathione S transferase; 8-oxoguanine

glycosylase 1; Liver fluke; *Opisthorchis viverrini*; Thailand

Core tip: Cholangiocarcinoma (CCA) is an intractable cancer due to the difficulty of diagnosis in its early stage. The incidence of CCA in the northeast of Thailand is the highest in the world. It is of the utmost importance to analyze the risk factors and the mechanism to prevent the disease and to also detect the disease in its early stage to save patients' lives. We analyzed the genetic and environmental determinants of risks for CCA and discussed this with the findings already published by other researchers. It is of the utmost importance to develop a non-invasive, specific and sensitive method to detect CCA.

Miwa M, Honjo S, You G, Tanaka M, Uchida K, Srivatanakul P, Khuhaprema T, Loilome W, Techasen A, Wongkham C, Limpaiboon T, Yongvanit P, Wongkham S. Genetic and environmental determinants of risk for cholangiocarcinoma in Thailand. *World J Gastrointest Pathophysiol* 2014; 5(4): 570-578 Available from: URL: http://www.wjgnet.com/2150-5330/full/v5/i4/570.htm DOI: http://dx.doi.org/10.4291/wjgp.v5.i4.570

INTRODUCTION

The age standardized rates (world ASR) of the incidence of liver and bile duct cancer in Thailand between 2001 and 2003 are 38.6 and 14.6 for men and women respectively. Most remarkably, world ASR of liver and bile duct cancer in Udon Thani, Khon Kaen, Nakorn Phanom, Ubon Ratchathani, Bangkok and Songkhla provinces for men are 115.0, 87.7, 78.4, 74.9, 21.5 and 10.9, respectively, and for women are 52.7, 36.3, 43.2, 34.7, 6.4 and 2.9, respectively. Cholangiocarcinoma (CCA) among the liver and bile duct cancer in the above provinces for men are 80.5%, 81.1%, 55.9%, 81.0%, 32.6% and 33.3%, respectively, and for women are 86.7%, 82.3%, 60.8%, 76.6%, 56.7% and 43.5%, respectively[1]. Thus, the incidence of CCA in the northeast of Thailand is extremely high in comparison to the rest of the world.

It was previously reported that the liver fluke, *Opisthorchis viverrini* (OV), and endogenous nitrosamines are the important risk factors for CCA in Thailand[2,3]. Multiple pathways on the tumorigenic OV infection to cause CCA from Thailand are nicely summarized in the recent review[4].

ENVIRONMENTAL DETERMINANTS

From the epidemiological study, it was previously known that the infection of the liver fluke, OV, is an important risk factor of CCA[2] (Table 1). In addition to OV infection, some of the chemical carcinogens like nitrosamine are also suggested to be factors in the risk for CCA[3]. We performed a population-based case-control study in which sex, age and place of residence were matched individually. We confirmed that the presence of the antibody against OV significantly increased the risk for CCA; odds ratio (OR) = 27.09 [95% confidence interval (CI): 6.30-116.57]. The results confirmed the previously reported data by Parkin *et al*[2]. In addition, we found that alcohol drinking is another risk factor for CCA. Ex-regular and regular alcohol drinkers showed OR = 6.23 (95%CI: 1.23-31.57) and OR = 4.31 (95%CI: 1.12-16.57), respectively (Table 1)[5]. We examined the possibility that alcohol consumption affects the risk for CCA due to OV infection, as well as smoking and dietary habits during the past 10 years, and found only the risks due to smoking and eating fermented fish (*pla-ra* and/or *pla-chao*) were altered with alcohol consumption (P for interaction < 0.01 and 0.07, respectively). The interactions between alcohol drinking and selected variables are shown in Table 2. The odds ratios are slightly different from those appearing in our previous paper[5] due to a typing error although the conclusion is materially the same. The increased risk for CCA due to ever-smoking was more prominent among ever-drinkers than among never-drinkers and a similar observation was made for the risk by eating *pla-ra* and/or *pla-chao*. Conversely, vitamin C was suggested to reduce the risk[3]. Recently, Songserm *et al*[6] confirmed that alcohol drinking increased the risk for CCA and they reported that the consumption of fruits and vegetables decreased the risk for CCA (Table 1). Manwong *et al*[7] also reported that a family history of cancer was a significant risk factor (Table 1).

INTERACTION BETWEEN GENETIC AND ENVIRONMENTAL DETERMINANTS

Since not all patients with CCA are infected with OV and not all individuals infected by OV develop CCA, it is possible that some other environmental and genetic determinants are involved in the pathogenesis of CCA. We examined the genetic polymorphism on the risk for CCA. We first examined the effect of carcinogen detoxification enzyme gene polymorphisms, namely *GSTM1* and *GSTT1*, which are well-known. DNA polymorphism of *GSTM1* or *GSTT1* alone was not associated with the risk of CCA. However, the null genotype of *GSTM1* enhanced OR of the risk for CCA in anti-OV antibody positive subjects was 18.00 (95%CI: 3.33-97.40) compared to that of *GSTM1* wild in anti-OV antibody positive subjects of 10.34 (95%CI: 1.31-81.63) and the null genotype of *GSTT1* enhanced OR in ex-regular alcohol drinkers was OR = 27.93 (95%CI: 1.84-424.60) compared to that of *GSTT1* wild in ex-regular drinkers of OR = 1.28 (95%CI: 0.12-14.08)[5].

Songserm *et al*[6] analyzed methylenetetrahydrofolate reductase gene polymorphism (*MTHFR*) at 677 and at 1298 for interaction with beef sausage consumption (Table 3). They found that *MTHFR*677 TT variants and *MTHFR*1298 CC variants showed increased risks when the individuals ate beef sausage daily. The data attained by the above researchers which showed an interaction are listed in Table 3.

Table 1 Effects of environmental determinants on risks for cholangiocarcinoma

Environmental determinants		Cases	Controls	OR	95%CI		*P* value	Ref.	Ethnic group
					LL	UL			
Anti-OV Ab	ref: < 1/40	101 matched case-control pairs		5.00	2.30	11.00	< 0.001	Parkin *et al*[2] 1991	Thai
				Adjusted OR					
Anti-OV Ab (ELISA)	< 0.200	61	119	1.00	Reference			Honjo *et al*[5] 2005	Thai
	⩾ 0.200	65	8	27.09	6.30	116.57	< 0.01		
Alcohol drinking	Never	30	46	1.00	Reference		-		
	Occasional	41	54	2.20	0.65	7.45	0.21		
	Ex-regular	15	7	6.23	1.23	31.57	0.03		
	Regular	41	21	4.31	1.12	16.57	0.03		
	Missing	2	-	-	-	-	-		
Raw fish	0	30	57	1.00	Reference				
	< 2/mo	54	41	2.70	1.28	5.68	< 0.01		
	⩾ 2/mo	45	31	2.94	1.24	6.96	0.01		
Fermented fish or pork	0	28	41	1.00	Reference				
	< 2/mo	58	63	2.95	0.98	8.90	0.06		
	⩾ 2/mo	43	25	4.50	1.30	15.54	0.02		
				Adjusted OR					
Alcohol drinking	Non-drinker	57	254	1.00	Reference			Songserm *et al*[6] 2012	Thai
(Units of alcohol per month)	< 14	79	92	5.60	2.85	10.95	< 0.001		
	⩾ 14	83	92	9.50	4.55	19.79	< 0.001		
Total vegetables (average times/ month)	< 52	136	214	1.00	Reference				
	⩾ 52	83	224	0.40	0.23	0.76	0.004		
Total fruits (average times/ month)	< 35	131	217	1.00	Reference				
	⩾ 35	88	221	0.60	0.33	0.98	0.04		
Family history of cancer	No	85	107	1.00	Reference			Manwong *et al*[7] 2013	Thai
	Yes	38	16	4.34	1.80	10.43	0.001		

OR: Odds ratio; CI: Confidence interval; LL: Lower limit; UL: Upper limit.

EFFECTS OF GENETIC DETERMINANTS AND DNA POLYMORPHISM ON RISK FOR CCA

There are several reports of the effects of DNA polymorphisms on the risk of CCA. Among various enzymes involved in carcinogen metabolism, CYP1A2, one of the phase I enzymes in the activation of such a carcinogen in cigarette smoke, has a DNA polymorphism. *CYP1A2* polymorphism, found in intron 1, might be involved in the risk of CCA. Prawan *et al*[8] found that *CYP1A2*1A/*1A* polymorphism had a protective effect on the risk of CCA in men but not in women (Table 4). Since men smoke more than women in Thailand, it is considered that in the individuals with *CYP1A2*1A* polymorphism, the CYP1A2 enzyme might be less inducible compared to that with *CYP1A2*1F*, although the effect of these mutations on the induction of the enzyme is not clear.

Arylamine *N*-acetyltransferase (NAT) catalyzes *N*- and *O*-acetylation of various arylamines and heterocyclic amines, thereby regulating the metabolic activation and detoxification of xenobiotics and carcinogens. Individuals with three *NAT2* alleles, *NAT2*13*, **6B* and **7A*, are associated with a decreased risk for CCA, while those with *NAT2*4, *5, *6A* and **7B* were not, suggesting that the *NAT2* polymorphism may modify the risk of CCA (Table 4)[8].

Glutathione *S*-transferases (GSTs), a family of Phase II detoxifying enzymes, can conjugate reduced glutathione to various compounds. Concerning polymorphism of *GSTO1* and *GSTO2*, Marahatta *et al*[9] found that individuals with *GSTO1*D140* had a significantly increased risk for CCA, hepatocellular carcinoma and breast cancer (Table 4). A study with a larger sample size will better clarify the function of GSTO1.

Natural killer cell receptor G2D (NKG2D) haplotypes were found to be associated with the natural cytotoxic activity of individuals. NKG2D triggers cell-mediated cytotoxicity in natural killer cells. Various NKG2D haplotype alleles showed a significant difference between cases and controls[10]. Primary sclerosing cholangitis (PSC) is an inflammatory bowel disease suggested to be a predisposing disease to hepatobiliary malignancy.

Table 2 Effect of modification of alcohol drinking on relationships between smoking, eating fermented fish and risk for cholangiocarcinoma

Variable	Category	Alcohol drinking							
		Never drinkers				Ever[1] drinkers			
		Adjusted[2] OR	95%CI		*P* value	Adjusted[2] OR	95% CI		*P* value
			LL	UL			LL	UL	
Smoking	Never	1	Reference			4.25	1.02	17.63	0.05
	Occasional	4.36	0.4	47.49	0.23	1.07	0.06	20.66	0.96
	Ex-regular					9.09	1.27	65.18	0.03
	Regular	3.64	0.19	71.41	0.39	7.99	1.56	40.94	0.01
Pla-ra,	< 3/d	1	Reference			14.07	1.46	135.36	0.02
Pla-chao	≥ 3/d	12.34	1.22	124.75	0.03	20.88	2.27	192.06	< 0.01

[1]Including occasional, ex- and currently regular drinkers; [2]Adjusted for anti-OV Ab when calculating the OR of smoking, and adjusted for anti-OV Ab and smoking when calculating the OR of eating of fermented fish (pla-ra and/or pla-chao). Nakorn Phanom (Thailand): based on the conditional logistic regression model. CI: Confidence interval; LL: Lower limit; UL: Upper limit; OR: Odds ratio. Adapted from Honjo *et al*[5] 2005. Allowing for absence of control subject in the category for occasional smoking and absence of case subject in the category for ex-regular smoking among never drinkers, we combined these two categories and confirmed the conclusion in the table is the materially unchanged from that in the table in our previous paper (Honjo *et al*[5] 2005).

Table 3 Interaction between genetic and environmental determinants on risks for cholangiocarcinoma

Genetic determinants		Environmental determinants		OR	95%CI		*P* value	Ref.	Ethnic group
					LL	UL			
				Adjusted OR					
GSTMI	Wild	Anti-OV antibody	Negative	1.00	Reference			Honjo *et al*[5] 2005	Thai
	Wild		Positive	10.34	1.31	81.63	0.03		
	Null		Negative	0.48	0.21	1.11	0.09		
	Null		Positive	18.00	3.33	97.40	< 0.01		
GSTMI	Wild	Toilet	Inside the house	1.00	Reference				
	Wild		Outside or none	0.20	0.04	1.02	0.05		
	Null		Inside the house	0.22	0.06	0.88	0.03		
	Null		Outside or none	0.25	0.07	0.91	0.04		
GSTTI	Wild	Alcohol drinking	Never	1.00	Reference				
	Wild		Occasional	3.58	0.71	17.95	0.12		
	Wild		Ex-regular	1.28	0.12	14.08	0.84		
	Wild		Regular	4.69	0.93	23.51	0.06		
	Null		Never	0.75	0.23	2.43	0.63		
	Null		Occasional	1.12	0.22	5.80	0.89		
	Null		Ex-regular	27.93	1.84	424.60	0.02		
	Null		Regular	3.28	0.35	30.91	0.30		
				Crude OR					
MTHFR 677	CC	Beef sausage	< 1/mo	1.0	Reference			Songserm *et al*[6] 2012	Thai
	CT		< 1/mo	1.1	0.51	2.37	0.82		
	TT		< 1/mo	0.6	0.25	1.53	0.32		
	CC		Weekly	0.9	0.45	1.83	0.80		
	CT		Weekly	1.2	0.57	2.43	0.65		
	TT		Weekly	1.6	0.80	3.31	0.18		
	CC		Daily	3.3	1.51	7.07	0.003		
	CT		Daily	3.2	1.33	7.62	0.01		
	TT		Daily	8.3	2.23	30.82	0.002		
MTHFR 1298	AA	Beef sausage	< 1/mo	1.0	Reference				
	AC		< 1/mo	1.3	0.63	2.55	0.51		
	CC		< 1/mo	0.8	0.28	2.15	0.63		
	AA		Weekly	1.3	0.71	2.45	0.39		
	AC		Weekly	1.0	0.49	1.79	0.84		
	CC		Weekly	3.8	1.48	9.89	0.01		
	AA		Daily	3.8	1.71	8.62	0.001		
	AC		Daily	3.5	1.56	7.85	0.002		
	CC		Daily	18.3	3.68	90.80	< 0.001		

Thirteen percent of patients with primary sclerosing cholangitis developed CCA[11]. When NKG2D single nucleotide polymorphisms (SNPs) were compared between the PSC patients with CCA and the PSC patients

Table 4 Effects of genetic determinants on risks for cholangiocarcinoma

Genotype		No. CCA (%)	No. control (%)	OR	95%CI		*P* value	Ref.	Ethnic group
					LL	UL			
				Adjusted OR					
CYP1A2, Male	*1F/*1F	85 (57.4)	88 (51.2)	1.0	Reference			Prawan *et al*[8] 2005	Thai
	*1A/*1F	59 (39.9)	69 (40.1)	0.9	0.55	1.47	0.677		
	*1A/*1A	4 (2.7)	15 (8.7)	0.28	0.08	0.94	0.039		
NAT2	All, except *6B, *7A and *13	193 (89.4)	162 (69.5)	1.0	Reference				
	One or two alleles (All, except *6B, *7A and *13)	23 (10.6)	71 (30.5)	0.26	0.15	0.44	< 0.001		
				Crude OR					
GST01	A140/A140	13 (43.33)	26 (86.67)	1.0	Reference			Marahatta *et al*[9] 2006	Thai
	A140/D140 + D140/D140	17 (56.67)	4 (13.33)	0.86	2.07	37.85			
		Minor allele frequency							
	Alleles	PSC[2] with CCA (n = 49)	PSC without CCA (n = 316)	OR			Corrected P		
NKG2D[1]	rs11053781 (Intron 5) G *vs* A	0.66	0.49	2.08	1.31	3.29	0.011	Melum *et al*[12] 2008	Norwegian
	rs2617167 (Intron 1) A *vs* G	0.39	0.22	2.32	1.47	3.66	0.002		
		PSC with CCA (n = 49)	Healthy controls (n = 368)						
	rs11053781 (Intron 5) G *vs* A	0.66	0.5	1.95	1.23	3.07	0.021		
	rs2617167 (Intron 1) A *vs* G	0.39	0.23	2.2	1.40	3.44	0.003		
		Counts (frequencies) of alleles/genotypes							
		2n = 120	2n = 146	Crude OR				Hoeblinger *et al*[13] 2009	Caucasian
MRP2/ ABCC2[3]	*ABCC2 c.3972* C (exon 28, synonymous SNP)	73 (0.61)	108 (0.74)						
	ABCC2 c.3972 T	47 (0.39)	38 (0.26)	1.83	1.087	3.08	0.022		
				OR					
MYH rs3219476	T/T	25 (42.4)	26 (26.0)	1.0	Reference			You *et al*[14] 2013	Han Chinese
	T/G	20 (33.9)	58 (58.0)	0.359	0.17	0.758	0.006		
	G/G	14 (23.7)	16 (16.0)	0.91	0.369	2.246	0.838		
	T/G + G/G	34 (57.6)	74 (74.0)	0.478	0.241	0.946	0.033		
MYH rs3219472	G/G	28 (47.5)	46 (46.0)	1.0	Reference				
	G/A	19 (32.2)	47 (47.0)	0.664	0.326	1.351	0.258		
	A/A	12 (20.3)	7 (7.0)	2.816	0.992	7.999	0.047		
	G/A + A/A	31 (52.5)	54 (54.0)	0.943	0.495	1.797	0.859		

[1]Natural killer cell receptor G2D; [2]Primary sclerosing cholangitis; [3]Multidrug resistance-associated protein 2 gene. OR: Odds ratio.

without CCA in a Norwegian population, there was significantly increased allele frequencies in two SNPs, namely rs11053781 and rs11053781, both of which are non-coding. The odds ratio for G *vs* A in the rs11053781 was 2.08 (95%CI: 1.31-3.29) and that for A *vs* G in rs2617167 was 2.32 (95%CI: 1.47-3.66). When they were compared between PSC patients with CCA and healthy controls, there was also a significant increase of allele frequencies in the above two SNPs. The odds ratio for G *vs* A in the rs11053781 was 1.95 (95%CI: 1.23-3.07) and that for A *vs* G in rs2617167 was 2.20 (95%CI: 1.40-3.44) (Table 4)[12]. The functional role of the changes of these SNPs on the susceptibility to CCA remains to be elucidated.

Multidrug resistance-associated protein 2 (MRP2/ABCC2), one of the ATP-binding cassette transporter proteins, is suggested to be involved in the excretion of the conjugates of carcinogens into bile, a metabolic step classified as so called "Phase Ⅲ metabolism". Thus, it might play an important role in cellular defense against toxic substances. The frequency of the *c.3972C > T ABCC2* gene variant (synonymous SNP) was compared between patients with CCA and healthy individuals.

Table 5 Interaction among genetic determinants on risks for cholangiocarcinoma

Genetic determinant		Genetic determinant		OR	95%CI		*P* value	Ref.	Ethnic group
					LL	UL			
MTHFR C677T[1]	CC	*TSER*[2]	*2R* (-)	1	Reference			Ko *et al*[17] 2006	South Korean
	CC		*2R* (+)[3]	5.38	1.23	23.56	0.026		
	CT		*2R* (-)	1.08	0.68	1.07			
	CT		*2R* (+)[3]	1.19	0.71	2.01			
	TT		*2R* (-)	1.02	0.7	1.5			
	TT		*2R* (+)[3]	1.24	0.9	1.71			
hOGG1(*Codon326*)	Ser/Ser	*GSTM1*	wild	1	Reference			Zeng *et al*[18] 2013	Thai
	Ser/Ser + Cys/Cys		wild	0.06	0.01	0.54	0.01		
	Ser/Ser		null	0.06	0.01	0.53	0.01		
	Ser/Ser + Cys/Cys		null	0.14	0.02	1.08	0.06		

[1]5,10-Methylenetetrahydrofolate reductase; [2]Thymidylate synthase enhancer region; [3]Including 2R2R and 2R3R.

There was a significant association between the SNP and the risk in a Caucasian population (Table 4)[13].

The DNA repair mechanism is protecting DNA damage caused by various kinds of carcinogenic factors. Among them, base excision repair (BER) plays an important role in the oxidative DNA damage caused by reactive oxygen species. MutY homolog, MYH, is involved in BER and functions as a DNA glycosylase which removes adenine paired with 8-hydroxy-2'-deoxyguanine residue. Individuals with T/G genotype in MYH rs3219476 had a reduced risk (OR = 0.478, 95%CI: 0.17-0.758, $P = 0.006$). Individuals with A/A genotype in MYHrs3219472 had an increased risk (OR = 2.816, 95%CI: 0.992-7.999, $P = 0.047$) (Table 4)[14].

Concerning other variants or mutations related to the risk for CCA, a mutation in bile salt export pump (ABCB11) was found in two children with progressive familial intrahepatic cholestasis and cholangiocarcinoma[15]. Biliary papillomatosis is considered to be a premalignant lesion with a high probability to develop to CCA, although the genetic changes have not been clarified[16].

INTERACTION AMONG GENETIC DETERMINANTS

Susceptibility to cancer might be regulated not only by one gene or one environmental determinant. Thus, interaction of genetic determinants could easily be imagined in regulating various cellular processes. However, there are few reports on the interaction among genetic determinants. Ko *et al*[17] reported the interaction of polymorphisms of 5,10-methylenetetrahydrofolate reductase (MTHFR *C677T*) and thymidylate synthase enhancer region (TSER) and the risk for CCA in a South Korean population (Ko *et al*[17] 2006). MTHFR is involved in the pathway of folate metabolism and DNA methylation. Thymidylate synthase (TS) catalyzes the formation of dTMP from dUMP, an important step for production of dTTP for use in DNA synthesis. Both TS and MTHFR use the common substrate 5,10-methylanetetrahydrofolate and might affect DNA synthesis and repair. Therefore, the interaction between *MTHFR C677T* and *TSER* polymorphisms were analyzed. Ko *et al*[17] found that the individuals with *MTHFR 677CC* with *TSER 2R(+)* genotypes (2R2R, 2R3R, 2R5R) showed an increased risk for CCA compared to *677CC* with *TSER 2R(-)* genotypes (3R3R, 3R4R, 3R5R) ($P = 0.0257$) (Table 5)[17]. There was no association between *MTHFR C677T* polymorphism or *TSER* polymorphism alone and the risk for CCA.

Human 8-oxoguanine glycosylase 1 (hOGG1) is involved in the repair of 8-hydroxy-2'deoxyguanine residue in oxidatively damaged DNA, one of the most mutagenic lesions among base modification produced by reactive oxygen species. While polymorphisms of DNA repair enzymes, including hOGG1 (codon 326), XRCC1 (codon 194, 280 and 399) and PARP1 (codon 762), alone had no association with the risk for CCA[18], there is a significant interaction between hOGG1 and *GSTM1* polymorphisms for the risk for CCA. When *GSTM1* polymorphism was considered, the hOGG1 codon 326 polymorphism was related to the decreased risk: OR = 1.00 (reference), OR = 0.06 (95%CI: 0.01-0.53), OR = 0.06 (95%CI: 0.01-0.54) and OR = 0.14 (95%CI: 0.02-1.08) for subjects with hOGG1 Ser/Ser and *GSTM1* wild, ones with Ser/Ser and *GSTM1* null, ones with Ser/Cys or Cys/Cys and *GSTM1* wild, and ones with Ser/Cys or Cys/Cys and *GSTM1* null, respectively (P for interaction < 0.01) (Table 5). Although the effect of hOGG1 polymorphism is not clear when amino acid Ser 326 is changed to Cys, the DNA repair capacity might decrease. However, the above data showed the decreased risk of CCA. It could be considered that if DNA repair capacity is inhibited when relatively abundant DNA damage is present in the presence or absence of *GSTM1* enzyme, the cells would die before malignant transformation[18]. Kim *et al*[19] reported that hOGG1 326 Cys/Cys genotypes were associated with lowered risk of bladder cancer occurrence and recurrence in South Korean subjects, while hOGG1 326 Ser/Cys genotype was a risk factor. The protective effect of *GSTM1* null variant could be

due to the slow metabolism caused by *GSTM1* deficiency of some dietary materials, such as isothiocyanates contained in cruciferous vegetables, known to be a chemopreventive compound. The protective effects of *GSTM1* null variant were reported in breast carcinoma[20] and hepatocellular carcinoma[21]. The concerted action of a DNA-repair enzyme and *GSTM1* on the risk for CCA should give a new insight in understanding the mechanism of the carcinogenesis of CCA.

ETIOLOGICAL AND ENHANCING AGENTS FOR CHOLANGIOCARCINOGENESIS AND THEIR PATHOGENICITY

Concerning the etiological agents for CCA, epidemiological studies implicated various chemicals and occupational risks. One of the examples is thorium dioxide (thorotrast) used for radiological examination[22]. Animal experiments showed that *N*-nitrosodimethylamine could induce CCA in the Syrian Golden hamster[23]. Although OV infection alone did not induce CCA, the OV infection enhanced CCA production by *N*-nitrosodimethylamine in the hamsters[24,25]. Actually, a small amount of nitrosamine was detected in the food[4]. Quite recently, 1, 2-dichloropropane and/or dichloromethane used in the color proof-printing factory were considered to be the etiological agents from a precise epidemiological study in Japan[26]. Other than the liver fluke, viral infections like hepatitis B and C virus infections are also related to the increased risk for CCA[27].

There have been many findings on the abnormalities of gene expression caused by the reorganization of the genome through endogenous and environmental factors in many types of cancers[28]. It is also true for CCA that many genetic changes are found in CCA. One of the examples from our laboratory is the mutation of the tumor suppressor protein genes, *p16Ink4/CDKN2* and *p15Ink4B/MTS2*[29]. However, the precise mechanisms of cholangiocarcinogenesis are not well clarified. We have been using a hamster model of cholangiocarcinoma and found that a molecule, protein kinase A regulatory subunit 1 α (Prkar1a), is overexpressed in the cholangiocarcinoma tissues[30,31]. *PRKAR1A* gene overexpression is also found in humans and this is associated with production of extracellular protein kinase A (ECPKA), especially its catalytic subunit (PRKACA)[31], as found in prostate cancer. Although the function of the extracellular protein kinase A is not clear, it might contribute to the development of cancer cells[32].

The precise mechanism of liver fluke infection causing CCA (cholangiocarcinogenesis) is also not known. OV produces mechanical injury to the biliary epithelia by attachment with suckers, inflammation caused by OV and mitogenic factors secreted by OV to help the biliary epithelial cells transform to CCA[33]. In particular, TGF-β and EGF signal transduction pathways are indicated as the possible pathways of OV-induced cell proliferation of fibroblasts[34]. It could be speculated that CCA-associated fibroblasts induce tumor progression of the initiated epithelium, as found in human prostate epithelium[35]. This would be a novel target for chemoprevention and treatment of fibrosis in CCA which might delay the formation of CCA. Gene expression profile of OV infection-related CCA and non-OV associated CCA was reported by Jinawath *et al*[36]. Enhanced expression of RAD51 associating protein-1 was also involved in the growth of CCA cells[37]. These genes upregulated in CCA would be expected to serve as diagnostic and therapeutic targets for CCA. The up-to-date findings of the mechanism of tumorigenesis by OV infection and the prevention of OV infection, including the education and trial for vaccine development against OV, is reviewed by Sripa *et al*[4].

FUTURE PROSPECTS FOR PREVENTION AND EARLY DETECTION OF CCA

The present work is intended to analyze the effects of environmental and genetic determinants on the risk of CCA and to know the mechanisms of CCA to prevent the disease. At the same time, it is also important to detect the disease during its early phase so that medical intervention could possibly prevent the death of patients with cholangiocarcinoma. Therefore, the method to detect the high risk population and patients with cholangiocarcinoma using a non-invasive procedure is quite important. To find the possible tumor marker of CCA, we use the sera and label the compounds with fluorescent chemicals to try and find a certain compound found in the serum of patients with cholangiocarcinoma. One of our preliminary results showed that a new peak (named peak B) was found in 50% of CCA patients but in 6.3% in normal individuals[38]. In addition, Loilome *et al*[31] found that in liver fluke-associated CCA, PRKR1A overexpression is associated with an increased extracellular PKA autoantibody. The antibody titers in the sera from patients with CCA (0.154 ± 0.077), adenocarcinoma (0.150 ± 0.061) and OV infected individuals with fibrosis (0.157 ± 0.045) were significantly higher than that in healthy control subjects (0.129 ± 0.028), while there was no significant difference between the sera from OV infected individuals without fibrosis (0.139 ± 0.053) and that of healthy control subjects[31]. Recently, Matsuda *et al*[39] found the *Wisteria floribunda* agglutinin-positive mucin 1 and the L1 cell adhesion molecule[40] were sensitive biliary biomarkers for CCA. Silsirivanit *et al*[41] reported a novel Lewis a associated carbohydrate epitope, CA-S27, as a diagnostic and prognostic biomarker for CCA.

Although the prognosis of CCA is not good, there are several reports on the relationship of genetic changes and the prognosis of patients with CCA. One example would be with the classical comparative genomic hybridization studies. It was suggested that amplification of the D22S283 region of the chromosome was a favorable prognostic marker[42].

With recent rapid advancement of DNA sequencing technology, it becomes possible to analyze the whole genome sequence relatively less expensively. Therefore, it should be possible to search the responsible chromosomal region involved in the genetic determinants of the risk for CCA and the progression or inhibition of the growth of CCA in more detail. With the technology of genomics, proteomics and glycobiology, one can expect to find the high risk population for CCA more easily, to help the population better adjust their lifestyles for prevention of CCA and to detect the patients with CCA in its early phase.

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REFERENCES

1 **Khuhaprema T**, Srivatanakul P, Attasara P, Sriplung H, Wiangnon S, Sumitsawan Y. Cancer in Thailand 2001-2003. vol. V. Bangkok: Bangkok Medical Publisher, 2010
2 **Parkin DM**, Srivatanakul P, Khlat M, Chenvidhya D, Chotiwan P, Insiripong S, L'Abbé KA, Wild CP. Liver cancer in Thailand. I. A case-control study of cholangiocarcinoma. *Int J Cancer* 1991; **48**: 323-328 [PMID: 1645697 DOI: 10.1002/ijc.2910480302]
3 **Srivatanakul P**, Ohshima H, Khlat M, Parkin M, Sukaryodhin S, Brouet I, Bartsch H. Opisthorchis viverrini infestation and endogenous nitrosamines as risk factors for cholangiocarcinoma in Thailand. *Int J Cancer* 1991; **48**: 821-825 [PMID: 1650329 DOI: 10.1002/ijc.2910480606]
4 **Sripa B**, Brindley PJ, Mulvenna J, Laha T, Smout MJ, Mairiang E, Bethony JM, Loukas A. The tumorigenic liver fluke Opisthorchis viverrini--multiple pathways to cancer. *Trends Parasitol* 2012; **28**: 395-407 [PMID: 22947297 DOI: 10.1016/j.pt.2012.07.006]
5 **Honjo S**, Srivatanakul P, Sriplung H, Kikukawa H, Hanai S, Uchida K, Todoroki T, Jedpiyawongse A, Kittiwatanachot P, Sripa B, Deerasamee S, Miwa M. Genetic and environmental determinants of risk for cholangiocarcinoma via Opisthorchis viverrini in a densely infested area in Nakhon Phanom, northeast Thailand. *Int J Cancer* 2005; **117**: 854-860 [PMID: 15957169 DOI: 10.1002/ijc.21146]
6 **Songserm N**, Promthet S, Sithithaworn P, Pientong C, Ekalaksananan T, Chopjitt P, Parkin DM. Risk factors for cholangiocarcinoma in high-risk area of Thailand: role of lifestyle, diet and methylenetetrahydrofolate reductase polymorphisms. *Cancer Epidemiol* 2012; **36**: e89-e94 [PMID: 22189445 DOI: 10.1016/j.canep.2011.11.007]
7 **Manwong M**, Songserm N, Promthet S, Matsuo K. Risk factors for cholangiocarcinoma in the lower part of Northeast Thailand: a hospital-based case-control study. *Asian Pac J Cancer Prev* 2013; **14**: 5953-5956 [PMID: 24289607 DOI: 10.7314/APJCP.2013.14.10.5953]
8 **Prawan A**, Kukongviriyapan V, Tassaneeyakul W, Pairojkul C, Bhudhisawasdi V. Association between genetic polymorphisms of CYP1A2, arylamine N-acetyltransferase 1 and 2 and susceptibility to cholangiocarcinoma. *Eur J Cancer Prev* 2005; **14**: 245-250 [PMID: 15901993 DOI: 10.1097/00008469-200506000-00008]
9 **Marahatta SB**, Punyarit P, Bhudisawasdi V, Paupairoj A, Wongkham S, Petmitr S. Polymorphism of glutathione S-transferase omega gene and risk of cancer. *Cancer Lett* 2006; **236**: 276-281 [PMID: 15992993 DOI: 10.1016/j.canlet.2005.05.020]
10 **Hayashi T**, Imai K, Morishita Y, Hayashi I, Kusunoki Y, Nakachi K. Identification of the NKG2D haplotypes associated with natural cytotoxic activity of peripheral blood lymphocytes and cancer immunosurveillance. *Cancer Res* 2006; **66**: 563-570 [PMID: 16397273 DOI: 10.1158/0008-5472.CAN-05-2776]
11 **Bergquist A**, Ekbom A, Olsson R, Kornfeldt D, Lööf L, Danielsson A, Hultcrantz R, Lindgren S, Prytz H, Sandberg-Gertzén H, Almer S, Granath F, Broomé U. Hepatic and extrahepatic malignancies in primary sclerosing cholangitis. *J Hepatol* 2002; **36**: 321-327 [PMID: 11867174 DOI: 10.1016/S0168-8278(01)00288-4]
12 **Melum E**, Karlsen TH, Schrumpf E, Bergquist A, Thorsby E, Boberg KM, Lie BA. Cholangiocarcinoma in primary sclerosing cholangitis is associated with NKG2D polymorphisms. *Hepatology* 2008; **47**: 90-96 [PMID: 18023027 DOI: 10.1002/hep.21964]
13 **Hoblinger A**, Grunhage F, Sauerbruch T, Lammert F. Association of the c.3972C& gt; T variant of the multidrug resistance-associated protein 2 Gene (MRP2/ABCC2) with susceptibility to bile duct cancer. *Digestion* 2009; **80**: 36-39 [PMID: 19451719 DOI: 10.1159/000212990]
14 **You SH**, Wang X, Huang S, Wang M, Ji GZ, Xia JR, Fan ZN. MYH rs3219476 and rs3219472 polymorphisms and risk of cholangiocarcinoma. *Mol Med Rep* 2013; **7**: 347-351 [PMID: 23138270 DOI: 10.3892/mmr.2012.1175]
15 **Scheimann AO**, Strautnieks SS, Knisely AS, Byrne JA, Thompson RJ, Finegold MJ. Mutations in bile salt export pump (ABCB11) in two children with progressive familial intrahepatic cholestasis and cholangiocarcinoma. *J Pediatr* 2007; **150**: 556-559 [PMID: 17452236 DOI: 10.1016/j.jpeds.2007.02.030]
16 **Lee SS**, Kim MH, Lee SK, Jang SJ, Song MH, Kim KP, Kim HJ, Seo DW, Song DE, Yu E, Lee SG, Min YI. Clinicopathologic review of 58 patients with biliary papillomatosis. *Cancer* 2004; **100**: 783-793 [PMID: 14770435 DOI: 10.1002/cncr.20031]
17 **Ko KH**, Kim NK, Yim DJ, Hong SP, Park PW, Rim KS, Kim S, Hwang SG. Polymorphisms of 5,10-methylenetetrahydrofolate reductase (MTHFR C677T) and thymidylate synthase enhancer region (TSER) as a risk factor of cholangiocarcinoma in a Korean population. *Anticancer Res* 2006; **26**: 4229-4233 [PMID: 17201138]
18 **Zeng L**, You G, Tanaka H, Srivatanakul P, Ohta E, Viwatthanasittiphong C, Matharit M, Chenvidhya D, Jedpiyawongse A, Tanaka M, Fujii T, Sripa B, Ohshima K, Miwa M, Honjo S. Combined effects of polymorphisms of DNA-repair protein genes and metabolic enzyme genes on the risk of cholangiocarcinoma. *Jpn J Clin Oncol* 2013; **43**: 1190-1194 [PMID: 24049014 DOI: 10.1093/jjco/hyt138]

19 **Kim EJ**, Jeong P, Quan C, Kim J, Bae SC, Yoon SJ, Kang JW, Lee SC, Jun Wee J, Kim WJ. Genotypes of TNF-alpha, VEGF, hOGG1, GSTM1, and GSTT1: useful determinants for clinical outcome of bladder cancer. *Urology* 2005; **65**: 70-75 [PMID: 15667866 DOI: 10.1016/j.urology.2004.08.005]

20 **Roodi N**, Dupont WD, Moore JH, Parl FF. Association of homozygous wild-type glutathione S-transferase M1 genotype with increased breast cancer risk. *Cancer Res* 2004; **64**: 1233-1236 [PMID: 14973116 DOI: 10.1158/0008-5472.CAN-03-2861]

21 **Kiran M**, Chawla YK, Kaur J. Glutathione-S-transferase and microsomal epoxide hydrolase polymorphism and viral-related hepatocellular carcinoma risk in India. *DNA Cell Biol* 2008; **27**: 687-694 [PMID: 18816171 DOI: 10.1089/dna.2008.0805]

22 **Rota AN**, Weindling HK, Goodman PG. Cholangiocarcinoma associated with thorium dioxide (thorotrast): report of a case. *Mich Med* 1971; **70**: 911-915 [PMID: 4329115]

23 **Tomatis L**, Magee PN, Shubik P. Induction of liver tumors in the syrian golden hamster by feeding dimethylnitrosamine. *J Natl Cancer Inst* 1964; **33**: 341-345 [PMID: 14207850]

24 **Thamavit W**, Bhamarapravati N, Sahaphong S, Vajrasthira S, Angsubhakorn S. Effects of dimethylnitrosamine on induction of cholangiocarcinoma in Opisthorchis viverrini-infected Syrian golden hamsters. *Cancer Res* 1978; **38**: 4634-4639 [PMID: 214229]

25 **Flavell DJ**, Lucas SB. Potentiation by the human liver fluke, Opisthorchis viverrini, of the carcinogenic action of N-nitrosodimethylamine upon the biliary epithelium of the hamster. *Br J Cancer* 1982; **46**: 985-989 [PMID: 6295426 DOI: 10.1038/bjc.1982.313]

26 **Kumagai S**, Kurumatani N, Arimoto A, Ichihara G. Cholangiocarcinoma among offset colour proof-printing workers exposed to 1,2-dichloropropane and/or dichloromethane. *Occup Environ Med* 2013; **70**: 508-510 [PMID: 23493378 DOI: 10.1136/oemed-2012-101246]

27 **Srivatanakul P**, Honjo S, Kittiwatanachot P, Jedpiyawongse A, Khuhaprema T, Miwa M. Hepatitis viruses and risk of cholangiocarcinoma in northeast Thailand. *Asian Pac J Cancer Prev* 2010; **11**: 985-988 [PMID: 21133611]

28 **Weinberg RA**. The biology of cancer. United States: Garland Science, Taylor & Francis Group, LLC, 2007

29 **Yoshida S**, Todoroki T, Ichikawa Y, Hanai S, Suzuki H, Hori M, Fukao K, Miwa M, Uchida K. Mutations of p16Ink4/CDKN2 and p15Ink4B/MTS2 genes in biliary tract cancers. *Cancer Res* 1995; **55**: 2756-2760 [PMID: 7796400]

30 **Loilome W**, Yongvanit P, Wongkham C, Tepsiri N, Sripa B, Sithithaworn P, Hanai S, Miwa M. Altered gene expression in Opisthorchis viverrini-associated cholangiocarcinoma in hamster model. *Mol Carcinog* 2006; **45**: 279-287 [PMID: 16550611 DOI: 10.1002/mc.20094]

31 **Loilome W**, Yooyuen S, Namwat N, Sithithaworn P, Puapairoj A, Kano J, Noguchi M, Miwa M, Yongvanit P. PRKAR1A overexpression is associated with increased ECPKA autoantibody in liver fluke-associated cholangiocarcinoma: application for assessment of the risk group. *Tumour Biol* 2012; **33**: 2289-2298 [PMID: 22922884 DOI: 10.1007/s13277-012-0491-3]

32 **Cvijic ME**, Kita T, Shih W, DiPaola RS, Chin KV. Extracellular catalytic subunit activity of the cAMP-dependent protein kinase in prostate cancer. *Clin Cancer Res* 2000; **6**: 2309-2317 [PMID: 10873081]

33 **Thuwajit C**, Thuwajit P, Kaewkes S, Sripa B, Uchida K, Miwa M, Wongkham S. Increased cell proliferation of mouse fibroblast NIH-3T3 in vitro induced by excretory/secretory product(s) from Opisthorchis viverrini. *Parasitology* 2004; **129**: 455-464 [PMID: 15521634 DOI: 10.1017/S0031182004005815]

34 **Thuwajit C**, Thuwajit P, Uchida K, Daorueang D, Kaewkes S, Wongkham S, Miwa M. Gene expression profiling defined pathways correlated with fibroblast cell proliferation induced by Opisthorchis viverrini excretory/secretory product. *World J Gastroenterol* 2006; **12**: 3585-3592 [PMID: 16773716]

35 **Olumi AF**, Grossfeld GD, Hayward SW, Carroll PR, Tlsty TD, Cunha GR. Carcinoma-associated fibroblasts direct tumor progression of initiated human prostatic epithelium. *Cancer Res* 1999; **59**: 5002-5011 [PMID: 10519415]

36 **Jinawath N**, Chamgramol Y, Furukawa Y, Obama K, Tsunoda T, Sripa B, Pairojkul C, Nakamura Y. Comparison of gene expression profiles between Opisthorchis viverrini and non-Opisthorchis viverrini associated human intrahepatic cholangiocarcinoma. *Hepatology* 2006; **44**: 1025-1038 [PMID: 17006947]

37 **Obama K**, Satoh S, Hamamoto R, Sakai Y, Nakamura Y, Furukawa Y. Enhanced expression of RAD51 associating protein-1 is involved in the growth of intrahepatic cholangiocarcinoma cells. *Clin Cancer Res* 2008; **14**: 1333-1339 [PMID: 18316552 DOI: 10.1158/1078-0432.CCR-07-1381]

38 **Miwa M**, You G, Tanaka H, Taniguchi S, Fujii T, Kamemura K, Suzaki M, Isono T, Tooyama I, Tanaka M, Srivatanakul P, Viwatthanasittiphong C, Sangrajrang S, Khuhaprema T. Analysis of new biomarkers for cholangiocarcinoma. *J Hepatobiliary Pancreat Sci* 2014; **21**: 397-398 [PMID: 24446393]

39 **Matsuda A**, Kuno A, Kawamoto T, Matsuzaki H, Irimura T, Ikehara Y, Zen Y, Nakanuma Y, Yamamoto M, Ohkohchi N, Shoda J, Hirabayashi J, Narimatsu H. Wisteria floribunda agglutinin-positive mucin 1 is a sensitive biliary marker for human cholangiocarcinoma. *Hepatology* 2010; **52**: 174-182 [PMID: 20578261 DOI: 10.1002/hep.23654]

40 **Matsuda A**, Kuno A, Matsuzaki H, Kawamoto T, Shikanai T, Nakanuma Y, Yamamoto M, Ohkohchi N, Ikehara Y, Shoda J, Hirabayashi J, Narimatsu H. Glycoproteomics-based cancer marker discovery adopting dual enrichment with Wisteria floribunda agglutinin for high specific glyco-diagnosis of cholangiocarcinoma. *J Proteomics* 2013; **85**: 1-11 [PMID: 23612463 DOI: 10.1016/j.jprot.2013.04.017]

41 **Silsirivanit A**, Araki N, Wongkham C, Vaeteewoottacharn K, Pairojkul C, Kuwahara K, Narimatsu Y, Sawaki H, Narimatsu H, Okada S, Sakaguchi N, Wongkham S. CA-S27: a novel Lewis a associated carbohydrate epitope is diagnostic and prognostic for cholangiocarcinoma. *Cancer Sci* 2013; **104**: 1278-1284 [PMID: 23809433 DOI: 10.1111/cas.12222]

42 **Thanasai J**, Limpaiboon T, Jearanaikoon P, Bhudhisawasdi V, Khuntikeo N, Sripa B, Miwa M. Amplification of D22S283 as a favorable prognostic indicator in liver fluke related cholangiocarcinoma. *World J Gastroenterol* 2006; **12**: 4338-4344 [PMID: 16865775]

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Management of acute severe ulcerative colitis

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Abstract

The management strategy of acute severe ulcerative colitis has evolved over the past decade from being entirely restricted to twin choices of intravenous steroids or colectomy to include colon rescue therapies like cyclosporin as well as infliximab. However it still remains a medical emergency requiring hospitalization and requires care from a multidisciplinary team comprising of a gastroenterologist and a colorectal surgeon. The frame shift in management has been the emphasis on time bound decision making with an attempt to curtail the mortality rate to below 1%. Intravenous corticosteroids are the mainstay of therapy. Response to steroids should be assessed at day 3 of admission and partial/non-responders should be considered for alternative medical therapy/surgery. Medical rescue therapies include intravenous cyclosporin and infliximab. Cyclosporin is administered in a dose of 2 mg/kg per day and infliximab is administered as a single dose intravenous infusion of 5 mg/kg. Approximately 75% patients have short term and 50% patients have long term response to cyclosporin. Long term response to cyclosporin is improved in patients who are thiopurine naïve and are started on thiopurines on day 7. Infliximab also has a response rate of approximately 70% in short term and 50% in long term. Both cyclosporin and infliximab are equally efficacious medical rescue therapies as demonstrated in a recent randomized control trial. Patients not responding to infliximab or cyclosporin should be considered for colectomy.

Key words: Ulcerative colitis; Acute severe colitis; Intravenous steroids; Cyclosporin; Infliximab

Core tip: The mortality of severe ulcerative colitis has drastically reduced from 30%-60% in pre steroid era to 1%-2.9% at present. However these figures are for specialist centers and at peripheral centers the mortality figures may be higher. The objective of this review is to provide in depth information for what can be categorized as a gastrointestinal medical emergency with the hope that informed clinical practices may translate to superior patient care at tertiary as well as peripheral centers treating ulcerative colitis. This review provides time bound framework, which looks at stepwise management of acute severe ulcerative colitis and explores the recent concepts of choice between biologics and cyclosporin colon rescue therapies in case of steroid refractory disease.

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INTRODUCTION

Acute severe ulcerative colitis (UC) is a medical emergency characterized by[1] (Table 1) presence of more than 6 bloody stools/d along with any one of the following: tachycardia > 90 bpm, fever > 37.8 ℃, Hb < 10.5 gm/dL, and/or ESR > 30 mm/h (Truelove and Witt's criteria). Other indices for defining severity include modified Mayo's classification[2], which is a combination of clinical and endoscopic findings, and Montreal classification[3], which is primarily based on Truelove and Witt's criteria.

Table 1 Modified Truelove and Witt's criteria for classification of severity of ulcerative colitis

	Mild	Moderate	Severe
Bloody stools per day	< 4	4-6	> 6
Pulse	< 90 bpm	≤ 90 bpm	> 90 bpm
Temperature	< 37.5 ℃	≤ 37.8 ℃	> 37.8 ℃
Hemoglobin	> 11.5 gm/dL	≥ 10.5 gm/dL	< 10.5 gm/dL
ESR	< 20 mm/h	≤ 30 mm/h	> 30 mm/h
CRP	Normal	≤ 30 mg/dL	> 30 mg/dL

However, Truelove and Witt's criteria is the most widely accepted disease severity index in clinical practice. The term acute severe colitis is preferred over fulminant colitis because the term fulminant is not well defined. It was coined in 1950 when it meant that single attack of UC could lead to mortality within 1 year[4], which is no longer relevant today. Approximately 20% UC patients with initial disease flares have severe UC[4], and about 15% patients have a severe attack at some stage of their disease[5]. Megacolon refers to presence of dilated colon (> 5.5 cm) on a plain abdominal X-ray film. Toxic megacolon is presence of megacolon with signs of systemic toxicity (fever, tachycardia, hypotension, leukocytosis). The overall lifetime incidence of toxic megacolon in patients with UC is 1%-2.5%[6]. Prior to introduction of corticosteroid therapy, mortality with acute severe UC was reported to be upto 22%-75% within first year of diagnosis[7]. First clinical trial of steroids for severe UC was performed in the 1950s and this trial reported a mortality of 7% in patients treated with steroids compared with 24% in the placebo group[8]. The mortality with severe UC has reduced to < 1% in specialist centers.

APPROACH TO MANAGEMENT

Investigations required at admission

In addition to monitoring patient's clinical feature and vital signs, all patients should have their full blood counts, liver and kidney function tests, electrolytes including serum magnesium and inflammatory markers [C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR)]. At least 3 stool samples for *Clostridium difficile* (*C. difficile*) toxin should be obtained to rule out superimposed pseudo-membranous colitis[9]. A plain abdominal X-ray should be done to exclude megacolon. Plain radiograph can also provide information about the extent of disease and can also predict response to treatment. The distal distribution of fecal residue can provide a rough estimate of disease extent as it correlates with the proximal extent of disease[10]. The predictors of poor response to treatment on a plain abdominal radiograph are presence of mucosal islands which are small, circular opacities that represent residual mucosa isolated by surrounding ulceration, or presence of more than two gas-filled loops of small bowel[11]. Flexible unprepared sigmoidoscopy with minimal air insufflation should be performed to confirm the diagnosis and exclude superimposed infection, especially cytomegalovirus (CMV) colitis[12]. Endoscopic markers of severe disease activity include hemorrhagic mucosa with deep ulceration, mucosal detachment on the edge of these ulcerations, and well like ulcerations[13].

Treatment

General management: In addition to specific therapy these supportive measures are very important in the management of patients with acute severe UC. These include: (1) Monitoring and replacement of intravenous fluid and electrolytes to correct and prevent dehydration or electrolyte imbalance as hypokalaemia/hypomagnesaemia can precipitate toxic dilatation[6]; (2) Anticholinergic, antidiarrheal, non-steroidal anti-inflammatory drugs and opioid drugs should be promptly withdrawn as these may precipitate colonic dilatation; (3) Malnourished patients should receive adequate nutritional support. Enteral nutrition is most appropriate and is preferred over parenteral nutrition as it is associated with significantly fewer complications than parenteral nutrition in acute colitis[14]. There is no evidence that bowel rest with parenteral nutrition alters the outcome[15]; (4) Flexible unprepared sigmoidoscopy and biopsy should be done to confirm the diagnosis of acute severe UC and exclude infections[16] such as CMV. Presence of active CMV infection is indicated by presence of cytomegalovirus inclusion bodies on colonic biopsies. However inclusion bodies are not very frequent even in patients with active disease with a sensitivity as low as 37.5%[17]. Special immunohistochemical staining against immediate early antigens of CMV increases the diagnostic sensitivity of histologic examination for CMV. In addition positive plasma real time PCR assays for CMV DNA at levels > 20 copies/100 μL is also an indicator of active CMV disease[18]. Presence of active CMV disease requires treatment with ganciclovir, especially if the patient is slow to respond to conventional therapy; (5) Stool analysis (in atleast 3 stool samples) to exclude co-existing *C. difficile* toxin is required especially in patients with history of prolonged hospitalization[19]. *C. difficile* infection co-existing with acute severe UC has been associated with increased morbidity and mortality, and requires appropriate antibiotic therapy (oral vancomycin or metronidazole)[20]; (6) There is increased risk of thromboembolic phenomena, in patients with active IBD compared to controls, especially during disease flares[21]. Therefore prophylaxis with subcutaneous low molecular weight heparin is indicated to reduce the risk of thromboembolism; (7) Topical corticosteroids or mesalazine may be administered if patient can tolerate and is able to retain them, although there have been no systematic studies in acute severe colitis; (8) Antibiotics are indicated only if infection is suspected or immediately prior to surgery. Controlled trials of antibiotics such as oral or intravenous metronidazole or ciprofloxacin in acute colitis have not shown any significant benefit in addition to conventional therapy[22,23]; and (9) Blood transfusion is indicated in patients with hemoglobin < 10 gm/dL[24].

In addition to these measures daily assessment

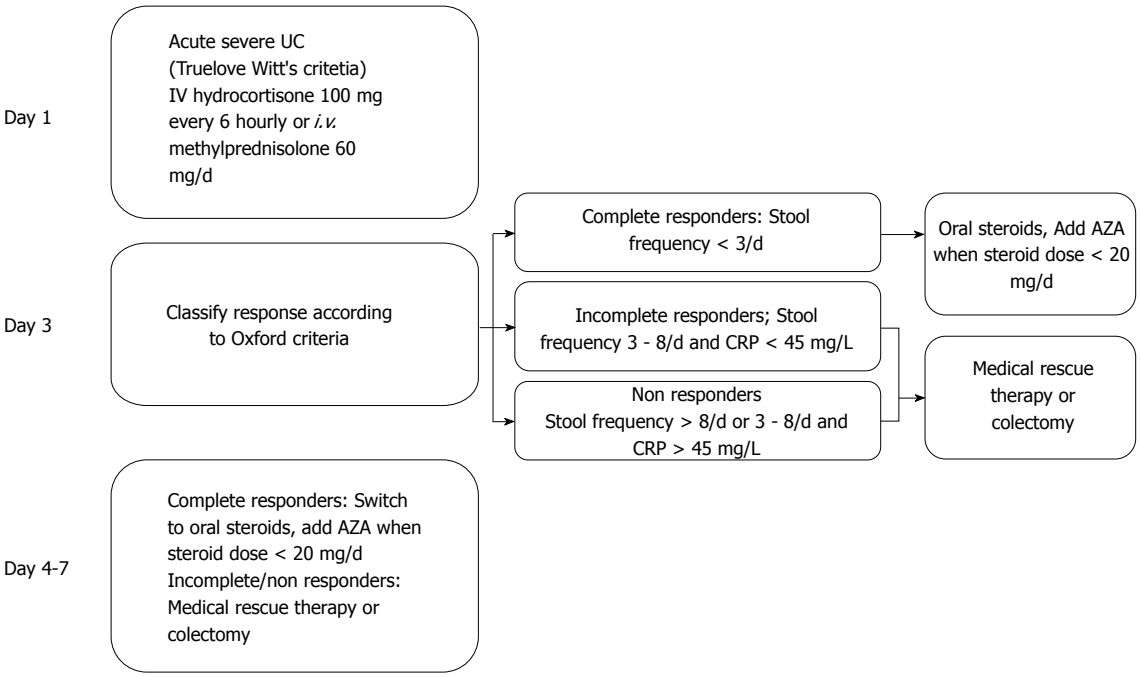

Figure 1 Algorithm for treatment decisions for patients with acute severe ulcerative colitis on intensive steroid therapy. AZA: Azathioprine.

of patients' clinical status should be done in following manner: (1) Physical examination is required daily to evaluate abdominal and rebound tenderness. Joint collaboration between medical and surgical team is required for appropriate management of such patients; (2) Vital signs should be recorded four times daily and more often if deterioration is noted; (3) A stool chart which records the number and character of bowel movements, including the presence or absence of blood and liquid versus solid stool should be properly maintained; (4) Measurement of blood count, CRP, serum electrolytes, serum albumin, liver function tests, and glucose should be done every 24 h; and (5) Abdominal radiographs should be done daily, especially in patients in whom there are signs of colonic distension and/or there is significant deterioration in clinical condition or laboratory parameters.

CORTICOSTEROIDS

Corticosteroids are the mainstay of therapy for acute severe UC. Steroids are given intravenously with methylprednisolone given in a dose of 60 mg/d or hydrocortisone 100 mg every 6 h. Treatment duration is usually limited to 7 to 10 d; continuing corticosteroid treatment beyond that period carries no additional benefit[25]. Truelove and Jewell published the first clinical trial of intravenous corticosteroids for acute severe UC in 1974[26]. Of 49 patients treated with intravenous steroids, 36 (73%) achieved complete remission by day 5. In a recently published systematic review of 1991 patients from 1974 to 2006[25], overall response to steroids was 67%. The overall short-term colectomy rate was 29% (565/1991) and mortality was 1%.

Predictors of response to steroids

Response to steroids is indicated by improvement in patients' symptoms (decreased stool frequency, urgency and rectal bleeding, improved stool consistency, reduction in abdominal pain, and improvement in general well being) and improved laboratory parameters (reduced CRP and ESR and improvement in hemoglobin and albumin).

At day 3 of admission, response to steroids should be measured by assessing stool frequency and CRP levels (Figure 1). In the landmark study by Travis *et al*[10], which included patients with 51 episodes of severe UC, presence of more than 8 stools/d or 3-8 stools/d plus a CRP > 45 mg/L at day 3 predicted a colectomy rate of 85%. In another prospective study by Lindgren *et al*[27] which included 97 episodes of severe UC, the following mathematical model was devised to predict colectomy: number of stools/d + 0.14 × CRP (mg/L) ≥ 8 predicted a colectomy rate of 72%.

Therefore regular assessment of response to steroids is of paramount importance in treating patients with acute severe UC. In a group of 80 patients who underwent emergency colectomy for severe UC between 1994 and 2000 in Oxford[28], patients with significantly longer duration of preoperative medical therapy (> 8 d) were more likely to have major post-operative complications.

Therefore at day 3 of admission, in cases of non response to steroids according to above mentioned criteria (stool frequency > 8/d or stool frequency 3-8/d and CRP > 45 mg/L) other treatment options or surgery should be considered. In cases of partial response, therapy should be continued till day 5-7, and if the patient still does not respond, other therapies/surgery should be considered (Table 2). In patients who respond to steroids, oral steroids should be started after 5-7 d of

Table 2 Ten year follow up of patients of Oxford cohort categorized at day 7 of intensive therapy

Parameter	Complete responders	Incomplete responders
Colectomy rate at 1 yr	5%	54%
Number requiring colectomy	6/19 (32%)	10/13 (76.9%)
Maximum steroid free remission	3.5 yr	< 1 yr

intensive therapy.

There are several other studies which have predicted response to steroids in acute severe UC. Ho *et al*[29], in a retrospective study found that, number of stools/day (score 1-4); hypoalbuminaemia < 3 mg/dL (score 1) and colonic dilatation > 5.5 cm (score 4) were useful in predicting colectomy as 85% of patients with a score ≥ 4 required colectomy. In another study by Ananthakrishnan *et al*[30], anemia, malnutrition, need for blood transfusion and total parenteral nutrition would independently predict colectomy. Radiological markers which can predict colectomy include the presence of mucosal islands on a plain abdominal radiograph which is associated with a 75% colectomy rate[31], and presence of an ileus (indicated by 3 or more small bowel loops of gas) which is associated with 73% colectomy rate[11]. In a study, presence of deep ulcers on endoscopy after gentle air insufflation identified 42/49 patients who required colectomy[32].

CYCLOSPORIN

Two controlled clinical trials established the efficacy of intravenous cyclosporin (fungal calcineurin inhibitor) as medical rescue therapy for acute severe UC not responding to intravenous corticosteroids. In the first landmark trial by Lichtiger *et al*[33] 9 out 11 patients in the cyclosporin (4 mg/kg per day) group had a response *vs* none of 9 placebo treated patients. The trial was terminated early for ethical reasons because of marked response to cyclosporin. Of nine placebo treated patients 5 patients were crossed over to cyclosporin and all five responded. In another study 73 patients were randomized to 4 mg/kg *vs* 2 mg/kg of intravenous cyclosporin[34]. Response rates at 8 d were similar in both groups (83% and 82% respectively), with 9% and 13% colectomy rate in 2 and 4 mg/kg group respectively. Therefore, cyclosporin dose of 2 mg/kg per day has become the standard in clinical practice. Another European study compared intravenous cyclosporin (4 mg/kg) with intravenous steroids and found similar response rates between the two groups (64% *vs* 53%)[35]. Therefore cyclosporin monotherapy may be preferred over steroids in patients who have high chances of side-effects with steroids including patients with osteoporosis, poorly controlled diabetes and those who are susceptible to steroid-psychosis. Overall, pooled results from controlled and non-controlled trials show response rates with intravenous cyclosporin to vary from 76% to 85%, with median time to response being 4 d[35].

Table 3 Long term response rates to cyclosporin[38]

Initial response	74%
1 yr	65% relapsed
3 yr	90% relapsed
7 yr	58% colectomy rates

However, one of the major limitations associated with cyclosporin use is its side effect profile. The short-term side effects are a cause of concern because cyclosporin is generally used as bridge to immunomodulators. These include minor side effects, which occur in 31%-51% patients, including tremors, paresthesias, malaise, headache, abnormal liver function tests, gingival hyperplasia and hirsutism. Major complications are reported in 0%-17%; including hypertension, renal impairment, infections and neurotoxicity[36]. Cyclosporin therapy in UC is associated with a mortality rate of approximately 1.8%-3.5%[36]. Therefore, following points should be considered before starting cyclosporin therapy.

Cyclosporin should not be used if cholesterol < 115 mg/dL or magnesium < 1.4 mEq/L. It should also be avoided in presence of hypertension, renal impairment, epilepsy, sepsis, age > 80 years. Magnesium, cholesterol, and creatinine should be measured at baseline and within 48 h of starting cyclosporin.

Cyclosporin should be administered in a dose of 2 mg/kg per day intravenously aiming for levels 150-250 ng/mL[37].

Oral microemulsion 4 mg/kg twice daily can be alternatively considered.

Blood Pressure and renal function should be monitored and cyclosporin should be stopped if serum creatinine rises > 25%.

Cyclosporin should be stopped if there is no improvement in 7 d.

In responders intravenous cyclosporin (Figure 2) should be switched to oral cyclosporin 4 mg/kg per day twice daily. Monitoring of trough levels (150-250 ng/mL) should be regularly done. Azathioprine should be started along with oral cyclosporin. Cyclosporin should be stopped after 3 mo.

Infective complications with cyclosporin can be avoided by minimizing concomitant immunosuppressants and by using prophylactic antibiotics when indicated.

Regarding long term efficacy of cyclosporin (Table 3) several cohorts have been evaluated long term colectomy in patients treated with cyclosporin. In the retrospective cohort from Oxford, 42% patients could avoid colectomy after 7 years[38]. Overall, approximately 50% patients will avoid colectomy over a period of 2-3 years[39,40]. Immunomodulators when used with cyclosporin can decrease the colectomy rate, thus improving the long term efficacy of cyclosporin. In a study by Cohen *et al*[41] probability of avoiding colectomy at long-term follow-up (5.5 years) was 66% in patients receiving cyclosporin and azathioprine/mercaptopurine compared with 40% in those who received cyclosporin alone. Further studies in this regard

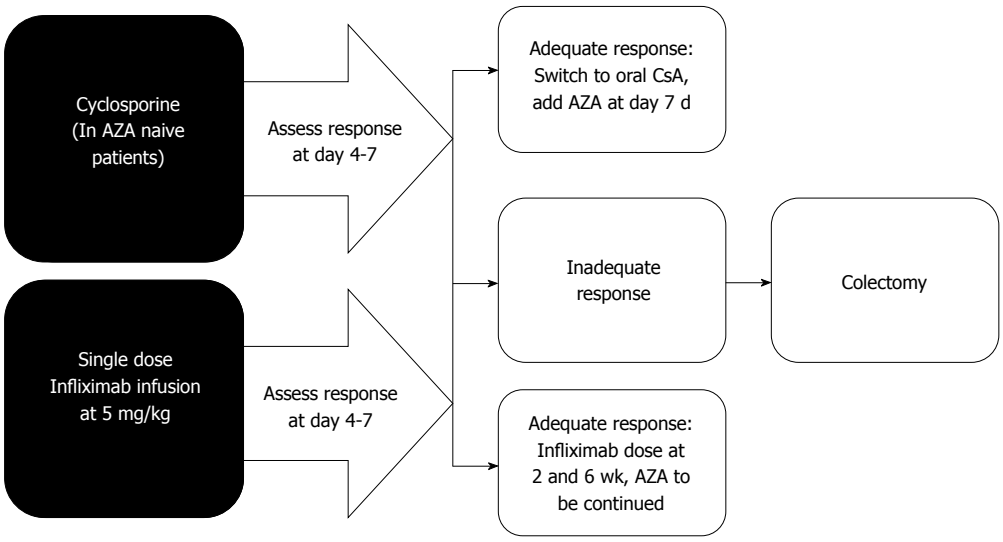

Figure 2 Algorithm for medical rescue therapy after failure of response to intravenous steroids.

have shown that in patients already on immunomodulators at the time of admission with acute severe UC, the likelihood of needing a colectomy following treatment with cyclosporin is higher than among those in whom immunomodulators are started after admission[40].

Therefore, cyclosporin is more beneficial in patients with acute severe UC who are thiopurine naïve at the time of admission. In patients who are already on thiopurine at the time of admission, the outcome with cyclosporin would be less favourable and other medical options or surgery needs to be considered.

INFLIXIMAB

Infliximab the chimeric monoclonal antibody against tumor necrosis factor (TNF) alpha has been found to have a favorable response in patients with steroid refractory acute severe UC. In an open label study of 6 steroid refractory severe UC patients[42], single infusion of infliximab in a dose of 5 mg/kg showed marked clinical improvement at day 7 in all patients. Four out of these 6 patients were in long term remission at median follow up of 5.5 mo. Later a randomized placebo controlled trial of 45 patients (24 infliximab and 21 placebo) showed that a colectomy rate at 3 mo was significantly lower in infliximab group as compared to placebo group (29% *vs* 67%, $P = 0.017$)[43]. The maximum benefit of infliximab was seen in patients with moderately severe disease than in those with most severe disease. Prior exposure to thiopurines does not seem to affect the outcome of patients treated with infliximab[43]. Other factors which may adversely affect outcome with infliximab include increased baseline CRP (> 20 mg/L), concomitant steroid use, disease duration ⩽ 3 years and baseline Mayo score ⩾ 10[44]. Screening for infections and immunization history should be obtained prior to initiating infliximab therapy. Screening tests which need to be done include hepatitis B serology, HIV serology, chest radiograph and tuberculin skin test or Interferon gamma release assays for latent tuberculosis.

Long term follow up data up to 3 years in infliximab treated severe UC patients are available. Two studies with follow up data of 1 year show colectomy rates of approximately 25% at 1 year in infliximab treated patients[45,46]. In another Swedish study, colectomy rate at 3 years in infliximab treated patients was 50% as compared to placebo (76%)[47].

There are no exclusive trials of other anti TNF agents for acute severe UC. However, there are few trials of adalimumab in moderate to severe active UC which showed efficacy of adalimumab over placebo. Reinisch *et al*[48] showed that adalimumab induced remission in 18.5% patients as compared to 9.2% patients in placebo group ($P = 0.031$). In another study Sandborn *et al*[49], in a similar group of patients showed efficacy of adalimumab over placebo (16.5% *vs* 9.3%, $P = 0.019$) in inducing remission.

CYCLOSPORIN *VS* INFLIXIMAB

Before the landmark randomized trial CYSIF (Cyclosporin With Infliximab in Steroid-refractory Severe Attacks of Ulcerative Colitis) between cyclosporin and infliximab there was limited evidence to suggest any difference in efficacy of cyclosporin and infliximab. In a retrospective review of two cohorts (43 treated with cyclosporin and 49 treated with infliximab) there was lower short term colectomy rate in the cyclosporin group[50]. The CYSIF trial[51] randomized 111 thiopurine naïve patients with severe UC after 5 d of IV steroids to cyclosporin (2 mg/kg per day for 8 d followed by 4 mg/kg per day orally) and infliximab (5 mg/kg *iv* infusion at 0, 2 and 6 wk). Patients who responded at day 7 received oral azathioprine and tapered steroids from day 8. The response to treatment at day 7 was seen in approximately 85% patients in both groups. Colectomy rates at day 98 were also similar be-

Table 4 Mortality according to day of surgery after intensive steroid therapy

Timing of surgery Total emergency surgeries = 72	Number of patients	Mortality
Overall	51	8
≤ 5 d	17	0/17
> 5 d	34	8/34

tween cyclosporin and infliximab (18% *vs* 21%, $P = 0.66$). Treatment failure at day 98 was also similar, seen in 60% patients in the cyclosporin group *vs* 54% in the infliximab group. There was no clear evidence of superiority of any one therapy over other.

Therefore choosing between cyclosporin and infliximab depends upon physician and patient preferences as both appear to be equally efficacious in the setting of acute severe colitis.

SWITCHING BETWEEN INFLIXIMAB AND CYCLOSPORIN

In cases of non-response to infliximab or cyclosporin, switching to either therapy is associated with significant morbidity and mortality and is not recommended. In the largest study of 86 patients on this aspect, 65 patients were administered infliximab after cyclosporin and 21 patients had cyclosporin after infliximab. Thirty three percent patients underwent colectomy within 3 mo and 1/3rd of the patients had adverse effects in form of infections[52].

TACROLIMUS

Tacrolimus is also a calcineurin inhibitor with mechanism of action similar to that of cyclosporin. A randomized trial of tacrolimus included 27/60 patients with severe UC[53]. In this trial partial response was seen in 67% patients, although complete remission was not seen on any patient. However, further case series have shown results similar to that of cyclosporin[54,55].

TOXIC MEGACOLON AND OTHER COMPLICATIONS OF SEVERE UC

Toxic megacolon may be defined as colonic dilatation of more than 5.5 cm along with signs of systemic toxicity. Lifetime incidence of toxic megacolon in patients with UC varies from 1%-2.5% and approximately 5% severe UC patients who are hospitalized may develop toxic megacolon[6]. Risk factors include dyselectrolytemia, full bowel preparation and medications (antidiarrheal, anticholinergic, and opiods)[6]. Earlier identification of this condition, prompt institution of medical therapy (nil per oral, intravenous broad spectrum antibiotics, fluid and electrolyte management, and intensive therapy) and low threshold of surgery in cases of non-response to medical therapy within 48 h will decrease the morbidity and mortality of this condition.

Other complications include perforation which is the most serious complication of severe UC. Risk factors include inappropriate total colonoscopy and delaying treatment of toxic megacolon. Diagnosis of perforation can often be delayed as abdominal signs can be masked when patient is on steroids. Therefore, patients with severe UC should be monitored closely for abdominal signs and on the slightest suspicion abdominal radiographs should be obtained. Other complication includes severe hemorrhage.

SURGERY

Surgery is the final option for patients with severe UC not responding to medical therapy. Other indications for surgery include toxic megacolon, perforation and severe haemorrhage. The decision for surgery should not be delayed as this increases the morbidity and mortality of surgery. In a study performed at our center, the mortality of emergency surgery was very high if the intervention was delayed beyond 5 d following non-response to intravenous steroid therapy (Table 4)[56]. In another study from Oxford, higher surgical complication was noted if surgery was delayed beyond 8 d of medical therapy[28]. Therefore management of severe UC requires close collaboration between surgeon and gastroenterologist so that appropriate decisions can be taken without delay.

Most centers advocate a 3 step surgery in emergency setting. The surgical procedure of choice in acute setting is sub-total colectomy and ileostomy, with the rectum left in situ. The whole of rectum and inferior mesenteric artery should be preserved, which facilitates further surgery. The bowel can either be closed in subcutaneous fat or brought forward as mucous fistula, depending upon the surgeon's decision. Subtotal colectomy is a safe procedure even in critically ill patients[57,58] and will relieve the patient from burden of severe colitis, thus allowing the patient to normalise health and nutrition. Reconstructive surgery is best performed approximately 6 mo after primary surgery[59]. The second step consists of ileal pouch formation and defuctioning temporary ileostomy. In the final step ileal pouch anal anastomosis (IPAA) is done restoring normal continuity.

There appears to be a strong association of prolonged use of immunosuppression and poor wound healing after surgery which may manifest as wound dehiscence, infection following intestinal leak or a pelvic abscess following anastomotic leak. Long-term preoperative steroid use has been found to be a significant risk factor for anastomotic leak. Immunosuppressive agents (azathioprine and 6-mercaptopurine) have not been associated with increased postoperative complications. When used alone, cyclosporin has not been associated with increased postoperative complications. The use of infliximab (IFX) and its impact on postoperative course is debatable and is a subject of intense interest. Two studies have identified a relationship between IFX and postoperative complica-

tions in IPAA patients. The first report came from Mayo Clinic[60] which included a retrospective survey of 47 patients who received preoperative IFX and 254 who did not. In the multivariate analysis, IFX was independently associated with increased risk of pouch-related and infectious complications. The authors concluded that IFX was a surrogate for critical patients who were at a higher risk for postoperative complications. The second study by Mor *et al*[61], had a case control design. It suggested that patients who had preoperative IFX were 3.5 times more likely to experience an early postoperative complication as compared to control patients. IFX-exposed patients were nearly 14 times more likely to suffer infectious complications. Other studies, which have not been in agreement with the conclusion of above-mentioned studies, include a large retrospective review of 413 patients with UC and CD over a 14-year period[62]. This study did not find any association between IFX and postoperative complications. The study faced certain criticisms, which included a heterogeneous population with > 50% of patients having CD and only 26 patients with UC who had a preoperative exposure to IFX. Another study[63], evaluating surgical outcomes in 141 UC patients over a 10-year period, found no association of IFX exposure with postoperative complications. In the same study, steroid use was related to increased infectious complications. The limitation of this study was that only 22 patients had IFX exposure prior to surgery. A recent meta-analysis concluded that infliximab use is not associated with increased risk of post-operative complications[64]. At present, no firm conclusions can be drawn. All these studies suffer from a retrospective design. Moreover, it is possible that patients who require IFX represent a patient population, which is at a higher risk for postoperative complications. At the same time, evidence exists that IFX may have a causal role in impairing wound healing and causing anastomotic failure and pelvis sepsis. The definite conclusion which can be drawn is that in patients who have received IFX, a three-stage procedure for IPAA should be considered rather than a two-stage procedure.

Mortality rates associated with emergency colectomy are higher as compared to elective colectomy[65]. In a study from England which included more than 20000 patients with IBD, mortality rates for patients with UC 3 years after colectomy was significantly lower with elective as compared to emergency colectomy (3.7% *vs* 13.2%)[66]. Surgery is not the preferred modality of therapy in young females as ileal pouch anal anastomosis has been associated with lower fertility and fecundity rates[67,68]. In patients with severe malnutrition, surgery may have to be deferred as the risk of post operative complications is significantly increased in this setting.

CONCLUSION

Acute severe ulcerative colitis as defined by Truelove Witt's criteria is a medical emergency that requires immediate hospitalization. Fluid and electrolyte balance, withdrawl of drugs promoting colonic dilatation and adequate nutritional support are important adjuncts in the management of severe UC. Intravenous corticosteroids are the first line therapy for severe UC, and approximately two thirds of patients respond. Response to steroids should be assessed at day 3, and in non-responders/partial responders, medical rescue therapy or surgery should be considered. Efficacy of both cyclosporin and infliximab in this setting is comparable as shown in a recent randomized trial. A close coordination between gastroenterologist and surgeon is required for optimal management of severe UC. Surgery is always an option after failure of IV steroids, and all patients should be given an option of surgery. A time bound strategy is required to manage such patients and surgery should not be delayed beyond 5 d of intensive therapy, as a delay increases surgical morbidity and mortality.

REFERENCES

1 **Truelove SC**, Witts LJ. Cortisone in ulcerative colitis; preliminary report on a therapeutic trial. *Br Med J* 1954; **2**: 375-378 [PMID: 13182220 DOI: 10.1136/bmj.2.4884.375]

2 **D'Haens G**, Sandborn WJ, Feagan BG, Geboes K, Hanauer SB, Irvine EJ, Lémann M, Marteau P, Rutgeerts P, Schölmerich J, Sutherland LR. A review of activity indices and efficacy end points for clinical trials of medical therapy in adults with ulcerative colitis. *Gastroenterology* 2007; **132**: 763-786 [PMID: 17258735 DOI: 10.1053/j.gastro.2006.12.038]

3 **Silverberg MS**, Satsangi J, Ahmad T, Arnott ID, Bernstein CN, Brant SR, Caprilli R, Colombel JF, Gasche C, Geboes K, Jewell DP, Karban A, Loftus EV, Peña AS, Riddell RH, Sachar DB, Schreiber S, Steinhart AH, Targan SR, Vermeire S, Warren BF. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. *Can J Gastroenterol* 2005; **19** Suppl A: 5A-36A [PMID: 16151544]

4 **Rice-Oxley JM**, Truelove SC. Ulcerative colitis course and prognosis. *Lancet* 1950; **255**: 663-666 [DOI: 10.1016/S0140-6736(50)90550-2]

5 **Edwards FC**, Truelove SC. The Course And Prognosis Of Ulcerative Colitis. *Gut* 1963; **4**: 299-315 [PMID: 14084741 DOI: 10.1136/gut.4.4.299]

6 **Gan SI**, Beck PL. A new look at toxic megacolon: an update and review of incidence, etiology, pathogenesis, and management. *Am J Gastroenterol* 2003; **98**: 2363-2371 [PMID: 14638335]

7 **Hardy TL**, Bulmer E. Ulcerative colitis: a survey of ninety-five cases. *Br Med J* 1933; **2**: 812-815 [PMID: 20777868 DOI: 10.1136/bmj.2.3800.812]

8 **Jakobovits SL**, Travis SP. Management of acute severe colitis. *Br Med Bull* 2005; **75-76**: 131-144 [PMID: 16847166 DOI: 10.1093/bmb/ldl001]

9 **García Rodríguez LA**, González-Pérez A, Johansson S, Wallander MA. Risk factors for inflammatory bowel disease in the general population. *Aliment Pharmacol Ther* 2005; **22**: 309-315 [PMID: 16097997 DOI: 10.1111/j.1365-2036.2005.02564.x]

10 **Travis SP**, Farrant JM, Ricketts C, Nolan DJ, Mortensen NM, Kettlewell MG, Jewell DP. Predicting outcome in severe ulcerative colitis. *Gut* 1996; **38**: 905-910 [PMID: 8984031 DOI: 10.1136/gut.38.6.905]

11 **Chew CN**, Nolan DJ, Jewell DP. Small bowel gas in severe ulcerative colitis. *Gut* 1991; **32**: 1535-1537 [PMID: 1773962 DOI: 10.1136/gut.32.12.1535]

12 **Criscuoli V**, Casà A, Orlando A, Pecoraro G, Oliva L, Traina

M, Rizzo A, Cottone M. Severe acute colitis associated with CMV: a prevalence study. *Dig Liver Dis* 2004; **36**: 818-820 [PMID: 15646428 DOI: 10.1016/j.dld.2004.05.013]
13 **Carbonnel F**, Lavergne A, Lémann M, Bitoun A, Valleur P, Hautefeuille P, Galian A, Modigliani R, Rambaud JC. Colonoscopy of acute colitis. A safe and reliable tool for assessment of severity. *Dig Dis Sci* 1994; **39**: 1550-1557 [PMID: 8026269 DOI: 10.1007/BF02088063]
14 **González-Huix F**, Fernández-Bañares F, Esteve-Comas M, Abad-Lacruz A, Cabré E, Acero D, Figa M, Guilera M, Humbert P, de León R. Enteral versus parenteral nutrition as adjunct therapy in acute ulcerative colitis. *Am J Gastroenterol* 1993; **88**: 227-232 [PMID: 8424426]
15 **McIntyre PB**, Powell-Tuck J, Wood SR, Lennard-Jones JE, Lerebours E, Hecketsweiler P, Galmiche JP, Colin R. Controlled trial of bowel rest in the treatment of severe acute colitis. *Gut* 1986; **27**: 481-485 [PMID: 3084344 DOI: 10.1136/gut.27.5.481]
16 **Papadakis KA**, Tung JK, Binder SW, Kam LY, Abreu MT, Targan SR, Vasiliauskas EA. Outcome of cytomegalovirus infections in patients with inflammatory bowel disease. *Am J Gastroenterol* 2001; **96**: 2137-2142 [PMID: 11467645 DOI: 10.1111/j.1572-0241.2001.03949.x]
17 **Kandiel A**, Lashner B. Cytomegalovirus colitis complicating inflammatory bowel disease. *Am J Gastroenterol* 2006; **101**: 2857-2865 [PMID: 17026558 DOI: 10.1111/j.1572-0241.2006.00869.x]
18 **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]
19 **Issa M**, Ananthakrishnan AN, Binion DG. Clostridium difficile and inflammatory bowel disease. *Inflamm Bowel Dis* 2008; **14**: 1432-1442 [PMID: 18484669 DOI: 10.1002/ibd.20500]
20 **Jen MH**, Saxena S, Bottle A, Aylin P, Pollok RC. Increased health burden associated with Clostridium difficile diarrhoea in patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2011; **33**: 1322-1331 [PMID: 21517920 DOI: 10.1111/j.1365-2036.2011.04661.x]
21 **Grainge MJ**, West J, Card TR. Venous thromboembolism during active disease and remission in inflammatory bowel disease: a cohort study. *Lancet* 2010; **375**: 657-663 [PMID: 20149425 DOI: 10.1016/S0140-6736(09)61963-2]
22 **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]
23 **Mantzaris GJ**, Petraki K, Archavlis E, Amberiadis P, Kourtessas D, Christidou A, Triantafyllou G. A prospective randomized controlled trial of intravenous ciprofloxacin as an adjunct to corticosteroids in acute, severe ulcerative colitis. *Scand J Gastroenterol* 2001; **36**: 971-974 [PMID: 11521989 DOI: 10.1080/00365520120413]
24 **Gasche C**, Berstad A, Befrits R, Beglinger C, Dignass A, Erichsen K, Gomollon F, Hjortswang H, Koutroubakis I, Kulnigg S, Oldenburg B, Rampton D, Schroeder O, Stein J, Travis S, Van Assche G. Guidelines on the diagnosis and management of iron deficiency and anemia in inflammatory bowel diseases. *Inflamm Bowel Dis* 2007; **13**: 1545-1553 [PMID: 17985376 DOI: 10.1002/ibd.20285]
25 **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]
26 **Truelove SC**, Jewell DP. Intensive intravenous regimen for severe attacks of ulcerative colitis. *Lancet* 1974; **1**: 1067-1070 [PMID: 4135487 DOI: 10.1016/S0140-6736(74)90552-2]
27 **Lindgren SC**, Flood LM, Kilander AF, Löfberg R, Persson TB, Sjödahl RI. Early predictors of glucocorticosteroid treatment failure in severe and moderately severe attacks of ulcerative colitis. *Eur J Gastroenterol Hepatol* 1998; **10**: 831-835 [PMID: 9831403 DOI: 10.1097/00042737-199810000-00003]
28 **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]
29 **Ho GT**, Mowat C, Goddard CJ, Fennell JM, Shah NB, Prescott RJ, Satsangi J. Predicting the outcome of severe ulcerative colitis: development of a novel risk score to aid early selection of patients for second-line medical therapy or surgery. *Aliment Pharmacol Ther* 2004; **19**: 1079-1087 [PMID: 15142197 DOI: 10.1111/j.1365-2036.2004.01945.x]
30 **Ananthakrishnan AN**, McGinley EL, Binion DG, Saeian K. Simple score to identify colectomy risk in ulcerative colitis hospitalizations. *Inflamm Bowel Dis* 2010; **16**: 1532-1540 [PMID: 20091926 DOI: 10.1002/ibd.21225]
31 **Lennard-Jones JE**, Ritchie JK, Hilder W, Spicer CC. Assessment of severity in colitis: a preliminary study. *Gut* 1975; **16**: 579-584 [PMID: 1183857 DOI: 10.1136/gut.16.8.579]
32 **Almer S**, Bodemar G, Franzén L, Lindström E, Nyström P, Ström M. Use of air enema radiography to assess depth of ulceration during acute attacks of ulcerative colitis. *Lancet* 1996; **347**: 1731-1735 [PMID: 8656906 DOI: 10.1016/S0140-6736(96)90808-9]
33 **Lichtiger S**, Present DH, Kornbluth A, Gelernt I, Bauer J, Galler G, Michelassi F, Hanauer S. Cyclosporine in severe ulcerative colitis refractory to steroid therapy. *N Engl J Med* 1994; **330**: 1841-1845 [PMID: 8196726 DOI: 10.1056/NEJM199406303302601]
34 **Van Assche G**, D'Haens G, Noman M, Vermeire S, Hiele M, Asnong K, Arts J, D'Hoore A, Penninckx F, Rutgeerts P. Randomized, double-blind comparison of 4 mg/kg versus 2 mg/kg intravenous cyclosporine in severe ulcerative colitis. *Gastroenterology* 2003; **125**: 1025-1031 [PMID: 14517785 DOI: 10.1016/S0016-5085(03)01214-9]
35 **D'Haens G**, Lemmens L, Geboes K, Vandeputte L, Van Acker F, Mortelmans L, Peeters M, Vermeire S, Penninckx F, Nevens F, Hiele M, Rutgeerts P. Intravenous cyclosporine versus intravenous corticosteroids as single therapy for severe attacks of ulcerative colitis. *Gastroenterology* 2001; **120**: 1323-1329 [PMID: 11313301 DOI: 10.1053/gast.2001.23983]
36 **Sternthal MB**, Murphy SJ, George J, Kornbluth A, Lichtiger S, Present DH. Adverse events associated with the use of cyclosporine in patients with inflammatory bowel disease. *Am J Gastroenterol* 2008; **103**: 937-943 [PMID: 18177449 DOI: 10.1111/j.1572-0241.2007.01718.x]
37 **Rayner CK**, McCormack G, Emmanuel AV, Kamm MA. Long-term results of low-dose intravenous ciclosporin for acute severe ulcerative colitis. *Aliment Pharmacol Ther* 2003; **18**: 303-308 [PMID: 12895214 DOI: 10.1046/j.1365-2036.2003.01618.x]
38 **Campbell S**, Travis S, Jewell D. Ciclosporin use in acute ulcerative colitis: a long-term experience. *Eur J Gastroenterol Hepatol* 2005; **17**: 79-84 [PMID: 15647646 DOI: 10.1097/00042737-200501000-00016]
39 **Bojic D**, Radojicic Z, Nedeljkovic-Protic M, Al-Ali M, Jewell DP, Travis SP. Long-term outcome after admission for acute severe ulcerative colitis in Oxford: the 1992-1993 cohort. *Inflamm Bowel Dis* 2009; **15**: 823-828 [PMID: 19145641 DOI: 10.1002/ibd.20843]
40 **Moskovitz DN**, Van Assche G, Maenhout B, Arts J, Ferrante M, Vermeire S, Rutgeerts P. Incidence of colectomy during long-term follow-up after cyclosporine-induced remission of severe ulcerative colitis. *Clin Gastroenterol Hepatol* 2006; **4**: 760-765 [PMID: 16716758 DOI: 10.1016/j.cgh.2006.04.001]
41 **Cohen RD**, Stein R, Hanauer SB. Intravenous cyclosporin in ulcerative colitis: a five-year experience. *Am J Gastroen-*

terol 1999; **94**: 1587-1592 [PMID: 10364029 DOI: 10.1111/j.1572-0241.1999.01149.x]
42 **Kaser A**, Mairinger T, Vogel W, Tilg H. Infliximab in severe steroid-refractory ulcerative colitis: a pilot study. *Wien Klin Wochenschr* 2001; **113**: 930-933 [PMID: 11802508]
43 **Järnerot G**, Hertervig E, Friis-Liby I, Blomquist L, Karlén P, Grännö C, Vilien M, Ström M, Danielsson A, Verbaan H, Hellström PM, Magnuson A, Curman B. Infliximab as rescue therapy in severe to moderately severe ulcerative colitis: a randomized, placebo-controlled study. *Gastroenterology* 2005; **128**: 1805-1811 [PMID: 15940615 DOI: 10.1053/j.gastro.2005.03.003]
44 **Sandborn WJ**, Rutgeerts P, Feagan BG, Reinisch W, Olson A, Johanns J, Lu J, Horgan K, Rachmilewitz D, Hanauer SB, Lichtenstein GR, de Villiers WJ, Present D, Sands BE, Colombel JF. Colectomy rate comparison after treatment of ulcerative colitis with placebo or infliximab. *Gastroenterology* 2009; **137**: 1250-1260; quiz 1520 [PMID: 19596014 DOI: 10.1053/j.gastro.2009.06.061]
45 **Monterubbianesi R**, Armuzzi A, Papi C, Daperno M, Marrollo M, Biancone L, Cappello M, Lavagna A, Annese V, Orlando A, Viscido A, Riegler G, Meucci G, Sostegni R, Guidi L, Petruzziello C, Peralta S, Prantera C, Kohn A. Infliximab for severe ulcerative colitis: short-term and one year outcome of three dose regimen. An Italian multicentre open-label study. *Gastroenterology* 2009; **138** (Suppl 1): S685
46 **Venu M**, Naik AS, Ananthakrishnan AN. Early infliximab infusion in hospitalised severe UC patients: one year outcome. *Gastroenterology* 2009; **136** (Suppl1): A201 [DOI: 10.1016/S0016-5085(09)60901-X]
47 **Gustavsson A**, Jarnerot G, Hertevig E. A 2-year follow up study of the Swedish-Danish infliximab trial in steroid resistant acute ulcerative colitis. *Gastroenterology* 2007; **132**: 983-984
48 **Reinisch W**, Sandborn WJ, Hommes DW, D'Haens G, Hanauer S, Schreiber S, Panaccione R, Fedorak RN, Tighe MB, Huang B, Kampman W, Lazar A, Thakkar R. Adalimumab for induction of clinical remission in moderately to severely active ulcerative colitis: results of a randomised controlled trial. *Gut* 2011; **60**: 780-787 [PMID: 21209123 DOI: 10.1136/gut.2010.221127]
49 **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 DOI: 10.1053/j.gastro.2011.10.032]
50 **Sjöberg M**, Walch A, Meshkat M, Gustavsson A, Järnerot G, Vogelsang H, Hertervig E, Novacek G, Friis-Liby I, Blomquist L, Angelberger S, Karlen P, Grännö C, Vilien M, Ström M, Verbaan H, Hellström PM, Dejaco C, Magnuson A, Halfvarson J, Reinisch W, Tysk C. Infliximab or cyclosporine as rescue therapy in hospitalized patients with steroid-refractory ulcerative colitis: a retrospective observational study. *Inflamm Bowel Dis* 2012; **18**: 212-218 [PMID: 21438096 DOI: 10.1002/ibd.21680]
51 **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]
52 **Leblanc S**, Allez M, Seksik P, Flourie B, Peeters H, Dupas JL, Bouguen G, Biroulet LP, Bourreille A, Dewit O, Bouhnik Y, Michetti PF, Chaussade S, de Saussure P, Colombel JF, Lemann M. Successive treatment with cyclosporin and infliximab in severe ulcerative colitis. *Gastroenterology* 2009; **136** (Suppl 1): A88 [DOI: 10.1016/S0016-5085(09)60396-6]
53 **Ogata H**, Matsui T, Nakamura M, Iida M, Takazoe M, Suzuki Y, Hibi T. A randomised dose finding study of oral tacrolimus (FK506) therapy in refractory ulcerative colitis. *Gut* 2006; **55**: 1255-1262 [PMID: 16484504 DOI: 10.1136/gut.2005.081794]
54 **Baumgart DC**, Wiedenmann B, Dignass AU. Rescue therapy with tacrolimus is effective in patients with severe and refractory inflammatory bowel disease. *Aliment Pharmacol Ther* 2003; **17**: 1273-1281 [PMID: 12755840 DOI: 10.1046/j.1365-2036.2003.01534.x]
55 **Baumgart DC**, Pintoffl JP, Sturm A, Wiedenmann B, Dignass AU. Tacrolimus is safe and effective in patients with severe steroid-refractory or steroid-dependent inflammatory bowel disease--a long-term follow-up. *Am J Gastroenterol* 2006; **101**: 1048-1056 [PMID: 16573777 DOI: 10.1111/j.1572-0241.2006.00524.x]
56 **Pal S**, Sahni P, Pande GK, Acharya SK, Chattopadhyay TK. Outcome following emergency surgery for refractory severe ulcerative colitis in a tertiary care centre in India. *BMC Gastroenterol* 2005; **5**: 39 [PMID: 16316474 DOI: 10.1186/1471-230X-5-39]
57 **Alves A**, Panis Y, Bouhnik Y, Maylin V, Lavergne-Slove A, Valleur P. Subtotal colectomy for severe acute colitis: a 20-year experience of a tertiary care center with an aggressive and early surgical policy. *J Am Coll Surg* 2003; **197**: 379-385 [PMID: 12946792 DOI: 10.1016/S1072-7515(03)00434-4]
58 **Hyman NH**, Cataldo P, Osler T. Urgent subtotal colectomy for severe inflammatory bowel disease. *Dis Colon Rectum* 2005; **48**: 70-73 [PMID: 15690660 DOI: 10.1007/s10350-004-0750-5]
59 **Andersson P**, Söderholm JD. Surgery in ulcerative colitis: indication and timing. *Dig Dis* 2009; **27**: 335-340 [PMID: 19786761 DOI: 10.1159/000228570]
60 **Selvasekar CR**, Cima RR, Larson DW, Dozois EJ, Harrington JR, Harmsen WS, Loftus EV, Sandborn WJ, Wolff BG, Pemberton JH. Effect of infliximab on short-term complications in patients undergoing operation for chronic ulcerative colitis. *J Am Coll Surg* 2007; **204**: 956-962; discussion 962-963 [PMID: 17481518 DOI: 10.1016/j.jamcollsurg.2006.12.044]
61 **Mor IJ**, Vogel JD, da Luz Moreira A, Shen B, Hammel J, Remzi FH. Infliximab in ulcerative colitis is associated with an increased risk of postoperative complications after restorative proctocolectomy. *Dis Colon Rectum* 2008; **51**: 1202-1207; discussion 1207-1210 [PMID: 18536964 DOI: 10.1007/s10350-008-9364-7]
62 **Kunitake H**, Hodin R, Shellito PC, Sands BE, Korzenik J, Bordeianou L. Perioperative treatment with infliximab in patients with Crohn's disease and ulcerative colitis is not associated with an increased rate of postoperative complications. *J Gastrointest Surg* 2008; **12**: 1730-1736; discussion 1736-1737 [PMID: 18709514 DOI: 10.1007/s11605-008-0630-8]
63 **Ferrante M**, D'Hoore A, Vermeire S, Declerck S, Noman M, Van Assche G, Hoffman I, Rutgeerts P, Penninckx F. Corticosteroids but not infliximab increase short-term postoperative infectious complications in patients with ulcerative colitis. *Inflamm Bowel Dis* 2009; **15**: 1062-1070 [PMID: 19161179 DOI: 10.1002/ibd.20863]
64 **Yang Z**, Wu Q, Wang F, Wu K, Fan D. Meta-analysis: effect of preoperative infliximab use on early postoperative complications in patients with ulcerative colitis undergoing abdominal surgery. *Aliment Pharmacol Ther* 2012; **36**: 922-928 [PMID: 23002804 DOI: 10.1111/apt.12060]
65 **Kaplan GG**, McCarthy EP, Ayanian JZ, Korzenik J, Hodin R, Sands BE. Impact of hospital volume on postoperative morbidity and mortality following a colectomy for ulcerative colitis. *Gastroenterology* 2008; **134**: 680-687 [PMID: 18242604 DOI: 10.1053/j.gastro.2008.01.004]
66 **Roberts SE**, Williams JG, Yeates D, Goldacre MJ. Mortality in patients with and without colectomy admitted to hospital

for ulcerative colitis and Crohn's disease: record linkage studies. *BMJ* 2007; **335**: 1033 [PMID: 17977817 DOI: 10.1136/bmj.39345.714039.55]

67 **Johnson P**, Richard C, Ravid A, Spencer L, Pinto E, Hanna M, Cohen Z, McLeod R. Female infertility after ileal pouch-anal anastomosis for ulcerative colitis. *Dis Colon Rectum* 2004; **47**: 1119-1126 [PMID: 15164254 DOI: 10.1007/s10350-004-0570-7]

68 **Oresland T**, Palmblad S, Ellström M, Berndtsson I, Crona N, Hultén L. Gynaecological and sexual function related to anatomical changes in the female pelvis after restorative proctocolectomy. *Int J Colorectal Dis* 1994; **9**: 77-81 [PMID: 8064194 DOI: 10.1007/BF00699417]

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LETTERS TO THE EDITOR

Antiviral treatment in patients with *cytomegalovirus* positive ulcerative colitis

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Abstract

Cytomegalovirus (CMV) is a common virus in patients with ulcerative colitis receiving immunosuppressive drugs. Many studies suggested that CMV infection is an exacerbating factor in patients with ulcerative colitis. The role of CMV in exacerbations of ulcerative colitis has been discussed. One of studies starting this discussion is an article entitled "CMV positive ulcerative colitis: A single center experience and literature review" by Kopylov *et al*. However, we think that there are some points that should be emphasized about the study. Especially, the small number of patients in the study has led to meaningless results. Large controlled prospective trials are needed to clarify the benefit of antiviral therapy for active ulcerative colitis patients.

Key words: *Cytomegalovirus*; Ulcerative colitis; Antiviral treatment; Steroid resistant; Colonoscopy

Core tip: Many studies suggested that *cytomegalovirus* (CMV) infection is an exacerbating factor in patients with ulcerative colitis. The role of CMV in exacerbations of ulcerative colitis has been discussed. We believe that large controlled prospective trials are needed to clarify the benefit of antiviral therapy for active ulcerative colitis patients.

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TO THE EDITOR

We read with great interest the recently published article entitled "Cytomegalovirus (CMV) positive ulcerative colitis: A single center experience and literature review" by Kopylov *et al*[1] in the February 15, 2013 issue of *World Journal of Gastrointestinal Pathophysiology*. In this retrospective study, the authors compared the clinical outcomes of CMV-positive ulcerative colitis patients with and without antiviral therapy (gancyclovir). They concluded that patients with obvious histological evidence of CMV in the colonic mucosa may not universally require antiviral therapy and may respond to conventional anti-inflammatory therapy. This study reveals the indications for antiviral therapy in CMV-positive patients with ulcerative colitis. Moreover, it provides some new information that represents educational "take-home messages" for readers. We believe that further studies will be performed in light of these findings. However, we think that there are some points that should be emphasized about the study.

First, in the discussion section of the paper, the authors reported that patients in the antiviral-treated group "are in greater need of hospitalization" than patients without antiviral treatment. However, as shown in Table 1, no statistically significant difference could be seen between these two groups. As we know that the *P* value is revealed below a certain significance level, often 0.05, this elucidates a strong presumption against the null hypothesis[2,3]. In light of this, we suggest that the conclusion of

Table 1 Clinical and demographic characteristics of the included patients (mean ± SD)

Patient characteristic	Treated (n = 7)	Untreated (n = 6)	P value
Age (yr)	50.0 ± 14.6	45.0 ± 13.6	0.540
Gender (male/female)	4/3	3/3	0.400
Extent of disease			
Pancolitis	6	5	0.540
Left-sided	1	1	0.540
Age at diagnosis of UC, yr	35.7 ± 13.3	41.5 ± 13.3	0.530
Duration of disease, yr	14.2 ± 9.3	3.5 ± 1.8	0.008
Hospitalized patients	6	4	0.560
Prehospitalization treatment			
SC	4	2	0.560
Thiopurines	3	2	1.000
Infliximab	1[1]	0	1.000
5-asa	5	4	1.000
SC + thiopurines	2	1	1.000
Treatment during hospitalization			
SC	6	3	0.400
Infliximab	1	0	1.000
Cyclosporine	3	0	0.200
Timing of colonoscopy (d)	3.8 ± 2.4	2.7 ± 3.4	0.600
Positive cytopathic changes on HE staining	2	0	0.460
Hospitalization outcome			
Death	1	0	1.000
Colectomy	1	0	1.000
Outcome by the end of the follow-up			
Colectomy	3	0	0.190
Death	1	0	1.000

[1]Combined with systemic corticosteroids and thiopurine. Treated: Patients who received antiviral therapy; Untreated: Patients who did not receive antiviral therapy; Timing of colonoscopy: Number of days from hospital admission; SC: Systemic corticosteroids; HE: Hematoxylin and eosin; IHC: Immunohistochemistry; UC: Ulcerative colitis.

the present study should be reviewed.

Second, the authors mentioned in the discussion section that only three patients without antiviral therapy were hospitalized. However, four patients in the group without antiviral therapy were hospitalized, according to Table 1. Finally, there are conflicting data regarding the staining method of the histopathological examination. Consequently, we conclude that, before making certain interpretations, this work should be rearranged in light of the above-mentioned suggestions. This could provide the readers of the journal clearer information regarding the role of CMV infection in the pathogenesis and clinical course of ulcerative colitis.

REFERENCES

1 **Kopylov U**, Sasson G, Geyshis B, Oikawa MT, Barshack I, Eliakim R, Ben-Horin S. Cytomegalovirus positive ulcerative colitis: A single center experience and literature review. *World J Gastrointest Pathophysiol* 2013; **4**: 18-23 [PMID: 23596551 DOI: 10.4291/wjgp.v4.i1.18]

2 **Stigler S**. Fisher and the 5% level. *Chance* 2008; **21**: 12 [DOI: 10.1007/s00144-008-0033-3]

3 **Goodman SN**. Toward evidence-based medical statistics. 2: The Bayes factor. *Ann Intern Med* 1999; **130**: 1005-1013 [PMID: 10383350 DOI: 10.7326/0003-4819-130-12-199906150-00019]

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Format

Journals

English journal article (list all authors and include the PMID where applicable)

1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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