ISSN 2150-5349 (online)

World Journal of *Gastrointestinal Pharmacology and Therapeutics*

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 469-586

Published by Baishideng Publishing Group Inc

WJGPT

World Journal of Gastrointestinal Pharmacology and Therapeutics

A peer-reviewed, online, open-access journal of gastrointestinal pharmacology and therapeutics

Editorial Board

2016-2019

The *World Journal of Gastrointestinal Pharmacology and Therapeutics* Editorial Board consists of 394 members, representing a team of worldwide experts in surgery research. They are from 45 countries, including Argentina (3), Australia (12), Austria (3), Belarus (1), Belgium (4), Brazil (10), Canada (10), China (39), Czech Repoublic (1), Egypt (3), Estonia (1), Finland (1), France (6), Germany (19), Greece (7), Hungary (4), India (17), Iran (5), Ireland (1), Israel (5), Italy (32), Japan (34), Lebanon (1), Lithuania (3), Mexico (2), Netherlands (10), New Zealand (2), Norway (2), Pakistan (1), Philippines (1), Poland (3), Portugal (2), Romania (1), Russia (1), Saudi Arabia (3), Singapore (3), Slovenia (1), South Africa (1), South Korea (14), Spain (13), Sweden (3), Thailand (4), Turkey (6), United Kingdom (19) and United States (80).

Duo Li, *Hangzhou*
Yu-Yuan Li, *Guangzhou*
Xiong Ma, *Shanghai*
Qin Pan, *Shanghai*
Guo-Ping Sun, *Hefei*
Xue-Ying Sun, *Harbin*
Ming-Fu Wang, *Hong Kong*
Che-Yuen Justin Wu, *Hong Kong*
De Xiang Xu, *Hefei*
Ruian Xu, *Xiamen*
Ming-Xian Yan, *Jinan*
Yong-Feng Yang, *Nanjing*
Thomas Yau, *Hong Kong*
Win-Nei Yeo, *Hong Kong*
Long Yu, *Guangzhou*
Jian-Ping Yuan, *Guangzhou*
Man-Fung Yuen, *Hong Kong*
Liqun Zhang, *Beijing*
Min-Sheng Zhu, *Nanjing*

Czech Republic

Rene Kizek, *Brno*

Denmark

Rene Kizek, *Brno*

Egypt

Omar ME Abdel-Salam, *Cairo*
Ahmed Osman Abdel-Zaher, *Assiut*
Osama Ahmed Badary, *Cairo*

Estonia

Riin Tamm, *Tartu*

Finland

Riitta Korpela, *Helsinki*

France

Tarik Asselah, *Clichy*
Ferrand Audrey, *Toulouse*
Frederic Batteux, *Paris*
Thierry Capiod, *Paris*
Frederic Lagarce, *Angers*
Hang Thi Thu Nguyen, *Clermont-Ferrand*

Germany

Susanne Beckebaum, *Essen*
Jürgen Borlak, *Hannover*
Güralp Onur Ceyhan, *Munich*
Walee Chamulitrat, *Heidelberg*
Anton Gillessen, *Munster*
Dirk Heitzmann, *Muenster*
Jens Michael Heyn, *München*
Joachim Labenz, *Siegen*
Thomas Müller, *Berlin*
Belal Naser, *Salzgitter*
Beate Niesler, *Heidelberg*
Matthias Ocker, *Marburg*
Andreas Marc Palmer, *Konstanz*
Dirk Rades, *Luebeck*
Fuat Hakan Saner, *Essen*
Manfred V Singer, *Mannheim*
Konrad Ludwig Streetz, *Aachen*
Frank Tacke, *Aachen*
Gerhard Treiber, *Balingen*

Greece

Moses S Elisaf, *Ioannina*
Anastasios Koulaouzidis, *Greek*
Ioannis E Koutroubakis, *Heraklion*
Spilios Manolakopoulos, *Athens*
Konstantinos Christou Mountzouris, *Athens*
George V Papatheodoridis, *Athens*
Konstantinos Tziomalos, *Thessaloniki*

Hungary

Zsolt Barta, *Debrecen*
László Czakó, *Szeged*
Bela Molnar, *Budapest*
Gyula Mózsik, *Pecs*

India

Anil Kumar Agarwal, *New Delhi*
Sandip Basu, *Bombay*
Chiranjib Chakraborty, *Vellore*
Rukhsana Chowdhury, *Kolkata*
C M Habibullah, *Hyderabad*
Mohandas K Mallath, *Mumbai*
Balraj Mittal, *Lucknow*
Rama Devi Mittal, *Lucknow*
Asish K Mukhopadhyay, *Kolkata*
Lekha Saha, *Chandigarh*
Darisetty Santosh, *Hyderabad*
Shiv Kumar Sarin, *New Delhi*
Jayshri Ankur Shah, *Mumbai*
Sonu Sundd Singh, *Gurgaon*
Sikta Swarnakar, *Calcutta*
Rakesh Kumar Tandon, *New Delhi*
Asna Urooj, *Mysore*

Iran

Seyed Mohsen Dehghani, *Shiraz*
Ahmad Reza Dehpour, *Tehran*
Sara Farhang, *Tabriz*
Parisa Hasanein, *Hamadan*
Amir Mohammad Mortazavian, *Tehran*

Ireland

Zaid S Heetun, *Dublin*

Israel

Nimer Assy, *Safed*
Simon Bar-Meir, *Tel-Hashomer*
Rami Eliakim, *Haifa*
Tiberiu Hershcovici, *Jerusalem*
Haim Shmuel Odes, *Beer Sheva*

Italy

Giovanni C Actis, *Turin*
Bruno Annibale, *Roma*
Leonardo Baiocchi, *Rome*
Gabrio Bassotti, *San Sisto*
Francesca Borrelli, *Napoli*
Giuseppe Brisinda, *Rome*
Renzo Caprilli, *Rome*
Mauro Antonio Maria Carai, *Cagliari*
Renato Caviglia, *Rome*
Carolina Ciacci, *Naples*
Mario Cottone, *Trabucco*
Roberto De Giorgio, *Bologna*
Luca Elli, *Milano*
Alessandro Granito, *Bologna*
Francesco William Guglielmi, *Bari*
Mario Guslandi, *Milano*
Pietro Invernizzi, *Monza*
Mariano Malaguarnera, *Catania*
Massimo C Mauri, *Milan*
Massimo Montalto, *Rome*
Giovanni Monteleone, *Rome*
Gerardo Nardone, *Naples*
Fabio Pace, *Seriate*
Raffaele Pezzilli, *Bologna*
Rita Rezzani, *Brescia*
Carmelo Scarpignato, *Parma*
Generoso Uomo, *Napoli*
Paolo Usai-Satta, *Cagliari*
Maurizio Vecchi, *Milan*
Massimiliano Veroux, *Catania*

Japan

Akira Andoh, *Otsu*
Yuichiro Eguchi, *Saga*
Munechika Enjoji, *Fukuoka*
Norihiro Furusyo, *Fukuoka*
Naoki Hotta, *Nagoya*
Shigeo Ikegawa, *Higashi-Osaka*
Susumu Ito, *Okinawa*
Satoru Kakizaki, *Maebashi*
Terumi Kamisawa, *Tokyo*
Motoyori Kanazawa, *Miyagi*
Takuma Kato, *Mie*
Takashi Kawai, *Tokyo*
Shirao Kuniaki, *Oita*
Nobuyuki Matsuhashi, *Tokyo*
Tatsuya Matsura, *Yonago*
Teruo Murakami, *Hiroshima*
Yuji Naito, *Kyoto*
Katsuyuki Nakajima, *Maebashi Gunma*
Hiroshi Nakase, *Kyoto*
Nobuhiro Ohkohchi, *Tsukuba*
Shogo Ohkoshi, *Niigata*
Tomohiko Shimatani, *Kure*
Yasuhiko Sugawara, *Tokyo*
Yoshitaka Takuma, *Okayama*
Tatsuhiro Tsujimoto, *Nara*
Takato Ueno, *Fukuoka*
Kenji Watanabe, *Osaka*
Toshiaki Watanabe, *Tokyo*

Jiro Watari, *Nishinomiya*
Satoshi Yamagiwa, *Niigata*
Takayuki Yamamoto, *Mie*
Norimasa Yoshida, *Kyoto*
Hitoshi Yoshiji, *Kashihara*
Katsutoshi Yoshizato, *Higashihiroshima*

Lebanon

AIa Sharara, *Beirut*

Lithuania

Dalia Adukauskiene, *Kaunas*
Giedrius Barauskas, *Kaunas*
Laimas Jonaitis, *Kaunas*

Mexico

Pablo Muriel, *Mexico City*
Guillermo Benito Robles-Díaz, *Mexico City*

Netherlands

Judith Elisabeth Baars, *Rotterdam*
Albert J Bredenoord, *Nieuwegein*
Nanne KH De Boer, *Amsterdam*
Pieter Jan Floris De Jonge, *Rotterdam*
Wouter J de Jonge, *Amsterdam*
Mireille A Edens, *Groningen*
Chris JJ Mulder, *Amsterdam*
Paul E Sijens, *Groningen*
Vera Esther Valkhoff, *Rotterdam*

New Zealand

Momir M Mikov, *Dunedin*
Maxim Petrov, *Auckland*

Norway

Guanglin Cui, *Tromso*
Reidar Fossmark, *Trondheim*

Pakistan

Anwar Hassan Gilani, *Karachi*

Philippines

Mark Anthony Ayuyao De Lusong, *Quezon City*

Poland

Halina Cichoz-Lach, *Lublin*
Jaroslaw Czyz, *Cracow*
Julian Teodor Swierczynski, *Gdansk*

Portugal

Cristina Freire, *Hull*
Ana Isabel Lopes, *Lisbon*

Romania

Dan Lucian Dumitrascu, *Cluj*

Russia

Tatyana A Korolenko, *Novosibirsk*

Saudi Arabia

Mohammad S Khuroo, *Riyadh*
Moamen Salah Refat, *Taif*
Shahab Uddin, *Riyadh*

Singapore

Kok-Ann Gwee, *Singapore*
Khek-Yu Ho, *Singapore*
Kok-Yuen Ho, *Singapore*

Slovenia

Rok Orel, *Ljubljana*

South Africa

Christoffel Van Rensburg, *Cape Town*

South Korea

Ki Baik Hahm, *Inchon*
Seok Joo Han, *Seoul*
Jeong Won Jang, *Seoul*
Dong Joon Kim, *Gangwon-do*
Jae J Kim, *Seoul*
Kyoung Mee Kim, *Seoul*
Nayoung Kim, *Songnam*
Sung-Bae Kim, *Seoul*
Byung-Hoon Lee, *Seoul*
Kwan S Lee, *Seoul*
Sang-Han Lee, *Daegu*
Yun Jeong Lim, *Goyang*
Ji-Young Park, *Seoul*
Uy Dong Sohn, *Seoul*

Spain

Matias Antonio Avila, *Pamplona*
Luis Bujanda, *San Sebastián*
Maria Carmen Collado, *Valencia*
Conrado M Fernandez-Rodriguez, *Madrid*
ángel Lanas, *Zaragoza*
Juan-R Malagelada, *Barcelona*
Jose JG Marin, *Salamanca*
Antonio Ruiz Medina, *Jaén*
Maria J Monte, *Salamanca*
Miguel Munoz, *Seville*
Jesus Prieto, *Pamplona*
Victor Manuel Victor, *Valencia*
Maria D Yago, *Granada*

Sweden

Bodil Ohlsson, *Malmo*
Henrik Thorlacius, *Malmo*
Curt Tysk, *Orebro*

Thailand

Weekitt Kittisupamongkol, *Bangkok*
Abhasnee Sobhonslidsuk, *Bangkok*
Suporn Treepongkaruna, *Bangkok*
Sombat Treeprasertsuk, *Bangkok*

Turkey

Fusun Acarturk, *Etiler-Ankara*
Engin Altintas, *Mersin*
Güldeniz K Cakmak, *Kozlu Zonguldak*
Hayrullah Derici, *Balkesir*
Mukaddes Esrefoglu, *Istanbul*
Ilker Tasci, *Ankara*

United Kingdom

Nadeem Ahmad Afzal, *Hampshire*
Qasim Aziz, *London*
Hugh Barr, *Gloucester*
Ian Leonard Phillip Beales, *Norwich*
Barbara Braden, *Oxford*
Susan J Duthie, *Aberdeen*
Eyad Elkord, *Manchester*
Anton Vignaraj Emmanuel, *London*
Konstantinos C Fragkos, *London*
Nusrat Husain, *Manchester*
Jin-Yong Kang, *London*
Mariusz Madalinski, *Ipswich*
Srinivasan Madhusudan, *Nottingham*
Subramanian Mahadevan, *Birmingham*
John Francis Mayberry, *Leicester*
Chuka Uche Nwokolo, *Coventry*
Ajith Kumar Siriwardena, *Manchester*
Her-Hsin Tsai, *Hull*
Craig LC Williams, *Glasgow*

United States

Zubair H Aghai, *Camden*
Shrikant Anant, *Oklahoma*
Kondala R Atkuri, *Stanford*
Cheryl Hunt Baker, *Orlando*
James M Becker, *Boston*
Qiang Cai, *Atlanta*
Jian-De Chen, *Galveston*
Liang Cheng, *Indianapolis*

WJGPT

World Journal of Gastrointestinal Pharmacology and Therapeutics

Contents

Quarterly Volume 7 Number 4 November 6, 2016

World Journal of Gastrointestinal Pharmacology and Therapeutics
Volume 7 Number 4 November 6, 2016

Contents

NAME OF JOURNAL
World Journal of Gastrointestinal Pharmacology and Therapeutics

ISSN
ISSN 2150-5349 (online)

LAUNCH DATE
May 6, 2010

FREQUENCY
Quarterly

EDITORIAL OFFICE
Xiu-Xia Song, Director
Fang-Fang Ji, Vice Director
World Journal of Gastrointestinal Pharmacology and Therapeutics
Baishideng Publishing Group Inc
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Inc
8226 Regency Drive,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLICATION DATE
November 6, 2016

INSTRUCTIONS TO AUTHORS
http://www.wjgnet.com/bpg/gerinfo/204

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

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i4.469

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 469-476
ISSN 2150-5349 (online)

REVIEW

Bilirubin in coronary artery disease: Cytotoxic or protective?

Nancy Gupta, Tavankit Singh, Rahul Chaudhary, Sushil K Garg, Gurprataap Singh Sandhu, Varun Mittal, Rahul Gupta, Roxana Bodin, Sachin Sule

Nancy Gupta, Sachin Sule, Department of Medicine, Westchester Medical Center, New York Medical College, Valhalla, NY 10595, United States

Tavankit Singh, Department of Medicine, Cleveland Clinic, Cleveland, OH 44195, United States

Rahul Chaudhary, Department of Medicine, Sinai Hospital of Baltimore, Johns Hopkins University, Baltimore, MD 21218, United States

Sushil K Garg, Department of Medicine, University of Minnesota, Minneapolis, MN 55455, United States

Gurprataap Singh Sandhu, Department of Medicine, University of Pittsburgh, Pittsburgh, PA 15260, United States

Varun Mittal, Einstein Healthcare Network, Philadelphia, PA 19141, United States

Rahul Gupta, Government Medical College, Amritsar, Punjab 143001, India

Roxana Bodin, Division of Gastroenterology and Hepatobiliary Disease, New York Medical College, Valhalla, NY 10595, United States

Author contributions: All authors contributed to the manuscript; Gupta N, Singh T and Chaudhary R have contributed equally for the paper.

Conflict-of-interest statement: No potential conflicts of interest relevant to this article were reported.

Manuscript source: Invited manuscript

Correspondence to: Nancy Gupta, MD, Department of Medicine, Westchester Medical Center, New York Medical College, 40 Sunshine Cottage Rd, Valhalla, NY 10595, United States. drnancygupta87@gmail.com
Telephone: +1-914-5361563
Fax: +1-914-4935827

Received: April 2, 2016
Peer-review started: April 7, 2016
First decision: June 6, 2016
Revised: June 7, 2016
Accepted: August 27, 2016
Article in press: August 29, 2016
Published online: November 6, 2016

Abstract

Bilirubin has traditionally been considered a cytotoxic waste product. However, recent studies have shown bilirubin to have anti-oxidant, anti-inflammatory, vaso-dilatory, anti-apoptotic and anti-proliferative functions. These properties potentially confer bilirubin a new role of protection especially in coronary artery disease (CAD), which is a low grade inflammatory process exacerbated by oxidative stress. In fact, recent literature reports an inverse relationship between serum concentration of bilirubin and the presence of CAD. In this article, we review the current literature exploring the association between levels of bilirubin and risk of CAD. We conclude that current evidence is inconclusive regarding the protective effect of bilirubin on CAD. A causal relationship between low serum bilirubin level and increased risk of CAD is not currently established.

Key words: Bilirubin; Cytotoxic; Protective; Anti-oxidant; Anti-inflammatory; Anti coronary artery disease; Lipid peroxidation; Gilbert

Core tip: Bilirubin has traditionally been considered a cytotoxic waste product. However, recent studies have shown bilirubin to have anti-oxidant, anti-inflammatory, vasodilatory, anti-apoptotic and anti-proliferative functions. These properties potentially confer bilirubin a new role of protection especially in coronary artery disease (CAD), which is a low grade inflammatory process exacerbated by oxidative stress. In fact, recent literature reports an inverse relationship between serum concentration of bilirubin and the presence of CAD. In this article, we review the current literature exploring the association between levels of bilirubin and risk of CAD.

Gupta N, Singh T, Chaudhary R, Garg SK, Sandhu GS, Mittal V, Gupta R, Bodin R, Sule S. Bilirubin in coronary artery disease: Cytotoxic or protective? *World J Gastrointest Pharmacol Ther* 2016; 7(4): 469-476 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i4/469.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i4.469

INTRODUCTION

Traditionally, bilirubin, a product of heme metabolism was thought to be a cytotoxic waste product, toxic to neurons[1]. However, it was later observed to possess other properties: Vasodilatory, anti-oxidant, anti-inflammatory, anti-mutagenic, immune-modulatory, antiproliferative and anti-apoptotic on vascular cells[2,3]. It has also been suggested to have a lipid lowering effect by reducing plasma and low-density lipid peroxidation[3]. By virtue of these properties, bilirubin was hypothesized to have a protective effect in coronary artery disease (CAD)[4]. Vitek *et al*[5] studied this relation in patients with Gilbert syndrome (a hereditary disorder leading to unconjugated hyperbilirubinemia with normal liver chemistries) and reported a 2% prevalence of ischemic CAD with Gilbert syndrome (n = 50) compared to 12.1% in the general population (n = 2296, P < 0.05)[5].

BILIRUBIN METABOLISM

Hemoglobin is released from the senescent red blood cells and non-erythroid hemoproteins. Hemoglobin is broken down into heme pigment and globin chains. Heme pigment is oxidatively metabolized by heme oxygenase into biliverdin, carbon monoxide and free iron. Biliverdin is further degraded by biliverdin reductase into unconjugated bilirubin. Following its uptake by the hepatocytes, unconjugated bilirubin is converted to conjugated bilirubin by the action of uridine-diphosphoglucuronate glucuronosyltransferase (UDP-GT). The gene that codes for UDP-GT is *UGT1A1* gene and a genetic variation in the promoter region of *UGT1A1* gene is associated with Gilbert syndrome. This genetic variation involves insertion of an additional thymine-adenine base pair in the TATA box of the *UGT1A1* gene instead of the normal 6 pairs[4,6] (Figure 1). This leads to deficiency of the enzyme that leads to accumulation of unconjugated bilirubin within the blood[4]. Patients with Gilbert have otherwise normal serum liver chemistries[6]. However, another condition called Crigler-Najjar syndrome with unconjugated hyperbilirubinemia stems from complete or near complete loss of UGT1A1 activity. Type 1 Crigler Najjar is a rare and lethal recessive disorder compared to type 2 Crigler Najjar where the UGT1A1 activity is still maintained, albeit at a minimal level[7]. Patients with Criggler Najjar Type 1 develop severe neurological impairment and carry a high early mortality unless they receive liver transplantation.

Conjugated bilirubin is excreted into the bile canaliculus by the canalicular membrane transporter multidrug resistance related protein 2 (MRP2). Mutations in the gene that affects this transport protein leads to conjugated hyperbilirubinemia. This condition is called Dubin-Johnson syndrome[8]. Another autosomal recessive disorder, in which patients have multiple defects in hepatocyte uptake and excretion of bilirubin, leads to increase in conjugated bilirubin and is called Rotor syndrome.

PROTECTIVE PROPERTIES OF BILIRUBIN

Several mechanisms have been proposed highlighting the protective effects of bilirubin: (1) Bilirubin has anti-oxidant properties independent of whether it is free or albumin bound, conjugated or unconjugated. Bilirubin increases in response to the oxidative stress and acts as a scavenger of the reactive oxygen species[9,10]. Bilirubin sub-fractions (Bu and Bc) have demonstrated inhibition of low-density lipoproteins oxidation, which in turn retards the peroxidation of lipids, hence could potentially restrict the progression of atherosclerosis[10]. Of note, unconjugated bilirubin in concentrations as low as 10 nmol/L has been reported to protect neuronal cultures from the oxidative stress generated by 10000 times higher concentrations of hydrogen peroxide[11]. This anti-oxidative effect of bilirubin is amplified by the recycling of bilirubin to biliverdin and so forth *via* redox reactions (Figure 1)[3]. This recycling of bilirubin amplifies its anti-oxidant potential up to 10000 times; (2) Bilirubin has been shown to be inversely associated with increased arterial stiffness[12,13]. Pre-clinical studies have observed this effect to be mediated by preservation of vascular nitric oxide, which mediates endothelial relaxation[2,13]. Decreased levels of nitric oxide impair the ability of the coronary vessels to dilate during exercise or stress, thus, provoking myocardial ischemia in patients with CAD[14]. Besides vaso-relaxation, nitric oxide also inhibits leukocyte adhesion to endothelium, vascular smooth muscle cell migration and proliferation, platelet aggregation and neointimal formation[2,15]. Thus, preservation of nitric oxide offers a significant protection against atherosclerosis[12]; (3) Bilirubin has

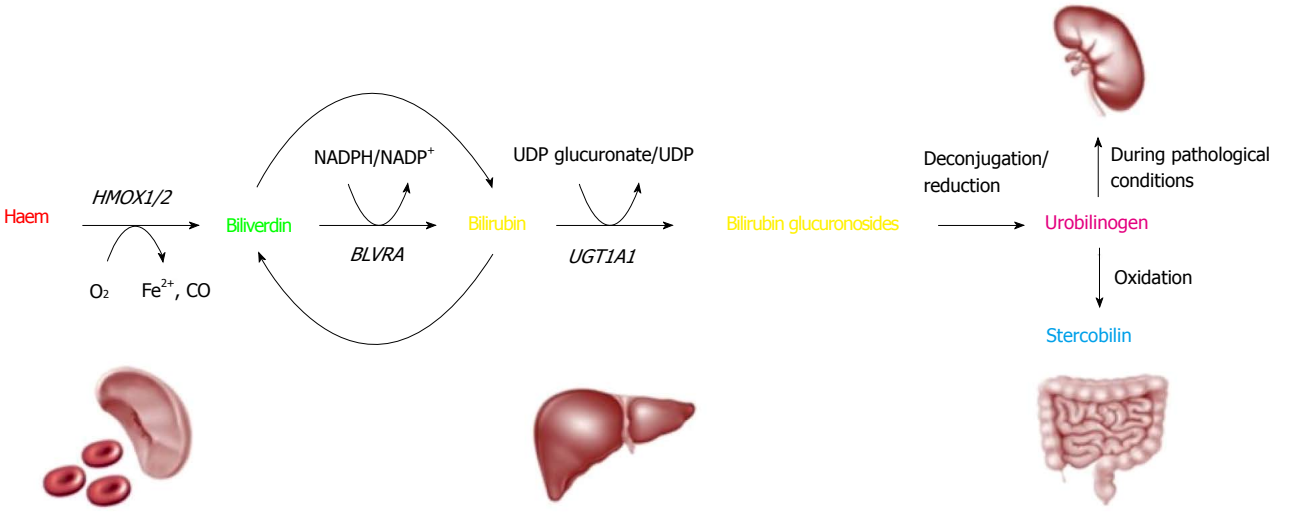

Figure 1 Bilirubin metabolism (from heme to bilirubin). Hemoglobin is cleaved to yield globin and heme (red). Heme is enzymatically converted to biliverdin (green) by liberating iron, *via* oxidation with loss of a carbon atom (CO). This, in turn, yields bilirubin (orange) after enzymatic reduction of biliverdin. In the liver, bilirubin is conjugated to enable excretion, requiring the enzyme UGT1A1[4].

also been seen to reduce platelet aggregation. Kundur *et al*[3] showed that when unconjugated bilirubin is added to platelet rich plasma at concentrations seen in Gilbert syndrome (0.99-5.85 mg/dL), it inhibits both - collagen induced and adenosine diphosphate induced platelet aggregation, in a dose dependent fashion for the latter. P and E-selectin are markers of platelet activation and released from activated platelets and endothelium. They predominantly mediate adhesion of platelets and inflammatory cells to endothelium and facilitate the formation of large stable platelet-leukocyte aggregates that can lead to thrombus formation. Bilirubin, biliverdin and inducible heme oxygenase have an inhibitory effect on P- and E-selectin[16]. This is supported by studies showing reduced levels of circulating inflammatory biomarkers, P selectin and CD 40 ligand in patients with Gilbert syndrome[3]; (4) Bilirubin and heme oxygenase exhibit anti-inflammatory properties and prevent oxidant induced microvascular leukocyte adhesion. Heme oxygenase (the rate-limiting enzyme in bilirubin production) also functions as a vasodilatory and anti- proliferative agent during vascular injury[15]. An inverse association has been demonstrated between total bilirubin levels and the markers of inflammation, namely, C-reactive protein (direct marker); neutrophil to lymphocyte ratio and red cell distribution width (indirect markers)[17]. In animal models, bilirubin has also been seen to exhibit anti-complement effect *in vitro*, thus, conferring protection against increased thrombogenicity and clot formation[3,17-19]. This anti-inflammatory effect has been hypothesized to antagonize the process of atherosclerosis, which is a low-grade chronic inflammatory state; (5) preclinical studies on mice have demonstrated a protective effect of bilirubin on angiotensin-Ⅱ induced hypertension. Angiotensin-Ⅱ is known to cause superoxide production, which is attenuated by induction of heme oxygenase *via* redox reactions. This reaction leads to production of bilirubin and carbon monoxide; hence an increased bilirubin level is associated with increased attenuation of superoxide production[20]. This protective effect, in conjunction with carbon monoxide, by inhibition of angiotensin-Ⅱ has also been suggested and extrapolated in the cardiomyocytes preventing left ventricular hypertrophy[21-23]; and (6) Bilirubin has also been described to solubilize cholesterol and promotes its clearance through the bile[24,25]. This finding is also supported by the evidence of reduced levels of total cholesterol, low-density lipoprotein (LDL) and apolipoprotein B/apolipoprotein-A1 ratio and elevated high density lipoprotein (HDL) to LDL ratio in patients with Gilbert syndrome[3].

EVIDENCE SUPPORTING THE PROTECTIVE EFFECT OF BILIRUBIN ON CAD

Several studies have suggested a cardioprotective role of bilirubin. Schwertner *et al*[26] was the first to report this protective effect of high level of bilirubin in CAD. They compared 619 subjects in training set (complete data on all risk factors considered was available) *vs* 258 subjects in test set (some risk factor data was not available). They observed a statistically significant inverse association between bilirubin and CAD. Fifty percent reduction in total bilirubin was associated with 47% increased odds of CAD, both univariate and multivariate after adjustment for other risk factors[26]. In 1996, Hopkins *et al*[27] evaluated 161 patients with early familial CAD and compared them to 155 control subjects. Patients with familial CAD had significantly lower bilirubin concentration as compared to controls (8.9 ± 6.1 micromol/L *vs* 12.4 ± 8.1 micromol/L, P = 0.0001). After adjustment for other risk factors, bilirubin

was found to be an independent protective factor with an odds ratio of 0.25 (P = 0.001) for an increase of 1 mg/dL. The benefits of elevated bilirubin were seen to be comparable to those of HDL. In a meta-analysis published in 2003 by Novotny *et al*[28] a significant inverse relationship was shown between serum bilirubin levels and the severity of atherosclerosis. Eleven relevant studies were used for analysis and the subjects involved were males. The relation between serum bilirubin levels and severity of atherosclerosis had a spearman rank coefficient of r = -0.31 (P < 0.0001).

Subsequently, in another study by Erdogan *et al*[29] in 2012, 179 patients undergoing angiography were analyzed to evaluate for CAD. Out of them, 110 patients had good collateral formation and 69 had poor collateral development. Higher serum bilirubin levels were associated with good collateral development as compared to poor collateral development (0.80 ± 0.27 mg/dL *vs* 0.53 ± 0.19 mg/dL, P < 0.001). These findings suggest a possible protective effect of elevated serum bilirubin levels against myocardial ischemia in patients with chronic total coronary occlusion with collaterals limiting infarct size and providing additional blood flow to the ischemic area[29]. Wei *et al*[30] also showed similar results with a significant inverse correlation between CAD and total bilirubin (n = 1260) in patients who underwent coronary angiography**.**

In 2013, Stojanov *et al*[31] reported cardioprotective effects of increased levels of bilirubin in 628 healthy subjects. The subjects were 442 men and 186 women aged 18 to 22 years. They divided the subjects into 2 groups based on levels of bilirubin. Subjects with level below the upper limit of reference were classified as "low bilirubin" (⩽ 0.95 mg/dL in women and ⩽ 1.4 mg/dL in men) and those with value above the upper limit of reference were classified as "high bilirubin". Men with high bilirubin concentration (> 1.4 mg/dL) had higher concentration of albumin and uric acid (P < 0.001) and lower level of oxidized LDL (P < 0.05). In females, high bilirubin (> 0.95 mg/dL) was associated with significantly higher albumin (P < 0.05) and lower thiobarbituric acid-reacting substances (TBARS) (P < 0.05). These findings support the evidence of an anti-oxidant effect of bilirubin secondary to inverse association with ox-LDL and anti-inflammatory effect secondary to direct correlation with albumin, which is a negative acute phase reactant in inflammatory response[31]. Shortly after that study, Canpolat *et al*[18] used computed tomographic angiography (CTA) to evaluate the relationship between bilirubin levels and nature of coronary plaques. The study included 1115 subjects who underwent CTA for evaluation of CAD. Patients were divided into 4 quartiles depending on the total bilirubin level. Patients with any coronary plaque were observed to have statistically significant lower levels of serum bilirubin (P = 0.002). Patients with critical stenosis (> 50% obstruction) had lower bilirubin levels compared to non-critical stenosis (0.57 ± 0.18 mg/dL *vs* 0.70 ± 0.24 mg/dL, P < 0.001). The authors concluded that lower serum levels of bilirubin were significantly associated with the presence, severity and the noncalcified morphology of atherosclerotic plaques.

Later, Song *et al*[32] designed a prospective cohort study with 8593 subjects followed over a period of 4 years. Low bilirubin levels (< 0.32 mg/dL) were observed to be an independent risk factor associated with an increased risk of CAD development (n = 80, 0.9% of total subjects) with adjusted hazard ratio (HR) of 1.890 (95%CI: 1.088-3.284, P = 0.024). Low bilirubin levels were shown to further increase the risk of CAD development six fold in patients with metabolic syndrome with HR of 2.016 (95%CI: 1.069-3.800, P = 0.030). The authors concluded by suggesting addition of bilirubin level to the risk assessment tool for assessing CAD in patients. Similar results showing an inverse association of bilirubin levels with coronary artery calcification were reported by Mahabadi *et al*[33]. However, they attributed the cardioprotective effects from bilirubin to a more favorable cardiovascular risk profile observed in their patients with CAD and elevated bilirubin levels in their study[33].

Akboga *et al*[17] conducted another study evaluating anti-inflammatory properties of bilirubin. In a retrospective cross-sectional study, they included 1501 patients who underwent coronary angiography. They divided them into 3 groups based on Gensini scores: No CAD (control group, n = 380), mild CAD (n = 497) and severe CAD (n = 624), with the objective of establishing anti-inflammatory effects of bilirubin in addition to its anti-oxidant effects. A significant inverse correlation between total bilirubin and C-reactive protein (r = -0.112, P < 0.001), neutrophil to lymphocyte ratio (r = -0.070, P = 0.026) and red cell distribution width (r = -0.074, P = 0.027) was observed. These findings helped establish anti-inflammatory properties of bilirubin in addition to their anti-oxidant effects. They also re-confirmed the inverse association of bilirubin with CAD severity [spearman's rank correlation coefficient (r) = -0.173, P < 0.001].

GENETIC POLYMORPHISMS OF *UGT1A1*28* AND THEIR RELATION WITH CAD

Polymorphisms in the *UGT1A1* gene (also known as *UGT1A1*28*) leads to unconjugated hyperbilirubinemia due to deficiency or decreased activity of *UGT1A1*[34]. Thus, patients with *UGT1A1*28* allele have shown to have higher levels of bilirubin[35]. Whereas, patients with wild type allele, *i.e.,* normal genotype have normal levels of bilirubin. To understand the true role of bilirubin it is prudent to look into the association between *UGT1A1*28* and CAD. Establishing an inverse association between the two would strengthen the hypothesis of bilirubin being protective in CAD.

In 2003, the Rotterdam study (case control study of 114 patients) hypothesized that since individuals

homozygous for *UGT1A1*28* have higher serum bilirubin, they would have a lower risk of CAD. They found that the relative risk of myocardial infarction (MI) for heterozygous genotype was 0.9 (95%CI: 0.7-1.3) and with homozygous *UGT1A1*28* was 1.3 (95%CI: 0.8-2.2). After adjusting for factors like age, gender, smoking, body mass index, diabetes mellitus, systolic blood pressure, total cholesterol, and HDL-cholesterol, the risk estimate was 1.0 (0.7-1.4) for heterozygotes and 1.2 (0.7-2.1) for homozygotes. Authors argued that the protective effect could have been missed because of the lack of power to detect such an effect. However, no association was seen between low serum bilirubin and the risk for CAD[36].

The Framingham offspring study (a prospective cohort study) in 2006 observed 1780 participants over 24 years and found that subjects homozygous for the gene *UGT1A1*28* had approximately one-third the risk for CAD compared to individuals homozygous for the wild type allele. However, the association between the incidence of myocardial infarction (MI) and gene polymorphism was not significant although the trend was similar. It was concluded that the carriers homozygous for *UGT1A1*28* allele with higher bilirubin concentrations exhibited a strong association with lower risk of cardiovascular disease[34].

Lingenhel *et al*[37] studied two polymorphisms in the promoter region of *UGT1A1* gene to analyze whether *UGTA1A* gene polymorphisms or bilirubin levels are independently associated with risk of CAD development. This case-control study enlisted 477 patients with premature, familial CAD and 619 controls that were matched for age and gender. Bilirubin levels were found to be significantly lower in the familial CAD group as compared to the controls ($P = 1.2 \times 10^{-10}$ in men and 1.9×10^{-9} in women). The low bilirubin levels were found to be significantly associated to CAD whereas *UGTA1A* polymorphisms were not, with odds ratio of 0.9 (CI: 0.86-0.94, $P = 2.6 \times 10^{-6}$) for men and 0.77 (CI: 0.68-0.87, $P = 3.2 \times 10^{-5}$) for women respectively for each 0.1mg/dl increase in bilirubin levels[37]. Hence, indicating that increased bilirubin levels and not genetic polymorphisms are associated with reduced risk of CAD.

Hsieh *et al*[38] sought to explore the association of UGTA1A polymorphisms with risk of CAD development. A case-control design was set up ($n = 135$; cases = 61, controls = 74) and although bilirubin levels in the control group were found to be significantly higher than CAD group, no significant differences were observed in the polymorphism of UGTA1A between the two groups.

Rantner *et al*[39] in their prospective case control study, investigated plasma bilirubin concentration and UGT1A1 promoter TA repeat polymorphism in a cohort of patients with peripheral arterial disease and age and diabetes matched control group. They observed significantly lower bilirubin concentrations in patients than in controls. UGT1A1 polymorphism was strongly associated with bilirubin concentration in both patients and controls. However, UGT1A1 polymorphism was not associated with peripheral arterial disease.

EVIDENCE NOT SUPPORTING THE PROTECTIVE EFFECT OF BILIRUBIN ON CAD

Contrary to the evidence presented above, several studies have negated the protective effect of bilirubin on CAD. British Regional Health Study (BRHS) was a prospective study designed to examine the relationship between the level of bilirubin and risk of ischemic CAD. Subjects ($n = 7685$) were followed up for a mean of 11.5 years, out of which 737 individuals were seen to develop major ischemic CAD. A U-shaped relationship was observed between serum bilirubin and risk of ischemic CAD with increased risk at bilirubin concentrations < 0.4 mg/dL and at > 0.7 mg/dL (RR = 0.99, CI: 0.73-1.34)[40]. A similar U-shaped relationship between serum bilirubin level and risk of developing CAD was observed in the Prospective Epidemiological Study of Myocardial Infarction (PRIME) study. In the PRIME study, 216 individuals who had developed CAD at 5-year follow up were designated as cases, and 434 individuals as matched controls. Individuals with bilirubin levels < 0.33 mg/dL and > 0.8 mg/dL were seen to have higher incidence of development of CAD, even after adjustment of other risk factors. However, the association between bilirubin levels > 0.8 mg/dL and the incidence of CAD was not statistically significant (OR = 0.68, CI: 0.34-1.39, $P = 0.29$). This U-shaped association was suggested as being due to reduced endogenous antioxidants with insufficient dietary intake[41]. The clinical significance of this U-shaped relation remains unclear.

Acet *et al*[42] investigated patients ($n = 360$) undergoing percutaneous coronary intervention (PCI) within 12 h of symptom onset with the aim to establish a relation between bilirubin levels and infarct-related artery patency in the setting of ST-segment elevation myocardial infarction (STEMI). The group with elevated total bilirubin was seen to have higher impaired flow (defined as pre-PCI TIMI $\leqslant$ 2 flow) than normal flow (Pre-PCI TIMI > 3) ($P < 0.001$). Furthermore, the in-hospital mortality and major adverse cardiac events were significantly higher in the high total bilirubin group ($P = 0.002$, $P < 0.001$ respectively). However, an important point to note is that the study did not exclude patients with elevated markers of hepatocellular injury thus making the true relationship between isolated elevated bilirubin levels and CAD difficult to interpret.

Ayaz *et al*[43] reported an independent positive association between bilirubin and left ventricular mass/hypertrophy in a population with untreated hypertension ($n = 114$). After performing a linear and logistical regression, total bilirubin ($P = 0.011$) was shown to be an independent risk factor for CAD. The authors attribute this to the suppression of reactive oxygen species[44]. A similar effect has been seen in pre-clinical studies

in rats, which showed a protective effect of elevated bilirubin on left ventricular hypertrophy in spontaneously hypertensive rats. They hypothesized the role of liver growth factor inhibition by bilirubin. However, this relationship still needs to be better elucidated.

In another study, 221 patients who were evaluated for CAD by coronary angiography showed a moderate but significant positive correlation between direct bilirubin levels and the Gensini score (r = 0.158, P = 0.019). However, no such significant correlation was demonstrated between total bilirubin and the Gensini score. This study was limited by its small sample size (n = 221)[5]. Authors interpreted that the relationship between bilirubin and CAD was unlikely causal. However, the cardiovascular risk factors in CAD have shown to be additive and in presence of several risk factors the beneficial effects of bilirubin might get masked. Thus, despite the results of this study, elevated serum bilirubin levels might confer protective effects in patients with favorable risk profile for CAD[5].

EXPERT COMMENTARY

While the available evidence regarding the effects of bilirubin are not definitive and different studies provide contradictory findings, some useful conclusions can be drawn.

First, it is possible that the protective effects seen with higher bilirubin levels are possibly mediated through heme oxygenase or by other substrates involved in the pathway of bilirubin production, namely, biliverdin and carbon monoxide. Although few studies have reported an inverse association between bilirubin and the risk of CAD, no such association was seen with *UGT1A1* gene polymorphism and the risk of CAD. Thus, a conclusion can be safely inferred that if at all bilirubin is protective in CAD, it is likely that bilirubin production (by induction of heme oxygenase and accompanied by production of carbon monoxide) and not just its excretion indirectly confers the protective effect observed with CAD. This would reflect as bilirubin having a protective effect on CAD whereas, in reality it is only a mediator or a marker.

Second, low bilirubin levels can be indicative of decreased heme oxygenase activity (a powerful anti-oxidant) or could be indicative of high oxidative stress in patients leading to consumption of the natural anti-oxidants including bilirubin. Hence, lower levels of bilirubin are perhaps not the causal factor for CAD but may indicate patients at an increased risk of developing CAD[45].

Third, bilirubin requires vitamin E as the co-oxidant, hence patients with a high bilirubin and deficiency of vitamin E, have less atheroprotective effect that weakens the inverse association between elevated bilirubin levels and the risk of CAD[46].

Also, Grosser *et al*[47-49] have reported induction of heme oxygenase with statin and aspirin therapy. Induction of heme oxygenase increases the bilirubin production. Individuals with *UGT1A1* gene variants have a lower capability of exclusion of bilirubin; hence, bilirubin accumulation is to be expected in individuals on statins and aspirin. Hence as per this hypothesis, patients with CAD or at an increased risk of CAD with *UGT1A1* gene polymorphism, on aspirin and statin should have increased levels of bilirubin. In that case, bilirubin might be looked upon as a marker of the drug activity.

Mendelian randomization is done to establish a causal relationship[50]. As mentioned above, a lack of significant association between the gene polymorphisms of UGT1A1 and risk for CAD goes in favor of bilirubin being a marker than a primary mediator for the cardioprotective effects observed with CAD. Moreover, it also points out towards incomplete penetrance of the *UGT1A1* gene. Inconsistent results further support the need for further exploration of the underlying mechanisms and a prospective study with a high power to establish a definite causal relationship between bilirubin levels and CAD.

REFERENCES

1 **Tenhunen R**, Marver HS, Schmid R. The enzymatic conversion of heme to bilirubin by microsomal heme oxygenase. *Proc Natl Acad Sci USA* 1968; **61**: 748-755 [PMID: 4386763 DOI: 10.1073/pnas.61.2.748]

2 **Pae HO**, Son Y, Kim NH, Jeong HJ, Chang KC, Chung HT. Role of heme oxygenase in preserving vascular bioactive NO. *Nitric Oxide* 2010; **23**: 251-257 [PMID: 20713168 DOI: 10.1016/j.niox.2010.08.002]

3 **Kundur AR**, Singh I, Bulmer AC. Bilirubin, platelet activation and heart disease: a missing link to cardiovascular protection in Gilbert's syndrome? *Atherosclerosis* 2015; **239**: 73-84 [PMID: 25576848 DOI: 10.1016/j.atherosclerosis.2014.12.042]

4 **Wagner KH**, Wallner M, Mölzer C, Gazzin S, Bulmer AC, Tiribelli C, Vitek L. Looking to the horizon: the role of bilirubin in the development and prevention of age-related chronic diseases. *Clin Sci* (Lond) 2015; **129**: 1-25 [PMID: 25881719 DOI: 10.1042/CS20140566]

5 **Vítek L**, Jirsa M, Brodanová M, Kalab M, Marecek Z, Danzig V, Novotný L, Kotal P. Gilbert syndrome and ischemic heart disease: a protective effect of elevated bilirubin levels. *Atherosclerosis* 2002; **160**: 449-456 [PMID: 11849670 DOI: 10.1016/S0021-9150(01)00601-3]

6 **VanWagner LB**, Green RM. Evaluating elevated bilirubin levels in asymptomatic adults. *JAMA* 2015; **313**: 516-517 [PMID: 25647209 DOI: 10.1001/jama.2014.12835]

7 **Erlinger S**, Arias IM, Dhumeaux D. Inherited disorders of bilirubin transport and conjugation: new insights into molecular mechanisms and consequences. *Gastroenterology* 2014; **146**: 1625-1638 [PMID: 24704527 DOI: 10.1053/j.gastro.2014.03.047]

8 **Strassburg CP**. Hyperbilirubinemia syndromes (Gilbert-Meulengracht, Crigler-Najjar, Dubin-Johnson, and Rotor syndrome). *Best Pract Res Clin Gastroenterol* 2010; **24**: 555-571 [PMID: 20955959 DOI: 10.1016/j.bpg.2010.07.007]

9 **Liao SL**. The role of bilirubin and phototherapy in the oxidative/antioxidant balance. *Pediatr Neonatol* 2015; **56**: 77-78 [PMID: 25735960 DOI: 10.1016/j.pedneo.2015.01.001]

10 **Wu TW**, Fung KP, Wu J, Yang CC, Weisel RD. Antioxidation of human low density lipoprotein by unconjugated and conjugated bilirubins. *Biochem Pharmacol* 1996; **51**: 859-862 [PMID: 8602883 DOI: 10.1016/0006-2952(95)02395-X]

11 **Baranano DE**, Rao M, Ferris CD, Snyder SH. Biliverdin reductase: a major physiologic cytoprotectant. *Proc Natl Acad Sci USA* 2002; **99**: 16093-16098 [PMID: 12456881 DOI: 10.1073/pnas.252626999]

12 **Tanındı A**, Erkan AF, Alhan A, Töre HF. Arterial stiffness and

central arterial wave reflection are associated with serum uric acid, total bilirubin, and neutrophil-to-lymphocyte ratio in patients with coronary artery disease. *Anatol J Cardiol* 2015; **15**: 396-403 [PMID: 25430407 DOI: 10.5152/akd.2014.5447]

13 **Liu J**, Wang L, Tian XY, Liu L, Wong WT, Zhang Y, Han QB, Ho HM, Wang N, Wong SL, Chen ZY, Yu J, Ng CF, Yao X, Huang Y. Unconjugated bilirubin mediates heme oxygenase-1-induced vascular benefits in diabetic mice. *Diabetes* 2015; **64**: 1564-1575 [PMID: 25475440 DOI: 10.2337/db14-1391]

14 **Cannon RO**. Role of nitric oxide in cardiovascular disease: focus on the endothelium. *Clin Chem* 1998; **44**: 1809-1819 [PMID: 9702990]

15 **Kang SJ**, Lee C, Kruzliak P. Effects of serum bilirubin on atherosclerotic processes. *Ann Med* 2014; **46**: 138-147 [PMID: 24720602 DOI: 10.3109/07853890.2014.895588]

16 **Vachharajani TJ**, Work J, Issekutz AC, Granger DN. Heme oxygenase modulates selectin expression in different regional vascular beds. *Am J Physiol Heart Circ Physiol* 2000; **278**: H1613-H1617 [PMID: 10775141]

17 **Akboga MK**, Canpolat U, Sahinarslan A, Alsancak Y, Nurkoc S, Aras D, Aydogdu S, Abaci A. Association of serum total bilirubin level with severity of coronary atherosclerosis is linked to systemic inflammation. *Atherosclerosis* 2015; **240**: 110-114 [PMID: 25770689 DOI: 10.1016/j.atherosclerosis.2015.02.051]

18 **Canpolat U**, Aytemir K, Yorgun H, Hazırolan T, Kaya EB, Şahiner L, Sunman H, Tokgözoğlu L, Kabakcı G, Oto A. Association of serum total bilirubin levels with the severity, extent and subtypes of coronary atherosclerotic plaques detected by coronary CT angiography. *Int J Cardiovasc Imaging* 2013; **29**: 1371-1379 [PMID: 23546648 DOI: 10.1007/s10554-013-0209-7]

19 **Bisht K**, Wegiel B, Tampe J, Neubauer O, Wagner KH, Otterbein LE, Bulmer AC. Biliverdin modulates the expression of C5aR in response to endotoxin in part via mTOR signaling. *Biochem Biophys Res Commun* 2014; **449**: 94-99 [PMID: 24814708 DOI: 10.1016/j.bbrc.2014.04.150]

20 **Kelsen S**, Patel BJ, Parker LB, Vera T, Rimoldi JM, Gadepalli RS, Drummond HA, Stec DE. Heme oxygenase attenuates angiotensin II-mediated superoxide production in cultured mouse thick ascending loop of Henle cells. *Am J Physiol Renal Physiol* 2008; **295**: F1158-F1165 [PMID: 18701634 DOI: 10.1152/ajprenal.00057.2008]

21 **Hu CM**, Chen YH, Chiang MT, Chau LY. Heme oxygenase-1 inhibits angiotensin II-induced cardiac hypertrophy in vitro and in vivo. *Circulation* 2004; **110**: 309-316 [PMID: 15226216 DOI: 10.1161/01.CIR.0000135475.35758.23]

22 **Li Volti G**. Letter regarding article by Hu et al, "heme oxygenase-1 inhibits angiotensin II-induced cardiac hypertrophy in vitro and in vivo". *Circulation* 2005; **111**: 100 [PMID: 15710770 DOI: 10.1161/01.CIR.0000155250.27345.02]

23 **Otterbein LE**, Soares MP, Yamashita K, Bach FH. Heme oxygenase-1: unleashing the protective properties of heme. *Trends Immunol* 2003; **24**: 449-455 [PMID: 12909459 DOI: 10.1016/S1471-4906(03)00181-9]

24 **Ockner RK**. Laboratory tests in liver disease. In: Wyngaarden JB, Smith LI (eds.). Cecil textbook of medicine. 16th ed. Philadelphia: WB Saunders, 1982: 775-778

25 **Routh JI.** Liver function. 1n: Tietz NW (eds.). Fundamentals of clinical chemistry. Philadelphia: WB Saunders, 1976: 1026-1062

26 **Schwertner HA**, Jackson WG, Tolan G. Association of low serum concentration of bilirubin with increased risk of coronary artery disease. *Clin Chem* 1994; **40**: 18-23 [PMID: 8287538]

27 **Hopkins PN**, Wu LL, Hunt SC, James BC, Vincent GM, Williams RR. Higher serum bilirubin is associated with decreased risk for early familial coronary artery disease. *Arterioscler Thromb Vasc Biol* 1996; **16**: 250-255 [PMID: 8620339 DOI: 10.1161/01.ATV.16.2.250]

28 **Novotný L**, Vítek L. Inverse relationship between serum bilirubin and atherosclerosis in men: a meta-analysis of published studies. *Exp Biol Med* (Maywood) 2003; **228**: 568-571 [PMID: 12709588]

29 **Erdogan T**, Ciçek Y, Kocaman SA, Canga A, Cetin M, Durakoglugil E, Satiroglu O, Temiz A, Ergül E, Sahin I, Bostan M. Increased serum bilirubin level is related to good collateral development in patients with chronic total coronary occlusion. *Intern Med* 2012; **51**: 249-255 [PMID: 22293798 DOI: 10.2169/internalmedicine.51.6417]

30 **Wei S**, Gao C, Wei G, Chen Y, Zhong L, Li X. The level of serum bilirubin associated with coronary lesion types in patients with coronary artery disease. *J Cardiovasc Med* (Hagerstown) 2012; **13**: 432-438 [PMID: 21799439 DOI: 10.2459/JCM.0b013e32834a3967]

31 **Stojanov M**, Stefanovic A, Dzingalasevic G, Ivanisevic J, Miljkovic M, Mandic-Radic S, Prostran M. Total bilirubin in young men and women: association with risk markers for cardiovascular diseases. *Clin Biochem* 2013; **46**: 1516-1519 [PMID: 23827733 DOI: 10.1016/j.clinbiochem.2013.06.020]

32 **Song YS**, Koo BK, Cho NH, Moon MK. Effect of low serum total bilirubin levels (≤0.32 mg/dl) on risk of coronary artery disease in patients with metabolic syndrome. *Am J Cardiol* 2014; **114**: 1695-1700 [PMID: 25288499 DOI: 10.1016/j.amjcard.2014.08.043]

33 **Mahabadi AA**, Lehmann N, Möhlenkamp S, Kälsch H, Bauer M, Schulz R, Moebus S, Jöckel KH, Erbel R, Heusch G. Association of bilirubin with coronary artery calcification and cardiovascular events in the general population without known liver disease: the Heinz Nixdorf Recall study. *Clin Res Cardiol* 2014; **103**: 647-653 [PMID: 24633678 DOI: 10.1007/s00392-014-0697-z]

34 **Lin JP**, O'Donnell CJ, Schwaiger JP, Cupples LA, Lingenhel A, Hunt SC, Yang S, Kronenberg F. Association between the UGT1A1*28 allele, bilirubin levels, and coronary heart disease in the Framingham Heart Study. *Circulation* 2006; **114**: 1476-1481 [PMID: 17000907 DOI: 10.1161/CIRCULATIONAHA.106.633206]

35 **Hunt SC,** Kronenberg F, Eckfeldt JH, Hopkins PN, Myers RH, Heiss G. Association of plasma bilirubin with coronary heart disease and segregation of bilirubin as a major gene trait: the NHLBI family heart study. *Atherosclerosis* 2001; **154**: 747-754 [PMID: 11257278 DOI: 10.1016/S0021-9150(00)00420-2]

36 **Bosma PJ**, van der Meer IM, Bakker CT, Hofman A, Paul-Abrahamse M, Witteman JC. UGT1A1*28 allele and coronary heart disease: the Rotterdam Study. *Clin Chem* 2003; **49**: 1180-1181 [PMID: 12816916 DOI: 10.1373/49.7.1180]

37 **Lingenhel A**, Kollerits B, Schwaiger JP, Hunt SC, Gress R, Hopkins PN, Schoenborn V, Heid IM, Kronenberg F. Serum bilirubin levels, UGT1A1 polymorphisms and risk for coronary artery disease. *Exp Gerontol* 2008; **43**: 1102-1107 [PMID: 18790042 DOI: 10.1016/j.exger.2008.08.047]

38 **Hsieh CJ**, Chen MJ, Liao YL, Liao TN. Polymorphisms of the uridine-diphosphoglucuronosyltransferase 1A1 gene and coronary artery disease. *Cell Mol Biol Lett* 2008; **13**: 1-10 [PMID: 17952380 DOI: 10.2478/s11658-007-0030-1]

39 **Rantner B**, Kollerits B, Anderwald-Stadler M, Klein-Weigel P, Gruber I, Gehringer A, Haak M, Schnapka-Köpf M, Fraedrich G, Kronenberg F. Association between the UGT1A1 TA-repeat polymorphism and bilirubin concentration in patients with intermittent claudication: results from the CAVASIC study. *Clin Chem* 2008; **54**: 851-857 [PMID: 18375480 DOI: 10.1373/clinchem.2007.102046]

40 **Breimer LH**, Wannamethee G, Ebrahim S, Shaper AG. Serum bilirubin and risk of ischemic heart disease in middle-aged British men. *Clin Chem* 1995; **41**: 1504-1508 [PMID: 7586525]

41 **Troughton JA**, Woodside JV, Young IS, Arveiler D, Amouyel P, Ferrières J, Ducimetière P, Patterson CC, Kee F, Yarnell JW, Evans A. Bilirubin and coronary heart disease risk in the Prospective Epidemiological Study of Myocardial Infarction (PRIME). *Eur J Cardiovasc Prev Rehabil* 2007; **14**: 79-84 [PMID: 17301631 DOI: 10.1097/01.hjr.0000230097.81202.9f]

42 **Acet H**, Ertş F, Akıl MA, Polat N, Aydın M, Akyüz A, Ayçiçek H, Alan S. A novel predictor of infarct-related artery patency before percutaneous intervention and in-hospital outcomes for ST-segment elevation myocardial infarction patients: serum bilirubin level. *Postepy Kardiol Interwencyjnej* 2014; **10**: 91-97 [PMID: 25061454 DOI: 10.5114/pwki.2014.43513]

43 **Ayaz T**, Durakoğlugil ME, Kocaman SA, Durakoğlugil T, Erdoğan T, Sahin OZ, Sahin SB, Çiçek Y, Satiroğlu O. Bilirubin Level is Associated with Left Ventricular Hypertrophy Independent of Blood Pressure in Previously Untreated Hypertensive Patients. *Korean Circ J* 2014; **44**: 336-343 [PMID: 25278987 DOI: 10.4070/

kcj.2014.44.5.336]
44 **Ndisang JF**, Jadhav A. Upregulating the heme oxygenase system suppresses left ventricular hypertrophy in adult spontaneously hypertensive rats for 3 months. *J Card Fail* 2009; **15**: 616-628 [PMID: 19700139 DOI: 10.1016/j.cardfail.2009.02.003]
45 **Lin R**, Wang Y, Wang Y, Fu W, Zhang D, Zheng H, Yu T, Wang Y, Shen M, Lei R, Wu H, Sun A, Zhang R, Wang X, Xiong M, Huang W, Jin L. Common variants of four bilirubin metabolism genes and their association with serum bilirubin and coronary artery disease in Chinese Han population. *Pharmacogenet Genomics* 2009; **19**: 310-318 [PMID: 19238116 DOI: 10.1097/FPC.0b013e328328f818]
46 **Neuzil J**, Stocker R. Free and albumin-bound bilirubin are efficient co-antioxidants for alpha-tocopherol, inhibiting plasma and low density lipoprotein lipid peroxidation. *J Biol Chem* 1994; **269**: 16712-16719 [PMID: 8206992]
47 **Grosser N**, Abate A, Oberle S, Vreman HJ, Dennery PA, Becker JC, Pohle T, Seidman DS, Schröder H. Heme oxygenase-1 induction may explain the antioxidant profile of aspirin. *Biochem Biophys Res Commun* 2003; **308**: 956-960 [PMID: 12927812 DOI: 10.1016/S0006-291X(03)01504-3]
48 **Grosser N**, Hemmerle A, Berndt G, Erdmann K, Hinkelmann U, Schürger S, Wijayanti N, Immenschuh S, Schröder H. The antioxidant defense protein heme oxygenase 1 is a novel target for statins in endothelial cells. *Free Radic Biol Med* 2004; **37**: 2064-2071 [PMID: 15544924 DOI: 10.1016/j.freeradbiomed.2004.09.009]
49 **Grosser N**, Erdmann K, Hemmerle A, Berndt G, Hinkelmann U, Smith G, Schröder H. Rosuvastatin upregulates the antioxidant defense protein heme oxygenase-1. *Biochem Biophys Res Commun* 2004; **325**: 871-876 [PMID: 15541371 DOI: 10.1016/j.bbrc.2004.10.123]
50 **Relton CL**, Davey Smith G. Two-step epigenetic Mendelian randomization: a strategy for establishing the causal role of epigenetic processes in pathways to disease. *Int J Epidemiol* 2012; **41**: 161-176 [PMID: 22422451 DOI: 10.1093/ije/dyr233]

P- Reviewer: Canpolat U, Jorgensen ME, Zhou T **S- Editor**: Qi Y
L- Editor: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i4.477

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 477-489
ISSN 2150-5349 (online)

REVIEW

Local ablative treatments for hepatocellular carcinoma: An updated review

Antonio Facciorusso, Gaetano Serviddio, Nicola Muscatiello

Antonio Facciorusso, Nicola Muscatiello, Gastroenterology Unit, Department of Medical and Surgical Sciences, University of Foggia, 71100 Foggia, Italy

Gaetano Serviddio, Internal Medicine Unit, University of Foggia, 71100 Foggia, Italy

Author contributions: Muscatiello N performed the bibliographic research; Facciorusso A wrote the paper; Serviddio G revised the manuscript; all the authors contributed to the article.

Conflict-of-interest statement: None of the authors have received fees for serving as a speaker or are consultant/advisory board member for any organizations. None of the authors have received research funding from any organizations. None of the authors are employees of any organizations. None of the authors own stocks and/or share in any organizations. None of the authors own patents. None of the authors has conflicts of interest to declare.

Manuscript source: Invited manuscript

Correspondence to: Antonio Facciorusso, MD, Gastroenterology Unit, Department of Medical and Surgical Sciences, University of Foggia, Viale L. Pinto, 1, 71100 Foggia, Italy. antonio.facciorusso@virgilio.it
Telephone: +39-0881-732154
Fax: +39-0881-732135

Received: May 22, 2016
Peer-review started: May 23, 2016
First decision: July 4, 2016
Revised: July 16, 2016
Accepted: August 17, 2016
Article in press: August 19, 2016

Published online: November 6, 2016

Abstract

Ablative treatments currently represent the first-line option for the treatment of early stage unresectable hepatocellular carcinoma (HCC). Furthermore, they are effective as bridging/downstaging therapies before orthotopic liver transplantation. Contraindications based on size, number, and location of nodules are quite variable in literature and strictly dependent on local expertise. Among ablative therapies, radiofrequency ablation (RFA) has gained a pivotal role due to its efficacy, with a reported 5-year survival rate of 40%-70%, and safety. Although survival outcomes are similar to percutaneous ethanol injection, the lower local recurrence rate stands for a wider application of RFA in hepato-oncology. Moreover, RFA seems to be even more cost-effective than liver resection for very early HCC (single nodule $\leqslant$ 2 cm) and in the presence of two or three nodules $\leqslant$ 3 cm. There is increasing evidence that combining RFA to transarterial chemoembolization may increase the therapeutic benefit in larger HCCs without increasing the major complication rate, but more robust prospective data is still needed to validate these pivotal findings. Among other thermal treatments, microwave ablation (MWA) uses high frequency electromagnetic energy to induce tissue death *via* coagulation necrosis. In comparison to RFA, MWA has several theoretical advantages such as a broader zone of active heating, higher temperatures within the targeted area in a shorter treatment time and the lack of heat-sink effect. The safety concerns raised on the risks of this procedure, due to the broader and less predictable necrosis areas, have been recently overcome. However, whether MWA ability to generate a larger ablation zone will translate into a survival gain remains unknown. Other treatments, such as high-intensity focused ultrasound ablation, laser ablation, and cryoablation, are less investigated but showed promising results in early HCC patients and could be a valuable therapeutic option in the

next future.

Key words: Liver cancer; Hepatocellular carcinoma; Radiofrequency ablation; Microwave ablation; Radiofrequency ablation

Core tip: Ablative treatments currently represent the first-line option for the treatment of early stage unresectable hepatocellular carcinoma. Among ablative therapies, radiofrequency ablation has gained a pivotal role due to its efficacy, with a reported 5-year survival rate of 40%-70%, and safety. Among other thermal treatments, microwave ablation, high-intensity focused ultrasound ablation, laser ablation, and cryoablation, are less investigated but showed promising results.

Facciorusso A, Serviddio G, Muscatiello N. Local ablative treatments for hepatocellular carcinoma: An updated review. *World J Gastrointest Pharmacol Ther* 2016; 7(4): 477-489 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i4/477.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i4.477

INTRODUCTION

Hepatocellular carcinoma (HCC) represents a life-threatening condition and constitutes the main cause of death among cirrhotic patients[1,2]. In the last years, the accurate screening programs and more refined diagnostic imaging have made early HCC diagnosis feasible in 30%-60% of cases[3].

Local ablation represents the standard of care for patients at early stage, who are not suitable to surgery or orthotopic liver transplantation (OLT). Among ablative treatments, thermal ablative therapies have gained an increasing role in the last decade due to their efficacy in preventing local recurrence as well as in prolonging overall survival (OS). Thermal ablative treatments are classified as hyperthermic, such as radiofrequency ablation (RFA), microwave ablation (MWA), high-intensity focused ultrasound (HIFU) or laser therapy, or hypothermic such as cryoablation.

These procedures are usually performed by means of a percutaneous approach but in particular conditions (for instance in cases of nodules in "at-risk" location) laparoscopic ablation may be recommended.

In this review we aim to provide a comprehensive overview on the main thermal therapies for HCC with the up-to-date data on their efficacy and safety.

INDICATION TO TREATMENT

Thermal ablative treatments represent the standard of care for unresectable HCC in very early/early stage according to Barcelona Clinic Liver Cancer (BCLC) system[2,4]. The term "unresectable" covers a broad spectrum of pathological conditions, from single nodule in a deep location (therefore not easy to treat by surgery) to multinodular disease in patients with deteriorated liver function. Therefore, percutaneous therapies are a valuable option in non-optimal candidates to surgery due to tumor size, number, location, liver function, or comorbidities.

Another indication to thermal treatment is the pre-transplant setting, where RFA has been proved to be effective both as downstaging and as bridging therapy[5-7].

Main absolute and relative contraindications to thermal treatments are described in Table 1. Absolute contraindications, shared with other locoregional treatments, are the presence of extrahepatic liver disease, altered mental status, active infection, tumor abutting a major hepatic duct, impaired liver function (particularly in presence of ascites); relative contraindications are more than 4 nodules or at least one lesion > 5 cm, severe cardiopulmonary disease and refractory coagulopathy[8].

MECHANISM OF ACTION AND EQUIPMENT OF RFA

The mechanism of action of RFA relies on the destruction of tumoral tissue by the radiofrequency-generated heat. In particular, the injury is due to frictional heat produced by the ionic agitation of particles within tissue as a consequence of the application of alternating current[9-13].

The electrical current in the radiofrequency range (200-1200 MHz) is transmitted by a needle electrode under imaging guidance (usually ultrasonography) and the electrical circuit is completed through grounding pads attached to the thighs or back of the patient. The needle is partially insulated and presents an activated tip that is not insulated. This tip varies in length with the most common size being 3 cm long. Tips may be singular and straight or consisting of an array of expandable tines that form an umbrella fully encompassing the nodule when deployed.

An important aim of the treatment should be to ensure thermal destruction not only of the tumoral nodule but also of a surrounding margin about 1 cm long in order to ablate eventual microsatellites thus preventing local recurrence.

In order to reach this target, multiple electrodes can be applied thus achieving a broader ablation zone and allowing ablation of nodules up to 4-5 cm.

Another aspect to be considered is the "heat-sink effect", namely the dissipation of the thermal output by blood flowing through adjacent vessels thereby decreasing the efficacy of the procedure[14]. This is the reason why nodules close to major vessel are considered a suboptimal target and constitute a relative contraindication for RFA.

Table 1 Contraindications to thermal ablative treatments

Absolute contraindications
Extrahepatic disease
Altered mental status
Active infection
Tumor abutting a major hepatic duct
Liver decompensation (particularly in presence of ascites)
Relative contraindications
Lesions > 5 cm
More than four lesions
Severe pulmonary or cardiac disease
Refractory coagulopathy

The procedure is usually performed under sedation when the percutaneous approach is preferred. In cases of laparoscopic RFA, to be considered in cases of nodules close to the liver capsule or other organs, general anesthesia is needed[15].

SURVIVAL OUTCOMES AFTER RFA FOR HCC

A large number of studies have confirmed the efficacy of RFA in early HCC patients suggesting this procedure as viable therapeutic option in unresectable early stage. Considering the state-of-art of the literature, RFA provided 5-year survival rates of 40%-70% and beyond in HCC series[9,10].

A recent Chinese study reported OS rates of 96.6%, 60.2%, and 27.3% at 1-, 5-, and 10-year[16], similar to those reported by Kim *et al*[17] which were 95.5%, 59.7% and 32.3%, respectively. These results are concordant with other recent Western studies conducted in Milan-in patients (87.0%-99.0% at 1 year, 60.0%-87.4% at 3 years, and 42.3%-74.8% at 5 years)[18,19].

Several studies pointed out different predictors of survival, such as Child-Pugh (CP) score, initial response, serum ferritin, number or size of nodules and AFP levels[19-21].

Our group has recently analyzed predictors of post-recurrence survival (PRS) after RFA, namely the survival time elapsed after tumor recurrence[18]. We found, in line with other studies, baseline CP score, AFP levels and performance status (PS) as predictors of OS in multivariate analysis. However, analysis of PRS showed that in addition to CP score and PS, also tumor burden at the time of recurrence and recurrence pattern significantly influenced PRS[18]. Interestingly, AFP level, one of the main predictors of survival at baseline, became non-significant when evaluated at tumor relapse, confirming the difference between predictors of OS assessed at baseline and at tumor relapse[18].

Of note, local recurrence (LR) did not impact significantly on OS in our study[18] as well as in other reports[17,21,22], probably due to the frequent multi-focality of distant recurrences that makes more difficult the therapeutic approach, while local recurrences, even when multifocal, are confined in one liver segment (namely the same as that previously treated) and may be more easily treated with RFA or a single selective transarterial chemoembolization (TACE) session.

Unlike OS, reported rates of LR after RFA are not univocal ranging from 3.2% to 27% at 5 years[16-21], maybe because of different etiologies of HCC in the published series, different approaches to the problem of insufficient ablative margins, use of combined treatment with TACE and, above all, different definition of radiologic tumor recurrence at imaging. As expected, tumor features such as nodules number, size, histopathological grading, and AFP have been found to be predictors of recurrence[16-21]. Moreover, an insufficient ablation margin after the treatment appear to be an important prognostic factor for LR[23,24].

Intrahepatic distant recurrences are common, ranging from 68% to 74% at 5 years[16-19,21], and are usually associated to poorer prognosis. This type of recurrence is mostly induced by underlying hepatic disease and is often observed after 2 years, which is the time point considered able to differentiate between real recurrences from de novo tumors occurred in the pro-tumorigenic milieu of liver cirrhosis[25].

Therefore, because of their high frequency and aggressive behavior, distal recurrences are a major determinant of patient survival.

PREVENTION OF RECURRENCE AFTER RFA

The issue of the high rates of post-RFA tumor relapse has recently pushed great efforts in studying adjuvant drugs aimed at decreasing the heavy burden of HCC recurrence after ablation.

Although earlier reports showed interesting results[26,27] and in spite of the theoretical beneficial role of sorafenib (Nexavar®, Bayer, Leverkusen, Germany) as adjuvant therapy, an important multicenter randomised controlled trial (RCT) [Sorafenib as Adjuvant Treatment in the Prevention Of Recurrence of Hepatocellular Carcinoma (STORM)], recruiting 1114 HCC patients after surgery or radiofrequency ablation, failed to meet its primary endpoint, namely recurrence-free survival [hazard ratio (HR): 0.940, 95%CI: 0.78-1.13, P = 0.26] and OS (HR: 0.99, 95%CI: 0.76-1.30, P = 0.48)[28]. This daunting finding was at least in part due to the high treatment discontinuation rate (24% *vs* 7% of placebo) and consent withdrawal (17% *vs* 6%) in the sorafenib arm, mainly because of severe adverse events[28].

Similarly, interferon was proven unhelpful as adjuvant treatment because of the high cost and the narrow therapeutic window[29,30].

Therefore, most of the recent research in this field has focused on other drugs. On the basis of the well-described pro-tumorigenic and pro-fibrogenic properties of angiotensin Ⅱ, due to the induction of vascular endothelial growth factor and transforming growth

factor-beta 1 release[31,32], a number of studies have reported significantly reduced HCC relapse rates after RFA when angiotensin converting enzyme inhibitor (ACE Ⅰ) were used in combination with other agents such as branched-chain amino acids or vitamin K[33-35]. However, ACE I did not prove effective in monotherapy and, above all, no significant difference in OS was registered as compared to the control arm[33-35].

Our group has recently published a retrospective report conducted in 153 HCC patients treated with RFA finding a significant benefit both in terms of recurrence and OS in hypertensive subjects in treatment with angiotensin Ⅱ type 1 receptor blockers (sartans) as compared to those under ACE Ⅰ therapy and to non-hypertensive subjects[36]. The apparent superiority of sartans over ACE Ⅰ may be due to the selective inhibition of angiotensin Ⅱ receptor 1, responsible of the pro-fibrogenic and pro-angiogenic activity of angiotensin, while pro-apoptotic and anti-tumorigenic activity of receptor 2 is preserved and even enhanced in patients administered sartans unlike ACE Ⅰ which prevent the binding of angiotensin Ⅱ to both receptors[37]. However, these preliminary results still need further confirmation.

In conclusion, in spite of the great amount of published reports and in absence of broad RCTs, clear evidence in favor of an adjuvant treatment after RFA is still lacking.

ADVERSE EVENTS OF RFA

In a recent systematic review of 9531 patients treated with RFA, treatment-related severe adverse events were registered in 4.1% of cases with a mortality rate of 0.15%[38].

Adverse events include gastrointestinal tract injury with/without perforation (0.06%-0.3%), diaphragm injury (0.03%), pleural effusion (0.2%-2.3%), bile duct stricture (0.06%-0.5%), biloma (0.06%-0.96%), gallbladder injury (0.06%-0.1%), and hepatic infarction (0.03%-0.06%). Other complications, related to direct mechanical injury, are tumor seeding (0.27%), tumor rupture (0.3%), hemoperitoneum (0.3%-1.6%), and hemo/pneumothorax (0.15%-0.8%). Events not related to mechanical or thermal injury to the liver are hepatic abscess (0.1%), grounding pad burn (0.6%), and vasovagal reflex (0.1%)[39]. However, all these complications are not common and RFA can be considered a safe procedure in high-volume centers when proper indications to treatment are followed.

RFA IN PRE-TRANSPLANT SETTING

RFA has gained increasing interest either as bridging and as downstaging therapy prior to transplantation in hepatocarcinoma patients. A number of papers have reported complete pathological response rates (*i.e.*, complete nodule assessed by the pathologist on the explanted liver) up to 47%-75%[5-7,40,41].

In particular, this response was observed in 50%-78% of nodules within 3 cm and between 13% and 43% in larger nodules[5-7,40,41] *vs* 27%-57% of TACE in Milan-in patients[42,43].

Safety concerns previously raised by some authors due to the theoretical risk of tumoral seeding, reported to occur in about 3% of cases[44], have been recently overcome[45]. Therefore, although TACE remains the most used treatment before OLT, RFA has to be preferred in cases of single nodules under 3 cm as provides higher complete necrosis rates and lower risk of recurrence after transplantation[46].

RFA *VS* LIVER RESECTION FOR HCC

Surgery is the first-line option in very early/early patients not fulfilling transplant criteria[2-4]. By the way, no more than 10%-35% of patients are actually suitable to surgery due to tumoral burden, inadequate liver reserve, or poor performance status[2-4]. These patients may be offered RFA as viable option because of its proven efficacy.

The aforementioned striking results of RFA have recently opened debates on whether RFA can be offered particularly in very early patients (namely, those with a single nodule less than 2 cm) as first-line therapy instead of surgery. To address this point, many research groups have conducted retrospective or randomized controlled studies directly comparing the two treatments.

Table 2 reports the main characteristics of the four RCTs[47-50] comparing the two treatments published so far. As one can read in Table 2, the available RCTs report discordant results with the sole study by Huang *et al*[48] demonstrating a superiority of hepatic resection over RFA. However, the different proportions of nodules larger than 2 cm are likely to be responsible of these discordant results, as RFA is recognized as less effective beyond very early stage.

None of the aforementioned RCTs restricted their analysis to single nodules ⩽ 2 cm, while there are five observational studies focused on this specific setting[51-55]. Unfortunately, most of these retrospective studies suffer from selection bias as RFA patients tended to be older and to present more deteriorated liver function than surgical ones, while larger nodules were more likely to be treated with resection. Therefore, OS and relapse outcomes can be biased by covariate distribution. Two of these studies, which tried to obviate to such a bias through propensity score one-to-one match, reported better DFS in surgical patients ($P = 0.031$ and $P < 0.001$) but discordant results with regard to overall survival ($P = 0.296$ and $P = 0.034$, respectively)[52,55]. However, several concerns have been raised on the rigorousness of the statistical procedure adopted, hence such findings require further confirmation[56]. The low level of evidence impairs the findings of several meta-analyses published in this field, which mostly support the superiority of hepatic resection over RFA in early stage without significant differences in single nodules less than 2 cm[57,58].

An interesting study conducted by the Bologna

Table 2 Randomized controlled trials comparing radiofrequency ablation and surgery in hepatocellular carcinoma patients

Ref.	Liver function	Tumor features	Treatment	3-yr SR	5-yr SR	3-yr DFS	5-yr DFS
Chen *et al*[47]	CP A	Single < 5 cm	HR 90	73.40%	NA	69.00%	NA
	ICG-R15 < 30%		RFA 71	71.40%	NA	64.10%	NA
	PLT > 40000/mm^3						
Huang *et al*[48]	CP A/B	Within MC	HR 115	92.20%	75.70%	60.90%	51.30%
	ICG-R15 < 20%		RFA 115	69.60%	54.80%	46.10%	28.70%
	PLT > 50000/mm^3						
		Single ⩽ 3 cm	HR 45	95.60%	82.20%	NA	NA
			RFA 57	77.20%	61.40%	NA	NA
		Single 3-5 cm	HR 44	95.50%	72.30%	NA	NA
			RFA 27	66.70%	51.50%	NA	NA
		Multifocal < 3 cm	HR 26	80.80%	69.20%	NA	NA
			RFA 31	58.10%	45.20%	NA	NA
Feng *et al*[49]	CP A/B	Up to 2 nodules < 4 cm	HR 84	74.80%	NA	61.10%	NA
	ICG-R15 < 30%		RFA 84	67.20%	NA	49.60%	NA
	PLT > 50000 mm^3						
Fang *et al*[50]	CP A/B	Up to 3 nodules ⩽ 3 cm	HR 60	77.50%	NA	41.30%	NA
	PLT > 50000 mm^3		RFA 60	82.50%	NA	55.40%	NA

SR: Survival rate; DFS: Disease-free survival; CP: Child-Pugh; ICG-R15: Indocyanin green retention at 15 min; PLT: Platelets; HR: Hepatic resection; RFA: Radiofrequency ablation; NA: Not available; MC: Milan criteria.

group, based on a Markov model and a Monte Carlo probabilistic sensitivity analysis, demonstrated that in a 10-year perspective RFA provided similar life-expectancy and quality-adjusted life-expectancy (QALY) at a lower cost than surgery in very early HCC patients, hence it was the most cost-effective therapeutic strategy for this stage[59]. In the case of 2 or 3 tumors ⩽ 3 cm, life-expectancy and QALY were very similar between surgery and RFA, but cost-effectiveness was again in favor of RFA[59]. Therefore, the authors concluded that RFA is more cost-effective than surgery in cases of single nodule under 2 cm or 2/3 nodules ⩽ 3 cm, while liver surgery still represents the most valuable option for single larger early stage HCCs[59].

In conclusion, as supported by a decision-making analysis performed by the same group, the superiority or equivalence of a treatment over the other is strictly dependent on the non-linear relationship among tumor number, size and liver function, with RFA to be preferred in cases of smaller tumors and impaired liver function[60].

RFA *VS* PERCUTANEOUS ETHANOL INJECTION IN EARLY HCC PATIENTS

Percutaneous ethanol injection (PEI) is a well-established technique for the treatment of small HCCs and induces coagulative necrosis as a result of cellular dehydration and protein denaturation. However, ethanol diffusion is likely to be impaired by intratumoral fibrotic septa in cases of nodules > 2 cm.

In fact, the efficacy of such a technique in early stage (namely, multiple nodules or single nodule larger than 2 cm) is considerably inferior as compared to RFA with a complete necrosis rate of 70% in nodules of 2-3 cm and 50% in those between 3 and 5 cm[61,62]. On the other hand, RFA showed a significantly higher necrosis rate, up to 71% in non-infiltrating medium-size (*i.e.*, between 3 and 5 cm) nodules[63]. In our recently published experience, overall complete necrosis rate after RFA was 84.4% in a series whose median tumor size was 3 cm[18,20].

However, if it is widely recognized the superiority of RFA over PEI in medium-size and large nodules, a clear advantage in term of survival in small HCCs (less than 3 cm) is still unclear.

In fact, a recent meta-analysis of 8 RCTs found better survival outcomes (HR: 0.67, 95%CI: 0.51-0.87, P < 0.001) and a lower 3-year LR rate [risk ratio (RR): 0.41, 95%CI: 0.30-0.57, P < 0.01] after RFA as compared to PEI[64], but sensitivity analysis confirmed the superiority of RFA only in Asian studies[65-69] while the three included Italian studies[70-72] found only a non-significant trend in favor of RFA as for survival (HR: 0.82, 95%CI: 0.56-1.20, P = 0.30)[64]. Table 3 summarizes the main findings of the aforementioned trials. Quite interestingly, RFA provided similar if not better results as compared to PEI requiring a significant lower number of sessions (Table 3). This aspect has to be taken into account since, although a single PEI treatment has significantly lower costs than RFA, the higher number of PEI sessions makes this benefit vanish and increases the risk of tumoral seeding.

The above described results are in keeping with another systematic review of four RCTs comparing the two techniques in small HCCs under 3 cm which, however, found RFA associated to higher major complication rates and to be more costly than PEI[73].

In conclusion, although whether RFA leads to better survival rates than PEI in small HCCs is still matter of debate, the lower local recurrence rate stands for a wider

Table 3 Randomized controlled trials comparing radiofrequency ablation and percutaneous ethanol injection in hepatocellular carcinoma patients

Ref.	Region	Patients (*n*)	Nodules *n* (1/>1)	Tumor size, cm	No. of sessions	Complete response (%)	3-yr survival (%)	3-yr recurrence (%)
Lin *et al*[65]	Taiwan	RFA (52)	38/14	2.9 ± 0.8	1.6 ± 0.4	96.0	74	18.0
		PEI (52)	40/12	2.8 ± 0.8	6.5 ± 1.6	88.0	50	45.0
Lin *et al*[66]	Taiwan	RFA (62)	49/13	2.5 ± 1.0	1.3 ± 0.3	96.1	74	14.0
		PEI (62)	49/13	2.3 ± 0.8	4.9 ± 1.3	88.1	51	34.0
Shiina *et al*[67]	Japan	RFA (118)	72/46	NA	2.1 ± 1.3	100.0	81	1.7
		PEI (114)	60/54	NA	6.4 ± 2.6	100.0	66	11.0
Wang *et al*[68]	China	RFA (49)	NA	2.4 ± 1.2	NA	93.8	NA	NA
		PEI (49)	NA	2.3 ± 1.4	NA	77.5	NA	NA
Azab *et al*[69]	Egypt	RFA (30)	NA	NA	1.45	85.0	NA	NA
		PEI (30)	NA	NA	7.68	75.0	NA	NA
Giorgio *et al*[70]	Italy	RFA (128)	128/0	2.3 ± 0.4	5.00	100.0	83	7.8
		PEI (143)	143/0	2.2 ± 0.5	8.00	100.0	78	9.4
Lencioni *et al*[71]	Italy	RFA (52)	40/12	2.8 ± 0.6	1.1 ± 0.5	91.0	NA	21.0
		PEI (50)	31/19	2.8 ± 0.8	5.4 ± 1.6	82.0	NA	59.0
Brunello *et al*[72]	Italy	RFA (70)	54/16	2.4 ± 0.5	NA	95.7	59	NA
		PEI (69)	54/15	2.2 ± 0.5	NA	65.6	56	NA

RFA: Radiofrequency ablation; PEI: Percutaneous ethanol injection; NA: Not available.

application of RFA in hepato-oncology.

COMBINED TREATMENT

There is increasing evidence that combining RFA to TACE may increase the therapeutic benefit in larger HCCs. In fact, the two techniques may exert a synergistic effect on inducing nodule necrosis: Occlusion of the tumor arterial supply by TACE would increase the area of coagulation necrosis obtained by RFA minimizing heat loss whereas the heating-related reactive hyperemia induced by RFA would concentrate the chemotherapeutic agent released during TACE in the peripheral residual viable neoplastic tissue and would reduce cell resistance to the drug[74].

A recent meta-analysis of eight RCTs[75-82] including 598 patients indicated that RFA plus TACE determines a significantly higher 3-year OS rate [odds ratio (OR): 2.65, 95%CI: 1.81-3.86, $P < 0.001$] and 3-year RFS rate (OR: 3.00, 95%CI: 1.75-5.13, $P < 0.001$) than RFA alone, with no difference in major complications (OR: 1.20, 95%CI: 0.31-4.62, $P = 0.79$)[83]. Subgroups analysis revealed that most of this benefit was obtained in patients with intermediate- and large-size HCCs, which are likely to be the optimal setting for the combined treatment[83]. These results should be considered with caution as all the included studies had been conducted in Asia with conventional TACE (Table 4), hence the applicability of such findings in the West is still unclear, although a recent small Italian retrospective report confirmed the superiority of RFA combined to drug-eluting beads TACE over RFA alone in single HCCs beyond 3 cm[84].

OTHER THERMAL ABLATION TECHNIQUES

Microwave ablation

MWA aims to induce tumor necrosis by using high frequency (> 900 MHz, usually 2450 MHz) electromagnetic energy which determines continuous rotation of dipole molecules in the microwave's oscillating electric field. This vigorous movement of dipoles (mainly water molecules) generates friction and heat, thus inducing tissue death *via* coagulation necrosis[85].

In comparison to RFA, MWA has several theoretical advantages: It induces a wider area of active heating and warmer temperatures into the target zone in a shorter treatment time as it is not impaired by tissue desiccation and charring[86]; its efficacy is less impaired by heat-sink effect, due to the more pronounced cooling effect of blood flow and the conductive rather than active nature of heating[87]; multiple antennae can be simultaneously activated without the electrical interference phenomena observed in RFA, thus allowing more rapid treatment of large or multifocal tumours[87]. On these premises, MWA mostly shares the applications of RFA, with the above cited advantages in larger nodules and/or close to blood vessel.

Complete ablation rates of 89%-94% and 5-year survival rates of 51%-57% are reported in 3 retrospective studies enrolling mainly CP B patients[88-90].

The safety concerns raised on the risks of the procedure, due to the broader and less predictable necrosis areas induced by MWA, have been recently overcome by a large multicenter Italian study conducted in a series of 736 patients, of which 522 with HCC, where MWA determined a major complication rate of 2.9% with a peri-procedural mortality rate of < 0.01%[91].

There are actually 7 studies (of which one RCT) directly comparing MWA and RFA in HCC patients[92-98] (Table 5). Unfortunately, the sole RCT published did not report long-term survival data but only complete necrosis rates, which were similar in the two treatment groups (89% for MWA *vs* 96% for RFA)[92]. Retrospective studies reported heterogeneous results, particularly with regard

Table 4 Randomized controlled trials comparing transarterial chemoembolization combined to radiofrequency ablation *vs* radiofrequency ablation alone in hepatocellular carcinoma patients

Ref.	Region	Patients (*n*)	Tumor size, cm	CP A/B/C	3-yr survival (%)	3-yr recurrence (%)
Peng *et al*[75]	China	TACE + RFA (69)	≤ 5.01	60/9/0	69.0	45.0
		RFA (70)	-	59/11/0	47.0	18.0
Cheng *et al*[76]	China	TACE + RFA (96)	≤ 7.5	NA	55.0	NA
		RFA (100)	-	NA	32.0	NA
Yang *et al*[77]	China	TACE + RFA (24)	6.6 ± 0.6	NA	NA	NA
		RFA (12)	5.2 ± 0.4	NA	NA	NA
Shibata *et al*[78]	Japan	TACE + RFA (46)	1.7 ± 0.6	32/14/0	84.8	48.8
		RFA (43)	1.6 ± 0.5	33/10/0	84.5	29.7
Morimoto *et al*[79]	Japan	TACE + RFA (19)	3.6 ± 0.7	12/7/0	93.0	NA
		RFA (18)	3.7 ± 0.6	16/2/0	80.0	28.0
Kang *et al*[80]	China	TACE + RFA (19)	6.7 ± 1.1	12/7/0	36.8	NA
		RFA (18)	6.2 ± 1.2	12/6/0	16.7	NA
Shen *et al*[81]	China	TACE + RFA (18)	5.6 (2.2-15.8)	4/14/0	73.3	50.0
		RFA (16)	5.0 (2.3-12.3)	6/10/0	20.4	18.7
Zhang *et al*[82]	China	TACE + RFA (15)	4.6 (2.3-7.1)	NA	NA	NA
		RFA (15)	4.1 (2.4-6.0)	NA	NA	NA

CP: Child-Pugh; TACE: Transarterial chemoembolization; RFA: Radiofrequency ablation; NA: Not available.

Table 5 Studies comparing radiofrequency ablation and microwave ablation in hepatocellular carcinoma patients

Ref.	Arm (*n*)	Study design	Region	CP (A/B/C)	Tumor size (cm)	Number nodules	3-yr survival (%)	Local tumor recurrence (%)
Shibata *et al*[92]	RFA (36)	RCT	Japan	21/15/0	1.6 (0.7-2)	1.08	NA	8.3
	MWA (36)			19/17/0	1.7 (0.8-2)	1.14	NA	17.4
Lu *et al*[93]	RFA (53)	R	China	49/4/0	2.6 (1-6.1)	1.35	37.6	20.9
	MWA (49)			39/10/0	2.5 (0.9-7.2)	2.00	50.5	11.8
Ohmoto *et al*[94]	RFA (34)	R	Japan	20/11/3	1.6 (0.7-2)	1.08	49.0	9.0
	MWA (49)			31/14/4	1.7 (0.8-2)	1.14	70.0	19.0
Ding *et al*[95]	RFA (85)	R	China	49/36/0	2.38 (1-4.8)	1.15	77.6	5.2
	MWA (113)			75/38/0	2.55 (0.8-5)	1.15	82.7	10.9
Zhang *et al*[96]	RFA (78)	R	China	78/0/0	NA	1.24	64.1	11.8
	MWA (77)			77/0/0	NA	1.36	51.7	10.5
Abdelaziz *et al*[97]	RFA (45)	R	Egypt	24/21/0	2.95 ± 1.03+	1.00	NA	13.5
	MWA (66)			25/41/0	2.9 ± 0.97	1.00	NA	3.9
Vogl *et al*[98]	RFA (25)	R	Germany	NA	NA	1.28	72.0	9.4
	MWA (28)			NA	NA	1.28	79.0	8.3

CP: Child-Pugh; RFA: Radiofrequency ablation; MWA: Microwave ablation; RCT: Randomized controlled trial; R: Retrospective.

to local recurrence probably because of different follow-up time length or radiologic criteria adopted (Table 5).

The two meta-analysis published so far in this field reported no difference 3-year OS with MWA outperforming RFA in terms of LR for treatment of larger tumours[99,100]. However, further RCTs are needed to verify whether MWA efficacy in determining broader ablation areas will translate into a real survival benefit.

HIFU ablation

HIFU ablation aims to elevate tissue temperature by focusing high energy ultrasound (US) waves into one small spot[39]. The main advantage of HIFU ablation is the safety and the less invasiveness with, on the other hand, the limitation of a longer procedure time and acoustic shadowing by the rib cage, which may also cause thermal injury of the overlying soft tissue as a result of high US absorption by the bony cortex[39]. This drawback has been partially overcome by novel equipment using a larger transducer to spread the US beams out, thus decreasing the superficial energy wasting, or a multi-element phased-array transducer able to selectively activate only elements corresponding to the intercostal spaces[101]. There are actually few studies on HIFU, mainly conducted in advanced or recurrent cases for palliative purposes. A retrospective study by Chan *et al*[102] did not find any difference in terms of 3-year survival between HIFU and RFA for recurrent HCCs (69.8% *vs* 64.2%, $P = 0.19$). The same group compared the outcomes of HIFU ablation to those of TACE as bridging therapy before OLT and found similar results as for tumor necrosis in explanted livers ($P = 0.35$)[103]. The authors concluded that HIFU ablation was safe even for CP C patients and increased the number of subject receiving bridging therapy from 39.2% to 80.4%[103].

In our opinion, because of the scarce data currently

available and in attendance of further reliable results in the clinical setting, HIFU represents a promising option to be performed in highly-experienced centers and in selected cases.

LA

LA is one of the least investigated ablative treatments.

In this case, ablation is induced by the interaction of light energy (derived by electrical energy) and tissue[104]. Because laser light is coherent and monochromatic, it can be selectively collimated and focused and large amounts of energy can be transmitted over long distances without significant losses. Light is delivered *via* multiple flexible quartz fibers which have flat or cylindrical diffusing tips. The use of water-cooled laser application sheaths enables a higher laser power output (up to 50 W compared with 5 W of previous devices) while preventing carbonization, thus allowing ablative zones of up to 80 mm diameter[105].

Several retrospective cohort studies have shown that LA is a safe and feasible procedure for the treatment of HCC with a complete response rate ranging from 82% to 97%[105-108].

In an Italian multicenter retrospective study, 5-year cumulative survival was 41%, median survival times were 65 and 68 mo in patients with tumor size ⩽ 3 cm and ⩽ 2 cm, respectively, while median time to recurrence was 24 mo[109].

In a recent RCT with 140 Milan-in patients, complete response was observed in 97.4% of patients treated with RFA and 95.7% with LA and mean time-to local progression and overall survival were comparable between the two study groups (P = 0.129 and 0.693, respectively)[110]. The authors concluded that LA resulted non-inferior to RFA and therefore it should be considered as a valuable alternative for thermal ablation of small HCC in cirrhotic patients[110].

However, in spite of the apparently excellent results in terms of safety and of the described efficacy of LA, the low experience available worldwide currently restricts its application to a limited number of high-volume centers.

CRYOABLATION

Cryoablation induces cytotoxicity based on cyclic applications of extremely low temperatures (-20 ℃ to -40 ℃) within the tumour[39]. Multiple cryoprobes of 2-3 mm in diameter are inserted into the target lesion *via* a dilation catheter to ensure the rapid freezing of the nodule. Cryotherapy is delivered by means of multiple cycles and between two consecutive cycles the cryoprobes are rewarmed by an heating system.

Despite being widely used in various other cancers, the application of percutaneous cryoablation in HCC was sparsely reported. Compared to RFA, cryoablation endows several unique advantages including larger ablative zones, more clearly discernible treatment margin, less pain and good visualization by imaging[111,112]. Main drawbacks are: (1) smaller ablation areas generated by each single probe, hence multiple cryoprobes applications are needed; (2) unpredictable area of ablation (4-10 mm or more); and (3) concerns on the risk of complications such as massive haemorrhage due to ice ball fracture, cold injury to adjacent organs, and cryoshock syndrome[113,114].

Nevertheless, with the recent improvements in technology and the increasing experience acquired worldwide, cryoablation represents a promising therapeutic tool in the field of HCC ablation.

An Asian series of 866 patients within Milan criteria who underwent percutaneous cryoablation was recently analyzed: Complete response was achieved in 96.1% of patients with a major complication rate of 2.8% and no treatment-related mortality[115]. Five-year local tumor recurrence rate was 24.2% and 5-year survival rate was 59.5%[115].

A recent meta-analysis including 4 retrospective studies comparing the effect of cryoablation and RFA on hepatic neoplastic lesions concluded that RFA was significantly superior in terms of safety and local recurrence[116]. However, these studies referred not only to HCC but also to other liver malignancies, used several different equipments as laparoscopic or even surgical cryoablation[116] and were mostly conducted several years ago when experience with cryoablation was still low. In a multicenter Asian RCT enrolling 360 patients with one or two HCC lesions ⩽ 4 cm, cryoablation proved superior to RFA according to 3-year local tumor progression (7% *vs* 11%, P = 0.043) while 5-year overall survival was similar between the two groups (40% *vs* 38%, P = 0.747)[117]. Major complications occurred in seven patients (3.9%) following cryoablation and in six patients (3.3%) following RFA (P = 0.776)[117]. These results have been confirmed in an interesting retrospective study comparing cryoablation and RFA combined to microwave coagulation therapy, where hypothermal therapy proved superior to combined regimen as for 2-year local recurrence-free survival (HR: 0.3, 95%CI: 0.1-0.9; P = 0.02) with no difference in safety outcomes[118].

Although further RCTs are needed in order to confirm these promising results, appropriate use of cryoablation could represent a valuable therapeutic option in early stage HCC patients.

CONCLUSION

Ablative treatments, particularly RFA, currently represent the first-line option for early stage unresectable HCC patients. Main indications to ablative treatments are BCLC 0/A patients not suitable to surgical therapies, namely liver resection and OLT, and bridging/down-staging setting before transplantation. Considering the state-of-art of the literature, RFA provided 5-year survival rates of 40%-70% and beyond in HCC series and, although survival rates are similar to PEI, the lower local recurrence rate stands for a wider application of RFA in

hepato-oncology.

In comparison to RFA, MWA has several theoretical advantages such as a wider ablation area, warmer temperatures into the target area in a shorter treatment time and it is not impaired by heat-sink effect. The safety concerns raised on the risks of this procedure, due to the broader and less predictable necrosis areas, have been recently overcome. However, whether MWA ability to induce a broader ablation zone will lead to a real survival benefit is still unclear.

Other treatments, such as HIFU, LA and cryoablation, are less investigated but showed promising results in early HCC patients and could be a valuable therapeutic option in the next future.

REFERENCES

1 **El-Serag HB**. Hepatocellular carcinoma. *N Engl J Med* 2011; **365**: 1118-1127 [PMID: 21992124 DOI: 10.1056/NEJMra1001683]

2 **European Association For The Study Of The Liver**; European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; **56**: 908-943 [PMID: 22424438 DOI: 10.1016/j.jhep.2011.12.001]

3 **Llovet JM**, Zucman-Rossi J, Pikarsky E, Sangro B, Schwartz M, Sherman M, Gores G. Hepatocellular carcinoma. *Nat Rev Dis Primers* 2016; **2**: 16018 [PMID: 27158749 DOI: 10.1038/nrdp.2016.18]

4 **Bruix J**, Sherman M; American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]

5 **Mazzaferro V**, Battiston C, Perrone S, Pulvirenti A, Regalia E, Romito R, Sarli D, Schiavo M, Garbagnati F, Marchianò A, Spreafico C, Camerini T, Mariani L, Miceli R, Andreola S. Radiofrequency ablation of small hepatocellular carcinoma in cirrhotic patients awaiting liver transplantation: a prospective study. *Ann Surg* 2004; **240**: 900-909 [PMID: 15492574 DOI: 10.1097/01.sla.0000143301.56154.95]

6 **Lu DS**, Yu NC, Raman SS, Lassman C, Tong MJ, Britten C, Durazo F, Saab S, Han S, Finn R, Hiatt JR, Busuttil RW. Percutaneous radiofrequency ablation of hepatocellular carcinoma as a bridge to liver transplantation. *Hepatology* 2005; **41**: 1130-1137 [PMID: 15841454 DOI: 10.1002/hep.20688]

7 **Pompili M**, Mirante VG, Rondinara G, Fassati LR, Piscaglia F, Agnes S, Covino M, Ravaioli M, Fagiuoli S, Gasbarrini G, Rapaccini GL. Percutaneous ablation procedures in cirrhotic patients with hepatocellular carcinoma submitted to liver transplantation: Assessment of efficacy at explant analysis and of safety for tumor recurrence. *Liver Transpl* 2005; **11**: 1117-1126 [PMID: 16123960 DOI: 10.1002/lt.20469]

8 **Lencioni R**, Crocetti L. Local-regional treatment of hepatocellular carcinoma. *Radiology* 2012; **262**: 43-58 [PMID: 22190656 DOI: 10.1148/radiol.11110144]

9 **Lencioni R**, Cioni D, Crocetti L, Franchini C, Pina CD, Lera J, Bartolozzi C. Early-stage hepatocellular carcinoma in patients with cirrhosis: long-term results of percutaneous image-guided radiofrequency ablation. *Radiology* 2005; **234**: 961-967 [PMID: 15665226 DOI: 10.1148/radiol.2343040350]

10 **Omata M**, Tateishi R, Yoshida H, Shiina S. Treatment of hepatocellular carcinoma by percutaneous tumor ablation methods: Ethanol injection therapy and radiofrequency ablation. *Gastroenterology* 2004; **127**: S159-S166 [PMID: 15508080 DOI: 10.1053/j.gastro.2004.09.030]

11 **Livraghi T**, Meloni F, Di Stasi M, Rolle E, Solbiati L, Tinelli C, Rossi S. Sustained complete response and complications rates after radiofrequency ablation of very early hepatocellular carcinoma in cirrhosis: Is resection still the treatment of choice? *Hepatology* 2008; **47**: 82-89 [PMID: 18008357 DOI: 10.1002/hep.21933]

12 **Pompili M**, Saviano A, de Matthaeis N, Cucchetti A, Ardito F, Federico B, Brunello F, Pinna AD, Giorgio A, Giulini SM, De Sio I, Torzilli G, Fornari F, Capussotti L, Guglielmi A, Piscaglia F, Aldrighetti L, Caturelli E, Calise F, Nuzzo G, Rapaccini GL, Giuliante F. Long-term effectiveness of resection and radiofrequency ablation for single hepatocellular carcinoma ≤3 cm. Results of a multicenter Italian survey. *J Hepatol* 2013; **59**: 89-97 [PMID: 23523578 DOI: 10.1016/j.jhep.2013.03.009]

13 **Chamberlain RS**, Fong Y. Radiofrequency thermal ablation of liver tumors. In: Blumgart LH, Fong Y. Surgery of the liver and biliary tract. 3rd ed. Toronto: W.B. Saunders, 2000: 1589-1595

14 **Jacobs A**. Radiofrequency Ablation for Liver Cancer. *Radiol Technol* 2015; **86**: 645-664; quiz 665-668 [PMID: 26199436]

15 **Facciorusso A**, Del Prete V, Antonino M, Neve V, Amoruso A, Crucinio N, Di Leo A, Barone M. Conditional survival analysis of hepatocellular carcinoma patients treated with radiofrequency ablation. *Hepatol Res* 2015; **45**: E62-E72 [PMID: 25472869 DOI: 10.1111/hepr.12458]

16 **Shiina S**, Tateishi R, Arano T, Uchino K, Enooku K, Nakagawa H, Asaoka Y, Sato T, Masuzaki R, Kondo Y, Goto T, Yoshida H, Omata M, Koike K. Radiofrequency ablation for hepatocellular carcinoma: 10-year outcome and prognostic factors. *Am J Gastroenterol* 2012; **107**: 569-577; quiz 578 [PMID: 22158026 DOI: 10.1038/ajg.2011.425]

17 **Kim YS**, Lim HK, Rhim H, Lee MW, Choi D, Lee WJ, Paik SW, Koh KC, Lee JH, Choi MS, Gwak GY, Yoo BC. Ten-year outcomes of percutaneous radiofrequency ablation as first-line therapy of early hepatocellular carcinoma: analysis of prognostic factors. *J Hepatol* 2013; **58**: 89-97 [PMID: 23023009 DOI: 10.1016/j.jhep.2012.09.020]

18 **Facciorusso A**, Del Prete V, Antonino M, Crucinio N, Neve V, Di Leo A, Carr BI, Barone M. Post-recurrence survival in hepatocellular carcinoma after percutaneous radiofrequency ablation. *Dig Liver Dis* 2014; **46**: 1014-1019 [PMID: 25085684 DOI: 10.1016/j.dld.2014.07.012]

19 **N'Kontchou G**, Mahamoudi A, Aout M, Ganne-Carrié N, Grando V, Coderc E, Vicaut E, Trinchet JC, Sellier N, Beaugrand M, Seror O. Radiofrequency ablation of hepatocellular carcinoma: long-term results and prognostic factors in 235 Western patients with cirrhosis. *Hepatology* 2009; **50**: 1475-1483 [PMID: 19731239 DOI: 10.1002/hep.23181]

20 **Facciorusso A**, Del Prete V, Antonino M, Neve V, Crucinio N, Di Leo A, Carr BI, Barone M. Serum ferritin as a new prognostic factor in hepatocellular carcinoma patients treated with radiofrequency ablation. *J Gastroenterol Hepatol* 2014; **29**: 1905-1910 [PMID: 24731153 DOI: 10.1111/jgh.12618]

21 **Lee DH**, Lee JM, Lee JY, Kim SH, Yoon JH, Kim YJ, Han JK, Choi BI. Radiofrequency ablation of hepatocellular carcinoma as first-line treatment: long-term results and prognostic factors in 162 patients with cirrhosis. *Radiology* 2014; **270**: 900-909 [PMID: 24475823 DOI: 10.1148/radiol.13130940]

22 **Ng KK**, Poon RT, Lo CM, Yuen J, Tso WK, Fan ST. Analysis of recurrence pattern and its influence on survival outcome after radiofrequency ablation of hepatocellular carcinoma. *J Gastrointest Surg* 2008; **12**: 183-191 [PMID: 17874276]

23 **Koda M**, Tokunaga S, Okamoto T, Hodozuka M, Miyoshi K, Kishina M, Fujise Y, Kato J, Matono T, Sugihara T, Oyama K, Hosho K, Okano J, Murawaki Y, Kakite S, Yamashita E. Clinical usefulness of the ablative margin assessed by magnetic resonance imaging with Gd-EOB-DTPA for radiofrequency ablation of hepatocellular carcinoma. *J Hepatol* 2015; **63**: 1360-1367 [PMID: 26232269 DOI: 10.1016/j.jhep.2015.07.023]

24 **Kim YS**, Lee WJ, Rhim H, Lim HK, Choi D, Lee JY. The minimal ablative margin of radiofrequency ablation of hepatocellular carcinoma (& gt; 2 and & lt; 5 cm) needed to prevent local tumor progression: 3D quantitative assessment using CT image fusion. *AJR Am J Roentgenol* 2010; **195**: 758-765 [PMID: 20729457 DOI: 10.2214/AJR.09.2954]

25 **Cucchetti A**, Piscaglia F, Caturelli E, Benvegnù L, Vivarelli M, Ercolani G, Cescon M, Ravaioli M, Grazi GL, Bolondi L, Pinna AD.

Comparison of recurrence of hepatocellular carcinoma after resection in patients with cirrhosis to its occurrence in a surveilled cirrhotic population. *Ann Surg Oncol* 2009; **16**: 413-422 [PMID: 19034578 DOI: 10.1245/s10434-008-0232-4]

26 **Feng X**, Xu R, Du X, Dou K, Qin X, Xu J, Jia W, Wang Z, Zhao H, Yang S, Guo C, Liu T, Ma K. Combination therapy with sorafenib and radiofrequency ablation for BCLC Stage 0-B1 hepatocellular carcinoma: a multicenter retrospective cohort study. *Am J Gastroenterol* 2014; **109**: 1891-1899 [PMID: 25403366 DOI: 10.1038/ajg.2014.343]

27 **Kan X**, Jing Y, Wan QY, Pan JC, Han M, Yang Y, Zhu M, Wang Q, Liu KH. Sorafenib combined with percutaneous radiofrequency ablation for the treatment of medium-sized hepatocellular carcinoma. *Eur Rev Med Pharmacol Sci* 2015; **19**: 247-255 [PMID: 25683938]

28 **Bruix J**, Takayama T, Mazzaferro V, Chau GY, Yang J, Kudo M, Cai J, Poon RT, Han KH, Tak WY, Lee HC, Song T, Roayaie S, Bolondi L, Lee KS, Makuuchi M, Souza F, Berre MA, Meinhardt G, Llovet JM. Adjuvant sorafenib for hepatocellular carcinoma after resection or ablation (STORM): a phase 3, randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2015; **16**: 1344-1354 [PMID: 26361969 DOI: 10.1016/S1470-2045(15)00198-9]

29 **Mazzaferro V**, Romito R, Schiavo M, Mariani L, Camerini T, Bhoori S, Capussotti L, Calise F, Pellicci R, Belli G, Tagger A, Colombo M, Bonino F, Majno P, Llovet JM. Prevention of hepatocellular carcinoma recurrence with alpha-interferon after liver resection in HCV cirrhosis. *Hepatology* 2006; **44**: 1543-1554 [PMID: 17133492]

30 **Hsu YC**, Ho HJ, Wu MS, Lin JT, Wu CY. Postoperative peg-interferon plus ribavirin is associated with reduced recurrence of hepatitis C virus-related hepatocellular carcinoma. *Hepatology* 2013; **58**: 150-157 [PMID: 23389758 DOI: 10.1002/hep.26300]

31 **Tamaki Y**, Nakade Y, Yamauchi T, Makino Y, Yokohama S, Okada M, Aso K, Kanamori H, Ohashi T, Sato K, Nakao H, Haneda M, Yoneda M. Angiotensin II type 1 receptor antagonist prevents hepatic carcinoma in rats with nonalcoholic steatohepatitis. *J Gastroenterol* 2013; **48**: 491-503 [PMID: 22886508 DOI: 10.1007/s00535-012-0651-7]

32 **Hirose A**, Ono M, Saibara T, Nozaki Y, Masuda K, Yoshioka A, Takahashi M, Akisawa N, Iwasaki S, Oben JA, Onishi S. Angiotensin II type 1 receptor blocker inhibits fibrosis in rat nonalcoholic steatohepatitis. *Hepatology* 2007; **45**: 1375-1381 [PMID: 17518368]

33 **Yoshiji H**, Noguchi R, Toyohara M, Ikenaka Y, Kitade M, Kaji K, Yamazaki M, Yamao J, Mitoro A, Sawai M, Yoshida M, Fujimoto M, Tsujimoto T, Kawaratani H, Uemura M, Fukui H. Combination of vitamin K2 and angiotensin-converting enzyme inhibitor ameliorates cumulative recurrence of hepatocellular carcinoma. *J Hepatol* 2009; **51**: 315-321 [PMID: 19501932 DOI: 10.1016/j.jhep.2009.04.011]

34 **Yoshiji H**, Noguchi R, Ikenaka Y, Kaji K, Aihara Y, Yamazaki M, Yamao J, Toyohara M, Mitoro A, Sawai M, Yoshida M, Morioka C, Fujimoto M, Uemura M, Fukui H. Combination of branched-chain amino acids and angiotensin-converting enzyme inhibitor suppresses the cumulative recurrence of hepatocellular carcinoma: a randomized control trial. *Oncol Rep* 2011; **26**: 1547-1553 [PMID: 21874260 DOI: 10.3892/or.2011.1433]

35 **Kaibori M**, Ishizaki M, Matsui K, Kitade H, Matsui Y, Kwon AH. Evaluation of metabolic factors on the prognosis of patients undergoing resection of hepatocellular carcinoma. *J Gastroenterol Hepatol* 2011; **26**: 536-543 [PMID: 21332549 DOI: 10.1111/j.1440-1746.2010.06439.x]

36 **Facciorusso A**, Del Prete V, Crucinio N, Muscatiello N, Carr BI, Di Leo A, Barone M. Angiotensin receptor blockers improve survival outcomes after radiofrequency ablation in hepatocarcinoma patients. *J Gastroenterol Hepatol* 2015; **30**: 1643-1650 [PMID: 25974743 DOI: 10.1111/jgh.12988]

37 **Du H**, Liang Z, Zhang Y, Jie F, Li J, Fei Y, Huang Z, Pei N, Wang S, Li A, Chen B, Zhang Y, Sumners C, Li M, Li H. Effects of angiotensin II type 2 receptor overexpression on the growth of hepatocellular carcinoma cells in vitro and in vivo. *PLoS One* 2013; **8**: e83754 [PMID: 24391821 DOI: 10.1371/journal.pone.0083754]

38 **Bertot LC**, Sato M, Tateishi R, Yoshida H, Koike K. Mortality and complication rates of percutaneous ablative techniques for the treatment of liver tumors: a systematic review. *Eur Radiol* 2011; **21**: 2584-2596 [PMID: 21858539 DOI: 10.1007/s00330-011-2222-3]

39 **Kim YS**, Lim HK, Rhim H, Lee MW. Ablation of hepatocellular carcinoma. *Best Pract Res Clin Gastroenterol* 2014; **28**: 897-908 [PMID: 25260316 DOI: 10.1016/j.bpg.2014.08.011]

40 **Brillet PY**, Paradis V, Brancatelli G, Rangheard AS, Consigny Y, Plessier A, Durand F, Belghiti J, Sommacale D, Vilgrain V. Percutaneous radiofrequency ablation for hepatocellular carcinoma before liver transplantation: a prospective study with histopathologic comparison. *AJR Am J Roentgenol* 2006; **186**: S296-S305 [PMID: 16632691]

41 **Rodríguez-Sanjuán JC**, González F, Juanco C, Herrera LA, López-Bautista M, González-Noriega M, García-Somacarrera E, Figols J, Gómez-Fleitas M, Silván M. Radiological and pathological assessment of hepatocellular carcinoma response to radiofrequency. A study on removed liver after transplantation. *World J Surg* 2008; **32**: 1489-1494 [PMID: 18373117 DOI: 10.1007/s00268-008-9559-z]

42 **Majno PE**, Adam R, Bismuth H, Castaing D, Ariche A, Krissat J, Perrin H, Azoulay D. Influence of preoperative transarterial lipiodol chemoembolization on resection and transplantation for hepatocellular carcinoma in patients with cirrhosis. *Ann Surg* 1997; **226**: 688-701; discussion 701-703 [PMID: 9409568]

43 **Golfieri R**, Cappelli A, Cucchetti A, Piscaglia F, Carpenzano M, Peri E, Ravaioli M, D'Errico-Grigioni A, Pinna AD, Bolondi L. Efficacy of selective transarterial chemoembolization in inducing tumor necrosis in small (& lt; 5 cm) hepatocellular carcinomas. *Hepatology* 2011; **53**: 1580-1589 [PMID: 21351114 DOI: 10.1002/hep.24246]

44 **Imamura J**, Tateishi R, Shiina S, Goto E, Sato T, Ohki T, Masuzaki R, Goto T, Yoshida H, Kanai F, Hamamura K, Obi S, Yoshida H, Omata M. Neoplastic seeding after radiofrequency ablation for hepatocellular carcinoma. *Am J Gastroenterol* 2008; **103**: 3057-3062 [PMID: 19086957 DOI: 10.1111/j.1572-0241.2008.02153.x]

45 **Lopez KT**, Kuwada SK, Wong LL. Consequences of needle tract seeding of hepatocellular cancer after liver transplant. *Clin Transplant* 2013; **27**: E400-E406 [PMID: 23837571 DOI: 10.1111/ctr.12160]

46 **Clavien PA**, Lesurtel M, Bossuyt PM, Gores GJ, Langer B, Perrier A; OLT for HCC Consensus Group. Recommendations for liver transplantation for hepatocellular carcinoma: an international consensus conference report. *Lancet Oncol* 2012; **13**: e11-e22 [PMID: 22047762 DOI: 10.1016/S1470-2045(11)70175-9]

47 **Chen MS**, Li JQ, Zheng Y, Guo RP, Liang HH, Zhang YQ, Lin XJ, Lau WY. A prospective randomized trial comparing percutaneous local ablative therapy and partial hepatectomy for small hepatocellular carcinoma. *Ann Surg* 2006; **243**: 321-328 [PMID: 16495695]

48 **Huang J**, Yan L, Cheng Z, Wu H, Du L, Wang J, Xu Y, Zeng Y. A randomized trial comparing radiofrequency ablation and surgical resection for HCC conforming to the Milan criteria. *Ann Surg* 2010; **252**: 903-912 [PMID: 21107100 DOI: 10.1097/SLA.0b013e3181efc656]

49 **Feng K**, Yan J, Li X, Xia F, Ma K, Wang S, Bie P, Dong J. A randomized controlled trial of radiofrequency ablation and surgical resection in the treatment of small hepatocellular carcinoma. *J Hepatol* 2012; **57**: 794-802 [PMID: 22634125 DOI: 10.1016/j.jhep.2012.05.007]

50 **Fang Y**, Chen W, Liang X, Li D, Lou H, Chen R, Wang K, Pan H. Comparison of long-term effectiveness and complications of radiofrequency ablation with hepatectomy for small hepatocellular carcinoma. *J Gastroenterol Hepatol* 2014; **29**: 193-200 [PMID: 24224779 DOI: 10.1111/jgh.12441]

51 **Peng ZW**, Lin XJ, Zhang YJ, Liang HH, Guo RP, Shi M, Chen MS. Radiofrequency ablation versus hepatic resection for the treatment of hepatocellular carcinomas 2 cm or smaller: a retrospective comparative study. *Radiology* 2012; **262**: 1022-1033 [PMID: 22357902 DOI: 10.1148/radiol.11110817]

52 **Wang JH**, Wang CC, Hung CH, Chen CL, Lu SN. Survival comparison between surgical resection and radiofrequency ablation for patients in BCLC very early/early stage hepatocellular carcinoma. *J Hepatol* 2012; **56**: 412-418 [PMID: 21756858 DOI: 10.1016/j.jhep.2011.05.020]

53 **Hung HH**, Chiou YY, Hsia CY, Su CW, Chou YH, Chiang JH, Kao

WY, Huo TI, Huang YH, Su YH, Lin HC, Lee SD, Wu JC. Survival rates are comparable after radiofrequency ablation or surgery in patients with small hepatocellular carcinomas. *Clin Gastroenterol Hepatol* 2011; **9**: 79-86 [PMID: 20831902 DOI: 10.1016/j.cgh.2010.08.018]

54 **Takayama T**, Makuuchi M, Hasegawa K. Single HCC smaller than 2 cm: surgery or ablation?: surgeon's perspective. *J Hepatobiliary Pancreat Sci* 2010; **17**: 422-424 [PMID: 19936598 DOI: 10.1007/s00534-009-0239-7]

55 **Liu PH**, Hsu CY, Hsia CY, Lee YH, Huang YH, Chiou YY, Lin HC, Huo TI. Surgical Resection Versus Radiofrequency Ablation for Single Hepatocellular Carcinoma ≤ 2 cm in a Propensity Score Model. *Ann Surg* 2016; **263**: 538-545 [PMID: 25775062 DOI: 10.1097/SLA.0000000000001178]

56 **Cucchetti A**, Piscaglia F, Cescon M, Ercolani G, Pinna AD. Systematic review of surgical resection vs radiofrequency ablation for hepatocellular carcinoma. *World J Gastroenterol* 2013; **19**: 4106-4118 [PMID: 23864773 DOI: 10.3748/wjg.v19.i26.4106]

57 **Cho YK**, Rhim H, Noh S. Radiofrequency ablation versus surgical resection as primary treatment of hepatocellular carcinoma meeting the Milan criteria: a systematic review. *J Gastroenterol Hepatol* 2011; **26**: 1354-1360 [PMID: 21679247 DOI: 10.1111/j.1440-1746.2011.06812.x]

58 **Wang Y**, Luo Q, Li Y, Deng S, Wei S, Li X. Radiofrequency ablation versus hepatic resection for small hepatocellular carcinomas: a meta-analysis of randomized and nonrandomized controlled trials. *PLoS One* 2014; **9**: e84484 [PMID: 24404166 DOI: 10.1371/journal.pone.0084484]

59 **Cucchetti A**, Piscaglia F, Cescon M, Colecchia A, Ercolani G, Bolondi L, Pinna AD. Cost-effectiveness of hepatic resection versus percutaneous radiofrequency ablation for early hepatocellular carcinoma. *J Hepatol* 2013; **59**: 300-307 [PMID: 23603669 DOI: 10.1016/j.jhep.2013.04.009]

60 **Cucchetti A**, Piscaglia F, Cescon M, Serra C, Colecchia A, Maroni L, Venerandi L, Ercolani G, Pinna AD. An explorative data-analysis to support the choice between hepatic resection and radiofrequency ablation in the treatment of hepatocellular carcinoma. *Dig Liver Dis* 2014; **46**: 257-263 [PMID: 24284006 DOI: 10.1016/j.dld.2013.10.015]

61 **Lencioni R**. Loco-regional treatment of hepatocellular carcinoma. *Hepatology* 2010; **52**: 762-773 [PMID: 20564355 DOI: 10.1002/hep.23725]

62 **Livraghi T**, Bolondi L, Lazzaroni S, Marin G, Morabito A, Rapaccini GL, Salmi A, Torzilli G. Percutaneous ethanol injection in the treatment of hepatocellular carcinoma in cirrhosis. A study on 207 patients. *Cancer* 1992; **69**: 925-929 [PMID: 1310435 DOI: 10.1002/1097-0142(19920215)69:4<925::AID-CNCR2820690415>3.0.CO;2-G]

63 **Livraghi T**, Goldberg SN, Lazzaroni S, Meloni F, Ierace T, Solbiati L, Gazelle GS. Hepatocellular carcinoma: radio-frequency ablation of medium and large lesions. *Radiology* 2000; **214**: 761-768 [PMID: 10715043 DOI: 10.1148/radiology.214.3.r00mr02761]

64 **Yang B**, Zan RY, Wang SY, Li XL, Wei ML, Guo WH, You X, Li J, Liao ZY. Radiofrequency ablation versus percutaneous ethanol injection for hepatocellular carcinoma: a meta-analysis of randomized controlled trials. *World J Surg Oncol* 2015; **13**: 96 [PMID: 25889181 DOI: 10.1186/s12957-015-0516-7]

65 **Lin SM**, Lin CJ, Lin CC, Hsu CW, Chen YC. Radiofrequency ablation improves prognosis compared with ethanol injection for hepatocellular carcinoma & lt; or =4 cm. *Gastroenterology* 2004; **127**: 1714-1723 [PMID: 15578509 DOI: 10.1053/j.gastro.2004.09.003]

66 **Lin SM**, Lin CJ, Lin CC, Hsu CW, Chen YC. Randomised controlled trial comparing percutaneous radiofrequency thermal ablation, percutaneous ethanol injection, and percutaneous acetic acid injection to treat hepatocellular carcinoma of 3 cm or less. *Gut* 2005; **54**: 1151-1156 [PMID: 16009687 DOI: 10.1136/gut.2004.045203]

67 **Shiina S**, Teratani T, Obi S, Sato S, Tateishi R, Fujishima T, Ishikawa T, Koike Y, Yoshida H, Kawabe T, Omata M. A randomized controlled trial of radiofrequency ablation with ethanol injection for small hepatocellular carcinoma. *Gastroenterology* 2005; **129**: 122-130 [PMID: 16012942 DOI: 10.1053/j.gastro.2005.04.009]

68 **Wang XW**, Yang S, Lai Z. Clinical effect of radiofrequency ablation therapy and percutaneous ethanol injection therapy on small hepatocellular carcinoma. *Med J West China* 2011; **23**: 1671-1673

69 **Azab M**, Zaki S, El-Shetey AG, Abdel-Moty MF, Alnoomani NM, Gomaa AA, Abdel-Fatah S, Mohiy S, Atia F. Radiofrequency ablation combined with percutaneous ethanol injection in patients with hepatocellular carcinoma. *Arab J Gastroenterol* 2011; **12**: 113-118 [PMID: 22055587 DOI: 10.1016/j.ajg.2011.07.005]

70 **Giorgio A**, Di Sarno A, De Stefano G, Scognamiglio U, Farella N, Mariniello A, Esposito V, Coppola C, Giorgio V. Percutaneous radiofrequency ablation of hepatocellular carcinoma compared to percutaneous ethanol injection in treatment of cirrhotic patients: an Italian randomized controlled trial. *Anticancer Res* 2011; **31**: 2291-2295 [PMID: 21737654]

71 **Lencioni RA**, Allgaier HP, Cioni D, Olschewski M, Deibert P, Crocetti L, Frings H, Laubenberger J, Zuber I, Blum HE, Bartolozzi C. Small hepatocellular carcinoma in cirrhosis: randomized comparison of radio-frequency thermal ablation versus percutaneous ethanol injection. *Radiology* 2003; **228**: 235-240 [PMID: 12759473]

72 **Brunello F**, Veltri A, Carucci P, Pagano E, Ciccone G, Moretto P, Sacchetto P, Gandini G, Rizzetto M. Radiofrequency ablation versus ethanol injection for early hepatocellular carcinoma: A randomized controlled trial. *Scand J Gastroenterol* 2008; **43**: 727-735 [PMID: 18569991 DOI: 10.1080/00365520701885481]

73 **Shen A**, Zhang H, Tang C, Chen Y, Wang Y, Zhang C, Wu Z. Systematic review of radiofrequency ablation versus percutaneous ethanol injection for small hepatocellular carcinoma up to 3 cm. *J Gastroenterol Hepatol* 2013; **28**: 793-800 [PMID: 23432154 DOI: 10.1111/jgh.12162]

74 **Ahrar K**, Newman RA, Pang J, Vijjeswarapu MK, Wallace MJ, Wright KC. 2004 Dr. Gary J. Becker Young Investigator Award: Relative thermosensitivity of cytotoxic drugs used in transcatheter arterial chemoembolization. *J Vasc Interv Radiol* 2004; **15**: 901-905 [PMID: 15361556]

75 **Peng ZW**, Zhang YJ, Liang HH, Lin XJ, Guo RP, Chen MS. Recurrent hepatocellular carcinoma treated with sequential transcatheter arterial chemoembolization and RF ablation versus RF ablation alone: a prospective randomized trial. *Radiology* 2012; **262**: 689-700 [PMID: 22157201 DOI: 10.1148/radiol.11110637]

76 **Cheng BQ**, Jia CQ, Liu CT, Fan W, Wang QL, Zhang ZL, Yi CH. Chemoembolization combined with radiofrequency ablation for patients with hepatocellular carcinoma larger than 3 cm: a randomized controlled trial. *JAMA* 2008; **299**: 1669-1677 [PMID: 18398079 DOI: 10.1001/jama.299.14.1669]

77 **Yang W**, Chen MH, Wang MQ, Cui M, Gao W, Wu W, Wu JY, Dai Y, Yan K. Combination therapy of radiofrequency ablation and transarterial chemoembolization in recurrent hepatocellular carcinoma after hepatectomy compared with single treatment. *Hepatol Res* 2009; **39**: 231-240 [PMID: 19054154 DOI: 10.1111/j.1872-034X.2008.00451.x]

78 **Shibata T**, Isoda H, Hirokawa Y, Arizono S, Shimada K, Togashi K. Small hepatocellular carcinoma: is radiofrequency ablation combined with transcatheter arterial chemoembolization more effective than radiofrequency ablation alone for treatment? *Radiology* 2009; **252**: 905-913 [PMID: 19567647 DOI: 10.1148/radiol.2523081676]

79 **Morimoto M**, Numata K, Kondou M, Nozaki A, Morita S, Tanaka K. Midterm outcomes in patients with intermediate-sized hepatocellular carcinoma: a randomized controlled trial for determining the efficacy of radiofrequency ablation combined with transcatheter arterial chemoembolization. *Cancer* 2010; **116**: 5452-5460 [PMID: 20672352 DOI: 10.1002/cncr.25314]

80 **Kang CB**, Xu HB, Wang SL, Rui B. Treatment of large hepatoma by TACE in combination with RFA. *Zhonghua Gandan Waike Zazhi* 2007; **13**: 828-830

81 **Shen SQ**, Xiang JJ, Xiong CL, Wu SM, Zhu SS. Intraoperative radiofrequency thermal ablation combined with portal vein infusion chemotherapy and transarterial chemoembolization for unresectable HCC. *Hepatogastroenterology* 2005; **52**: 1403-1407 [PMID: 16201083]

82 **Zhang Z**, Wu M, Chen H, Chen D, He J. [Percutaneous

radiofrequency ablation combined with transcatheter arterial chemoembolization for hepatocellular carcinoma]. *Zhonghua Waike Zazhi* 2002; **40**: 826-829 [PMID: 12487855]

83 **Ni JY**, Liu SS, Xu LF, Sun HL, Chen YT. Meta-analysis of radiofrequency ablation in combination with transarterial chemoembolization for hepatocellular carcinoma. *World J Gastroenterol* 2013; **19**: 3872-3882 [PMID: 23840128 DOI: 10.3748/wjg.v19.i24.3872]

84 **Iezzi R**, Pompili M, La Torre MF, Campanale MC, Montagna M, Saviano A, Cesario V, Siciliano M, Annicchiarico E, Agnes S, Giuliante F, Grieco A, Rapaccini GL, De Gaetano AM, Gasbarrini A, Bonomo L. Radiofrequency ablation plus drug-eluting beads transcatheter arterial chemoembolization for the treatment of single large hepatocellular carcinoma. *Dig Liver Dis* 2015; **47**: 242-248 [PMID: 25577299 DOI: 10.1016/j.dld.2014.12.007]

85 **Liang P**, Wang Y. Microwave ablation of hepatocellular carcinoma. *Oncology* 2007; **72** Suppl 1: 124-131 [PMID: 18087193 DOI: 10.1159/000111718]

86 **Skinner MG**, Iizuka MN, Kolios MC, Sherar MD. A theoretical comparison of energy sources--microwave, ultrasound and laser--for interstitial thermal therapy. *Phys Med Biol* 1998; **43**: 3535-3547 [PMID: 9869030 DOI: 10.1088/0031-9155/43/12/011]

87 **McWilliams JP**, Yamamoto S, Raman SS, Loh CT, Lee EW, Liu DM, Kee ST. Percutaneous ablation of hepatocellular carcinoma: current status. *J Vasc Interv Radiol* 2010; **21**: S204-S213 [PMID: 20656230 DOI: 10.1016/j.jvir.2009.11.025]

88 **Dong B**, Liang P, Yu X, Su L, Yu D, Cheng Z, Zhang J. Percutaneous sonographically guided microwave coagulation therapy for hepatocellular carcinoma: results in 234 patients. *AJR Am J Roentgenol* 2003; **180**: 1547-1555 [PMID: 12760916 DOI: 10.2214/ajr.180.6.1801547]

89 **Liang P**, Dong B, Yu X, Yu D, Wang Y, Feng L, Xiao Q. Prognostic factors for survival in patients with hepatocellular carcinoma after percutaneous microwave ablation. *Radiology* 2005; **235**: 299-307 [PMID: 15731369 DOI: 10.1148/radiol.2351031944]

90 **Lu MD**, Chen JW, Xie XY, Liu L, Huang XQ, Liang LJ, Huang JF. Hepatocellular carcinoma: US-guided percutaneous microwave coagulation therapy. *Radiology* 2001; **221**: 167-172 [PMID: 11568335 DOI: 10.1148/radiol.2211001783]

91 **Livraghi T**, Meloni F, Solbiati L, Zanus G; Collaborative Italian Group using AMICA system. Complications of microwave ablation for liver tumors: results of a multicenter study. *Cardiovasc Intervent Radiol* 2012; **35**: 868-874 [PMID: 21833809 DOI: 10.1007/s00270-011-0241-8]

92 **Shibata T**, Iimuro Y, Yamamoto Y, Maetani Y, Ametani F, Itoh K, Konishi J. Small hepatocellular carcinoma: comparison of radiofrequency ablation and percutaneous microwave coagulation therapy. *Radiology* 2002; **223**: 331-337 [PMID: 11997534]

93 **Lu MD**, Xu HX, Xie XY, Yin XY, Chen JW, Kuang M, Xu ZF, Liu GJ, Zheng YL. Percutaneous microwave and radiofrequency ablation for hepatocellular carcinoma: a retrospective comparative study. *J Gastroenterol* 2005; **40**: 1054-1060 [PMID: 16322950]

94 **Ohmoto K**, Yoshioka N, Tomiyama Y, Shibata N, Kawase T, Yoshida K, Kuboki M, Yamamoto S. Comparison of therapeutic effects between radiofrequency ablation and percutaneous microwave coagulation therapy for small hepatocellular carcinomas. *J Gastroenterol Hepatol* 2009; **24**: 223-227 [PMID: 18823439 DOI: 10.1111/j.1440-1746.2008.05596.x]

95 **Ding J**, Jing X, Liu J, Wang Y, Wang F, Wang Y, Du Z. Comparison of two different thermal techniques for the treatment of hepatocellular carcinoma. *Eur J Radiol* 2013; **82**: 1379-1384 [PMID: 23726122 DOI: 10.1016/j.ejrad.2013.04.025]

96 **Zhang L**, Wang N, Shen Q, Cheng W, Qian GJ. Therapeutic efficacy of percutaneous radiofrequency ablation versus microwave ablation for hepatocellular carcinoma. *PLoS One* 2013; **8**: e76119 [PMID: 24146824 DOI: 10.1371/journal.pone.0076119]

97 **Abdelaziz A**, Elbaz T, Shousha HI, Mahmoud S, Ibrahim M, Abdelmaksoud A, Nabeel M. Efficacy and survival analysis of percutaneous radiofrequency versus microwave ablation for hepatocellular carcinoma: an Egyptian multidisciplinary clinic experience. *Surg Endosc* 2014; **28**: 3429-3434 [PMID: 24935203 DOI: 10.1007/s00464-014-3617-4]

98 **Vogl TJ**, Farshid P, Naguib NN, Zangos S, Bodelle B, Paul J, Mbalisike EC, Beeres M, Nour-Eldin NE. Ablation therapy of hepatocellular carcinoma: a comparative study between radiofrequency and microwave ablation. *Abdom Imaging* 2015; **40**: 1829-1837 [PMID: 25601438 DOI: 10.1007/s00261-015-0355-6]

99 **Chinnaratha MA**, Chuang MY, Fraser RJ, Woodman RJ, Wigg AJ. Percutaneous thermal ablation for primary hepatocellular carcinoma: A systematic review and meta-analysis. *J Gastroenterol Hepatol* 2016; **31**: 294-301 [PMID: 26114968 DOI: 10.1111/jgh.13028]

100 **Facciorusso A**, Di Maso M, Muscatiello N. Microwave ablation versus radiofrequency ablation for the treatment of hepatocellular carcinoma: A systematic review and meta-analysis. *Int J Hyperthermia* 2016; In press

101 **Quesson B**, Merle M, Köhler MO, Mougenot C, Roujol S, de Senneville BD, Moonen CT. A method for MRI guidance of intercostal high intensity focused ultrasound ablation in the liver. *Med Phys* 2010; **37**: 2533-2540 [PMID: 20632565]

102 **Chan AC**, Cheung TT, Fan ST, Chok KS, Chan SC, Poon RT, Lo CM. Survival analysis of high-intensity focused ultrasound therapy versus radiofrequency ablation in the treatment of recurrent hepatocellular carcinoma. *Ann Surg* 2013; **257**: 686-692 [PMID: 23426335 DOI: 10.1097/SLA.0b013e3182822c02]

103 **Chok KS**, Cheung TT, Lo RC, Chu FS, Tsang SH, Chan AC, Sharr WW, Fung JY, Dai WC, Chan SC, Fan ST, Lo CM. Pilot study of high-intensity focused ultrasound ablation as a bridging therapy for hepatocellular carcinoma patients wait-listed for liver transplantation. *Liver Transpl* 2014; **20**: 912-921 [PMID: 24753206 DOI: 10.1002/lt.23892]

104 **Jacques SL**. Laser-tissue interactions. Photochemical, photothermal, and photomechanical. *Surg Clin North Am* 1992; **72**: 531-558 [PMID: 1589829]

105 **Giorgio A**, Tarantino L, de Stefano G N, Catalano O, Cusati B, Del Viscovo L A, Caturelli E. Interstitial laser photocoagulation under ultrasound guidance of liver tumors: results in 104 treated patients. *Eur J Ultrasound* 2000; **11**: 181-188 [PMID: 10874193 DOI: 10.1016/S0929-8266(00)00086-0]

106 **Pacella CM**, Bizzarri G, Francica G, Bianchini A, De Nuntis S, Pacella S, Crescenzi A, Taccogna S, Forlini G, Rossi Z, Osborn J, Stasi R. Percutaneous laser ablation in the treatment of hepatocellular carcinoma with small tumors: analysis of factors affecting the achievement of tumor necrosis. *J Vasc Interv Radiol* 2005; **16**: 1447-1457 [PMID: 16319150 DOI: 10.1097/01.RVI.90000172121.82299.38]

107 **Francica G**, Iodice G, Delle Cave M, Sarrantonio R, Lapiccirella G, Molese V, Smeraldo D, Scarano F, De Marino F. Factors predicting complete necrosis rate after ultrasound-guided percutaneous laser thermoablation of small hepatocellular carcinoma tumors in cirrhotic patients: a multivariate analysis. *Acta Radiol* 2007; **48**: 514-519 [PMID: 17520427 DOI: 10.1080/02841850701199942]

108 **Pacella CM**, Bizzarri G, Magnolfi F, Cecconi P, Caspani B, Anelli V, Bianchini A, Valle D, Pacella S, Manenti G, Rossi Z. Laser thermal ablation in the treatment of small hepatocellular carcinoma: results in 74 patients. *Radiology* 2001; **221**: 712-720 [PMID: 11719667 DOI: 10.1148/radiol.2213001501]

109 **Pacella CM**, Francica G, Di Lascio FM, Arienti V, Antico E, Caspani B, Magnolfi F, Megna AS, Pretolani S, Regine R, Sponza M, Stasi R. Long-term outcome of cirrhotic patients with early hepatocellular carcinoma treated with ultrasound-guided percutaneous laser ablation: a retrospective analysis. *J Clin Oncol* 2009; **27**: 2615-2621 [PMID: 19332729 DOI: 10.1200/JCO.2008.19.0082]

110 **Di Costanzo GG**, Tortora R, D'Adamo G, De Luca M, Lampasi F, Addario L, Galeota Lanza A, Picciotto FP, Tartaglione MT, Cordone G, Imparato M, Mattera S, Pacella CM. Radiofrequency ablation versus laser ablation for the treatment of small hepatocellular carcinoma in cirrhosis: a randomized trial. *J Gastroenterol Hepatol* 2015; **30**: 559-565 [PMID: 25251043 DOI: 10.1111/jgh.12791]

111 **Hutchinson M**, Shyn P, Silverman S. Cryoablation of Liver Tumors. In: Dupuy DE, Fong Y, McMullen WN, editors. Image-Guided

Cancer Therapy. New York: Springer Inc., 2013: 491-503
112 **Hu KQ**. Advances in clinical application of cryoablation therapy for hepatocellular carcinoma and metastatic liver tumor. *J Clin Gastroenterol* 2014; **48**: 830-836 [PMID: 25148553 DOI: 10.1097/MCG.0000000000000201]
113 **Mala T**, Samset E, Aurdal L, Gladhaug I, Edwin B, Søreide O. Magnetic resonance imaging-estimated three-dimensional temperature distribution in liver cryolesions: a study of cryolesion characteristics assumed necessary for tumor ablation. *Cryobiology* 2001; **43**: 268-275 [PMID: 11888220]
114 **Sheen AJ**, Siriwardena AK. The end of cryotherapy for the treatment of nonresectable hepatic tumors? *Ann Surg Oncol* 2005; **12**: 202-204 [PMID: 15827810]
115 **Rong G**, Bai W, Dong Z, Wang C, Lu Y, Zeng Z, Qu J, Lou M, Wang H, Gao X, Chang X, An L, Li H, Chen Y, Hu KQ, Yang Y. Long-term outcomes of percutaneous cryoablation for patients with hepatocellular carcinoma within Milan criteria. *PLoS One* 2015; **10**: e0123065 [PMID: 25849963 DOI: 10.1371/journal.pone.0123065]
116 **Wu S**, Hou J, Ding Y, Wu F, Hu Y, Jiang Q, Mao P, Yang Y. Cryoablation Versus Radiofrequency Ablation for Hepatic Malignancies: A Systematic Review and Literature-Based Analysis. *Medicine* (Baltimore) 2015; **94**: e2252 [PMID: 26656371 DOI: 10.1097/MD.0000000000002252]
117 **Wang C**, Wang H, Yang W, Hu K, Xie H, Hu KQ, Bai W, Dong Z, Lu Y, Zeng Z, Lou M, Wang H, Gao X, Chang X, An L, Qu J, Li J, Yang Y. Multicenter randomized controlled trial of percutaneous cryoablation versus radiofrequency ablation in hepatocellular carcinoma. *Hepatology* 2015; **61**: 1579-1590 [PMID: 25284802 DOI: 10.1002/hep.27548]
118 **Ei S**, Hibi T, Tanabe M, Itano O, Shinoda M, Kitago M, Abe Y, Yagi H, Okabayashi K, Sugiyama D, Wakabayashi G, Kitagawa Y. Cryoablation provides superior local control of primary hepatocellular carcinomas of & gt; 2 cm compared with radiofrequency ablation and microwave coagulation therapy: an underestimated tool in the toolbox. *Ann Surg Oncol* 2015; **22**: 1294-1300 [PMID: 25287439 DOI: 10.1245/s10434-014-4114-7]

P- Reviewer: Li Z, Senousy MA, Sheth RA **S- Editor**: Gong XM
L- Editor: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i4.490

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 490-502
ISSN 2150-5349 (online)

REVIEW

Treatment of pregnant women with a diagnosis of inflammatory bowel disease

Sule Poturoglu, Asli Ciftcibasi Ormeci, Ali Erkan Duman

Sule Poturoglu, Asli Ciftcibasi Ormeci, Ali Erkan Duman, Department of Gastroenterology, Haseki Training and Research Hospital, 34096 Istanbul, Turkey

Author contributions: Poturoglu S, Ormeci AC and Duman AE contributed equally to this work; Poturoglu S and Ormeci AC performed the literature search, designed and wrote the manuscript; Poturoglu S and Duman AE was responsible for critical revision of the manuscript for important intellectual content.

Conflict-of-interest statement: No conflicts of interest.

Manuscript source: Invited manuscript

Correspondence to: Sule Poturoglu, MD, Department of Gastroenterology, Haseki Training and Research Hospital, 34096 Istanbul, Turkey. sulepot@mynet.com
Telephone: +90-542-5257271
Fax: +90-212-5896229

Received: March 29, 2016
Peer-review started: April 4, 2016
First decision: May 23, 2016
Revised: August 20, 2016
Accepted: September 21, 2016
Article in press: September 22, 2016
Published online: November 6, 2016

Abstract

The frequency of diagnosis of inflammatory bowel disease (IBD) has increased in younger populations. For this reason, pregnancy in patients with IBD is a topic of interest, warranting additional focus on disease management during this period. The main objective of this article is to summarize the latest findings and guidelines on the management of potential problems from pregnancy to the breastfeeding stage. Fertility is decreased in patients with active IBD. Disease remission prior to conception will likely decrease the rate of pregnancy-related complications. Most of the drugs used for IBD treatment are safe during both pregnancy and breastfeeding. Two exceptions are methotrexate and thalidomide, which are contraindicated in pregnancy. Anti-tumor necrosis factor agents are not advised during the third trimester as they exhibit increased transplacental transmission and potentially cause immunosuppression in the fetus. Radiological and endoscopic examinations and surgical interventions should be performed only when absolutely necessary. Surgery increases the fetal mortality rate. The delivery method should be determined with consideration of the disease site and presence of progression or flare up. Treatment planning should be a collaborative effort among the gastroenterologist, obstetrician, colorectal surgeon and patient.

Key words: Pregnancy; Inflammatory bowel disease; Immunomodulators; Biologics; Breastfeeding; Treatment

Core tip: Active disease prior to conception and during pregnancy increases the rate of pregnancy-related complications; thus, special attention should be given to pregnancy during the disease remission period. The safest drugs for use during pregnancy and breastfeeding are 5-aminosalicylic acid complexes, thiopurines and corticosteroids. Methotrexate and thalidomide are contraindicated. Anti-tumor necrosis factor treatment should be avoided during the third trimester. The risk of venous thromboembolism is increased in patients with moderate-to-severe disease. The delivery method should be selected according to the region of the body involved and disease activity. In this article, the problems

encountered by patients with inflammatory bowel disease from pregnancy to breastfeeding are discussed, and appropriate management strategies are suggested.

Poturoglu S, Ormeci AC, Duman AE. Treatment of pregnant women with a diagnosis of inflammatory bowel disease. *World J Gastrointest Pharmacol Ther* 2016; 7(4): 490-502 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i4/490.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i4.490

INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC) are chronic, idiopathic diseases characterized by relapse and remission periods, and they constitute a major portion of the inflammatory bowel disease (IBD) spectrum. A multicentric epidemiological study performed in Europe found the incidence of UC to be 10.4/10000 and that of CD to be 5.6/10000[1]. The risk of IBD in children is increased 2-13-fold if one parent has IBD[2] and by 33%-36% if both parents have IBD[3,4]. The disease is most frequently diagnosed during the second and third decades of life. CD is diagnosed slightly more frequently in women than men (1.3:1), whereas the ratio is 1:1 for UC. Approximately 25% of female IBD patients are expected to become mothers during their disease period[5]. In other words, the disease affects prospective parents. For this reason, the effect of the disease on possible complications encountered during pregnancy and the effects of the treatment on the fetus, birth method selected and breastfeeding safety are sources of anxiety for patients. To alleviate such worries, physicians must increase their knowledge of and experience with such subjects and share this information with patients and their relatives.

Pregnancy does not cause IBD flare-ups; however, the disease can be exacerbated in IBD patients who become pregnant during the active phase of the disease[6]. Of those patients who become pregnant during the active phase, approximately two-thirds have active disease throughout their pregnancy term[7,8]. Approximately one-third of patients who become pregnant during the remission period experience a disease flare-up[9]. However, these proportions are identical to those in the general population. IBD flare-ups are often due to medication discontinuation during pregnancy, lactation and smoking resumption following birth[10]. Approximately one-third of patients have active disease during conception[11]. Nielsen *et al*[12] reported that the yearly exacerbation rate is 34% during pregnancy and 32% in non-pregnancy. Increased prevalences of premature birth, low birth weight, stillbirth, cesarean section and congenital anomalies have been reported in pregnant women with IBD[13,14]. Such complications are more frequent in patients with CD than those with UC. However, these meta-analyses did not take disease activity or medical treatments into consideration. Congenital malformation rates were increased in IBD patients in case-controlled studies conducted in Hungary[15] and Italy[16], as opposed to other studies that reported similar rates in IBD patients to those of the general population[17,18]. Some authors[19-21] suggest this difference is explained by disease activity, while others suggest it is due to the high numbers of low-activity patients included in the studies[10,22]. The prospective, case-controlled ECCO-EpiCom study[23] found no significant difference in pregnancy outcomes in pregnant IBD patients compared with the general pregnant population. In that study, logistic regression analyses showed that age > 35 years and tobacco smoking were risk factors for premature birth and congenital anomalies in CD patients and for premature birth in UC patients.

The chance of a normal birth is 85% in patients with UC and 83.5% in those with CD if the disease is in remission during conception[9]. Ideally, the patient should be in remission when trying to conceive.

The physician should inform the patient and her partner of the IBD-pregnancy interaction and its effects on pregnancy outcomes, the treatment risk to benefit ratio and the importance of remission maintenance in reducing fetal risk. It is essential to be aware of disease management protocols during pregnancy to alleviate patient fears.

In this article, our main objective is to evaluate the management of IBD patients during pregnancy by reviewing the effects of IBD treatment on the fetus and mother during pregnancy and lactation.

MEDICAL TREATMENT

Discontinuing medical treatment during pregnancy can further harm the fetus by causing a flare-up in the patient. The pregnancy risk profiles for conventional drugs used in IBD treatment, such as 5-aminosalicylic acid (5-ASA), steroids and immunomodulatory agents, are available. Experience with biological agents is also increasing. The United States Food and Drug Administration (FDA) has deemed almost all drugs safe for use during pregnancy and lactation, with the exception of methotrexate and thalidomide, which are pregnancy category X drugs (Tables 1 and 2).

5-ASA

All 5-ASAs (mesalazine, balsalazide, ipsalazide and sulfasalazine) are used to induce and maintain remission in patients with light-to-moderately active UC, and these drugs exert their effects by acting on the intestinal mucosa.

Sulfasalazine

Effect on pregnancy: Sulfasalazine and its metabolite sulfapyridine inhibit folate synthesis. Sulfasalazine and sulfapyridine cross the placental barrier and can be detected in umbilical cord blood at rates similar to those in maternal blood. For this reason, sulfasalazine

Table 1 Food and Drug Administration pregnancy categories

FDA category	Definition
A	Controlled studies in animals and women demonstrate no risks during the first trimester, and the possibility of fetal harm appears remote
B	Studies in animals have not demonstrated a fetal risk, but no controlled studies have been conducted in pregnant women, or animal studies have shown an adverse event that was not confirmed in controlled studies in women during the first trimester. Chance of fetal harm is remote but remains a possibility
C	No controlled studies have been conducted in women, and animal studies have shown adverse effects on the fetus, or studies in humans and animals are not available. Chance of fetal harm. Give only if potential benefit outweighs the risk
D	There are no controlled studies in women or animals, but positive evidence of fetal risk is available. It can still be used for life-threatening or serious diseases when there are no effective alternative drugs
X	Studies in animals or women have demonstrated fetal abnormalities. The drug is contraindicated in women who are pregnant or may become pregnant

FDA: Food and Drug Administration.

Table 2 Safety of inflammatory bowel disease medications during pregnancy and breastfeeding

Medication	FDA category	Comments during pregnancy	Comments during breastfeeding
Adalimumab	B	Low risk: Transported through the placenta late in the second and third trimester; avoid treatment in the last trimester	Compatible
5-Aminosalicylic acid preparations[1]	B	Low risk: Limited data for olsalazine; if using sulfasalazine, folic acid supplementation is mandatory	Enters breast milk; probably compatible
Amoxicillin/clavulanate	B	Low risk	Enters breast milk; probably compatible
Azathioprine/6-mercaptopurine	D	Low risk	Low transfer to infant; appears in the milk 4 h after ingestion
Budesonide/prednisone	C	Probably low risk, avoid during first trimester (potential risk of oral clefts)	Probably compatible; enters breast milk
Certolizumab	B	Low risk	Limited data; probably compatible
Ciprofloxacin	C	Limited data; not recommended	Compatible
Cyclosporine	C	Low risk	Contraindicated
Methotrexate	X	Contraindicated: Teratogenic	Contraindicated
Metronidazole	B	Low risk, avoid during the first trimester	Enters breast milk, not recommended
Natalizumab	C	Limited data; low risk	Limited data; probably compatible
Tacrolimus	C	Limited data; no increase in congenital anomalies	Contraindicated
Thalidomide	X	Contraindicated: Teratogenic	No data available; potential toxicity

[1]Asacol HD (due to the dibutyl phthalate content) and olsalazine are category C drugs. FDA: Food and Drug Administration.

treatment can be continued during pregnancy. However, since it results in folate deficiency, it must be supplemented with 2 mg folic acid daily[24].

A meta-analysis published in 2008 compared 642 pregnant women who used mesalazine, sulfasalazine or olsalazine with 1158 pregnant controls and found no increased risks of congenital anomalies, low birth weight or similar complications[25]. The rates of low birth weight, prematurity, spontaneous abortion, live births and birth defects were similar between sulfasalazine-using mothers and the general population[8].

Effect on breastfeeding: Sulfasalazine and its metabolite sulfapyridine pass into breast milk. However, although sulfasalazine replaces bilirubin, which is a serious issue, no clinically significant cases have been reported[26]. Diarrhea was reported in infants of mothers taking sulfasalazine[27]. In such cases, adjustment of the sulfasalazine dosage or discontinuing treatment is indicated.

Mesalazine

Effect on pregnancy: Although most 5-ASA drugs are pregnancy category B, olsalazine is category C. Mesalazine metabolites, especially N-acetyl mesalazine, cross the placental barrier[28,29]. The rate of congenital anomalies in babies of mesalazine-exposed mothers was no higher than that in babies of the general population[30,31]. Several studies have reported the use of 5-ASA during pregnancy to be generally safe[32-34].

Enteric-coated mesalamine with dibutyl phthalate (DBP) was reported to cause skeletal anomalies and negative effects on the male reproductive system in animal models[35]. Drugs that contain DBP and 5-ASA (Asacol or Asacol HD; Procter and Gamble Pharmaceuticals, Cincinnati, OH, United States) should be switched to another 5-ASA preparation during pregnancy. However, a study that compared 117 pregnant patients who used Asacol with 156 pregnant patients who used non-Asacol aminosalycilate drugs reported no significant differences in terms of congenital anomaly rates[36].

Effect on breastfeeding: 5-ASA is excreted at a low concentration *via* breast milk[37]. Infants of mothers using 5-ASA can develop diarrhea due to allergic reactions. In such cases, the treatment should be stopped immediately.

Steroids

Effect on pregnancy: Corticosteroids, particularly prednisolone, are classified as pregnancy category C drugs. Carmichael *et al*[38] reported increased incidences of cleft palate and cleft lip anomalies with the use of corticosteroids 1 mo before pregnancy or during the first trimester. However, other studies involving larger patient groups reported no such risk[31].

Several studies also suggest that high-dose corticosteroid usage might cause adrenal suppression by affecting the hypothalamus-pituitary-adrenal axis in newborns. However, one study also concluded that the long-term effects are unclear and the absolute effects on the fetus negligible[39].

The use of budesonide is also regarded as safe during pregnancy. Beaulieu *et al*[40] reported no side effects in eight pregnant patients with CD who used budesonide. Moreover, studies involving larger groups of patients who used budesonide for asthma treatment reported no increase in the rate of birth defects or stillbirths[41,42].

Effect on breastfeeding: As the concentrations of steroids that enter breast milk are low, steroid usage during breastfeeding is deemed safe[43]. However, no specific guidelines exist for prednisolone usage during lactation. If the mother is worried about breastfeeding during steroid treatment, she can stop breastfeeding during her steroid treatment and resume once the treatment is discontinued[44].

Thiopurines

Effect on pregnancy: Azathioprine is a pro-drug that is metabolized into 6-mercaptopurine (6-MP). Following its metabolism into 6-MP, it is again metabolized into its active 6-thioguanine (6-TG) and inactive 6-methylmercaptopurine (6-MMP) metabolites. These drugs damage chromosomes by disrupting nucleic acid synthesis. The FDA classifies these drugs as pregnancy category D, since animal models showed teratogenic effects at therapeutic or elevated dosages[45]. Yet, the significantly higher bioavailability of intraperitoneal or parenteral, compared with oral, thiopurines used for IBD treatment in animal models should not be overlooked. Intact azathioprine or 6-MP cannot cross the placental barrier, whereas 6-TG can[46]. In a prospective study that included 30 pregnant women, 6-TG levels decreased but 6-MMP levels increased during pregnancy; however, these changes did not cause myelotoxicity or hepatotoxicity[47]. After pregnancy, both metabolites returned to their pregestational levels. With the exception of a newborn whose mother had severe pre-eclampsia and pancytopenia during delivery and high alkaline phosphatase levels, 6-MMP was not detected in any of the newborns. No major congenital malformations were seen in those newborns. All newborns had normal Apgar scores, but 60% were diagnosed with anemia. Therefore, a complete blood count is advised for newborns whose mothers used thiopurines during pregnancy.

In daily clinical practice, gestational planning for IBD patients who take thiopurines and continuation of thiopurine usage during pregnancy pose a challenge for the physician. This is due to the numerous contradictory studies in the literature. Two more recent publications reported an increase in the risk of congenital anomalies with thiopurine usage[48,49]. However, these studies have been criticized for their small number of patients and other limitations, such as inclusion of both major and minor anomalies[50]. Other than the risk of congenital anomalies, other studies have reported a relationship between thiopurine usage and the incidences of preterm births and low birth weight[48,49,51].

However, a large number of recent studies showed no relationship between thiopurine usage and the risk of congenital anomalies. Goldstein[52] evaluated women who took azathioprine for various indications; after a review of birth defect records, no significant increases in malformation rates were found. The 20-year study by Ban *et al*[53] reported that neither MP nor any other drug is related to an increased risk of congenital anomalies. Beaugerie *et al*[54] and Coelho *et al*[55], *via* the CESAME study in France, compared 89 women exposed to thiopurine during pregnancy and 129 IBD patients without thiopurine exposure and found no increase in the risk of congenital anomalies in a sub-analysis. The meta-analysis published by Akbari *et al*[56] reported no increase in the risk of congenital anomalies or low birth weight but an increased risk of premature birth with thiopurine usage during pregnancy. Casanova *et al*[57] reported that thiopurine usage was not associated with pregnancy complications and actually predicted lower rates of obstetric complications and better pregnancy outcomes. The first results of the ongoing PIANO study[58], published in 2012, showed no increase in the rates of congenital anomalies or pregnancy complications in 317 pregnant women exposed to thiopurine during pregnancy. The infants of those exposed mothers were followed up and exhibited similar or better developmental parameters compared with infants of mothers who were not exposed to thiopurines[59]. This finding supports the results of another study in 2013 in which 30 babies of mothers taking thiopurines during pregnancy for both medical and psychosocial health reasons showed no differences compared with the control groups[60].

To summarize, thiopurine treatment should be continued during pregnancy to prevent flare-ups, as the risk of active disease outweighs the risk of thiopurine usage. A female patient using anti-tumor necrosis factor (TNF) therapy combined with thiopurines who

is planning to become pregnant can discontinue the thiopurines prior to pregnancy, considering the risk of infection[50]. Women who were not taking thiopurines prior to pregnancy are not advised to start thiopurine treatment during pregnancy, as thiopurines take a long time to act and pose a small risk of bone marrow suppression and pancreatitis[61].

Effect on breastfeeding: Thiopurines were detectable in breast milk 4 h after their ingestion, albeit at very low levels compared with serum plasma levels[62]. Thiopurine metabolites are almost undetectable in infants breastfed by mothers taking thiopurines[63]. Furthermore, no increase in the risk of infection was evident in babies of mothers treated with thiopurines[64]. Therefore thiopurine-using mothers have no issues during breastfeeding. However, mothers of infants with weak immune systems should exercise caution while breastfeeding during thiopurine treatment[10].

Methotrexate

Effect on pregnancy: When taken during the organogenesis period, methotrexate can cause methotrexate embryopathy or fetal/methotrexate syndrome, which is characterized by congenital extremity and craniofacial anomalies[65]. When taken during the third trimester, methotrexate can cause fetal toxicity, retardation of development and loss of the fetus[66,67]. Since it remains in the body for a prolonged period, the drug should be discontinued 3-6 mo before conception[68]. It is recommended that men also stop taking methotrexate 3 mo before conception. In addition, folic acid supplementation should commence 3 mo prior to and be continued during pregnancy.

While on methotrexate, the patient should be warned about its toxic effects on the fetus and advised to use at least two contraception methods to prevent pregnancy[69].

Effect on breastfeeding: Methotrexate is present in breast milk and can cause immunosuppression and neutropenia by accumulating in neonatal tissues. Therefore, it should not be used during pregnancy[70].

Cyclosporine

Effect on pregnancy: Cyclosporine is a pregnancy category C drug. Because cyclosporine can cross the placental barrier, it may exert adverse effects on the fetus[71]. In renal transplant patients, cyclosporine caused premature birth, low birth weight, gestational diabetes, maternal hypertension, pre-eclampsia and fluctuations in the levels of other drugs[72]. Another meta-analysis of the effects of cyclosporine usage on pregnancy (15 studies, 410 patients) reported the incidence of premature birth to be 56% and that of congenital anomalies to be 4.1%. However, the rate of congenital malformation is not significantly different from that in the normal population[73]. In a smaller study that included eight pregnant patients, seven were treated successfully for steroid-refractory ulcerative pancolitis, while one required infliximab (IFX) treatment and was treated successfully. Seven patients delivered healthy infants, and one fetus died in utero. Two newborns were premature, and no congenital anomalies were detected in any of the infants[74].

Effect on breastfeeding: Since very small amounts of cyclosporine pass the placental barrier, it is safe to use in nursing mothers. However, the possibility of immunosuppression in the infant must not be overlooked. If treatment with this drug is planned, the potential risks should be discussed with the mother.

Tacrolimus

Effect on pregnancy: Tacrolimus, like cyclosporine, is classified as a pregnancy category C drug by the FDA. A study that followed 37 female liver transplant patients for 13 years with 49 recorded births reported an increase in the rate of premature births but not in the rate of congenital anomalies[75]. In another report, a female patient treated with tacrolimus during pregnancy delivered a healthy baby[76]. As with cyclosporine, there are few data regarding tacrolimus, so the risk to benefit ratio should be evaluated before using this drug.

Effect on breastfeeding: Tacrolimus is reported to enter the breast milk at a rate of 0.05%. Therefore, there is no clear evidence that it must be stopped during breastfeeding[69].

Biological agents

The use of synthetic TNF inhibitors such as IFX, adalimumab, certolizumab pegol and golimumab for the treatment of IBD is increasing. These drugs are classified as pregnancy category B. Natalizumab, which is rarely used, is a pregnancy category C drug. Clinical experience with the use of anti-TNF agents during pregnancy is limited[77,78].

Effect on pregnancy: Transplacental transmission has been reported mostly for monoclonal antibodies (IFX, adalimumab and golimumab) and rarely for fusion proteins (etanercept). Transplacental transmission of monoclonal antibodies increases during pregnancy, and their concentration in umbilical blood becomes equal to or higher than that in maternal blood during the last trimester.

Infliximab

IFX is an IGG1-type monoclonal antibody that inhibits TNF-α. It cannot pass the placental barrier during the first trimester but is transmitted effectively during the third trimester[79]. Therefore, the fetus is not exposed to the drug during the organogenesis period, but following transplacental transmission during the third trimester, IFX remains in infant blood for a few months following

birth. Neither teratogenicity nor toxicity was detected in a study involving 35 pregnant women who used IFX[80]. No difference was seen in terms of pregnancy outcomes between patients using IFX and healthy controls[81]. However, neonatal death caused by intracerebral and pulmonary hemorrhage, premature birth and Fallot's tetralogy have been reported[82].

The TREAT Registry and IFX Safety Database are the two largest studies on this subject[83,84]. In the TREAT Registry, a prospective study involving CD patients, patients on IFX were compared with those not taking IFX. Thirty-six of 66 pregnant women were treated with IFX during pregnancy. Fetal malformations were not detected. In addition, there was no significant difference between the two groups in terms of neonatal complications and miscarriage.

The IFX Safety Database is a retrospective review of 96 patients using IFX. The treatment was generally stopped during the first trimester after the patients realized they were pregnant. Pregnancy outcomes were not different between patients who did and did not take IFX.

The IFX levels of six neonates of mothers taking IFX were higher than those of the mother, but IFX was cleared from the bloodstream of the infants after 2-7 mo[85]. This shows that IFX crosses the placental barrier easily during the third trimester. Moreover, the reticuloendothelial system of the infant is not sufficiently developed to clear the antibodies effectively.

The latest prospective PIANO study[58] included 1232 pregnant women, of whom 264 were treated with IFX, 151 with adalimumab, 67 with certolizumabpegol and 29 with combined biological agent-immunomodulatory therapy. No differences in parameters such as birth defects and infection rates were detected during the first year, but differences in weight and height were detected between infants who were exposed to anti-TNF therapy and those who were not[58]. The odds ratios for developing complications and for preterm birth with combined IFX-immunomodulatory therapy were 1.7 (1.0-2.2) and 2.4 (1.3-4.3), respectively. No difference was seen in the pregnancy outcomes of CD patients according to drug exposure, but increased risks of preterm birth and low birth weight and a prolonged stay in the intensive care unit were seen in UC patients taking combination therapy.

To summarize, IFX can be used during the first two trimesters of gestation. Patients are advised to stop IFX prior to the last trimester (30 wk). In patients with a flare-up caused by IFX treatment discontinuation, short-term corticosteroid treatment can be administered. Discontinuing IFX during the third trimester or at the end of the second trimester decreases IFX transportation to the placenta, reducing the level to which the neonate is exposed. Exposure to IFX during the third trimester can result in infections or a suboptimal response to vaccinations during the neonatal period. Live vaccines should not be administered during the first 6 mo in infants exposed to anti-TNF therapy[79]. Other vaccinations may proceed according to schedule.

Effect on breastfeeding: IFX is found in minute amounts in breast milk, and its oral absorption is minimal; thus, systemic adverse effects are rarely diagnosed[24,86]. IFX levels in an infant whose mother was taking IFX until 4 wk prior to birth decreased after 6 mo even though the mother continued to take IFX while breastfeeding[87].

However, data regarding the safety of IFX during breastfeeding and its local immunosuppression in the gastrointestinal system are insufficient.

Adalimumab

Adalimumab is an antibody targeting IGG1 that is actively transported through the placenta during pregnancy[79,88]. Similar to IFX, adalimumab crosses the placental barrier during the third trimester. Small observational studies on adalimumab during pregnancy have been conducted. It can be used from the time of conception through the first two trimesters of pregnancy. No increased risk of congenital malformation, spontaneous abortion or preterm birth was detected in pregnant women exposed to adalimumab. Although clinical experience with this drug in pregnant women is limited, the Organization for Teratology Information Specialists, which conducted a prospective study on adalimumab involving 27 pregnant women as well as a review of the birth outcomes of 47 pregnant women who used adalimumab during pregnancy, reported that the rates of spontaneous abortion, stillbirth, preterm birth and congenital anomalies were similar to those in the general population[89]. As this medication is used in weekly doses, it is difficult to discontinue treatment at the beginning of the third trimester as this might result in disease flare-ups. It is suggested to discontinue adalimumab 6-8 wk prior to birth.

Effect on breastfeeding: There are insufficient data on the safety of adalimumab use while breastfeeding. The drug passes into breast milk in small amounts, but no adverse effects have been reported[86]. However, discontinuing treatment while breastfeeding should be decided after reviewing the health of the mother and her IBD status.

Certolizumab

Certolizumab is a PEGylated Fab' fragment of an anti-TNFα monoclonal antibody. As opposed to IgG1 antibodies, Fab' fragments pass through the placenta by passive diffusion; therefore, placental transfer is minimal during the third trimester, unlike the cases with IFX and adalimumab[79]. Certolizumab levels in umbilical blood were very low (less than 2 ng/mL) in 10 pregnant women exposed to certolizumab[90]. In theory, it can be used from conception until birth, but the data are insufficient. Certolizumab excretion *via* breast milk is

minimal[78].

Natalizumab

Information regarding the safety of natalizumab use during pregnancy and lactation is insufficient. No increased risk of congenital malformation was detected in a study involving 164 pregnant women treated with natalizumab for CD or multiple sclerosis[91].

Golimumab

Golimumab is a new anti-TNF inhibitor, approved in 2013. Its effects on pregnancy are unclear. Lau *et al*[92] reviewed 42 pregnant women exposed to golimumab (10 pregnant UC patients) for congenital anomalies. These pregnancies resulted in 19 live births, 13 spontaneous abortions (miscarriages) and 6 elective abortions. Of the 13 mothers who experienced miscarriages, 30.8% received simultaneous methotrexate treatment. As golimumab is a new drug, information on the safety of its use during pregnancy is insufficient, and no evidence of its presence in breast milk is available.

Anti-diarrheal agents

Anti-diarrheal agents should be avoided during pregnancy, especially during the early period. Teratogenicity was detected in neonates exposed to diphenoxylate/atropine and loperamide, but whether this was due to chance or to the drugs was unclear[93,94].

Cathartics

A colon cleanse is necessary for sigmoidoscopy during pregnancy. No study has specifically addressed the teratogenic effects of cathartics; however, no congenital anomalies were seen among 22843 pregnant women treated with laxatives[95]. The FDA reclassified cathartics and laxative agents from category B to category X. Cathartics are associated with a risk of dehydration and electrolyte imbalance.

Magnesium citrate

Magnesium citrate has an FDA category B rating and thus is safe to use for constipation or prior to sigmoidoscopy. It might cause electrolyte imbalance and dehydration when used long term.

Polyethylene glycol solution

Polyethylene glycol (PEG) has a FDA category C rating. No data on the safety of PEG use during pregnancy are available.

COMPLICATIONS AND RISKY SITUATIONS IN PREGNANT IBD PATIENTS

Patients who underwent IBD-related surgery prior to pregnancy may experience a temporary increase in stool frequency. Incontinence can be seen especially during the third trimester, but this disappears after birth. Ileostomy can prolapse during the third trimester. Patients with a history of abdominal surgery and ileostomy reported subileus and ileus attacks[96]. The study by Nguyen *et al*[19], which used data from the Nationwide Inpatient Sample (NIS), compared pregnant women with CD or UC with healthy pregnant women without IBD. The frequencies of venous thromboembolism (VTE) and blood transfusions were increased in pregnant women with IBD. The risk of VTE is fourfold greater in pregnant than in non-pregnant IBD patients[97]. The risk of VTE is especially high during the first 6 wk postpartum[98]. Subcutaneous low-molecular-weight heparin is indicated during the peripartum period in high-risk (relapsed and hospitalized patients with moderate-to-severe disease activity) pregnant IBD patients. Heparin prophylaxis is also advised in pregnant IBD patients who are hospitalized for other reasons. VTE is common among UC patients, while the risk of antepartum hemorrhage is at least twofold higher in CD patients[99]. In another study, placental abruption was seen in 2% of IBD patients[19].

Gestational diabetes is another issue. The risk of gestational diabetes did not differ between IBD patients not on steroids and a control group[100]. However, the risk of gestational diabetes was increased in IBD patients who used steroids during pregnancy in the PIANO registry study, which included more than 1000 pregnant IBD patients[58]. Therefore, a pregnancy in a patient with IBD must be considered risky.

NUTRITION AND SUPPLEMENT TREATMENTS

Although nutrition is crucial for all pregnant women, it is especially so in pregnant women with IBD. The prevalence of malnutrition was sixfold higher in pregnant IBD patients compared with healthy controls[19]. A retrospective study in Canada reported less weight gain during pregnancy in IBD patients compared with the general pregnant population[17]. Since malnutrition during pregnancy poses a risk to the fetus, although enteral nutrition is preferred, parenteral nutrition should be used as soon as the need arises. There is no solid evidence of the benefits of specialized diets in terms of IBD remission[101]. A randomized controlled study reported that consumption of fish oil supplements increased the rate of pregnancy without affecting fetal growth[102]. In patients with anti-phospholipid antibody syndrome, fish oil supplements can be used to prevent miscarriage[103]. They also reduce the risk of preterm birth and miscarriage in pregnant IBD patients. Since fish oil is not classified as a drug, it has not been categorized by the FDA for use during pregnancy. Folic acid supplementation is essential during pregnancy for prevention of neural tube defects. If the patient is on sulfasalazine, daily folic acid intake should be increased accordingly (2-5 g/d)[96,102]. Calcium and vitamin D supplementation is advised in patients using steroids to prevent bone

loss. The patient should also be advised not to smoke. Smoking has a negative effect on pregnancy outcomes, particularly for patients with CD.

ENDOSCOPY

Endoscopic retrograde cholangiopancreatography can be performed if indicated[104].

SURGICAL TREATMENT

Surgical treatment indications in both UC (acute, severe or refractory colitis) and CD (perforation, abscess, severe hemorrhage or bowel obstruction) are identical in pregnant and non-pregnant patients. Surgical interventions should be conducted during the second trimester. However, surgeries performed due to acute indications in pregnant patients with IBD carry a high risk of losing the fetus[105]. Few studies have addressed the effect of surgery on maternal morbidity. Ileostomy should be performed in place of primary anastomosis during surgery[106]. Live and healthy births have been reported despite poor prognoses, intraperitoneal sepsis and surgical interventions[107].

There are case reports of colectomy surgery performed during the third trimester in combination with vaginal birth or cesarean section[108-110]. However, medical treatments are preferred to surgical treatments in non-emergency situations.

DELIVERY METHOD

Delivery by cesarean section is more frequent in IBD patients compared with the general population. Indeed, compared with the general population, the frequency of cesarean delivery is 1.5-fold higher in pregnant patients with CD but similar in those with UC[13,19]. Another study reported that the risk of elective cesarean section was twofold higher in pregnant patients with UC and even higher in those with CD[99]. This is likely due to the increased frequency of perianal diseases in CD patients. A retrospective questionnaire study reported that the risk of developing perianal disease was 18% in patients without prior perianal disease who gave birth vaginally (especially in episiotomy cases)[111]. This may also explain the increased preference for cesarean section. However, other studies involving larger patient groups did not support these findings[112,113]. There are insufficient data to recommend this delivery method for IBD patients[85]. Cesarean section is not thought to prevent disease flare-up or development of perianal diseases. It should be performed only when vaginal birth is contradicted in an individual patient. Vaginal birth exacerbates the disease in pregnant CD patients with active perianal disease and in UC patients with active rectal disease with a history of ileal pouch anal anastomosis (IPAA) with colectomy[85,114]. It is vital to prevent pouch dysfunction in IPAA patients and maintain sphincter functionality. Sphincter integrity can be disturbed by mechanical pressure during vaginal birth. Episiotomy has a risk of causing rectovaginal fistulas and non-healing wounds during periods of active perianal disease[115]. Forceps use and uncontrollable tears can negatively affect pelvic floor function. In general, vaginal birth is advised for pregnant IBD patients with light-to-moderate disease activity, whereas cesarean section is preferred for patients with IPAA or with fulminant or active perianal disease[102]. Performance of a cesarean section for reasons other than the above should be decided by the obstetrician for obstetric reasons.

Although there is a risk of complications caused by adhesions in patients who underwent previous pelvic or abdominal surgery or ileostomy or colostomy, vaginal birth is considered safe. Episiotomy can also be performed in such patients[96,116]. A questionnaire administered to 232 pregnant women with IPAA reported no difference in the rates of pouch-related complications or functional problems between vaginal birth and cesarean section[117]. The European Crohn's and Colitis Organization (ECCO) recommends cesarean section for patients with active perianal disease or active rectal involvement[114]. Furthermore, the presence of an ileo-anal pouch or ileo-rectal anastomosis is reported to be relative indications for cesarean section[114].

The delivery method should be decided through collaboration among the patient, obstetrician, gastroenterologist and colorectal surgeon.

CONCLUSION

Since active IBD can have negative effects on both the pregnant patient and the fetus, treatment should be performed in a conscious and energetic manner[118]. In the case of a planned pregnancy, disease remission should be maintained prior to conception. Most studies report increased rates of preterm, stillbirths, low birth weight and spontaneous abortions in pregnant patients with active disease during pregnancy[13,19]. For this reason, maintenance of disease remission is essential.

The drugs used to treat IBD are generally recognized as safe during both pregnancy and lactation, with the exception of methotrexate and thalidomide. Methotrexate and thalidomide should be discontinued in both men and women at least 3 mo prior to conception. Some clinicians tend to stop treatment during the first trimester. Moskovitz *et al*[30] evaluated 207 pregnancies and reported no significant difference in the effects of medication used during the first trimester *vs* any time during pregnancy on pregnancy outcomes. Although they are low risk, methotrexate and 6-MP should not be used in first pregnancies due to a possible risk of bone marrow suppression and pancreatitis. Cyclosporine can be used successfully during pregnancy but can cause preterm birth and low birth weight. As anti-TNF agents cross the placental barrier during the third trimester and might cause immunosuppression in newborns, the ECCO guidelines suggest discontinuing IFX and

adalimumab at 24-26 wk of gestation[114]. In cases in which discontinuing anti-TNF treatment can cause a disease flare-up, certolizumab can be used, as it has a low rate of transplacental transmission during the third trimester.

In the last decade, pregnancy outcomes in IBD patients who became pregnant during a remission period and maintained remission throughout pregnancy were reported to be normal. Patients should be informed of the importance of remission maintenance, and the risk to benefit ratio of continuing treatment during pregnancy should be discussed. The benefits of remission outweigh possible harm to the fetus caused by any potential drugs used.

Since malnutrition occurs more frequently in pregnant patients with IBD, nutritional supplements should be taken. Preventative measures for VTE should be implemented in hospitalized pregnant patients. Endoscopic retrograde cholangiopancreatography should be performed only if absolutely necessary. Surgical treatment indications are identical to those for non-pregnant patients with IBD but carry a high risk of fetal mortality. As previous surgical intervention can negatively affect fertility, laparoscopic methods should be used whenever possible.

The delivery method should be decided in collaboration with the patient. Vaginal birth is deemed safe in patients without perianal disease or severe active rectal involvement who have no complications.

The ECCO guidelines state that 5-ASA preparations, thiopurines, anti-TNF and corticosteroids carry a low risk for the infant[114]. When used during the third trimester, IFX can be transferred to the newborn through the placenta. Therefore, live vaccines are not advised during the first 6 mo after birth. IFX has not been detected in breast milk. However, IBD treatment planning in pregnant patients requires special attention, and decisions should be made on a case-by-case basis. Pregnant patients should be treated more aggressively than non-pregnant patients, as maintaining remission is crucial for pregnancy outcomes. The treatment method should be decided by consensus among the obstetrician, gastroenterologist and colorectal surgeon to reassure the patient.

ACKNOWLEDGMENTS

We would like to thank Yenal Dundar (Department of Health Services Research, University of Liverpool and Mersey Care NHS Trust) for revising the language of the manuscript.

REFERENCES

1 **Binder V**. Epidemiology of IBD during the twentieth century: an integrated view. *Best Pract Res Clin Gastroenterol* 2004; **18**: 463-479 [PMID: 15157821 DOI: 10.1016/j.bpg.2003.12.002]

2 **Orholm M**, Fonager K, Sørensen HT. Risk of ulcerative colitis and Crohn's disease among offspring of patients with chronic inflammatory bowel disease. *Am J Gastroenterol* 1999; **94**: 3236-3238 [PMID: 10566721 DOI: 10.1111/j.1572-0241.1999.01526.x]

3 **Bennett RA**, Rubin PH, Present DH. Frequency of inflammatory bowel disease in offspring of couples both presenting with inflammatory bowel disease. *Gastroenterology* 1991; **100**: 1638-1643 [PMID: 2019369]

4 **Laharie D**, Debeugny S, Peeters M, Van Gossum A, Gower-Rousseau C, Bélaïche J, Fiasse R, Dupas JL, Lerebours E, Piotte S, Cortot A, Vermeire S, Grandbastien B, Colombel JF. Inflammatory bowel disease in spouses and their offspring. *Gastroenterology* 2001; **120**: 816-819 [PMID: 11231934 DOI: 10.1053/gast.2001.22574]

5 **Malgarinos G**, Gikas A, Delicha E, Stamataki A, Georgopoulos F, Papadimitriou A, Stanciu C, Triantafillidis JK. Pregnancy and inflammatory bowel disease: a prospective case-control study. *Rev Med Chir Soc Med Nat Iasi* 2007; **111**: 613-619 [PMID: 18293689]

6 **Ferguson CB**, Mahsud-Dornan S, Patterson RN. Inflammatory bowel disease in pregnancy. *BMJ* 2008; **337**: a427 [PMID: 18599468 DOI: 10.1136/bmj.39566.681458.BE]

7 **Khosla R**, Willoughby CP, Jewell DP. Crohn's disease and pregnancy. *Gut* 1984; **25**: 52-56 [PMID: 6140209 DOI: 10.1136/gut.25.1.52]

8 **Mogadam M**, Dobbins WO, Korelitz BI, Ahmed SW. Pregnancy in inflammatory bowel disease: effect of sulfasalazine and corticosteroids on fetal outcome. *Gastroenterology* 1981; **80**: 72-76 [PMID: 6108894]

9 **Miller JP**. Inflammatory bowel disease in pregnancy: a review. *J R Soc Med* 1986; **79**: 221-225 [PMID: 2871190]

10 **Mahadevan U**, Sandborn WJ, Li DK, Hakimian S, Kane S, Corley DA. Pregnancy outcomes in women with inflammatory bowel disease: a large community-based study from Northern California. *Gastroenterology* 2007; **133**: 1106-1112 [PMID: 17764676 DOI: 10.1053/j.gastro.2007.07.019]

11 **Hosseini-Carroll P**, Mutyala M, Seth A, Nageeb S, Soliman D, Boktor M, Sheth A, Chapman J, Morris J, Jordan P, Manas K, Becker F, Alexander JS. Pregnancy and inflammatory bowel diseases: Current perspectives, risks and patient management. *World J Gastrointest Pharmacol Ther* 2015; **6**: 156-171 [PMID: 26558150 DOI: 10.4292/wjgpt.v6.i4.156]

12 **Nielsen OH**, Andreasson B, Bondesen S, Jarnum S. Pregnancy in ulcerative colitis. *Scand J Gastroenterol* 1983; **18**: 735-742 [PMID: 6669937 DOI: 10.3109/00365528309182088]

13 **Cornish J**, Tan E, Teare J, Teoh TG, Rai R, Clark SK, Tekkis PP. A meta-analysis on the influence of inflammatory bowel disease on pregnancy. *Gut* 2007; **56**: 830-837 [PMID: 17185356 DOI: 10.1136/gut.2006.108324]

14 **Dominitz JA**, Young JC, Boyko EJ. Outcomes of infants born to mothers with inflammatory bowel disease: a population-based cohort study. *Am J Gastroenterol* 2002; **97**: 641-648 [PMID: 11926208 DOI: 10.1111/j.1572-0241.2002.05543.x]

15 **Nørgård B**, Puho E, Pedersen L, Czeizel AE, Sørensen HT. Risk of congenital abnormalities in children born to women with ulcerative colitis: a population-based, case-control study. *Am J Gastroenterol* 2003; **98**: 2006-2010 [PMID: 14499779 DOI: 10.1111/j.1572-0241.2003.07578.x]

16 **Bortoli A**, Saibeni S, Tatarella M, Prada A, Beretta L, Rivolta R, Politi P, Ravelli P, Imperiali G, Colombo E, Pera A, Daperno M, Carnovali M, de Franchis R, Vecchi M. Pregnancy before and after the diagnosis of inflammatory bowel diseases: retrospective case-control study. *J Gastroenterol Hepatol* 2007; **22**: 542-549 [PMID: 17376049 DOI: 10.1111/j.1440-1746.2006.04754.x]

17 **Moser MA**, Okun NB, Mayes DC, Bailey RJ. Crohn's disease, pregnancy, and birth weight. *Am J Gastroenterol* 2000; **95**: 1021-1026 [PMID: 10763954 DOI: 10.1111/j.1572-0241.2000.01852.x]

18 **Naganuma M**, Kunisaki R, Yoshimura N, Nagahori M, Yamamoto H, Kimura H, Sako M, Kawaguchi T, Takazoe M, Yamamoto S, Matsui T, Hibi T, Watanabe M. Conception and pregnancy outcome in women with inflammatory bowel disease: A multicentre study from Japan. *J Crohns Colitis* 2011; **5**: 317-323 [PMID: 21683301 DOI: 10.1016/j.crohns.2011.02.003]

19 **Nguyen GC**, Boudreau H, Harris ML, Maxwell CV. Outcomes of obstetric hospitalizations among women with inflammatory bowel disease in the United States. *Clin Gastroenterol Hepatol* 2009; **7**:

329-334 [PMID: 19027089 DOI: 10.1016/j.cgh.2008.10.022]
20 **Morales M**, Berney T, Jenny A, Morel P, Extermann P. Crohn's disease as a risk factor for the outcome of pregnancy. *Hepatogastroenterology* 2000; **47**: 1595-1598 [PMID: 11149010]
21 **Bush MC**, Patel S, Lapinski RH, Stone JL. Perinatal outcomes in inflammatory bowel disease. *J Matern Fetal Neonatal Med* 2004; **15**: 237-241 [PMID: 15280131 DOI: 10.1080/14767050410001668662]
22 **Nørgård B**, Hundborg HH, Jacobsen BA, Nielsen GL, Fonager K. Disease activity in pregnant women with Crohn's disease and birth outcomes: a regional Danish cohort study. *Am J Gastroenterol* 2007; **102**: 1947-1954 [PMID: 17573787 DOI: 10.1111/j.1572-0241.2007.01355.x]
23 **Bortoli A**, Pedersen N, Duricova D, D'Inca R, Gionchetti P, Panelli MR, Ardizzone S, Sanroman AL, Gisbert JP, Arena I, Riegler G, Marrollo M, Valpiani D, Corbellini A, Segato S, Castiglione F, Munkholm P. Pregnancy outcome in inflammatory bowel disease: prospective European case-control ECCO-EpiCom study, 2003-2006. *Aliment Pharmacol Ther* 2011; **34**: 724-734 [PMID: 21815900 DOI: 10.1111/j.1365-2036.2011.04794.x]
24 **Dignass A**, Van Assche G, Lindsay JO, Lémann M, Söderholm J, Colombel JF, Danese S, D'Hoore A, Gassull M, Gomollón F, Hommes DW, Michetti P, O'Morain C, Oresland T, Windsor A, Stange EF, Travis SP. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Current management. *J Crohns Colitis* 2010; **4**: 28-62 [PMID: 21122489 DOI: 10.1016/j.crohns.2009.12.002]
25 **Rahimi R**, Nikfar S, Rezaie A, Abdollahi M. Pregnancy outcome in women with inflammatory bowel disease following exposure to 5-aminosalicylic acid drugs: a meta-analysis. *Reprod Toxicol* 2008; **25**: 271-275 [PMID: 18242053 DOI: 10.1016/j.reprotox.2007.11.010]
26 **Khan AK**, Truelove SC. Placental and mammary transfer of sulphasalazine. *Br Med J* 1979; **2**: 1553 [PMID: 43760 DOI: 10.1136/b mj.2.6204.1553]
27 **Branski D**, Kerem E, Gross-Kieselstein E, Hurvitz H, Litt R, Abrahamov A. Bloody diarrhea--a possible complication of sulfasalazine transferred through human breast milk. *J Pediatr Gastroenterol Nutr* 1986; **5**: 316-317 [PMID: 2870147 DOI: 10.1097/00005176-198605020-00028]
28 **Järnerot G**, Into-Malmberg MB, Esbjörner E. Placental transfer of sulphasalazine and sulphapyridine and some of its metabolites. *Scand J Gastroenterol* 1981; **16**: 693-697 [PMID: 6119765 DOI: 10.3109/00365528109182032]
29 **Nørgård B**, Fonager K, Pedersen L, Jacobsen BA, Sørensen HT. Birth outcome in women exposed to 5-aminosalicylic acid during pregnancy: a Danish cohort study. *Gut* 2003; **52**: 243-247 [PMID: 12524407 DOI: 10.1136/gut.52.2.243]
30 **Moskovitz DN**, Bodian C, Chapman ML, Marion JF, Rubin PH, Scherl E, Present DH. The effect on the fetus of medications used to treat pregnant inflammatory bowel-disease patients. *Am J Gastroenterol* 2004; **99**: 656-661 [PMID: 15089898 DOI: 10.1111/j.1572-0241.2004.04140.x]
31 **Nørgård B**, Pedersen L, Christensen LA, Sørensen HT. Therapeutic drug use in women with Crohn's disease and birth outcomes: a Danish nationwide cohort study. *Am J Gastroenterol* 2007; **102**: 1406-1413 [PMID: 17437503 DOI: 10.1111/j.1572-0241.2007.01216.x]
32 **Habal FM**, Hui G, Greenberg GR. Oral 5-aminosalicylic acid for inflammatory bowel disease in pregnancy: safety and clinical course. *Gastroenterology* 1993; **105**: 1057-1060 [PMID: 8405849]
33 **Marteau P**, Tennenbaum R, Elefant E, Lémann M, Cosnes J. Foetal outcome in women with inflammatory bowel disease treated during pregnancy with oral mesalazine microgranules. *Aliment Pharmacol Ther* 1998; **12**: 1101-1108 [PMID: 9845399 DOI: 10.1046/j.1365-2036.1998.00417.x]
34 **Trallori G**, d'Albasio G, Bardazzi G, Bonanomi AG, Amorosi A, Del Carlo P, Palli D, Galli M, Pacini F. 5-Aminosalicylic acid in pregnancy: clinical report. *Ital J Gastroenterol* 1994; **26**: 75-78 [PMID: 8032081]
35 **US Food and Drug Administration.** Asacol (mesalamine) delayed release tablets. May 2010
36 **Aparajita S**, Martin CF, Kane SV, Dubinsky M, Nguyen DD, McCabe RP, Rubin DT, Scherl EJ, Mahadevan U. Is Asacol use associated with congenital anomalies? Results from a nationwide prospective pregnancy registry. *Gastroenterology* 2013; **5**: S-379 [DOI: 10.1016/S0016-5085(13)61398-0]
37 **Habal FM**, Huang VW. Review article: a decision-making algorithm for the management of pregnancy in the inflammatory bowel disease patient. *Aliment Pharmacol Ther* 2012; **35**: 501-515 [PMID: 22221203 DOI: 10.1111/j.1365-2036.2011.04967.x]
38 **Carmichael SL**, Shaw GM. Maternal corticosteroid use and risk of selected congenital anomalies. *Am J Med Genet* 1999; **86**: 242-244 [PMID: 10482873 DOI: 10.1002/(SICI)1096-8628(19990917)86:3<242: : AID-AJMG9>3.0.CO; 2-U]
39 **Tegethoff M**, Pryce C, Meinlschmidt G. Effects of intrauterine exposure to synthetic glucocorticoids on fetal, newborn, and infant hypothalamic-pituitary-adrenal axis function in humans: a systematic review. *Endocr Rev* 2009; **30**: 753-789 [PMID: 19837868 DOI: 10.1210/er.2008-0014]
40 **Beaulieu DB**, Ananthakrishnan AN, Issa M, Rosenbaum L, Skaros S, Newcomer JR, Kuhlmann RS, Otterson MF, Emmons J, Knox J, Binion DG. Budesonide induction and maintenance therapy for Crohn's disease during pregnancy. *Inflamm Bowel Dis* 2009; **15**: 25-28 [PMID: 18680195 DOI: 10.1002/ibd.20640]
41 **Källén B**, Rydhstroem H, Aberg A. Congenital malformations after the use of inhaled budesonide in early pregnancy. *Obstet Gynecol* 1999; **93**: 392-395 [PMID: 10074986 DOI: 10.1097/00006250-199903000-00016]
42 **Norjavaara E**, de Verdier MG. Normal pregnancy outcomes in a population-based study including 2,968 pregnant women exposed to budesonide. *J Allergy Clin Immunol* 2003; **111**: 736-742 [PMID: 12704351 DOI: 10.1067/mai.2003.1340]
43 **McKenzie SA**, Selley JA, Agnew JE. Secretion of prednisolone into breast milk. *Arch Dis Child* 1975; **50**: 894-896 [PMID: 1211963 DOI: 10.1136/adc.50.11.894]
44 **Ng SW**, Mahadevan U. Management of inflammatory bowel disease in pregnancy. *Expert Rev Clin Immunol* 2013; **9**: 161-173; quiz 174 [PMID: 23390947 DOI: 10.1586/eci.12.103]
45 **Polifka JE**, Friedman JM. Teratogen update: azathioprine and 6-mercaptopurine. *Teratology* 2002; **65**: 240-261 [PMID: 11967923 DOI: 10.1097/00007890-200104270-00006]
46 **Nielsen OH**, Bjerrum JT, Herfarth H, Rogler G. Recent advances using immunomodulators for inflammatory bowel disease. *J Clin Pharmacol* 2013; **53**: 575-588 [PMID: 23408468 DOI: 10.1002/jcph.2]
47 **Jharap B**, de Boer NK, Stokkers P, Hommes DW, Oldenburg B, Dijkstra G, van der Woude CJ, de Jong DJ, Mulder CJ, van Elburg RM, van Bodegraven AA. Intrauterine exposure and pharmacology of conventional thiopurine therapy in pregnant patients with inflammatory bowel disease. *Gut* 2014; **63**: 451-457 [PMID: 23424097 DOI: 10.1136/gutjnl-2012-303615]
48 **Nørgård B**, Pedersen L, Fonager K, Rasmussen SN, Sørensen HT. Azathioprine, mercaptopurine and birth outcome: a population-based cohort study. *Aliment Pharmacol Ther* 2003; **17**: 827-834 [PMID: 12641505 DOI: 10.1046/j.1365-2036.2003.01537.x]
49 **Cleary BJ**, Källén B. Early pregnancy azathioprine use and pregnancy outcomes. *Birth Defects Res A Clin Mol Teratol* 2009; **85**: 647-654 [PMID: 19343728 DOI: 10.1002/bdra.20583]
50 **McConnell RA**, Mahadevan U. Use of Immunomodulators and Biologics Before, During, and After Pregnancy. *Inflamm Bowel Dis* 2016; **22**: 213-223 [PMID: 26444103 DOI: 10.1097/MIB.0000000000000596]
51 **Bröms G**, Granath F, Linder M, Stephansson O, Elmberg M, Kieler H. Birth outcomes in women with inflammatory bowel disease: effects of disease activity and drug exposure. *Inflamm Bowel Dis* 2014; **20**: 1091-1098 [PMID: 24810137 DOI: 10.1097/MIB.0000000000000060]
52 **Goldstein LH**, Dolinsky G, Greenberg R, Schaefer C, Cohen-Kerem R, Diav-Citrin O, Malm H, Reuvers-Lodewijks ME, Rost

van Tonningen-van Driel MM, Arnon J, Ornoy A, Clementi M, Di Gianantonio E, Koren G, Braunstein R, Berkovitch M. Pregnancy outcome of women exposed to azathioprine during pregnancy. *Birth Defects Res A Clin Mol Teratol* 2007; **79**: 696-701 [PMID: 17847119 DOI: 10.1002/bdra.20399]

53 **Ban L**, Tata LJ, Fiaschi L, Card T. Limited risks of major congenital anomalies in children of mothers with IBD and effects of medications. *Gastroenterology* 2014; **146**: 76-84 [PMID: 24126096 DOI: 10.1053/j.gastro.2013.09.061]

54 **Beaugerie L**, Brousse N, Bouvier AM, Colombel JF, Lémann M, Cosnes J, Hébuterne X, Cortot A, Bouhnik Y, Gendre JP, Simon T, Maynadié M, Hermine O, Faivre J, Carrat F. Lymphoproliferative disorders in patients receiving thiopurines for inflammatory bowel disease: a prospective observational cohort study. *Lancet* 2009; **374**: 1617-1625 [PMID: 19837455 DOI: 10.1016/S0140-6736(09)61302-7]

55 **Coelho J**, Beaugerie L, Colombel JF, Hébuterne X, Lerebours E, Lémann M, Baumer P, Cosnes J, Bourreille A, Gendre JP, Seksik P, Blain A, Metman EH, Nisard A, Cadiot G, Veyrac M, Coffin B, Dray X, Carrat F, Marteau P. Pregnancy outcome in patients with inflammatory bowel disease treated with thiopurines: cohort from the CESAME Study. *Gut* 2011; **60**: 198-203 [PMID: 21115547 DOI: 10.1136/gut.2010]

56 **Akbari M**, Shah S, Velayos FS, Mahadevan U, Cheifetz AS. Systematic review and meta-analysis on the effects of thiopurines on birth outcomes from female and male patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2013; **19**: 15-22 [PMID: 22434610 DOI: 10.1002/ibd.22948]

57 **Casanova MJ**, Chaparro M, Domènech E, Barreiro-de Acosta M, Bermejo F, Iglesias E, Gomollón F, Rodrigo L, Calvet X, Esteve M, García-Planella E, García-López S, Taxonera C, Calvo M, López M, Ginard D, Gómez-García M, Garrido E, Pérez-Calle JL, Beltrán B, Piqueras M, Saro C, Botella B, Dueñas C, Ponferrada A, Mañosa M, García-Sánchez V, Maté J, Gisbert JP. Safety of thiopurines and anti-TNF-α drugs during pregnancy in patients with inflammatory bowel disease. *Am J Gastroenterol* 2013; **108**: 433-440 [PMID: 23318480 DOI: 10.1038/ajg.2012.430]

58 **Mahadevan U**, Martin CF, Sandler RS, Kane SV, Dubinsky M, Lewis JD, Sands B E. 865 PIANO: a 1000 patient prospective registry of pregnancy outcomes in women with IBD exposed to immunomodulators and biologic therapy. *Gastroenterology* 2012; **142**: 149

59 **Lin K**, Martin CF, Chambers C, Kane SV, Dubinsky M, Sandborn W, Sands BE. Achievement of developmental milestones among offspring of women with inflammatory bowel disease: the PIANO registry. *Gastroenterology* 2014; **5**: S-1 [DOI: 10.1016/S0016-5085(14)60002-0]

60 **de Meij TG**, Jharap B, Kneepkens CM, van Bodegraven AA, de Boer NK. Long-term follow-up of children exposed intrauterine to maternal thiopurine therapy during pregnancy in females with inflammatory bowel disease. *Aliment Pharmacol Ther* 2013; **38**: 38-43 [PMID: 23675854 DOI: 10.1111/apt.12334]

61 **Ng SW**, Mahadevan U. My treatment approach to management of the pregnant patient with inflammatory bowel disease. *Mayo Clin Proc* 2014; **89**: 355-360 [PMID: 24582194 DOI: 10.1016/j.mayocp.2013.11.012]

62 **Christensen LA**, Dahlerup JF, Nielsen MJ, Fallingborg JF, Schmiegelow K. Azathioprine treatment during lactation. *Aliment Pharmacol Ther* 2008; **28**: 1209-1213 [PMID: 18761704 DOI: 10.1111/j.1365-2036.2008.03843.x]

63 **Gardiner SJ**, Gearry RB, Roberts RL, Zhang M, Barclay ML, Begg EJ. Exposure to thiopurine drugs through breast milk is low based on metabolite concentrations in mother-infant pairs. *Br J Clin Pharmacol* 2006; **62**: 453-456 [PMID: 16995866 DOI: 10.1111/j.1365-2125.2006.02639.x]

64 **Angelberger S**, Reinisch W, Messerschmidt A, Miehsler W, Novacek G, Vogelsang H, Dejaco C. Long-term follow-up of babies exposed to azathioprine in utero and via breastfeeding. *J Crohns Colitis* 2011; **5**: 95-100 [PMID: 21453877 DOI: 10.1016/j.crohns.2010.10.005]

65 **Milunsky A**, Graef JW, Gaynor MF. Methotrexate-induced congenital malformations. *J Pediatr* 1968; **72**: 790-795 [PMID: 5652604]

66 **Briggs GG**, Wan SR. Drug therapy during labor and delivery, part 1. *Am J Health Syst Pharm* 2006; **63**: 1038-1047 [PMID: 16709890]

67 **Del Campo M**, Kosaki K, Bennett FC, Jones KL. Developmental delay in fetal aminopterin/methotrexate syndrome. *Teratology* 1999; **60**: 10-12 [PMID: 10413333]

68 **Gisbert JP**. Safety of immunomodulators and biologics for the treatment of inflammatory bowel disease during pregnancy and breast-feeding. *Inflamm Bowel Dis* 2010; **16**: 881-895 [PMID: 19885906 DOI: 10.1002/ibd.21154]

69 **Nielsen OH**, Maxwell C, Hendel J. IBD medications during pregnancy and lactation. *Nat Rev Gastroenterol Hepatol* 2014; **11**: 116-127 [PMID: 23897285 DOI: 10.1038/nrgastro.2013.135]

70 **Johns DG**, Rutherford LD, Leighton PC, Vogel CL. Secretion of methotrexate into human milk. *Am J Obstet Gynecol* 1972; **112**: 978-980 [PMID: 5042796]

71 **van der Aa EM**, Peereboom-Stegeman JH, Noordhoek J, Gribnau FW, Russel FG. Mechanisms of drug transfer across the human placenta. *Pharm World Sci* 1998; **20**: 139-148 [PMID: 9762726]

72 **Paziana K**, Del Monaco M, Cardonick E, Moritz M, Keller M, Smith B, Coscia L, Armenti V. Ciclosporin use during pregnancy. *Drug Saf* 2013; **36**: 279-294 [PMID: 23516008 DOI: 10.1007/s40264-013-0034-x]

73 **Bar Oz B**, Hackman R, Einarson T, Koren G. Pregnancy outcome after cyclosporine therapy during pregnancy: a meta-analysis. *Transplantation* 2001; **71**: 1051-1055 [PMID: 11374400]

74 **Branche J**, Cortot A, Bourreille A, Coffin B, de Vos M, de Saussure P, Seksik P, Marteau P, Lemann M, Colombel JF. Cyclosporine treatment of steroid-refractory ulcerative colitis during pregnancy. *Inflamm Bowel Dis* 2009; **15**: 1044-1048 [PMID: 19137604 DOI: 10.1002/ibd.20858]

75 **Ecevit Ç**, Ünal F, Baran M, Aydoğdu S. Parenthood in pediatric liver transplant patients. *Pediatr Transplant* 2012; **16**: 346-349 [PMID: 22404458 DOI: 10.1111/j.1399-3046.2012.01671.x]

76 **Baumgart DC**, Sturm A, Wiedenmann B, Dignass AU. Uneventful pregnancy and neonatal outcome with tacrolimus in refractory ulcerative colitis. *Gut* 2005; **54**: 1822-1823 [PMID: 16120758 DOI: 10.1136/gut.2005.078972]

77 **O'Donnell S**, O'Morain C. Review article: use of antitumour necrosis factor therapy in inflammatory bowel disease during pregnancy and conception. *Aliment Pharmacol Ther* 2008; **27**: 885-894 [PMID: 18284649 DOI: 10.1111/j.1365-2036.2008.03648.x]

78 **Mahadevan U**, Cucchiara S, Hyams JS, Steinwurz F, Nuti F, Travis SP, Sandborn WJ, Colombel JF. The London Position Statement of the World Congress of Gastroenterology on Biological Therapy for IBD with the European Crohn's and Colitis Organisation: pregnancy and pediatrics. *Am J Gastroenterol* 2011; **106**: 214-223; quiz 224 [PMID: 21157441 DOI: 10.1038/ajg.2010.464]

79 **Mahadevan U**, Wolf DC, Dubinsky M, Cortot A, Lee SD, Siegel CA, Ullman T, Glover S, Valentine JF, Rubin DT, Miller J, Abreu MT. Placental transfer of anti-tumor necrosis factor agents in pregnant patients with inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2013; **11**: 286-292; quiz e24 [PMID: 23200982 DOI: 10.1016/j.cgh.2012.11.011]

80 **Katz JA**, Lichtenstein GR, Keenan GF, Healy D, Jacobs SJ. Outcome of pregnancy in women receiving REMICADE® (Infliximab) for the treatment of Crohn's disease or rheumatoid arthritis. *Gastroenterology* 2001; **120**: A69 [DOI: 10.1016/S0016-5085(08)80343-5]

81 **Friedman S**, Regueiro MD. Pregnancy and nursing in inflammatory bowel disease. *Gastroenterol Clin North Am* 2002; **31**: 265-273, xii [PMID: 12122737 DOI: 10.1016/S0889-8553(01)00015-2]

82 **Srinivasan R**. Infliximab treatment and pregnancy outcome in active Crohn's disease. *Am J Gastroenterol* 2001; **96**: 2274-2275 [PMID: 11467677 DOI: 10.1111/j.1572-0241.2001.03987.x]

83 **Lichtenstein GR**, Feagan BG, Cohen RD, Salzberg BA, Diamond RH, Chen DM, Pritchard ML, Sandborn WJ. Serious infections and

mortality in association with therapies for Crohn's disease: TREAT registry. *Clin Gastroenterol Hepatol* 2006; **4**: 621-630 [PMID: 16678077 DOI: 10.1016/j.cgh.2006.03.002]

84 **Katz JA**, Antoni C, Keenan GF, Smith DE, Jacobs SJ, Lichtenstein GR. Outcome of pregnancy in women receiving infliximab for the treatment of Crohn's disease and rheumatoid arthritis. *Am J Gastroenterol* 2004; **99**: 2385-2392 [PMID: 15571587 DOI: 10.1111/j.1572-0241.2004.30186.x]

85 **Dubinsky M**, Abraham B, Mahadevan U. Management of the pregnant IBD patient. *Inflamm Bowel Dis* 2008; **14**: 1736-1750 [PMID: 18626967 DOI: 10.1002/ibd.20532]

86 **Ben-Horin S**, Yavzori M, Kopylov U, Picard O, Fudim E, Eliakim R, Chowers Y, Lang A. Detection of infliximab in breast milk of nursing mothers with inflammatory bowel disease. *J Crohns Colitis* 2011; **5**: 555-558 [PMID: 22115374 DOI: 10.1016/j.crohns.2011.05.006]

87 **Vasiliauskas EA**, Church JA, Silverman N, Barry M, Targan SR, Dubinsky MC. Case report: evidence for transplacental transfer of maternally administered infliximab to the newborn. *Clin Gastroenterol Hepatol* 2006; **4**: 1255-1258 [PMID: 17045211 DOI: 10.1016/j.cgh.2006.07.018]

88 **Zelinkova Z**, van der Ent C, Bruin KF, van Baalen O, Vermeulen HG, Smalbraak HJ, Ouwendijk RJ, Hoek AC, van der Werf SD, Kuipers EJ, van der Woude CJ. Effects of discontinuing anti-tumor necrosis factor therapy during pregnancy on the course of inflammatory bowel disease and neonatal exposure. *Clin Gastroenterol Hepatol* 2013; **11**: 318-321 [PMID: 23103819 DOI: 10.1016/j.cgh.2012.10.024]

89 **Osting VC**, Carter JD. A safety assessment of tumor necrosis factor antagonists during pregnancy. *Expert Opin Drug Saf* 2010; **9**: 421-429 [PMID: 20367495 DOI: 10.1517/14740330903578639]

90 **Wolf D**, Mahadevan U. Certolizumab pegol use in pregnancy: low levels detected in cord blood. *Arthritis Rheu* 2010; **62**: 718 [DOI: 10.1002/art.28486]

91 **Mahadevan U**, Nazareth M, Cristiano L, Kooijmans M, Hogge G. Natalizumab use during pregnancy. *Am J Gastroenterol* 2008; **103**: S449-S450 [DOI: 10.1111/j.1572-0241.2008.02139_9.x]

92 **Lau AG**, Clark M, Harrison DD, Geldho A, Nissinen R, Sanders M. Pregnancy outcomes in women exposed to golimumab. *Arthritis Rheum* 2013; **65**: S870-S871 [DOI: 10.1002/art.38216]

93 **Lewis JH**, Weingold AB. The use of gastrointestinal drugs during pregnancy and lactation. *Am J Gastroenterol* 1985; **80**: 912-923 [PMID: 2864852]

94 **Briggs GG**, Freeman R K, Yaffe S J. Drugs in pregnancy and lactation: a reference guide to fetal and neonatal risk. USA: Lippincott Williams & Wilkins, 2012

95 **Acs N**, Bánhidy F, Puhó EH, Czeizel AE. No association between severe constipation with related drug treatment in pregnant women and congenital abnormalities in their offspring: A population-based case-control study. *Congenit Anom* (Kyoto) 2010; **50**: 15-20 [PMID: 20201964 DOI: 10.1111/j.1741-4520.2009.00252.x]

96 **Tilson RS**, Friedman S. Inflammatory Bowel Disease During Pregnancy. *Curr Treat Options Gastroenterol* 2003; **6**: 227-236 [PMID: 12744822 DOI: 10.1007/s11938-003-0004-y]

97 **Nguyen GC**, Sam J. Rising prevalence of venous thromboembolism and its impact on mortality among hospitalized inflammatory bowel disease patients. *Am J Gastroenterol* 2008; **103**: 2272-2280 [PMID: 18684186 DOI: 10.1111/j.1572-0241.2008.02052.x]

98 **Pomp ER**, Lenselink AM, Rosendaal FR, Doggen CJ. Pregnancy, the postpartum period and prothrombotic defects: risk of venous thrombosis in the MEGA study. *J Thromb Haemost* 2008; **6**: 632-637 [PMID: 18248600 DOI: 10.1111/j.1538-7836.2008.02921.x]

99 **Bröms G**, Granath F, Linder M, Stephansson O, Elmberg M, Kieler H. Complications from inflammatory bowel disease during pregnancy and delivery. *Clin Gastroenterol Hepatol* 2012; **10**: 1246-1252 [PMID: 22922307 DOI: 10.1016/j.cgh.2012.08.018]

100 **Leung YP**, Kaplan GG, Coward S, Tanyingoh D, Kaplan BJ, Johnston DW, Barkema HW, Ghosh S, Panaccione R, Seow CH. Intrapartum corticosteroid use significantly increases the risk of gestational diabetes in women with inflammatory bowel disease. *J Crohns Colitis* 2015; **9**: 223-230 [PMID: 25576754 DOI: 10.1093/ecco-jcc/jjv006]

101 **Zachos M**, Tondeur M, Griffiths AM. Enteral nutritional therapy for inducing remission of Crohn's disease. *Cochrane Database Syst Rev* 2001; **(3)**: CD000542 [PMID: 11686966 DOI: 10.1002/14651858.cd000542.pub2]

102 **Habal FM**, Ravindran NC. Management of inflammatory bowel disease in the pregnant patient. *World J Gastroenterol* 2008; **14**: 1326-1332 [PMID: 18322943 DOI: 10.3748/wjg.14.1326]

103 **Rossi E,** Costa M. Fish oil derivatives as a prophylaxis of recurrent miscarriage associated with antiphospholipid antibodies (APL): a pilot study. *Lupus* 1993; **2**: 319-323 [PMID: 8305926 DOI: 10.1177/096120339300200508]

104 **Cappell MS**, Colon VJ, Sidhom OA. A study at 10 medical centers of the safety and efficacy of 48 flexible sigmoidoscopies and 8 colonoscopies during pregnancy with follow-up of fetal outcome and with comparison to control groups. *Dig Dis Sci* 1996; **41**: 2353-2361 [PMID: 9011442 DOI: 10.1007/BF02100127]

105 **Ferrero S**, Ragni N. Inflammatory bowel disease: management issues during pregnancy. *Arch Gynecol Obstet* 2004; **270**: 79-85 [PMID: 12721680 DOI: 10.1007/s00404-003-0489-6]

106 **Visser BC**, Glasgow RE, Mulvihill KK, Mulvihill SJ. Safety and timing of nonobstetric abdominal surgery in pregnancy. *Dig Surg* 2001; **18**: 409-417 [PMID: 11721118 DOI: 10.1159/000050183]

107 **Hill J**, Clark A, Scott NA. Surgical treatment of acute manifestations of Crohn's disease during pregnancy. *J R Soc Med* 1997; **90**: 64-66 [PMID: 9068432 DOI: 10.1177/014107689709000203]

108 **Watson WJ**, Gaines TE. Third-trimester colectomy for severe ulcerative colitis. A case report. *J Reprod Med* 1987; **32**: 869-872 [PMID: 3430496]

109 **Bohe MG**, Ekelund GR, Genell SN, Gennser GM, Jiborn HA, Leandoer LJ, Klaus GL, Svanberg LK. Surgery for fulminating colitis during pregnancy. *Dis Colon Rectum* 1983; **26**: 119-122 [DOI: 10.1007/BF02562590]

110 **Boulton R**, Hamilton M, Lewis A, Walker P, Pounder R. Fulminant ulcerative colitis in pregnancy. *Am J Gastroenterol* 1994; **89**: 931-933 [PMID: 8198108]

111 **Kornfeld D**, Cnattingius S, Ekbom A. Pregnancy outcomes in women with inflammatory bowel disease--a population-based cohort study. *Am J Obstet Gynecol* 1997; **177**: 942-946 [PMID: 9369849 DOI: 10.1016/S0002-9378(97)70298-9]

112 **Ilnyckyji A**, Blanchard JF, Rawsthorne P, Bernstein CN. Perianal Crohn's disease and pregnancy: role of the mode of delivery. *Am J Gastroenterol* 1999; **94**: 3274-3278 [PMID: 10566729 DOI: 10.1111/j.1572-0241.1999.01537.x]

113 **Beniada A**, Benoist G, Maurel J, Dreyfus M. [Inflammatory bowel disease and pregnancy: report of 76 cases and review of the literature]. *J Gynecol Obstet Biol Reprod* (Paris) 2005; **34**: 581-588 [PMID: 16208201]

114 **van der Woude CJ**, Ardizzone S, Bengtson MB, Fiorino G, Fraser G, Katsanos K, Kolacek S, Juillerat P, Mulders AG, Pedersen N, Selinger C, Sebastian S, Sturm A, Zelinkova Z, Magro F. The second European evidenced-based consensus on reproduction and pregnancy in inflammatory bowel disease. *J Crohns Colitis* 2015; **9**: 107-124 [PMID: 25602023 DOI: 10.1093/ecco-jcc/jju006]

115 **Brandt LJ**, Estabrook SG, Reinus JF. Results of a survey to evaluate whether vaginal delivery and episiotomy lead to perineal involvement in women with Crohn's disease. *Am J Gastroenterol* 1995; **90**: 1918-1922 [PMID: 7484992 DOI: 10.1097/00006254-199606000-00006]

116 **Biedermann L**, Rogler G, Vavricka SR, Seibold F, Seirafi M. Pregnancy and breastfeeding in inflammatory bowel disease. *Digestion* 2012; **86** Suppl 1: 45-54 [PMID: 23051726 DOI: 10.1159/000341941]

117 **Hahnloser D**, Pemberton JH, Wolff BG, Larson D, Harrington J, Farouk R, Dozois RR. Pregnancy and delivery before and after ileal pouch-anal anastomosis for inflammatory bowel disease: immediate and long-term consequences and outcomes. *Dis Colon Rectum* 2004; **47**: 1127-1135 [PMID: 15164253 DOI: 10.1007/s10350-004-0569-0]

118 **Mountifield RE**, Prosser R, Bampton P, Muller K, Andrews JM. Pregnancy and IBD treatment: this challenging interplay from a patients' perspective. *J Crohns Colitis* 2010; **4**: 176-182 [PMID: 21122502 DOI: 10.1016/j.crohns.2009.10.001]

P- Reviewer: Actis GC, Desai DC, Gheita TA **S- Editor**: Gong ZM
L- Editor: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i4.503

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 503-512
ISSN 2150-5349 (online)

REVIEW

Logical hypothesis: Low FODMAP diet to prevent diverticulitis

Yoshiharu Uno, Jennifer C van Velkinburgh

Yoshiharu Uno, Office Uno Column (Medical Science Publisher), Onoe-Cho, Hyogo 675-0025, Japan

Jennifer C van Velkinburgh, van Velkinburgh Initiative for Collaboratory Biomedical Research, Santa Fe, NM 87501, United States

Author contributions: Uno Y contributed to creation of theory, reference collection, and creation of the table and figures; Uno Y and van Velkinburgh JC contributed equally to the writing of the manuscript.

Conflict-of-interest statement: Uno Y was granted a trademark of low FODMAP diet from the Japan Patent Office. van Velkinburgh JC declares no conflicts of interest in relation to the publication of this review.

Manuscript source: Unsolicited manuscript

Correspondence to: Yoshiharu Uno, MD, PhD, Office Uno Column (Medical Science Publisher), Onoe-Cho, Yota 419-2, Hyogo 675-0025, Japan. yoshiharu333@hotmail.com
Telephone: +81-79-4394697
Fax: +81-79-4394697

Received: April 27, 2016
Peer-review started: April 28, 2016
First decision: July 4, 2016
Revised: August 4, 2016
Accepted: August 15, 2016
Article in press: August 17, 2016
Published online: November 6, 2016

Abstract

Despite little evidence for the therapeutic benefits of a high-fiber diet for diverticulitis, it is commonly recommended as part of the clinical management. The ongoing uncertainty of the cause(s) of diverticulitis confounds attempts to determine the validity of this therapy. However, the features of a high-fiber diet represent a logical contradiction for colon diverticulitis. Considering that Bernoulli's principle, by which enlarged diameter of the lumen leads to increased pressure and decreased fluid velocity, might contribute to development of the diverticulum. Thus, theoretically, prevention of high pressure in the colon would be important and adoption of a low FODMAP diet (consisting of fermentable oligosaccharides, disaccharides, monosaccharides, and polyols) may help prevent recurrence of diverticulitis.

Key words: Diverticular disease; High-fiber diet; Low FODMAP diet; Bernoulli's principle; Irritable bowel syndrome

Core tip: The ongoing uncertainty of the cause(s) of diverticulitis confounds attempts to determine the validity of this therapy; however, the features of a high-fiber diet represent a logical contradiction for colon diverticulitis. Prevention of high pressure in the colon may help to avoid or correct diverticulitis, and this may be achieved by adoption of a low FODMAP diet (restriction of fermentable oligosaccharides, disaccharides, monosaccharides, and polyols).

Uno Y, van Velkinburgh JC. Logical hypothesis: Low FODMAP diet to prevent diverticulitis. *World J Gastrointest Pharmacol Ther* 2016; 7(4): 503-512 Available from: URL: http://www.

wjgnet.com/2150-5349/full/v7/i4/503.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i4.503

INTRODUCTION

The theory of colon diverticulitis and diet association was expanded upon by Painter[1], first in 1969 when he reported that diverticular disease (DD) occurred in people who ate a low residue diet with refined flour and sugar, then again in 1970 when he stated there was no DD in Africa[2]. Painter went on to explain that Denis Parsons Burkitt, the famed United Kingdom surgeon and dietary fiber proponent, provided a personal communication of his observation of short oro-anal transit time in Africans. This information helped to inspire Painter to theorize that a low residue intake related to, what he described as, the "civilized diet" would lead to a viscous stool that passes through the colon more slowly, and that this difference in fecal consistency would explain the incidence of DD in the civilized nations. Moreover, Painter recommended a diet with high residue intake, such as that consisting of wholemeal bread, unprocessed bran, porridge and fruit, replace the traditional low-residue diet of the civilized nations to reduce risk of DD. In 1971, Painter and Burkitt[3] jointly published their "fiber hypothesis" for DD, suggesting that a diet based on unrefined, natural foods with adequate fiber may prevent DD.

Subsequent studies found that intake of a higher fiber diet led to increased volume and less viscous feces accompanied by a shorter transit time[4-6], thereby preventing the rise of internal pressure in the large intestine. Advocates of the fiber diet suggested that it would help to spread the lumen of the large intestine, thereby suppressing the excessive contraction that would otherwise be caused by large amounts of compacted feces. These findings have led to the widely accepted theory that DD is strongly related to constipation[7].

The most important data published so far in support of the fiber hypothesis is that showing a correlation between amount of feces and transit time. In particular, the relationship between stool volume and transit time is not inverse, but is exponential [*i.e.,* log(time) = 2.81633 - 0.56057log(weight)][4]. It is not feasible to shorten transit time for stools over 300 g; therefore, theoretically, the effectiveness of high-fiber diet is limited. Methanogenesis has been linked to the presence of diverticulosis[8], and cellulose, which is contained in dietary fibers, is fermented by methane-producing bacteria[9]. A very recent study used a gas-sensing capsule to measure gas produced by diets of various fiber content found that the high-fiber diet produced more gas in the large intestine than the low-fiber diet[10]. Therefore, ingestion of excess dietary fiber may exacerbate the conditions that support accumulation of feces and gas in the intestine.

Although the mechanism underlying diverticula generation remains unknown, the fiber hypothesis has been widely adopted as an appropriate intervention. Indeed, over the past 45 years, the fiber hypothesis itself has become the basis for dietary advice of DD. The most current patient guide published by the American Gastroenterology Association (AGA) formally recommends a daily fiber intake of at least 25 g[11].

Yet, findings from several recent studies have cast doubt on the validity of the high-fiber hypothesis[12-16]. A subsequent study by Peery *et al*[17] found no association between a low-fiber diet and DD. More concerning, however, were their results from the cross-sectional study of 2104 participants between the ages of 30 and 80 years old, and including 878 cases of DD and 1226 controls without DD, which indicated that high total fiber intake was associated with an increased prevalence of multiple diverticula. The particular fiber subtypes that showed significant association with the occurrence of multiple diverticula were grains, insoluble fiber, and soluble fiber. Another study by the same group a year later found no increased risk of diverticulosis in the descending or sigmoid colon associated with either less frequent bowel movements or symptoms of constipation, and no association between dietary fiber intake and diverticulosis[18]. A subsequent study by Braunschmid *et al*[19] found that colonic diverticular disease did not correlate with constipation symptoms.

PATHOPHYSIOLOGY OF DIVERTICULA

Muscle layer of the colon and diverticula

Theoretically, formation of a mucosal hernia requires intraluminal high pressure and a pre-existing defect in the involved muscle layer (*i.e.,* weakened integrity). Normally, the muscle layer of the human colon is composed of circular muscle and longitudinal muscle, the latter of which is bound as three separate formations (*i.e.,* the taenia) that run from the cecum to the distal portion of the sigmoid colon[20]. When surgical specimens of diverticulitis in sigmoid colon were examined using electron microscopy, the taenia showed up-regulated elastin, with concentration levels greater than 200% compared to those in controls. Increased elastin in the region of the colonic tissue afflicted by diverticulitis may cause unequal elasticity and strength compared to the adjacent areas of unafflicted tissues[21]. Moreover, several conditions that cause the colon tissue to thicken, such as inflammatory or infectious conditions, affect elasticity, as increased thickness leads to reduced tensile strength. Finally, prevalence of colonic DD has been correlated with advancing age[22], presumably due to the large intestine wall becoming brittle. Intriguingly, studies of both European, Asians and African populations have shown similar findings of sigmoid colon strength decreasing with age[23]. Japanese have shown descending colon (lateral direction) strength decreasing with age (Figure 1)[24].

Investigation of colonic tearing during colonoscopy showed that when the sigmoid colon wall is affected by pressure forces from the inside the muscularis propria ruptures first, followed by the serosa and the mucosa

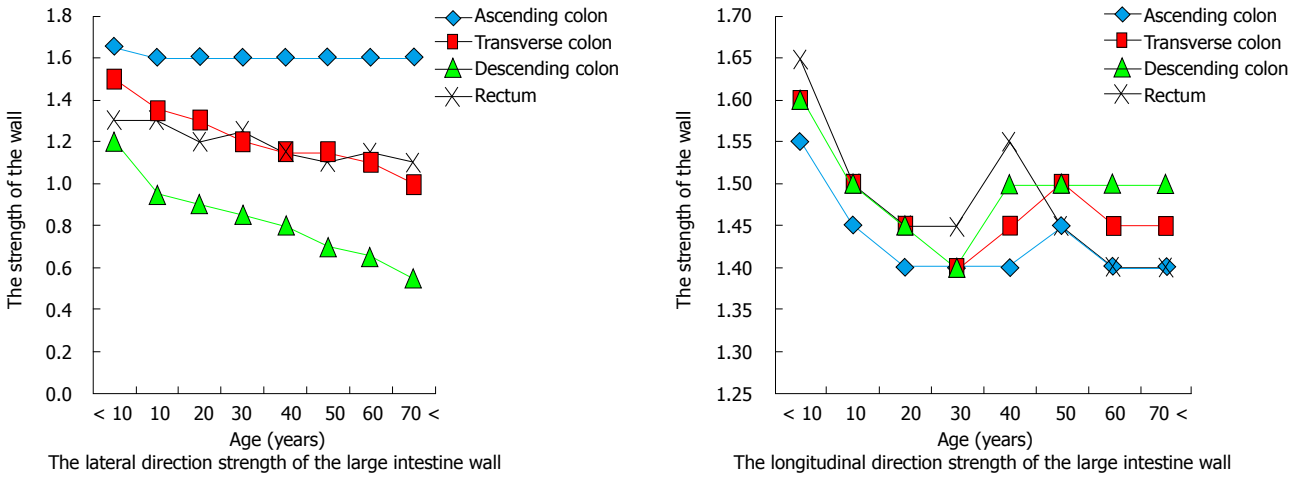

Figure 1 Change of strength with aging of the wall of the large intestine. The descending colon (lateral direction) shows reduction in strength related to aging. In contrast, the ascending colon shows no reduction in strength related to aging.

sequentially[25]. As such, the muscle layer appears to be the weakest point in the wall of the colon, even under physiologic conditions. Thus, considering the collective data of factors that affect the structural integrity of the distal colon, it can be hypothesized that development of diverticula in this area may be related to vulnerability of the intestinal wall that increases with age.

In contrast to the descending colon, the ascending colon shows no reduction in strength associated with aging (Figure 1). Hard stool is usually not present in the proximal colon, and this region does not form a closed space. Theoretically, herniation in the proximal colon requires the presence of a natural vulnerability.

Not all animals have taenia of the colon, and those that do are humans and monkeys among the primates, horses, and guinea pigs and rabbits among the rodents[26]. While natural occurrence of DD has been reported in monkeys and horses, the rodents are used for study of diverticula since the condition can be created experimentally and their small size facilitates convenient research investigation[27-30]. These features of diverticula in the animals with taenia have led to suspicion of a causal relationship between the two.

Colonic DD are most frequently located along the side of taenia[31]. It has thus been speculated that these sites represent the weakest points in the colon muscles, possibly explained by the fact that they are where penetrating vessels cross through[7,32-35]. It is theorized that until diverticulum formation is complete, the outpouching process is advanced by ongoing forces of pressure; although, this hypothesis has not yet been proven experimentally. In line with this theory, however, it is believed that DD does not occur between the tenia libera and tenia omentalis of the transverse colon because of the low vasculature at this site.

A report from 1925 by Lineback[36] demonstrates defects on both sides of the taenia. The circular muscle forms a convergence at the site that is in contact with the longitudinal muscle, and the most frequent site of diverticula is the cleft between the bundles of circular muscle (Figure 2).

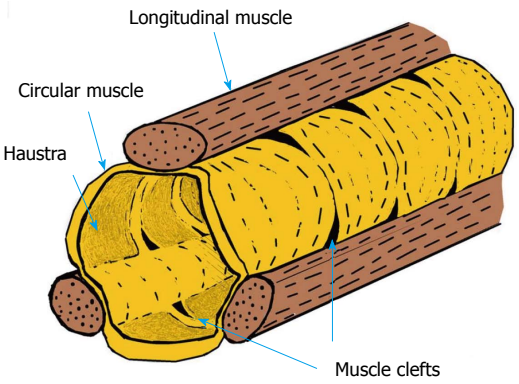

Figure 2 Muscle clefts.

Furthermore, since circular muscles and longitudinal muscles are connected, these clefts may be widened upon contraction of the muscle. This theory does not contradict the fact that multiple diverticula of the same size are present simultaneously. Moreover, these clefts may contain blood vessels[36], and may be present from birth. In line with this theory is the explanation as to DD not occurring between the tenia libera and tenia omentalis of the transverse colon because of the low influence of gas at this site.

Intraluminal pressure after diverticulitis

It is widely believed that feces produced by a high-fiber diet increases colon diameter and in turn decreases intraluminal pressure, in accordance with Laplace's law (wall tension = pressure × radius)[35,37]. This idea is based upon the theory of "segmentation hypothesis" that was first put forth by Painter, in which he described the colon obstructed at both ends as an enclosed space that acts as a series of "little bladders"[1,7]. However, the large intestine is functionally and structurally different from a bladder; indeed, it is a continuous space without

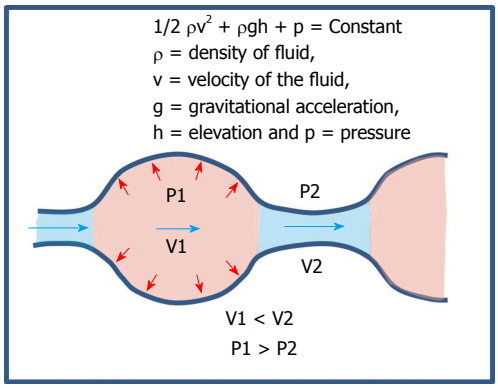

Figure 3 According to Bernoulli's principle, the pressure at the extended site is increased.

complete obstruction, with its outlet at the anus. Besides, in order for this theory to be feasible and valid the colon wall must be flexible enough to adapt the Laplace's law. In patients who have optimal contractility of the intestines, the high-fiber diet may be an effective prevention or intervention measure. However, in patients who have an intestinal wall that is stiff or has excessive contraction, the intestinal tract will not extend adequately in response to the high-fiber diet, leading to higher intraluminal pressure and consequent increased forces acting on the intestinal wall.

Colon affected by diverticulitis shows thickened wall and signs of post-inflammatory fibrosis[38]. *In vivo* studies using colon manometry in patients with diverticulitis showed motility of the intestinal tract as being increased in the descending colon and sigmoid colon but no concomitant increase in the rectum[39,40]. Another study using CT colonography (colonoscopy) to assess symptoms of patients with diverticula in the sigmoid colon showed that pain was associated with air pressure[41]. Considering these data, high bulk feces produced by a high-fiber diet would not be expected to prevent the recurrence of diverticulitis. Not only that but, theoretically, this diet might even promote the symptoms of diverticulitis and pose a risk of recurrence.

Dynamics of fluid and gas

High pressure in the lumen of the colon cannot be produced by small hard stool alone. Although multiple hard stools can accumulate in the intestinal lumen, they cannot exclusively explain the influences of pressure.

In theory, in order to obtain a pressure increase sufficient to affect the intestinal wall, a substance that can rapidly move in the colon is required; such a substance would be gaseous or liquid in form. In 1964, Painter[7,42] used a fluid (barium) to produce internal pressure in the intestinal tract and showed that pressures > 50 mmHg were reached in the closed sigmoid colon; additionally, the author theorized that the pressure levels may have reached > 90 mmHg, but limitations of the equipment precluded accurate measurement. The higher forces (specifically 56-80 mmHg) were confirmed by other studies using barium enema administration from a height of 3 feet (91 cm) above the examination table, and these pressures were considered safe[43]. However, when the barium enema was administered from a height of 6 feet (1.8 m) above the table the intraluminal colonic pressures reached 140-168 mmHg, which surpass the threshold of safety and can cause perforation[43].

Intestinal perforation caused by air pressure has been the subject of many studies[44-49]. The upper limit of the safety air pressure of the endoscope is 80 mmHg[50]. Brayko *et al*[51] studied the characteristics of high-pressure perforation in serosa and mucosa and found that the pressures required for rupture (202 mmHg and 226 mmHg respectively) translated to low risk of perforation of the diverticula in normal endoscopy[51]. Another study, however, showed that the lower pressure forces of CT colonography (38-40 mmHg) can induce abdominal pain[41]. Thus, the human colon can feel pain caused by air pressure at ⩾ 40 mmHg, but the risk of perforation occurs at ⩾ 80 mmHg. While the diverticulum may be induced in the cleft of the muscle layer by a pressure of < 80 mmHg, it is not easily ruptured due to the strength of the mucosa and serosa (which require pressures > 200 mmHg). Considering that most cases of diverticula present as asymptomatic (without abdominal pain), it is likely then that the pressure required for completion of a new diverticulum might be 40 mmHg or less.

Intestinal pressure is affected by the dynamics of liquid as well as air. Compressed gas, according to Boyle's law, has a higher energy than the uncompressed liquid. Therefore, the presence of liquid will increase air pressure in a confined space such as the intestine. A study using barium contrast showed that the transit time in the proximal sigmoid of patients with DD was twice as fast as that in the non-DD control group, but the total time of gastrointestinal emptying was similar in both groups[52]. These results can be explained by Bernoulli's principle, which states that if the diameter of the lumen is large, the pressure will be increased and the fluid velocity will be decreased (Figure 3). Considering this law, narrowing of the rectum will be expected to increase the internal pressure of the sigmoid colon, and when the descending colon is contracted, the pressure of the proximal colon will be expected to be increased. There are seven sphincters located along the length of the colon[53]. The hydrodynamics of each sphincter and influence of its contraction (including the Haustral type) may be explained by Bernoulli's principle (Figure 4). Thus, the difference in frequency of right DD and left DD may be related to differences in pressure at each site.

Japan has a high incidence of DD in the proximal colon[54]. Moreover, study of Japanese cases of DD, but specifically with the condition affecting the right side, showed a high intraluminal pressure (> 20 mmHg) and abnormal motility in the ascending colon[55]. When another group examined the dynamics of gas pressures by scintigraphy they found that gas generated in the right and left colon does not move, as evidenced by

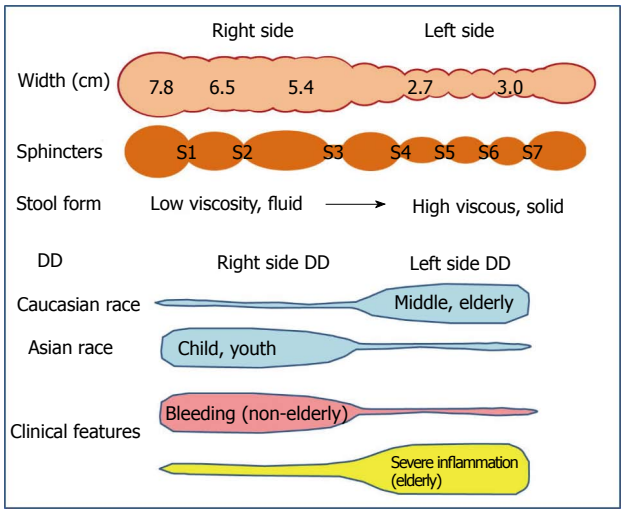

Figure 4 Distinctions in the background and symptoms of right and left diverticular disease. The width of the right colon is about twice that of the left colon[53]. The large intestine contains seven sphincters[53], including S1: Busi ring; S2: Hirsh ring; S3: Cannon ring; S4: Payr-Straus ring; S5: Balli ring; S6: Moultier ring; S7: Rossi ring. Blue-colored areas represent the frequency at the site. In Caucasians, diverticular disease (DD) most frequently occurs on the left side and after middle age. In Asians, DD most frequently occurs on the right side and during childhood. Right-side diverticula is more likely to bleed but less likely to develop the severe diverticula-associated complications of perforation, abscess formation, fistulation, or structuring[7,54].

observations at 60 min post-injection when gas injected into the jejunum remained in the cecum and the right colon and gas injected into the rectum remained in the recto-sigmoid colon[56]. Thus, the segmental location at which gas is fermented will be impacted by the corresponding pressure.

It has already been established that excessive pressure in the colon is related to the intraluminal concentrations of both gas and water. The next question of interest is then, what is the cause of increased levels of gas and the water in the colon?

Dietary fiber increases gas within the colon[10,57]. The primary dietary fiber contained in vegetable and fruit is inulin, and its intake leads to flatulence[58]. The 2015 AGA guidelines cited at the beginning of this article recommend that dietary fiber be obtained through intake of legumes (as lentils), yogurt, and fresh fruit[11]. However, yogurt and beans contain oligosaccharides, both of which are known to generate gas in the gut by fermentation[59,60]. In the case of individuals with fructose intolerance, eating of fresh fruit can result in abdominal pain, belching, bloating, an uncomfortable feeling of fullness, indigestion, and diarrhea[61]. Therefore, the diet components recommended by the AGA are expected to produce a substantial amount of gas in the intestines.

ALTERNATIVE DIET FOR DIVERTICULITIS

Recently, there has been increasing interest in developing diet-based therapies for IBS, and the diet consisting of low fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAP) appears a promising candidate. FODMAPs are not digested or absorbed in the small intestine[62,63]. Therefore, their intake causes increased fluid in the ileum due to the corresponding high osmotic pressure; in addition, they lead to a large amount of gas produced by fermentation in the colon. The daily adoption of the low FODMAP diet by patients with IBS has led to significant improvements in symptoms[64,65].

The detrimental impact of a high FODMAP diet on the intestinal tract has been shown by a study in which the participants drank lactulose[66], which has been demonstrated by MRI to increase fluid content in the ileum[67]. Since this study measured the lactulose-induced increase of gas indirectly, with the hydrogen breath test, a more recent study of the FODMAP diet obtained a direct confirmation of the increasing intestinal gas by abdominal X-ray[68]; these findings, thus, support the theory that the FODMAP diet increases the intraluminal volume of gas and fluid.

The breath test, however, remains a valid method of analysis. Jang *et al*[69] performed a breath test in patients with right DD at 180 min following ingestion of 10 g of lactulose and determined that methane gas was increased but hydrogen gas was decreased in these individuals as compared to controls. It is important to note here, though, that gas volume is known to significantly increase in response to lactulose ingestion at time points greater than the 180 min used in that study, specifically at 240 to 300 min after the ingestion[68]. Therefore, to more accurately investigate the influence of colon gas on clinical symptoms, it is necessary that the study design allow for adequate time for fermentation to occur in the large intestine.

The low FODMAP diet purports avoidance of foods that contain lactose. Lactose not only causes an increase in intestinal water content (*via* increased osmotic load in the ileum) but is also readily fermented by the colonic microbiota, which leads to production of short-chain fatty acids and gas: Mainly hydrogen (H_2), carbon dioxide (CO_2), and methane (CH_4)[70]. Thus, individuals with lactose intolerance can experience diarrhea and abdominal distension as a result of dietary intake.

Prevalence of lactose intolerance is high in Asia and Africa, and lower in Caucasian populations. From this point forward, the article will discuss the potential correlation between DD of right colon (RDD) and lactose intolerance. Among the nine countries with reports of RDD and lactose intolerance in the publicly available literature (Table 1)[34,71-84], all show a strong correlation (r^2 = 0.9524, Figure 5). Incidence of RDD is lowest in European countries, while the incidence of lactose intolerance is lowest in the United States; however, the Asian countries show high incidences for both RDD and lactose intolerance. These findings support a hypothesis of lactose intolerance and RDD.

In Japan, DD in young individuals almost exclusively involves the right side of the colon (Figure 6)[77]. LDD risk

Table 1 The proportion of diverticular disease of right colon and lactose intolerance

Country	Lactose intolerance (%)	RDD (%)[1]
Singapore	100[71]	42[72]
Thailand	98[73]	55[74]
China	95[73]	55[75]
Japan	90[76]	45[77]
South Korea	88[78]	52[79]
Poland	27[80]	15[81]
India	20[73]	12[82]
United Kingdom (White)	6[83]	3[84]
Australia (White)	4[74]	5[34]

[1]Not including pan-DD and bilateral DD. DD: Diverticular disease; RDD: DD of right colon.

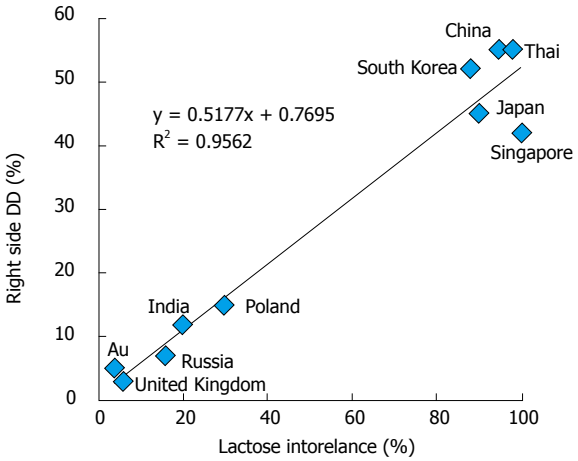

Figure 5 Relationship between lactose intolerance and the right-side diverticular disease. Au: Australia; DD: Diverticular disease.

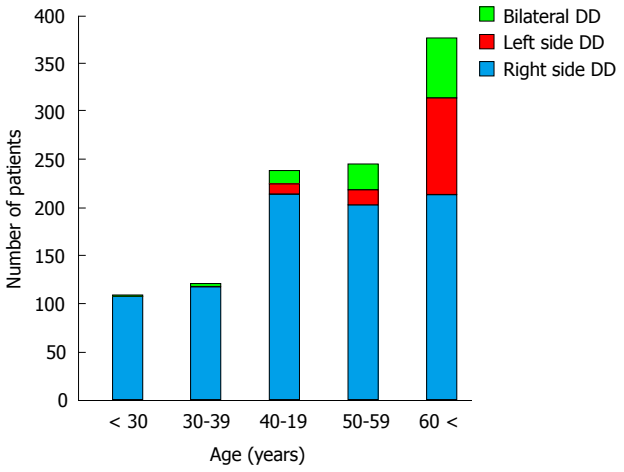

Figure 6 Relationship between the site of and age at onset for diverticular disease. DD: Diverticular disease.

was found to increase with age, likely due to increased vulnerability of the muscle layer over a person's lifespan. In adults under 29 years of age, 100% of the DD cases involved the right side. In Japan, pediatric DD between the ages of 7-15 years is not rare: These cases of pediatric DD occur in cecum and ascending colon[85,86]. This finding cannot be explained merely by age-related vulnerability of the muscle layer and may indicate factors related to childhood. It has been reported that up to 86% of Japanese children develop lactose intolerance by the age of 6 (30% in 3-year-old, 36% in 4-year-old, 58% in 5-year-old)[76]. The time that it takes for gas to increase in the intestines after lactose intake is 1-2 h[87-89], which is shorter than the times required for any of the other constituents of a high-FODMAP diet. Thus, lactose intake may induce a large amount of gas and liquid in the right colon of Japanese children, especially those with lactose intolerance. The physical pressure brought on by the increased gas and fluid will affect the mucous membrane, presumably pushing it outward into the physiological cleft that exists from birth. This may explain why diverticulum in young Japanese tends to be generated only on the right side.

Yet, many Europeans and Americans experience DD of the left colon LDD. This phenomenon may be related to the higher ingestion of wheat[90], compared to Asian societies historically. The fructan component of wheat is a part of the high-FODMAP. The time required for fermentation of fructan in the gut is relatively long, between 2 and 6 h[91,92], so that the high pressure would occur in the left colon. In Japan, however, consumption of wheat has increased since World War Ⅱ, and this change in dietary pattern - towards one that more closely resembles the European and American diets - has been accompanied by an increase in colonic DD. For example, DD was reportedly 2% in the 1960s[93] but had increased to 20% by the 1980s[94]. Additionally, the cases of LDD have increased in Japan as well[94-97]. This trend is similar to that reported in South Koreans[79].

Besides the change in eating habits, the increased longevity of the Japanese population in recent decades may also have contributed to the observed rise in LDD. In addition, the prevalence of IBS has remarkable increased in Japan; according to the various revised definitions of IBS made by the Rome diagnostic criteria, incidence was 3.6% in 1996 (Rome Ⅰ)[98], 10.7% in 2006 (Rome Ⅱ)[99], 14.0% in 2010 (Rome Ⅲ)[100]. Thus, not only may DD and IBS be correlated but they also may share an etiologic component of diet.

Several reports have addressed the potential correlation between IBS and DD[54,101-103]. Symptoms consistent with IBS are common among patients with DD, and this symptomology has been reported as significantly higher in the DD patients when compared to non-DD controls[103-105]. However, this overlap of symptoms can cause diverticulitis to be misdiagnosed as IBS[104].

IBS and DD are distinct conditions, the former having demonstrated characteristics of inflammation as a distinguishing feature; as such, it may be inappropriate to adapt the diagnostic criteria of IBS to patients with DD. Yet, the two share common symptoms of abdominal bloating accompanied by abdominal pain, which are presumed to be consequent to internal pressure in the

digestive tract. Regardless, if the cause of symptoms in either is an excess volume of gas and liquid content in the colon, reduction of either or both might help to prevent the chronic symptoms of diverticulosis.

IBS is classified as a functional disorder, while DD is classified as an organic disease. The most obvious difference between the two is that including a case having homeostatic stenosis after inflammation and/or inflammation among the group of DD. Shape change and inflammation is the result, the cause may be the same. Patient with DD will sustain severe symptoms than IBS without diverticulum. Cuomo *et al*[105] suggested that these symptoms may be used to differentiate the patients with DD from those with IBS. However, their study design was based upon a patient population presenting with fever and requiring hospitalization and treatment, so that cases of diverticulum without inflammation were not considered.

It is possible that inflammation related to diverticulitis may lead to excessive contraction and support development of IBS[106]. In both conditions, Bernoulli's principle may be at play, namely production of non-uniform pressure in the intestinal tract caused by any variety of factors. It is also possible that in patients with asymptomatic DD without stenosis, a high-fiber diet with high FODMAPs may be lead to IBS. In such a diet, the inulin and oligosaccharides may produce short-chain fatty acids and gases by fermentation at 6 to 48 h after ingestion, and pH of feces is reduced from 7 to 6[107,108].

It has been demonstrated that IBS patients have reduced colonic intraluminal pH, compared to healthy controls[109,110]; the lower pH is suggestive of higher colonic fermentation. Specifically, these studies used a wireless motility capsule (SmartPill™) to show that IBS patients had a pH of 6.8 in the colon (*vs* healthy controls who had a pH of 7.3) and showed that colonic low-pH levels were correlated with IBS symptom severity scores and abdominal pain. IBS is characterized by excessive contraction of the descending colon, starting from the sigmoid colon[111]. Interestingly, when another study found low-pH of the cecum in IBS patients, it was correlated with a reduction in right colon contraction[112]. These collective findings indicate that IBS is likely subject to the Bernoulli's principle, which is also inferred for the pathogenesis of DD.

CONCLUSION

The high fiber hypothesis represents a logical contradiction. A high-fiber diet is most likely not suitable for long-term management of diverticulitis. The anatomical clefts that are present in the musculature of the large intestine are prone to diverticula caused by gas and fluid-related force pressures following Bernoulli's principle. RDD, however, may also be related to lactose intolerance. The currently recommended diet of high fiber with high FODMAPs may bring about substantial amounts of gas in the colon and a low pH, which is linked with IBS symptoms. Theoretically, then, a low FODMAP diet will be valid for the prevention of recurrent diverticulitis.

REFERENCES

1 **Painter NS**. Diverticulosis of the colon and diet. *Br Med J* 1969; **2**: 764-765 [PMID: 5786772 DOI: 10.1136/bmj.2.5659.764-b]
2 **Painter NS**. Pressures in the colon related to diverticular disease. *Proc R Soc Med* 1970; **63** Suppl: 144-145 [PMID: 5525488]
3 **Painter NS**, Burkitt DP. Diverticular disease of the colon: a deficiency disease of Western civilization. *Br Med J* 1971; **2**: 450-454 [PMID: 4930390 DOI: 10.1136/bmj.2.5759.450]
4 **Burkitt DP**, Walker AR, Painter NS. Effect of dietary fibre on stools and the transit-times, and its role in the causation of disease. *Lancet* 1972; **2**: 1408-1412 [PMID: 4118696 DOI: 10.1016/S0140-6736(72)92974-1]
5 **Spiller GA**, Chernoff MC, Shipley EA, Beigler MA, Briggs GM. Can fecal weight be used to establish a recommended intake of dietary fiber (plantix) *Am J Clin Nutr* 1977; **30**: 659-661 [PMID: 855809]
6 **Saito T**, Hayakawa T, Nakamura K, Takita T, Suzuki K, Innami S. Fecal output, gastrointestinal transit time, frequency of evacuation and apparent excretion rate of dietary fiber in young men given diets containing different levels of dietary fiber. *J Nutr Sci Vitaminol* (Tokyo) 1991; **37**: 493-508 [PMID: 1666410 DOI: 10.3177/jnsv.37.493]
7 **Painter NS**. Diverticular disease of the colon: A deficiency disease of western civilization. USA: William Heinemann Medical Books Ltd, 1975 [DOI: 10.1016/b978-0-12-144750-2.50015-2]
8 **Yazici C**, Arslan DC, Abraham R, Cushing K, Keshavarzian A, Mutlu EA. Breath Methane Levels Are Increased Among Patients with Diverticulosis. *Dig Dis Sci* 2016; **61**: 2648-2654 [PMID: 27129485 DOI: 10.1007/s10620-016-4174-6]
9 **Robert C**, Bernalier-Donadille A. The cellulolytic microflora of the human colon: evidence of microcrystalline cellulose-degrading bacteria in methane-excreting subjects. *FEMS Microbiol Ecol* 2003; **46**: 81-89 [PMID: 19719585 DOI: 10.1016/S0168-6496(03)00207-1]
10 **Kalantar-Zadeh K**, Yao CK, Berean KJ, Ha N, Ou JZ, Ward SA, Pillai N, Hill J, Cottrell JJ, Dunshea FR, McSweeney C, Muir JG, Gibson PR. Intestinal Gas Capsules: A Proof-of-Concept Demonstration. *Gastroenterology* 2016; **150**: 37-39 [PMID: 26518389 DOI: 10.1053/j.gastro.2015.07.072]
11 **American Gastroenterological Association**. A Patient Guide: Managing Diverticulitis. *Gastroenterology* 2015; **149**: 1977-1978 [PMID: 26453775 DOI: 10.1053/j.gastro.2015.10.002]
12 **Tan KY**, Seow-Choen F. Fiber and colorectal diseases: separating fact from fiction. *World J Gastroenterol* 2007; **13**: 4161-4167 [PMID: 17696243 DOI: 10.3748/wjg.v13.i31.4161]
13 **Ünlü C**, Daniels L, Vrouenraets BC, Boermeester MA. A systematic review of high-fibre dietary therapy in diverticular disease. *Int J Colorectal Dis* 2012; **27**: 419-427 [PMID: 21922199 DOI: 10.1007/s00384-011-1308-3]
14 **Peery AF**, Sandler RS. Diverticular disease: reconsidering conventional wisdom. *Clin Gastroenterol Hepatol* 2013; **11**: 1532-1537 [PMID: 23669306 DOI: 10.1016/j.cgh.2013.04.048]
15 **Tursi A**, Papa A, Danese S. Review article: the pathophysiology and medical management of diverticulosis and diverticular disease of the colon. *Aliment Pharmacol Ther* 2015; **42**: 664-684 [PMID: 26202723 DOI: 10.1111/apt.13322]
16 **Elisei W**, Tursi A. Recent advances in the treatment of colonic diverticular disease and prevention of acute diverticulitis. *Ann Gastroenterol* 2016; **29**: 24-32 [PMID: 26752946]
17 **Peery AF**, Barrett PR, Park D, Rogers AJ, Galanko JA, Martin CF, Sandler RS. A high-fiber diet does not protect against asymptomatic diverticulosis. *Gastroenterology* 2012; **142**: 266-272.e1 [PMID: 22062360 DOI: 10.1053/j.gastro.2011.10.035]
18 **Peery AF**, Sandler RS, Ahnen DJ, Galanko JA, Holm AN, Shaukat A, Mott LA, Barry EL, Fried DA, Baron JA. Constipation and a low-fiber diet are not associated with diverticulosis. *Clin Gastroenterol Hepatol* 2013; **11**: 1622-1627 [PMID: 23891924 DOI: 10.1016/

j.cgh.2013.06.033]
19 **Braunschmid T**, Stift A, Mittlböck M, Lord A, Weiser FA, Riss S. Constipation is not associated with diverticular disease - Analysis of 976 patients. *Int J Surg* 2015; **19**: 42-45 [PMID: 25980396 DOI: 10.1016/j.ijsu.2015.04.045]
20 **Meyers MA**, Volberg F, Katzen B, Abbott G. Haustral anatomy and pathology: a new look. I. Roentgen identification of normal patterns and relationships. *Radiology* 1973; **108**: 497-504 [PMID: 4723648 DOI: 10.1148/108.3.497]
21 **Whiteway J**, Morson BC. Elastosis in diverticular disease of the sigmoid colon. *Gut* 1985; **26**: 258-266 [PMID: 3972272 DOI: 10.1136/gut.26.3.258]
22 **Almerie MQ**, Simpson J. Diagnosing and treating diverticular disease. *Practitioner* 2015; **259**: 29-33, 3 [PMID: 26591654]
23 **Watters DA**, Smith AN, Eastwood MA, Anderson KC, Elton RA, Mugerwa JW. Mechanical properties of the colon: comparison of the features of the African and European colon in vitro. *Gut* 1985; **26**: 384-392 [PMID: 3920126 DOI: 10.1136/gut.26.4.384]
24 **Iwasaki T**. Study on the strength of human intestinal walls. *J Kyoto Prefectural Univ Med* 1953; **52**: 673-702
25 **Uno Y**, Morita T. Colonic perforation and serosal tears associated with colonoscopy. *Lancet* 1997; **349**: 1888 [PMID: 9217768 DOI: 10.1016/S0140-6736(05)63879-2]
26 **Stevens CE**, Hume ID. Contributions of microbes in vertebrate gastrointestinal tract to production and conservation of nutrients. *Physiol Rev* 1998; **78**: 393-427 [PMID: 9562034]
27 **Hodgson J**. Animal models in the study of diverticular disease. Part I: aetiology and treatment. *Clin Gastroenterol* 1975; **4**: 201-219 [PMID: 1109816]
28 **Hare WC**. Observations On A Case Of A Diverticulum In The Small Colon Of The Horse. *Can J Comp Med Vet Sci* 1959; **23**: 272-273 [PMID: 17649171]
29 **Bunton TE**, Bacmeister CX. Diverticulosis and colonic leiomyosarcoma in an aged rhesus macaque. *Vet Pathol* 1989; **26**: 351-352 [PMID: 2763427 DOI: 10.1177/030098588902600415]
30 **Hayama SI**, Rika Akamatsu R, Kishimoto M, Suzuki M, Hideo Nigi H. A case of diverticular disease of the colon in a Japanese monkey (Macaca fuscata). *Primates* 1988; **29**: 423-426 [DOI: 10.1007/BF02380966]
31 **Meyers MA**, Volberg F, Katzen B, Abbott G. Haustral anatomy and pathology: a new look. II. Roentgen interpretation of pathological alterations. *Radiology* 1973; **108**: 505-512 [PMID: 4723649 DOI: 10.1148/108.3.505]
32 **Griffiths JD**. Extramural and intramural blood-supply of colon. *Br Med J* 1961; **1**: 323-326 [PMID: 13708616 DOI: 10.1136/bmj.1.5222.323]
33 **Slack WW**. The anatomy, pathology, and some clinical features of divericulitis of the colon. *Br J Surg* 1962; **50**: 185-190 [PMID: 13913774 DOI: 10.1002/bjs.18005022012]
34 **Hughes LE**. Postmortem survey of diverticular disease of the colon. I. Diverticulosis and diverticulitis. *Gut* 1969; **10**: 336-344 [PMID: 5771665]
35 **Maykel JA**, Opelka FG. Colonic diverticulosis and diverticular hemorrhage. *Clin Colon Rectal Surg* 2004; **17**: 195-204 [PMID: 20011276 DOI: 10.1055/s-2004-832702]
36 **Lineback PE**. Studies on the musculature of the human colon, with special reference to the taeniae. *Am J Anat* 1925; **36**: 357-383 [DOI: 10.1002/aja.1000360207]
37 **Sheth AA**, Longo W, Floch MH. Diverticular disease and diverticulitis. *Am J Gastroenterol* 2008; **103**: 1550-1556 [PMID: 18479497 DOI: 10.1111/j.1572-0241.2008.01879.x]
38 **Fenoglio-Preiser CM**, Lantz PL, Listrom MB, Davis M, Rike FO. Gastrointestinal pathology: An atlas and text. Raven Press, New York, 1989: 632-634
39 **Bassotti G**, Battaglia E, Spinozzi F, Pelli MA, Tonini M. Twenty-four hour recordings of colonic motility in patients with diverticular disease: evidence for abnormal motility and propulsive activity. *Dis Colon Rectum* 2001; **44**: 1814-1820 [PMID: 11742167 DOI: 10.1007/BF02234460]
40 **Viebig RG**, Pontes JF, Michelsohn NH. Electromanometry of the rectosigmoid in colonic diverticulosis. *Arq Gastroenterol* 1994; **31**: 135-144 [PMID: 7575173]
41 **Sosna J**, Bar-Ziv J, Libson E, Eligulashvili M, Blachar A. CT colonography: positioning order and intracolonic pressure. *AJR Am J Roentgenol* 2008; **191**: 1100 [PMID: 18806149 DOI: 10.2214/AJR.07.3303]
42 **Painter NS**. The aetiology of diverticulosis of the colon with special reference to the action of certain drugs on the behaviour of the colon. *Ann R Coll Surg Engl* 1964; **34**: 98-119 [PMID: 14120557]
43 **Noveroske RJ**. Intracolonic pressures during barium enema examination. *Am J Roentgenol Radium Ther Nucl Med* 1964; **91**: 852-863 [PMID: 14139913]
44 **Wojtalik RS**, Lindenauer SM, Kahn SS. Perforation of the colon associated with adynamic ileus. *Am J Surg* 1973; **125**: 601-606 [PMID: 4699203 DOI: 10.1016/0002-9610(73)90146-3]
45 **Kozarek RA**, Earnest DL, Silverstein ME, Smith RG. Air-pressure-induced colon injury during diagnostic colonoscopy. *Gastroenterology* 1980; **78**: 7-14 [PMID: 7350038]
46 **Sandhu KS**, Cohen H. Cecal perforation following fiberoptic flexible sigmoidoscopy. *Am J Med* 1987; **82**: 546-548 [PMID: 3826107 DOI: 10.1016/0002-9343(87)90459-1]
47 **Luchette FA**, Doerr RJ, Kelly K, Kulaylat M, Stephan RM, Hassett JM. Colonoscopic impaction in left colon strictures resulting in right colon pneumatic perforation. *Surg Endosc* 1992; **6**: 273-276 [PMID: 1448745 DOI: 10.1007/BF02498858]
48 **Foliente RL**, Chang AC, Youssef AI, Ford LJ, Condon SC, Chen YK. Endoscopic cecal perforation: mechanisms of injury. *Am J Gastroenterol* 1996; **91**: 705-708 [PMID: 8677933]
49 **Tzelepis GE**, Nasiff L, McCool FD, Hammond J. Transmission of pressure within the abdomen. *J Appl Physiol* (1985) 1996; **81**: 1111-1114 [PMID: 8889741]
50 **Woltjen JA**. A retrospective analysis of cecal barotrauma caused by colonoscope air flow and pressure. *Gastrointest Endosc* 2005; **61**: 37-45 [PMID: 15672054 DOI: 10.1016/S0016-5107(04)02453-8]
51 **Brayko CM**, Kozarek RA, Sanowski RA, Howells T. Diverticular rupture during colonoscopy. Fact or fancy? *Dig Dis Sci* 1984; **29**: 427-431 [PMID: 6425026 DOI: 10.1007/BF01296218]
52 **Leandro PA**, Cecconello I, Habr-Gama A, de Olivereira e Silva A, Pontes JF. Gastrointestinal motility in normal subjects and patients with diverticulosis of the colon. *Arq Gastroenterol* 1984; **21**: 157-163 [PMID: 6536253]
53 **Reeders JW**, Rodenbush G. Clinical radiology and endoscopy of the colon. New York: Thieme Medical Publishers, Inc., 1994
54 **Spiller R**. Editorial: new thoughts on the association between diverticulosis and irritable bowel syndrome. *Am J Gastroenterol* 2014; **109**: 1906-1908 [PMID: 25470583 DOI: 10.1038/ajg.2014.367]
55 **Sugihara K**, Muto T, Morioka Y. Motility study in right sided diverticular disease of the colon. *Gut* 1983; **24**: 1130-1134 [PMID: 6642277 DOI: 10.1136/gut.24.12.1130]
56 **Harder H**, Serra J, Azpiroz F, Passos MC, Aguadé S, Malagelada JR. Intestinal gas distribution determines abdominal symptoms. *Gut* 2003; **52**: 1708-1713 [PMID: 14633947 DOI: 10.1136/gut.52.12.1708]
57 **Marthinsen D**, Fleming SE. Excretion of breath and flatus gases by humans consuming high-fiber diets. *J Nutr* 1982; **112**: 1133-1143 [PMID: 6283045]
58 **Muir JG**, Shepherd SJ, Rosella O, Rose R, Barrett JS, Gibson PR. Fructan and free fructose content of common Australian vegetables and fruit. *J Agric Food Chem* 2007; **55**: 6619-6627 [PMID: 17625872 DOI: 10.1021/jf070623x]
59 **Suarez FL**, Springfield J, Furne JK, Lohrmann TT, Kerr PS, Levitt MD. Gas production in human ingesting a soybean flour derived from beans naturally low in oligosaccharides. *Am J Clin Nutr* 1999; **69**: 135-139 [PMID: 9925135]
60 **Cummings JH**, Macfarlane GT, Englyst HN. Prebiotic digestion and fermentation. *Am J Clin Nutr* 2001; **73**: 415S-420S [PMID: 11157351]
61 **Choi YK**, Kraft N, Zimmerman B, Jackson M, Rao SS. Fructose intolerance in IBS and utility of fructose-restricted diet. *J Clin Gastroenterol* 2008; **42**: 233-238 [PMID: 18223504 DOI: 10.1097/MCG.0b013e31802cbc2f]
62 **Gibson PR**, Shepherd SJ. Personal view: food for thought--western

lifestyle and susceptibility to Crohn's disease. The FODMAP hypothesis. *Aliment Pharmacol Ther* 2005; **21**: 1399-1409 [PMID: 15948806 DOI: 10.1111/j.1365-2036.2005.02506.x]
63 **Gibson PR**, Shepherd SJ. Food choice as a key management strategy for functional gastrointestinal symptoms. *Am J Gastroenterol* 2012; **107**: 657-666; quiz 667 [PMID: 22488077 DOI: 10.1038/ajg.2012.49]
64 **Halmos EP**, Power VA, Shepherd SJ, Gibson PR, Muir JG. A diet low in FODMAPs reduces symptoms of irritable bowel syndrome. *Gastroenterology* 2014; **146**: 67-75.e5 [PMID: 24076059 DOI: 10.1053/j.gastro.2013.09.046]
65 **Chumpitazi BP**, Cope JL, Hollister EB, Tsai CM, McMeans AR, Luna RA, Versalovic J, Shulman RJ. Randomised clinical trial: gut microbiome biomarkers are associated with clinical response to a low FODMAP diet in children with the irritable bowel syndrome. *Aliment Pharmacol Ther* 2015; **42**: 418-427 [PMID: 26104013 DOI: 10.1111/apt.13286]
66 **Clausen MR**, Jørgensen J, Mortensen PB. Comparison of diarrhea induced by ingestion of fructooligosaccharide Idolax and disaccharide lactulose: role of osmolarity versus fermentation of malabsorbed carbohydrate. *Dig Dis Sci* 1998; **43**: 2696-2707 [PMID: 9881502 DOI: 10.1023/A:1026659512786]
67 **Undseth R**, Berstad A, Kløw NE, Arnljot K, Moi KS, Valeur J. Abnormal accumulation of intestinal fluid following ingestion of an unabsorbable carbohydrate in patients with irritable bowel syndrome: an MRI study. *Neurogastroenterol Motil* 2014; **26**: 1686-1693 [PMID: 25271767 DOI: 10.1111/nmo.12449]
68 **Uno Y**. Pilot Study on Gas Patterns of Irritable Bowel Syndrome and Small Intestinal Bacterial Overgrowth Following Ingestion of Lactulose. *Open J Gastroenterol* 2015; **5**: 155-163 [DOI: 10.4236/ojgas.2015.511025]
69 **Jang SI**, Kim JH, Youn YH, Park H, Lee SI, Conklin JL. Relationship between intestinal gas and the development of right colonic diverticula. *J Neurogastroenterol Motil* 2010; **16**: 418-423 [PMID: 21103424 DOI: 10.5056/jnm.2010.16.4.418]
70 **Deng Y**, Misselwitz B, Dai N, Fox M. Lactose Intolerance in Adults: Biological Mechanism and Dietary Management. *Nutrients* 2015; **7**: 8020-8035 [PMID: 26393648 DOI: 10.3390/nu7095380]
71 **Bolin TD**, Davis AE, Seah CS, Chua KL, Yong V, Kho KM, Siak CL, Jacob E. Lactose intolerance in Singapore. *Gastroenterology* 1970; **59**: 76-84 [PMID: 5468089]
72 **Wong SK**, Ho YH, Leong AP, Seow-Choen F. Clinical behavior of complicated right-sided and left-sided diverticulosis. *Dis Colon Rectum* 1997; **40**: 344-348 [PMID: 9118752 DOI: 10.1007/BF02050427]
73 **Silanikove N**, Leitner G, Merin U. The Interrelationships between Lactose Intolerance and the Modern Dairy Industry: Global Perspectives in Evolutional and Historical Backgrounds. *Nutrients* 2015; **7**: 7312-7331 [PMID: 26404364 DOI: 10.3390/nu7095340]
74 **Rungjiratananon S**, Sirinthornpunya S. Prevalence of colonic diverticulosis in irritable bowel syndrome patients compared to other patients in Rajavithi hospital prevalence of colonic diverticulosis. *Thai J Gastroenterol* 2009; **10**: 148-151
75 **Chan CC**, Lo KK, Chung EC, Lo SS, Hon TY. Colonic diverticulosis in Hong Kong: distribution pattern and clinical significance. *Clin Radiol* 1998; **53**: 842-844 [PMID: 9833789 DOI: 10.1016/S0009-9260(98)80197-9]
76 **Nose O**, Iida Y, Kai H, Harada T, Ogawa M, Yabuuchi H. Breath hydrogen test for detecting lactose malabsorption in infants and children. Prevalence of lactose malabsorption in Japanese children and adults. *Arch Dis Child* 1979; **54**: 436-440 [PMID: 475426 DOI: 10.1136/adc.54.6.436]
77 **Kubo A**, Ishiwata J, Maeda Y, Kida T, Yamabe K, Shimosegawa T. Clinical studies on diverticular disease of the colon. *Jpn J Med* 1983; **22**: 185-189 [PMID: 6620706 DOI: 10.2169/internalmedicine1962.22.185]
78 **Park SH**, Chang YW, Kim SJ, Lee MH, Nam JH, Oh CH, Kim JW, Jang JY, Yang JO, Yoo JA, Chung JY. [Efficacy of Lactose-free Milk in Korean Adults with Lactose Intolerance]. *Korean J Gastroenterol* 2016; **67**: 22-27 [PMID: 26809628 DOI: 10.4166/kjg.2016.67.1.22]
79 **Oh HK**, Han EC, Ha HK, Choe EK, Moon SH, Ryoo SB, Jeong SY, Park KJ. Surgical management of colonic diverticular disease: discrepancy between right- and left-sided diseases. *World J Gastroenterol* 2014; **20**: 10115-10120 [PMID: 25110438 DOI: 10.3748/wjg.v20.i29.10115]
80 **Pawłowska K**, Umławska W, Iwańczak B. Prevalence of Lactose Malabsorption and Lactose Intolerance in Pediatric Patients with Selected Gastrointestinal Diseases. *Adv Clin Exp Med* 2015; **24**: 863-871 [PMID: 26768639 DOI: 10.17219/acem/26337]
81 **Blachut K**, Paradowski L, Garcarek J. Prevalence and distribution of the colonic diverticulosis. Review of 417 cases from Lower Silesia in Poland. *Rom J Gastroenterol* 2004; **13**: 281-285 [PMID: 15624024]
82 **Goenka MK**, Nagi B, Kochhar R, Bhasin DK, Singh A, Mehta SK. Colonic diverticulosis in India: the changing scene. *Indian J Gastroenterol* 1994; **13**: 86-88 [PMID: 8076987]
83 **Smith GD**, Lawlor DA, Timpson NJ, Baban J, Kiessling M, Day IN, Ebrahim S. Lactase persistence-related genetic variant: population substructure and health outcomes. *Eur J Hum Genet* 2009; **17**: 357-367 [PMID: 18797476 DOI: 10.1038/ejhg.2008.156]
84 **Golder M**, Ster IC, Babu P, Sharma A, Bayat M, Farah A. Demographic determinants of risk, colon distribution and density scores of diverticular disease. *World J Gastroenterol* 2011; **17**: 1009-1017 [PMID: 21448352 DOI: 10.3748/wjg.v17.i8.1009]
85 **Yoshimoto K**, Terakura H, Hiki S, Ogata H. Diverticulitis in children. *Stomach and Intestine* (Tokyo) 2012; **47**: 1135-1140 [DOI: 10.11477/mf.1403113527]
86 **Watanuki H**, Minota N, Takahara M, et al. Study on diverticulitis of the large intestine in children. *J Jpn Red Cross Himeji Hosp* 2015; **39**: 71-74
87 **Mummah S**, Oelrich B, Hope J, Vu Q, Gardner CD. Effect of raw milk on lactose intolerance: a randomized controlled pilot study. *Ann Fam Med* 2014; **12**: 134-141 [PMID: 24615309 DOI: 10.1370/afm.1618]
88 **Yang JF**, Fox M, Chu H, Zheng X, Long YQ, Pohl D, Fried M, Dai N. Four-sample lactose hydrogen breath test for diagnosis of lactose malabsorption in irritable bowel syndrome patients with diarrhea. *World J Gastroenterol* 2015; **21**: 7563-7570 [PMID: 26140004 DOI: 10.3748/wjg.v21.i24.7563]
89 **Schommer K**, Reljic D, Bärtsch P, Sauer P. Gastrointestinal complaints in runners are not due to small intestinal bacterial overgrowth. *J Negat Results Biomed* 2011; **10**: 8 [PMID: 21794099 DOI: 10.1186/1477-5751-10-8]
90 **De Giorgio R**, Volta U, Gibson PR. Sensitivity to wheat, gluten and FODMAPs in IBS: facts or fiction? *Gut* 2016; **65**: 169-178 [PMID: 26078292 DOI: 10.1136/gutjnl-2015-309757]
91 **Murray K**, Wilkinson-Smith V, Hoad C, Costigan C, Cox E, Lam C, Marciani L, Gowland P, Spiller RC. Differential effects of FODMAPs (fermentable oligo-, di-, mono-saccharides and polyols) on small and large intestinal contents in healthy subjects shown by MRI. *Am J Gastroenterol* 2014; **109**: 110-119 [PMID: 24247211 DOI: 10.1038/ajg.2013.386]
92 **Fedewa A**, Rao SS. Dietary fructose intolerance, fructan intolerance and FODMAPs. *Curr Gastroenterol Rep* 2014; **16**: 370 [PMID: 24357350 DOI: 10.1007/s11894-013-0370-0]
93 **Inoue M**. The epidemiologic and clinical features of diverticular disease of the colon. *J Jpn Soc Coloproctol* 1992; **45**: 904-913 [DOI: 10.3862/jcoloproctology.45.6_904]
94 **Miura S**, Kodaira S, Aoki H, Hosoda Y. Bilateral type diverticular disease of the colon. *Int J Colorectal Dis* 1996; **11**: 71-75 [PMID: 8739830 DOI: 10.1007/BF00342463]
95 **Takano M**, Yamada K, Sato K. An analysis of the development of colonic diverticulosis in the Japanese. *Dis Colon Rectum* 2005; **48**: 2111-2116 [PMID: 16228844 DOI: 10.1007/s10350-005-0111-z]
96 **Yamada E**, Inamori M, Uchida E, Tanida E, Izumi M, Takeshita K, Fujii T, Komatsu K, Hamanaka J, Maeda S, Kanesaki A, Matsuhashi N, Nakajima A. Association between the location of diverticular disease and the irritable bowel syndrome: a multicenter study in Japan. *Am J Gastroenterol* 2014; **109**: 1900-1905 [PMID: 25331347 DOI: 10.1038/ajg.2014.323]
97 **Tarao K**, Sekino Y, Nonaka T, Iida H, Inamori M, Nakajima A,

Maeda S, Natsumeda Y, Ikegami T, Ohshige K. Recent trends in colonic diverticulosis in Yokohama City: a possibility of changing to a more Western profile. *Intern Med* 2015; **54**: 2545-2550 [PMID: 26466687 DOI: 10.2169/internalmedicine.54.4813]

98 **Itasaka T**, Takahashi T. Epidemiological study of irritable bowel syndrome using population screening. *Ther Res* 1996; **17**: 4059-4063

99 **Shiotani A**, Miyanishi T, Takahashi T. Sex differences in irritable bowel syndrome in Japanese university students. *J Gastroenterol* 2006; **41**: 562-568 [PMID: 16868804]

100 **Miwa H**. Life style in persons with functional gastrointestinal disorders--large-scale internet survey of lifestyle in Japan. *Neurogastroenterol Motil* 2012; **24**: 464-471, e217 [PMID: 22292849 DOI: 10.1111/j.1365-2982.2011.01872.x]

101 **Annibale B**, Lahner E, Maconi G, Usai P, Marchi S, Bassotti G, Barbara G, Cuomo R. Clinical features of symptomatic uncomplicated diverticular disease: a multicenter Italian survey. *Int J Colorectal Dis* 2012; **27**: 1151-1159 [PMID: 22573184 DOI: 10.1007/s00384-012-1488-5]

102 **Sirinthornpunya S**, Rungjiratananon S. Association of colonic diverticular disease and irritable bowel syndrome in Thai patients. *J Med Assoc Thai* 2014; **97** Suppl 11: S18-S24 [PMID: 25509691]

103 **Jung HK**, Choung RS, Locke GR, Schleck CD, Zinsmeister AR, Talley NJ. Diarrhea-predominant irritable bowel syndrome is associated with diverticular disease: a population-based study. *Am J Gastroenterol* 2010; **105**: 652-661 [PMID: 19861955 DOI: 10.1038/ajg.2009.621]

104 **Longstreth GF**, Tieu RS. Clinically Diagnosed Acute Diverticulitis in Outpatients: Misdiagnosis in Patients with Irritable Bowel Syndrome. *Dig Dis Sci* 2016; **61**: 578-588 [PMID: 26441278 DOI: 10.1007/s10620-015-3892-5]

105 **Cuomo R**, Barbara G, Andreozzi P, Bassotti G, Casetti T, Grassini M, Ierardi E, Maconi G, Marchi S, Sarnelli G, Savarino V, Usai P, Vozzella L, Annibale B. Symptom patterns can distinguish diverticular disease from irritable bowel syndrome. *Eur J Clin Invest* 2013; **43**: 1147-1155 [PMID: 23992370 DOI: 10.1111/eci.12152]

106 **Cohen E**, Fuller G, Bolus R, Modi R, Vu M, Shahedi K, Shah R, Atia M, Kurzbard N, Sheen V, Agarwal N, Kaneshiro M, Yen L, Hodgkins P, Erder MH, Spiegel B. Increased risk for irritable bowel syndrome after acute diverticulitis. *Clin Gastroenterol Hepatol* 2013; **11**: 1614-1619 [PMID: 23524129 DOI: 10.1016/j.cgh.2013.03.007]

107 **Kaur A**, Rose DJ, Rumpagaporn P, Patterson JA, Hamaker BR. In vitro batch fecal fermentation comparison of gas and short-chain fatty acid production using "slowly fermentable" dietary fibers. *J Food Sci* 2011; **76**: H137-H142 [PMID: 22417432 DOI: 10.1111/j.1750-3841.2011.02172.x]

108 **Noack J**, Timm D, Hospattankar A, Slavin J. Fermentation profiles of wheat dextrin, inulin and partially hydrolyzed guar gum using an in vitro digestion pretreatment and in vitro batch fermentation system model. *Nutrients* 2013; **5**: 1500-1510 [PMID: 23645025 DOI: 10.3390/nu5051500]

109 **Choi CH**, Ringel-Kulka T, Temas DJ, Kim A, Scott K, Ringel Y. Altered colonic bacterial fermentation is a steadfast pathophysiological factor in irritable bowel syndrome. *Gastroenterology* 2014; **146**: S8 [DOI: 10.1016/S0016-5085(14)60028-7]

110 **Ringel-Kulka T**, Choi CH, Temas D, Kim A, Maier DM, Scott K, Galanko JA, Ringel Y. Altered Colonic Bacterial Fermentation as a Potential Pathophysiological Factor in Irritable Bowel Syndrome. *Am J Gastroenterol* 2015; **110**: 1339-1346 [PMID: 26303129 DOI: 10.1038/ajg.2015.220]

111 **Clemens CH**, Samsom M, Van Berge Henegouwen GP, Smout AJ. Abnormalities of left colonic motility in ambulant nonconstipated patients with irritable bowel syndrome. *Dig Dis Sci* 2003; **48**: 74-82 [PMID: 12645793]

112 **Farmer AD**, Mohammed SD, Dukes GE, Scott SM, Hobson AR. Caecal pH is a biomarker of excessive colonic fermentation. *World J Gastroenterol* 2014; **20**: 5000-5007 [PMID: 24803812 DOI: 10.3748/wjg.v20.i17.5000]

P- Reviewer: Horesh N, Montomoli J, Stam MAW
S- Editor: Gong ZM **L- Editor**: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i4.513

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 513-523
ISSN 2150-5349 (online)

REVIEW

Eosinophilic gastroenteritis: Approach to diagnosis and management

Antoine Abou Rached, Weam El Hajj

Antoine Abou Rached, Weam El Hajj, Division of Gastroenterology, Department of Internal Medicine, School of Medicine, Lebanese University, Hadath, Beirut 2903 1308, Lebanon

Author contributions: Abou Rached A and El Hajj W conceived and designed the study; El Hajj W performed the literature review and drafted the article; Abou Rached A critically revised the article for intellectual content and gave final approval of the manuscript.

Conflict-of-interest statement: Neither author has any personal or financial interests related to the publication of this study or its findings.

Manuscript source: Invited manuscript

Correspondence to: Antoine Abou Rached, MD, MBAIP, Division of Gastroenterology, Department of Internal Medicine, School of Medicine, Lebanese University, Hadath, Campus, PO Box #3, Hadath, Beirut 2903 1308, Lebanon. abourachedantoine@gmail.com
Telephone: +961-5-451100
Fax: +961-5-455131

Received: December 5, 2015
Peer-review started: December 7, 2015
First decision: February 15, 2016
Revised: July 23, 2016
Accepted: August 15, 2016
Article in press: August 17, 2016
Published online: November 6, 2016

Abstract

Eosinophilic gastroenteritis (EGE) is a rare and benign inflammatory disorder that predominantly affects the stomach and the small intestine. The disease is divided into three subtypes (mucosal, muscular and serosal) according to klein's classification, and its manifestations are protean, depending on the involved intestinal segments and layers. Hence, accurate diagnosis of EGE poses a significant challenge to clinicians, with evidence of the following three criteria required: Suspicious clinical symptoms, histologic evidence of eosinophilic infiltration in the bowel and exclusion of other pathologies with similar findings. In this review, we designed and applied an algorithm to clarify the steps to follow for diagnosis of EGE in clinical practice. The management of EGE represents another area of debate. Prednisone remains the mainstay of treatment; however the disease is recognized as a chronic disorder and one that most frequently follows a relapsing course that requires maintenance therapy. Since prolonged steroid treatment carries of risk of serious adverse effects, other options with better safety profiles have been proposed; these include budesonide, dietary restrictions and steroid-sparing agents, such as leukotriene inhibitors, azathioprine, anti-histamines and mast-cell stabilizers. Single cases or small case series have been reported in the literature for all of these options, and we provide in this review a summary of these various therapeutic modalities, placing them within the context of our novel algorithm for EGE management according to disease severity upon presentation.

Key words: Eosinophilic; Gastroenteritis; Diagnosis; Management; Algorithm; Review

Core tip: Eosinophilic gastroenteritis (EGE) is a heterogeneous inflammatory bowel disorder, which commonly follows a chronic and relapsing course. To date, only single cases or small case series provide insights into its diagnosis and management. This manuscript reviews the different diagnostic tools utilized in practice and provides an algorithm for diagnosis. It also provides a summary of

the therapeutic modalities applied in EGE management, which are placed within the context of an algorithm for systematic application of the different strategies according to the initial disease severity.

Abou Rached A, El Hajj W. Eosinophilic gastroenteritis: Approach to diagnosis and management. *World J Gastrointest Pharmacol Ther* 2016; 7(4): 513-523 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i4/513.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i4.513

INTRODUCTION

Eosinophilic gastroenteritis (EGE) is a rare inflammatory disorder characterized by eosinophilic infiltration of the intestinal wall. Since its first description, about 8 decades ago, reports of subsequent cases have revealed a widely variable and heterogeneous profile of physical manifestations. Studies from the United States have found a prevalence ranging between 8.4 and 28 per 100000[1,2], with a slightly increasing incidence over the past 50 years[3]; additionally, the disease is well known to be more common among the pediatric population, with afflicted adults typically between the 3rd and 5th decade of life[4]. Intriguingly, the more recent estimates of EGE in the United States have found a shift from male preponderance[4,5] to female predominance[2]. Higher socioeconomic status, Caucasian race and excess weight may be risk factors of EGE[3], and a possible hereditary component (genetic factor) is suggested by reports of familial cases[6].

Concomitant allergic disorders, including asthma, rhinitis, eczema and drug or food intolerances, are present in 45% to 63% of the reported EGE cases[1,3]; moreover, 64% of reported cases include a family history of atopic diseases[7]. Some studies have found an association with other autoimmune conditions, such as celiac disease[8], ulcerative colitis[9] and systemic lupus erythematosus[10]. These data collectively suggest that EGE may result from immune dysregulation in response to an allergic reaction; yet, a triggering allergen is not always identified. Indeed, about 50% of EGE cases involving the alimentary tract have been detected by allergy testing to address a suspected food allergy[3]. Other environmental factors, such as parasitic infestation and drugs, may act as predisposing agents as well[11].

Both immunoglobulin E (IgE) dependent and delayed TH2 cell-mediated allergic mechanisms have been demonstrated to be involved in the pathogenesis of EGE. Interleukin 5 (IL-5) has also been shown to play an essential role in the expansion of eosinophils and their recruitment to the gastrointestinal (GI) tract, the mechanism underlying the pathogenic hallmark of EGE. Chemokines, namely eotaxin 1 and $\alpha4\beta7$ integrin, are also known to contribute to eosinophilic homing inside the intestinal wall. Other mediators-most notably IL-3, IL-4, IL-13, leukotrienes and tumor necrosis factor (TNF)-alpha-act to enhance eosinophilic trafficking and have been proposed to help in prolonging lymphocytic and eosinophilic activity[11-13]. Many of these immune-related molecules are currently under consideration as potential targets for molecular therapy of EGE.

Once recruited to the GI tract, the activated eosinophils induce a significant inflammatory response by secreting a variety of mediators including the cytotoxic granules that lead to structural damage in the infiltrated intestinal layers[12]. Thus, EGE can affect any GI segment, but reports have shown that the small intestine and stomach are the most predominant areas[4]. In clinical practice, the Klein classification system[14] is used to categorize the disease type according to the involved intestinal layer; the 3 Klein categories are mucosal, muscular and serosal. The mucosal layer is the most commonly affected, as has been reported in the majority of case series in the literature, with prevalence ranging between 57% in older estimates[4] and 88% to 100% in more recent estimates[3,15]. Furthermore, the muscular and serosal types are commonly associated with concomitant mucosal eosinophilic infiltration, which raises the hypothesis of centrifugal disease progression from the deep mucosa toward the muscular and serosal layers[3].

DIAGNOSIS

Diagnosis of EGE requires three criteria, namely: (1) presence of GI symptoms; (2) histologic evidence of eosinophilic infiltration in one or more areas of the GI tract; and (3) exclusion of other causes of tissue eosinophilia[16] (Figure 1).

While EGE manifestations vary depending on the affected GI layer, abdominal pain is the predominant presenting symptom among all 3 of the disease types[5]. Involvement of the mucosal layer may cause diarrhea, vomiting, protein-losing enteropathy and malabsorption, which in turn can manifest as anemia, hypoalbuminemia and weight loss. Involvement of the muscular layer can lead to a partial or total intestinal obstruction. Involvement of the serosal layer may cause peritoneal irritation, which can lead to ascites, peritonitis and perforation in more severe cases; intestinal intussusception may occur in the serosal type as well[17]. An additional manifestation of the disease, peripapillary duodenal disease, which is secondary to the eosinophilic infiltration of the peripapillary duodenal region, might result in pancreatitis and biliary obstruction[18,19].

Some laboratory findings are sufficient to raise suspicion of EGE, although they are not adequate for an EGE diagnosis. About 70% of cases present with peripheral eosinophilia[4,20] and EGE cases with deep serosal involvement frequently have higher absolute eosinophilic counts (AECs)[20], the latter of which may also be associated with greater risk of relapse[20]. Elevated IgE is reportedly present in about two-thirds of EGE cases[5]

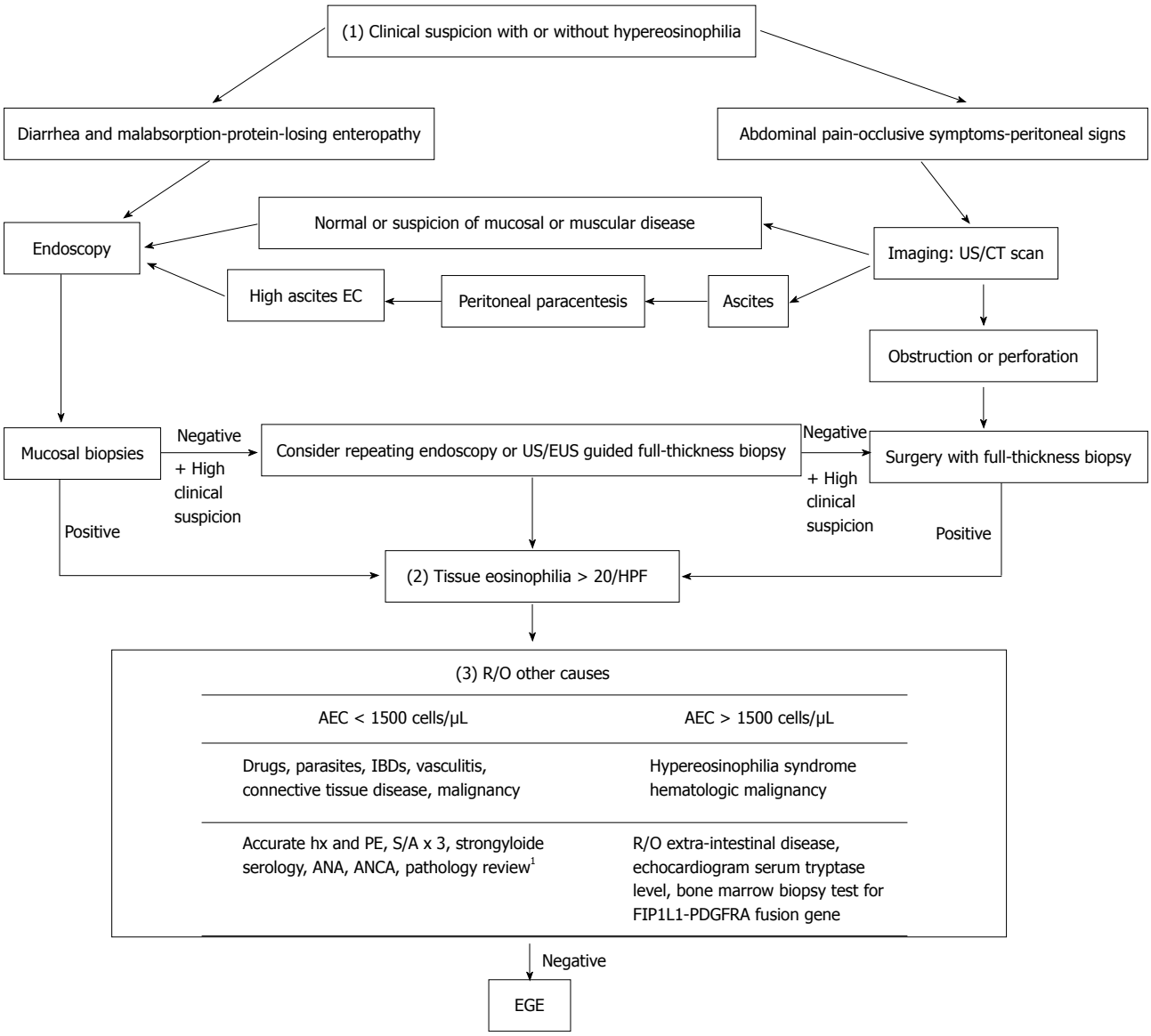

Figure 1 Algorithm for eosinophilic gastroenteritis diagnosis. [1]Histologic ascertainment for absence of malignant cells or findings suggestive of IBD, connective tissue diseases or vasculitis. AEC: Absolute eosinophilic count; ANA: Anti-nuclear antibody; ANCA: Anti-neutrophil cytoplasmic antibodies; EC: Eosinophilic count; EUS: Endoscopic ultrasound; Hx: History; IBD: Inflammatory bowel disease; PE: Physical examination; S/A: Stool analysis; US: Ultrasound; EGE: Eosinophilic gastroenteritis.

and a trend of increased erythrocyte sedimentation rate (ESR) values has been observed. Finally, some reports of EGE cases have demonstrated that peritoneal fluid analysis shows exudative fluid with a net eosinophilic predominance reaching about 90% of white blood cells (WBCs)[21].

Following assessment of the patient's initial presentation, the next step toward diagnosis will require either endoscopy or imaging studies (Figure 1). Endoscopic findings suggestive of EGE include normal aspect, erythematous friable mucosa, ulcers, pseudo-polyps and polyps[22,23], none of which are sensitive or specific for diagnosis of the disease. Thus, findings from endoscopic biopsies can play an essential role in diagnosis, as evidenced by the reported detection rate of 80% for this examination modality[24]. Unfortunately, however, the patchy distribution profile of the disease necessitates multiple biopsies, at least 5 or 6, be obtained from both endoscopically abnormal and normal mucosa, as the latter may mask about 60% of histologically proven disease[15]. Even in cases of negative initial biopsies, but with an otherwise high suspicion index, repeat endoscopy may be useful. Endoscopic ultrasound is also a useful tool for assessing muscular and sub-serosal involvement, as it facilitates access to these tissues for biopsy *via* fine needle aspiration[25,26].

Imaging studies are another diagnostic modality that has proven useful. In addition to guiding biopsy taking efforts, ultrasound can detect ascites and intestinal wall thickening[27]. Computed tomography (CT) scan can detect diffuse thickening of mucosal folds, intestinal wall thickening, ascites and obstruction. Two other

Table 1 Eosinophilic gastroenteritis severity upon presentation

Initial findings	Mild	Moderate	Severe	Complicated
Clinical				
Abdominal pain	Mild	Moderate	Severe	
Vomiting	Mild (< 3/d)	Moderate (3-7/d)	protracted (> 8/d)	
Diarrhea	< 6 BM/d	6-12 BM/d	> 12 BM/d	
Weight loss[1][35]	Non-significant	1 wk 1%-2% 1 mo 5% 3 mo 7.5% 6 mo 10%	1 wk > 2% 1 mo > 5% 3 mo > 7.5% 6 mo > 10%	
Laboratory				
Alb, g/dL	> 3	2.5-3	< 2.5	
HB, g/dL[36]	9.5-11	8-9.5	< 8	
AEC, cells/μL[37]	< 1500	1500-5000	> 5000	
Radiologic				
Ascites	None or mild	Moderate volume	Large volume	Perforation
Intestinal wall thickening[38]	Mild (1-2 cm) Focal (< 10 cm)	Marked (> 2 cm), segmental (10-30 cm)	Sub-occlusion, extensive (> 30 cm)	Occlusion Intussusception
Endoscopy				
Mucosal inflammation[39]	Normal or mild erythema	Moderate	Severe with pseudo-polyps/bleeding	GOO Pyloric stenosis
Histology				
Structural damage[2][34]	Minimal	Moderate	Severe	

[1]Percent weight change = [(usual weight - actual weight)/(usual weight)] × 100; [2]Subjective assessment by expert pathologist. AEC: Absolute eosinophilic count; Alb: Albumin; GOO: Gastric outlet obstruction; HB: Hemoglobin.

scanographic signs that may appear secondary to bowel wall layering are the "Halo sign" and the "araneid-limb-like sign", both of which can aid in differentiating between an inflammatory and a neoplastic lesion[28,29] and in ruling out extra-intestinal pathologies. The imaging modality of Tc-99m hexamethylpropyleneamineoxime (HMPAO)-labeled WBC scintigraphy provides a topographic description of the disease and allows for monitoring of therapeutic response[30]; however, this technology is not widely available and is not yet established as a reliable diagnostic tool for EGE.

While many tools can aid in obtainment of biopsies, the preferred method is still surgery, which provides a full thickness specimen for comprehensive pathology and the most accurate diagnosis, particularly for the muscular and serosal disease types[31].

Histologic examination remains the cornerstone of diagnosis. An absolute eosinophil count of at least 20 eosinophils/hpf has been set in most reports[7,23] as the threshold for fulfilling the second diagnostic criterion. The presence of intraepithelial eosinophils and eosinophils in the Peyer's patches[32], as well as of extracellular deposition of eosinophil major basic proteins (MBPs)[33], favor development of EGE. The latter finding, in particular, reflects the degree of degranulation in activated eosinophils, which is directly linked to greater structural damage[6]. Observation of villous atrophy, crypt hyperplasia or abscesses and epithelial degenerative/regenerative changes are also common findings of EGE. As such, some researchers have emphasized the importance of a subjective histological analysis, in addition to the eosinophilic count, as an important aspect for diagnosis[34].

Accordingly, we suggest dividing the disease into four classifications - mild, moderate, severe and complicated - based upon the initial clinical manifestations, initial laboratory findings, and severity of GI structural damage as assessed by radiologic, endoscopic and histologic examinations (Table 1)[34-39].

Following confirmation of eosinophilic infiltration to the GI tract, the exclusion of other possible causes of the initial clinical presentation is crucial for diagnosis of EGE (Figure 1). These other possible causes include parasitic infections (*i.e.*, *Strongyloides*, *Ascaris*, *Ancylostoma*, *Anisakis*, *Capillaria*, *Toxicara*, *Trichiura* and *Trichinella spp*), drugs, vasculitis (*i.e.,* Churg-Strauss syndrome, polyarteritis nodosa), connective tissue diseases, inflammatory bowel diseases (IBDs), celiac disease, lymphoma, leukemia and mastocytosis. Furthermore, ruling out of the hyper-eosinophilic syndrome is of special value as it is a myelo-proliferative disorder, characterized by idiopathic high peripheral eosinophilic count of > 1500 eos/hpf persisting for > 6 mo and having severe systemic implications due to its multisystem involvement, including heart, central nervous system, skin, lungs, liver and kidneys in addition to the GI tract[34,40].

It is also important to perform a food allergy evaluation in all patients with suspected EGE. Both IgE dependent (specific IgE and skin prick) and non-IgE TH2 dependent (skin patch) allergy tests may aid in identification of the specific allergen related to a case. However, these tests lack both sensitivity (missing about 40% of causative agents) and specificity (capable of overlapping detection of up to 14 allergens in some cases)[41]. A combination of both testing types, however, might enhance their overall predictive value for identifying the EGE-provoking

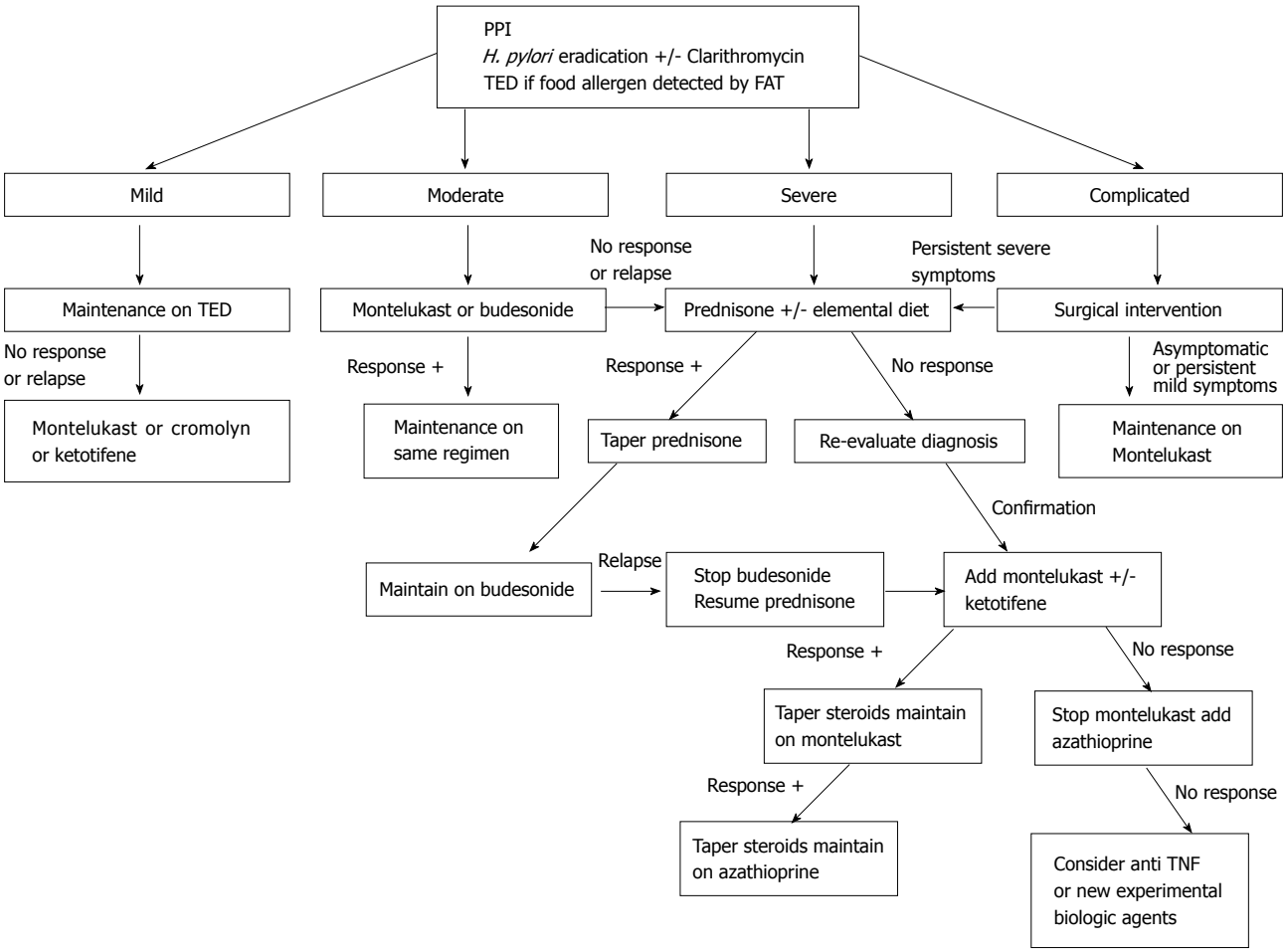

Figure 2 Eosinophilic gastroenteritis management based on initial disease severity. Anti-TNF: Anti-tumor necrosis factor; FAT: Food allergy testing; PPI: Proton pump inhibitor; TED: Targeted elimination diet.

agents[42].

MANAGEMENT

Although spontaneous remission reportedly occurs in around 30% to 40% of EGE cases[20,43], most patients require ongoing treatment. Many therapeutic options have been suggested, including dietary considerations, steroids, leukotrienes inhibitors and mast cells stabilizers. All of these treatment approaches have been described in small case series, but no randomized controlled or comparative trials have been published in the publicly available literature to describe the efficacies of different treatments or predictors of response to one or another option. Thus, no clear, systematic and practical strategy has been put forth for healthcare teams to follow in their management of EGE cases.

EGE is recognized as a chronic inflammatory disorder. Pineton de Chambrun *et al*[20] described three different long-term progression patterns: Non-relapsing disease (42%), commonly seen in patients with the serosal type; relapsing-remitting disease (37%), occurring primarily in patients with the muscular type; and chronic (persistent) disease (21%), predominantly observed in patients with the mucosal type. As mentioned above, a high AEC at diagnosis was found to be an independent predictor of relapses, as was extensive intestinal involvement. Some case series have found higher relapsing rates of 60% to 80%[7,26,44], while others have noted a possible association between younger age (under 20-year-old) and disease recurrence[24]. Unfortunately, research has not identified any other predictors of EGE disease evolution. Thus, it is worth contemplating maintenance treatment for patients after the initial induction phase has passed (Figure 2), taking into consideration the safety profile of the drug in use. It is important to remember, however, that the duration of such maintenance therapy cannot be predicted at this point.

TREATMENT MODALITIES

Proton pump inhibitor and Helicobacter pylori eradication

Proton pump inhibitor (PPI) treatment has been shown to improve the extent of duodenal eosinophilic infiltration in a patient with EGE, and the mechanism has been hypothesized to involve blockade of IL-4 and IL-13 activity[45]. *H. pylori* eradication has also been postulated as capable of inducing a cure of EGE disease[46]. The antibiotic clarithromycin, which is commonly used to

treat *H. pylori*-related ulcers, is also known to have immunomodulatory effect, whereby its actions cause inhibition of T cell proliferation and induction of eosinophil apoptosis; these mechanistic actions in the immune system have led to clarithromycin being applied as maintenance therapy for patients with steroid dependent EGE who are in remission[47].

Dietary therapy

Many dietary strategies have been proposed for management of EGE based on results from food allergy tests. In general, when a limited number of food allergens is detected, patients should be maintained on a "targeted elimination diet" (TED). When many or no allergens are identified, the more aggressive "empiric elimination diet" or "elemental diet" can be used. Lucendo *et al*[48] investigated dietary treatment efficacy in EGE through a systematic review and found significant improvement in most cases, especially in those who undertook the elemental diet, which induced clinical remission in > 75% of cases. However, the validity of such a high efficacy rate was questionable since no confirmation of histologic response was available for the majority of cases included in the review. On the other hand, the authors noted that dietary measures were predominantly considered in the setting of mucosal disease, which is well known to be associated with food allergy, while the efficacy in muscular and serosal types, which show weaker linkage to food allergy[4], was only rarely reported. In addition, patients' adherence and tolerability to such strategies remain an important drawback, especially when empiric elimination or elemental diets are used.

Thus, we suggest the TED for all EGE patients (Figure 2) who show few food allergens upon testing. The overall data in the literature is insufficient to recommend empiric and total elimination diets in routine management; however, an elemental diet can be used initially as adjunct treatment for severe cases.

Prednisone

Prednisone remains the mainstay for induction of remission of EGE. While most of the case series reported have shown a response rate to prednisone (up to 90%)[3,49], the most recent reports showed remarkably lower values (only 50%)[7]. This steroid acts by inducing eosinophil apoptosis and inhibiting chemotaxis. The recommended initial dose of 0.5 to 1 mg/kg usually induces remission within a 2 wk period, with the most dramatic response occurring in patients with the serosal type[50]. Thereafter, tapering dosage over a 6 to 8 wk period is recommended. Re-evaluation of the EGE diagnosis (and type) must be considered in cases of initial unresponsiveness[51]. Steroid dependent disease reportedly accounts for about 20% of cases[7] and, consequently, low doses of prednisone may be needed to maintain remission. Unfortunately, long-term steroid treatment predisposes some patients to serious side effects; in such cases, steroid-sparing agents can be of benefit.

Budesonide

Budesonide, a common steroid treatment of Crohn's disease and ulcerative colitis, has a high affinity for steroid receptors and produces fewer side effects due to its lower systemic impact. It has also been demonstrated as effective for induction and maintenance of remission in the majority of reported cases (Table 2)[15,26,52-59]. The usual dose is 9 mg/d, which can be tapered to 6 mg/d for use as prolonged maintenance therapy. The better safety profile of budesonide, compared to other steroid drugs, is of particular benefit for management of EGE cases over the long term, especially in the setting of steroid dependent disease.

Azathioprine

Azathioprine, a common immunosuppressive agent used in organ transplant and patients with autoimmune diseases, is an immunomodulator that induces apoptosis of T and B cells. The efficacy of this steroid-sparing agent has been demonstrated in patients with steroid dependent and refractory EGE disease. The usual dose for EGE patients is similar to that used in patients with IBD (2-2.5 mg/kg)[9,60,61]; lower doses may not be effective[62].

Montelukast sodium

Montelukast sodium, commonly used to treat asthma, is a selective leukotriene (LTD4) inhibitor with demonstrated efficacy for various eosinophilic disorders, including EGE. The majority of reports in the literature concerning its use in EGE (Table 3)[5,9,15,21,26,63-70] have shown significant clinical response in patients, either when the drug is used alone or in combination with steroids for induction and maintenance of remission in steroid dependent or refractory disease. The usual dose is 5-10 mg/d.

Oral cromolyn sodium

Oral cromolyn sodium is a mast cell stabilizer that blocks the release of immune mediators and the subsequent activation of eosinophils. While it has been shown to have significant efficacy in many of the reported cases of EGE, its effect was only modest in others, for unknown reasons (Table 4)[4,52,71-77]. The usual dose is 200 mg *tid* or *qid*.

Ketotifene

Ketotifene is a 2nd-generation H1-antihistamine agent that also modulates the release of mast cell mediators. Melamed *et al*[78] described 6 patients with EGE who responded clinically and histologically to ketotifen; however, Freeman *et al*[79] reported a single case in which the drug failed to maintain disease remission. This agent has also been proposed as an adjunct to steroids and montelukast for treating refractory EGE[5]. The usual dose is 1-2 mg twice daily.

Biologic agents

Biologic agents have also been reported in some case

Table 2 Published cases of eosinophilic gastroenteritis treated with budesonide

Ref.	Patient no.	Intestinal layer	Location	Previous treatment	Response
Russel *et al*[52], 1994	1	Mucosal	Ileum and cecum	Intolerant to steroids Failure of cromolyn sodium and mesalazine	Efficacy comparable to steroids over 5 mo
Tan *et al*[53], 2001	1	Full thickness with ascites	Antrum	Steroid dependent	Remission (+) over 2 yr
Siewert *et al*[54], 2006	1	Mucosal	Duodenum to ileum	None	Response (+)
Lombardi *et al*[55], 2007	1	Mucosal + submucosal	Ileum	Relapse after stopping budesonide and cromolyn sodium	Remission (+) on budesonide alone over 4 mo
Elsing *et al*[56], 2007	1	Muscular	Jejunum	Surgery + steroids for relapse	Remission (+) over 3 mo
Shahzad *et al*[57], 2011	1	Mucosal	Antrum + colon	None	Response (+)
Busoni *et al*[58], 2011	5	Mucosal	Lower + upper GI tract	Prednisone/methylprednisolone	Remission (+)
Lombardi *et al*[59], 2011	1	Muscular	Pyloric stenosis	Methylprednisolone	Remission (+) over 6 mo
Müller *et al*[26], 2014	1	Mucosal	Duodenum + colon + ileum	None	50% response (combined with 6-food elimination diet)
Wong *et al*[15], 2015	1	Mucosal +/- serosal or muscular	-	None	Recurrent symptoms

GI: Gastrointestinal.

Table 3 Published cases of eosinophilic gastroenteritis treated with montelukast

Ref.	Patient no.	Intestinal layer	Location	Previous treatment	Response
Neustrom *et al*[63], 1999	1	Mucosal	Esophagus + stomach + small intestine	Failure of response to elimination diet, cromolyn sodium, ranitidine and hydroxyzine	Clinical and histologic response (+)
Schwartz *et al*[64], 2001	1	Serosal	Duodenum	Steroid dependent	Remission (+) over 4 wk
Lu *et al*[65], 2003	2	Mucosal	-	Steroid dependent	1 → Not effective 2 → Partial response with tapering of prednisone to 10 mg/d
Vanderhoof *et al*[66], 2003	8	Mucosal	Esophagus (n = 4) Duodenum (n = 2) Colon (n = 2)	Failure of standard therapies	Clinical response (+) within 1 mo
Copeland *et al*[9], 2004	1	Mucosal	Stomach	Steroid refractory EGE (also receiving 6MP and 5ASA for UC)	Not effective
Friesen *et al*[67], 2004	40	Mucosal	Duodenum	None	Response (+) within 2 wk
Quack *et al*[68], 2005	1	Serosal	Ileum	Steroid dependent	Remission (+) over 2 yr
Urek *et al*[21], 2006	1	Serosal	Ileum	Steroid dependent	Response (+) within 4 wk
De Maeyer *et al*[69], 2011	1	-	-	Steroid dependent	Response (+)
Tien *et al*[5], 2011	12	Mucosal	Stomach + duodenum + colon + esophagus	4 → None 8 → Steroid dependent	Remission (+) over 12 mo 4/8 → Successful steroid tapering 3/8 → Not effective 1/8 → Lost to follow-up
Selva Kumar *et al*[70], 2011	1	Mucosal	Small intestine	Unresponsive to standard therapy	Response (+)
Müller *et al*[26], 2014	2	Mucosal (+/- serosal or muscular)	Stomach + small intestine	1 and 2 → Steroid dependent	1 → Remission (+) in combination with low-dose prednisone 2 → Remission (+) (off steroids)
Wong *et al*[15], 2015	2	Mucosal (+/- serosal or muscular)	-	1: Steroid dependent 2: None	Remission (+) for 36 mo (in combination with prednisone) Asymptomatic for 10 mo

5ASA: 5-Aminosalicylic acid; 6MP: 6 Mercaptopurine; UC: Ulcerative colitis.

studies of EGE. Mepolizumab (anti-IL5) was reported to have improved tissue and peripheral eosinophilia, but without relieving symptoms, in 4 patients with EGE[80]; unfortunately, another report associated its use

Table 4 Published cases of eosinophilic gastroenteritis treated with cromolyn sodium

Ref.	Patient no.	Intestinal layer	Location	Previous treatment	Response
Moots *et al*[71], 1988	1	Mucosal +/- muscular	Small intestine + colon	Prednisone, cyclophosphamide	Response (+) in 10 wk Maintenance over 2.5 yr
Talley *et al*[4], 1990	3	Mucosal	-	None	1 → Response (+) 2 → No response
Di Gioacchino *et al*[72], 1990	2	Mucosal	Stomach + duodenum	None	Clinical and histologic response (+) after 4-5 mo
Beishuizen *et al*[73], 1993	2	Mucosal	Upper gastrointestinal tract	Steroids	Prolonged response (+)
Van Dellen *et al*[74], 1994	1	Mucosal	Stomach + duodenum	Elemental diet (poorly tolerated)	Response (+)
Russel *et al*[52], 1994	1	Mucosal	Ileum + colon	Steroid dependent	None (failure to taper steroids)
Pérez-Millán *et al*[75], 1997	1	Serosal	Duodenum	None	Response (+) over 6 mo
Suzuki *et al*[76], 2003	1	Mucosal	Stomach + duodenum	Targeted elimination diet (poorly tolerated)	Response (+) (in combination with ketotifene)
Sheikh *et al*[77], 2009	3	Mucosal	Esophagus + stomach + duodenum	Steroid refractory	Not effective
		Mucosal	Stomach + duodenum + colon	None	Partial response
		Mucosal +/- muscular	Esophagus + stomach + duodenum + colon	Steroid dependent	Response (+) with tapering of prednisone over 6 mo

with rebound hypereosinophilia[81]. Omalizumab (anti-IgE) was reported to similarly result in a significant histologic response[82] but to be unlikely to efficiently treat EGE patients with a serum IgE level > 700 kIU/L[83]. Infliximab (anti-TNF) was reported as highly effective for inducing remission in refractory EGE, but its use is limited by the development of resistance and secondary loss of response, both of which can be managed by switching to adalimumab[84].

Surgery

Surgery is indicated in cases of severe disease that are complicated by perforation, intussusception or intestinal occlusion. It has been reported that about 40% of EGE patients may need surgery during the course of their disease, and about half of those may experience persistent symptoms postoperatively[85].

Other modalities

Other modalities include intravenous immunoglobulin and interferon-alpha, both of which appear to be effective in treating severe refractory and steroid dependent cases[10,65]. Suplatast tosilate, a TH2 cytokine inhibitor, can be beneficial as well[86]. Finally, fecal microbiota transplantation has also been reported to improve diarrhea in a patient with EGE, even before its application in combination with steroids[87].

FOLLOW UP AND TREATMENT END-POINTS

While most reported treatments of EGE aim to achieve clinical remission[48,67], histologic improvement remains the optimal way to assess a patient's response, even though it does not always correlate with clinical amelioration[79]. Biopsies can be obtained either endoscopically or under ultrasound guidance[27]. Other less invasive parameters may also be useful in monitoring of treatment response, such as reduction in peripheral eosinophilia[5] and improved radiologic aspects[88]. The choice of appropriate follow-up modality should always be individualized.

CONCLUSION

EGE is a chronic GI disease, having protean manifestations that mimic many other GI disorders. Its diagnosis requires a combination of clinical and pathologic criteria that are evaluated upon suspicious laboratory, radiologic and endoscopic findings. According to the disease severity at initial presentation, many therapeutic modalities can be applied, all of which have been reported in single and case series and have shown variable efficacy. A maintenance regimen is often needed, preferably based upon a safe steroid-sparing drug. Further studies are needed to compare the efficacy and safety profiles of the various treatments available as well as to select predictors of relapses, which might guide decision-making for the kind and duration of maintenance therapy.

REFERENCES

1 **Jensen ET**, Martin CF, Kappelman MD, Dellon ES. Prevalence of Eosinophilic Gastritis, Gastroenteritis, and Colitis: Estimates From a National Administrative Database. *J Pediatr Gastroenterol Nutr* 2016; **62**: 36-42 [PMID: 25988554 DOI: 10.1097/MPG.0000000000000865]

2 **Spergel JM**, Book WM, Mays E, Song L, Shah SS, Talley NJ, Bonis PA. Variation in prevalence, diagnostic criteria, and initial management options for eosinophilic gastrointestinal diseases in the United States. *J Pediatr Gastroenterol Nutr* 2011; **52**: 300-306 [PMID: 21057327 DOI: 10.1097/MPG.0b013e3181eb5a9f]

3 **Chang JY**, Choung RS, Lee RM, Locke GR, Schleck CD, Zinsmeister AR, Smyrk TC, Talley NJ. A shift in the clinical spectrum of eosinophilic gastroenteritis toward the mucosal disease type. *Clin Gastroenterol Hepatol* 2010; **8**: 669-675; quiz e88 [PMID: 20451664 DOI: 10.1016/j.cgh.2010.04.022]

4 **Talley NJ**, Shorter RG, Phillips SF, Zinsmeister AR. Eosinophilic gastroenteritis: a clinicopathological study of patients with disease of the mucosa, muscle layer, and subserosal tissues. *Gut* 1990; **31**: 54-58 [PMID: 2318432 DOI: 10.1136/gut.31.1.54]

5 **Tien FM**, Wu JF, Jeng YM, Hsu HY, Ni YH, Chang MH, Lin DT, Chen HL. Clinical features and treatment responses of children with eosinophilic gastroenteritis. *Pediatr Neonatol* 2011; **52**: 272-278 [PMID: 22036223 DOI: 10.1016/j.pedneo.2011.06.006]

6 **Keshavarzian A**, Saverymuttu SH, Tai PC, Thompson M, Barter S, Spry CJ, Chadwick VS. Activated eosinophils in familial eosinophilic gastroenteritis. *Gastroenterology* 1985; **88**: 1041-1049 [PMID: 3918913]

7 **Reed C**, Woosley JT, Dellon ES. Clinical characteristics, treatment outcomes, and resource utilization in children and adults with eosinophilic gastroenteritis. *Dig Liver Dis* 2015; **47**: 197-201 [PMID: 25547198 DOI: 10.1016/j.dld.2014.11.009]

8 **Butterfield JH**, Murray JA. Eosinophilic gastroenteritis and gluten-sensitive enteropathy in the same patient. *J Clin Gastroenterol* 2002; **34**: 552-553 [PMID: 11960068 DOI: 10.1097/00004836-200205000-00014]

9 **Copeland BH**, Aramide OO, Wehbe SA, Fitzgerald SM, Krishnaswamy G. Eosinophilia in a patient with cyclical vomiting: a case report. *Clin Mol Allergy* 2004; **2**: 7 [PMID: 15144561 DOI: 10.1186/1476-7961-2-7]

10 **Ciccia F**, Giardina AR, Alessi N, Rodolico V, Galia M, Ferrante A, Triolo G. Successful intravenous immunoglobulin treatment for steroid-resistant eosinophilic enteritis in a patient with systemic lupus erythematosus. *Clin Exp Rheumatol* 2011; **29**: 1018-1020 [PMID: 22132839]

11 **Daneshjoo R**, J Talley N. Eosinophilic gastroenteritis. *Curr Gastroenterol Rep* 2002; **4**: 366-372 [PMID: 12228038 DOI: 10.1007/s11894-002-0006-2]

12 **Rothenberg ME**. Eosinophilic gastrointestinal disorders (EGID). *J Allergy Clin Immunol* 2004; **113**: 11-28; quiz 29 [PMID: 14713902 DOI: 10.1016/j.jaci.2003.10.047]

13 **Jaffe JS**, James SP, Mullins GE, Braun-Elwert L, Lubensky I, Metcalfe DD. Evidence for an abnormal profile of interleukin-4 (IL-4), IL-5, and gamma-interferon (gamma-IFN) in peripheral blood T cells from patients with allergic eosinophilic gastroenteritis. *J Clin Immunol* 1994; **14**: 299-309 [PMID: 7814459 DOI: 10.1007/BF01540983]

14 **Klein NC**, Hargrove RL, Sleisenger MH, Jeffries GH. Eosinophilic gastroenteritis. *Medicine* (Baltimore) 1970; **49**: 299-319 [PMID: 5426746 DOI: 10.1097/00005792-197007000-00003]

15 **Wong GW**, Lim KH, Wan WK, Low SC, Kong SC. Eosinophilic gastroenteritis: Clinical profiles and treatment outcomes, a retrospective study of 18 adult patients in a Singapore Tertiary Hospital. *Med J Malaysia* 2015; **70**: 232-237 [PMID: 26358020]

16 **Cello JP**. Eosinophilic gastroenteritis--a complex disease entity. *Am J Med* 1979; **67**: 1097-1104 [PMID: 517550 DOI: 10.1016/0002-9343(79)90652-1]

17 **Shin WG**, Park CH, Lee YS, Kim KO, Yoo KS, Kim JH, Park CK. Eosinophilic enteritis presenting as intussusception in adult. *Korean J Intern Med* 2007; **22**: 13-17 [PMID: 17427639 DOI: 10.3904/kjim.2007.22.1.13]

18 **Maeshima A**, Murakami H, Sadakata H, Saitoh T, Matsushima T, Tamura J, Karasawa M, Naruse T. Eosinophilic gastroenteritis presenting with acute pancreatitis. *J Med* 1997; **28**: 265-272 [PMID: 9355030]

19 **Madhotra R**, Eloubeidi MA, Cunningham JT, Lewin D, Hoffman B. Eosinophilic gastroenteritis masquerading as ampullary adenoma. *J Clin Gastroenterol* 2002; **34**: 240-242 [PMID: 11873104 DOI: 10.1097/00004836-200203000-00009]

20 **Pineton de Chambrun G**, Gonzalez F, Canva JY, Gonzalez S, Houssin L, Desreumaux P, Cortot A, Colombel JF. Natural history of eosinophilic gastroenteritis. *Clin Gastroenterol Hepatol* 2011; **9**: 950-956.e1 [PMID: 21806952 DOI: 10.1016/j.cgh.2011.07.017]

21 **Urek MC**, Kujundzić M, Banić M, Urek R, Veic TS, Kardum D. Leukotriene receptor antagonists as potential steroid sparing agents in a patient with serosal eosinophilic gastroenteritis. *Gut* 2006; **55**: 1363-1364 [PMID: 16905705 DOI: 10.1136/gut.2006.099465]

22 **Chehade M**, Sicherer SH, Magid MS, Rosenberg HK, Morotti RA. Multiple exudative ulcers and pseudopolyps in allergic eosinophilic gastroenteritis that responded to dietary therapy. *J Pediatr Gastroenterol Nutr* 2007; **45**: 354-357 [PMID: 17873749 DOI: 10.1097/MPG.0b013e31803219d5]

23 **Manatsathit W**, Sermsathanasawadi R, Pongpaiboon A, Pongprasobchai S. Mucosal-type eosinophilic gastroenteritis in Thailand: 12-year retrospective study. *J Med Assoc Thai* 2013; **96** Suppl 2: S194-S202 [PMID: 23590042]

24 **Chen MJ**, Chu CH, Lin SC, Shih SC, Wang TE. Eosinophilic gastroenteritis: clinical experience with 15 patients. *World J Gastroenterol* 2003; **9**: 2813-2816 [PMID: 14669340 DOI: 10.3748/wjg.v9.i12.2813]

25 **Alnaser S**, Aljebreen AM. Endoscopic ultrasound and hisopathologic correlates in eosinophilic gastroenteritis. *Saudi J Gastroenterol* 2007; **13**: 91-94 [PMID: 19858621 DOI: 10.4103/1319-3767.32185]

26 **Müller M**, Keller K, Stallmann S, Eckardt AJ. Clinicopathologic Findings in Eosinophilic Gastroenteritis: A German Case Series. *J Genet Syndr Gene Ther* 2014; **5**: 1 [DOI: 10.4172/2157-7412.1000230]

27 **Marco-Doménech SF**, Gil-Sánchez S, Jornet-Fayos J, Ambit-Capdevila S, Gonzalez-Añón M. Eosinophilic gastroenteritis: percutaneous biopsy under ultrasound guidance. *Abdom Imaging* 1998; **23**: 286-288 [PMID: 9569298 DOI: 10.1007/s002619900341]

28 **Anuradha C**, Mittal R, Yacob M, Manipadam MT, Kurian S, Eapen A. Eosinophilic disorders of the gastrointestinal tract: imaging features. *Diagn Interv Radiol* 2012; **18**: 183-188 [PMID: 21948696]

29 **Zheng X**, Cheng J, Pan K, Yang K, Wang H, Wu E. Eosinophilic enteritis: CT features. *Abdom Imaging* 2008; **33**: 191-195 [PMID: 17387538 DOI: 10.1007/s00261-007-9209-1]

30 **Lee KJ**, Hahm KB, Kim YS, Kim JH, Cho SW, Jie H, Park CH, Yim H. The usefulness of Tc-99m HMPAO labeled WBC SPECT in eosinophilic gastroenteritis. *Clin Nucl Med* 1997; **22**: 536-541 [PMID: 9262899 DOI: 10.1097/00003072-199708000-00005]

31 **Solis-Herruzo JA**, de Cuenca B, Muñoz-Yagüe MT. Laparoscopic findings in serosal eosinophilic gastroenteritis. Report of two cases. *Endoscopy* 1988; **20**: 152-153 [PMID: 2972537 DOI: 10.1055/s-2007-1018162]

32 **Rothenberg ME**, Mishra A, Brandt EB, Hogan SP. Gastrointestinal eosinophils. *Immunol Rev* 2001; **179**: 139-155 [PMID: 11292017 DOI: 10.1034/j.1600-065X.2001.790114.x]

33 **Torpier G**, Colombel JF, Mathieu-Chandelier C, Capron M, Dessaint JP, Cortot A, Paris JC, Capron A. Eosinophilic gastroenteritis: ultrastructural evidence for a selective release of eosinophil major basic protein. *Clin Exp Immunol* 1988; **74**: 404-408 [PMID: 3233790]

34 **Hurrell JM**, Genta RM, Melton SD. Histopathologic diagnosis of eosinophilic conditions in the gastrointestinal tract. *Adv Anat Pathol* 2011; **18**: 335-348 [PMID: 21841404 DOI: 10.1097/PAP.0b013e318229bfe2]

35 **Blackburn GL**, Bistrian BR, Maini BS, Schlamm HT, Smith MF. Nutritional and metabolic assessment of the hospitalized patient. *JPEN J Parenter Enteral Nutr* 1977; **1**:11-22 [PMID: 98649]

36 **Carley A**. Anemia: when is it not iron deficiency? *Pediatr Nurs* 2003; **29**: 205-211 [PMID: 12836997]

37 **Tefferi A**. Blood eosinophilia: a new paradigm in disease classification, diagnosis, and treatment. *Mayo Clin Proc* 2005; **80**: 75-83 [PMID: 15667033 DOI: 10.4065/80.1.75]

38 **Macari M**, Balthazar EJ. CT of bowel wall thickening: significance and pitfalls of interpretation. *AJR Am J Roentgenol* 2001; **176**: 1105-1116 [PMID: 11312162 DOI: 10.2214/ajr.176.5.1761105]

39 **Weinstein WM**, Wiggett SD. The spectrum of endoscopic and histologic findings in eosinophilic gastroenteritis. A video capsule and push enteroscopic study. *Gastroenterology* 2003; **124**: A191 [DOI: 10.1016/s0016-5085(03)80957-5]

40 **Prussin C**. Eosinophilic gastroenteritis and related eosinophilic disorders. *Gastroenterol Clin North Am* 2014; **43**: 317-327 [PMID: 24813518 DOI: 10.1016/j.gtc.2014.02.013]

41 **Guajardo JR**, Plotnick LM, Fende JM, Collins MH, Putnam PE, Rothenberg ME. Eosinophil-associated gastrointestinal disorders: a world-wide-web based registry. *J Pediatr* 2002; **141**: 576-581 [PMID: 12378201 DOI: 10.1067/mpd.2002.127663]

42 **Spergel JM**, Brown-Whitehorn T, Beausoleil JL, Shuker M,

Liacouras CA. Predictive values for skin prick test and atopy patch test for eosinophilic esophagitis. *J Allergy Clin Immunol* 2007; **119**: 509-511 [PMID: 17291865 DOI: 10.1016/j.jaci.2006.11.016]
43 **Liao WH**, Wei KL, Po-Yen-Lin CS. A rare case of spontaneous resolution of eosinophilic ascites in a patient with primary eosinophilic gastroenteritis. *Chang Gung Med J* 2012; **35**: 354-359 [PMID: 22913863]
44 **Venkataraman S**, Ramakrishna BS, Mathan M, Chacko A, Chandy G, Kurian G, Mathan VI. Eosinophilic gastroenteritis--an Indian experience. *Indian J Gastroenterol* 1998; **17**: 148-149 [PMID: 9795503]
45 **Yamada Y**, Toki F, Yamamoto H, Nishi A, Kato M. Proton pump inhibitor treatment decreased duodenal and esophageal eosinophilia in a case of eosinophilic gastroenteritis. *Allergol Int* 2015; **64** Suppl: S83-S85 [PMID: 26344087 DOI: 10.1016/j.alit.2015.06.002]
46 **Soavi C**, Caselli M, Sioulis F, Cassol F, Lanza G, Zuliani G. Eosinophilic gastroenteritis cured with Helicobacter pylori eradication: case report and review of literature. *Helicobacter* 2014; **19**: 237-238 [PMID: 24650300 DOI: 10.1111/hel.12122]
47 **Ohe M**, Hashino S. Successful treatment of eosinophilic gastroenteritis with clarithromycin. *Korean J Intern Med* 2012; **27**: 451-454 [PMID: 23269887 DOI: 10.3904/kjim.2012.27.4.451]
48 **Lucendo AJ**, Serrano-Montalbán B, Arias Á, Redondo O, Tenias JM. Efficacy of Dietary Treatment for Inducing Disease Remission in Eosinophilic Gastroenteritis. *J Pediatr Gastroenterol Nutr* 2015; **61**: 56-64 [PMID: 25699593 DOI: 10.1097/MPG.0000000000000766]
49 **Khan S**. Eosinophilic gastroenteritis. *Best Pract Res Clin Gastroenterol* 2005; **19**: 177-198 [PMID: 15833687 DOI: 10.1016/j.bpg.2005.01.009]
50 **Zhang L**, Duan L, Ding S, Lu J, Jin Z, Cui R, McNutt M, Wang A. Eosinophilic gastroenteritis: clinical manifestations and morphological characteristics, a retrospective study of 42 patients. *Scand J Gastroenterol* 2011; **46**: 1074-1080 [PMID: 21623674 DOI: 10.3109/00365521.2011.579998]
51 **Stone KD**, Prussin C. Immunomodulatory therapy of eosinophil-associated gastrointestinal diseases. *Clin Exp Allergy* 2008; **38**: 1858-1865 [PMID: 19037962 DOI: 10.1111/j.1365-2222.2008.03122.x]
52 **Russel MG**, Zeijen RN, Brummer RJ, de Bruine AP, van Kroonenburgh MJ, Stockbrügger RW. Eosinophilic enterocolitis diagnosed by means of technetium-99m albumin scintigraphy and treated with budesonide (CIR). *Gut* 1994; **35**: 1490-1492 [PMID: 7959211 DOI: 10.1136/gut.35.10.1490]
53 **Tan AC**, Kruimel JW, Naber TH. Eosinophilic gastroenteritis treated with non-enteric-coated budesonide tablets. *Eur J Gastroenterol Hepatol* 2001; **13**: 425-427 [PMID: 11338074]
54 **Siewert E**, Lammert F, Koppitz P, Schmidt T, Matern S. Eosinophilic gastroenteritis with severe protein-losing enteropathy: successful treatment with budesonide. *Dig Liver Dis* 2006; **38**: 55-59 [PMID: 16326154]
55 **Lombardi C**, Salmi A, Savio A, Passalacqua G. Localized eosinophilic ileitis with mastocytosis successfully treated with oral budesonide. *Allergy* 2007; **62**: 1343-1345 [PMID: 17919149]
56 **Elsing C**, Placke J, Gross-Weege W. Budesonide for the treatment of obstructive eosinophilic jejunitis. *Z Gastroenterol* 2007; **45**: 187-189 [PMID: 17304405]
57 **Shahzad G**, Moise D, Lipka S, Rizvon K, Mustacchia PJ. Eosinophilic enterocolitis diagnosed by means of upper endoscopy and colonoscopy with random biopsies treated with budenoside: a case report and review of the literature. *ISRN Gastroenterol* 2011; **2011**: 608901 [PMID: 21991521 DOI: 10.5402/2011/608901]
58 **Busoni VB**, Lifschitz C, Christiansen S, G de Davila MT, Orsi M. [Eosinophilic gastroenteropathy: a pediatric series]. *Arch Argent Pediatr* 2011; **109**: 68-73 [PMID: 21283948 DOI: 10.1590/S0325-00752011000100019]
59 **Lombardi C**, Salmi A, Passalacqua G. An adult case of eosinophilic pyloric stenosis maintained on remission with oral budesonide. *Eur Ann Allergy Clin Immunol* 2011; **43**: 29-30 [PMID: 21409859]
60 **Redondo-Cerezo E**, Cabello MJ, González Y, Gómez M, García-Montero M, de Teresa J. Eosinophilic gastroenteritis: our recent experience: one-year experience of atypical onset of an uncommon disease. *Scand J Gastroenterol* 2001; **36**: 1358-1360 [PMID: 11761030]
61 **Netzer P**, Gschossmann JM, Straumann A, Sendensky A, Weimann R, Schoepfer AM. Corticosteroid-dependent eosinophilic oesophagitis: azathioprine and 6-mercaptopurine can induce and maintain long-term remission. *Eur J Gastroenterol Hepatol* 2007; **19**: 865-869 [PMID: 17873610 DOI: 10.1097/MEG.0b013e32825a6ab4]
62 **Kim YJ**, Chung WC, Kim Y, Chung YY, Lee KM, Paik CN, Chin HM, Hyun Choi HJ. A Case of Steroid Dependent Eosinophilic Gastroenteritis Presenting as a Huge Gastric Ulcer. *Korean J Helicobacter Up Gastrointest Res* 2012; **12**:103-107 [DOI: 10.7704/kjhugr.2012.12.2.103]
63 **Neustrom MR**, Friesen C. Treatment of eosinophilic gastroenteritis with montelukast. *J Allergy Clin Immunol* 1999; **104**: 506 [PMID: 10452782]
64 **Schwartz DA**, Pardi DS, Murray JA. Use of montelukast as steroid-sparing agent for recurrent eosinophilic gastroenteritis. *Dig Dis Sci* 2001; **46**: 1787-1790 [PMID: 11508684]
65 **Lu E**, Ballas ZK. Immuno-modulation in the treatment of eosinophilic gastroenteritis. *J Allergy Clin Immunol* 2003, **111**: S262 [DOI: 10.1016/S0091-6749(03)80936-3]
66 **Vanderhoof JA**, Young RJ, Hanner TL, Kettlehut B. Montelukast: use in pediatric patients with eosinophilic gastrointestinal disease. *J Pediatr Gastroenterol Nutr* 2003; **36**: 293-294 [PMID: 12548071]
67 **Friesen CA**, Kearns GL, Andre L, Neustrom M, Roberts CC, Abdel-Rahman SM. Clinical efficacy and pharmacokinetics of montelukast in dyspeptic children with duodenal eosinophilia. *J Pediatr Gastroenterol Nutr* 2004; **38**: 343-351 [PMID: 15076638]
68 **Quack I**, Sellin L, Buchner NJ, Theegarten D, Rump LC, Henning BF. Eosinophilic gastroenteritis in a young girl--long term remission under Montelukast. *BMC Gastroenterol* 2005; **5**: 24 [PMID: 16026609]
69 **De Maeyer N**, Kochuyt AM, Van Moerkercke W, Hiele M, Montelukast as a treatment modality for eosinophilic gastroenteritis. *Acta Gastroenterol Belg* 2011; **74**:570-575 [PMID: 22319970]
70 **Selva Kumar C**, Das RR, Balakrishnan CD, Balagurunathan K, Chaudhuri K. Malabsorption syndrome and leukotriene inhibitor. *J Trop Pediatr* 2011; **57**: 135-137 [PMID: 20571153]
71 **Moots RJ**, Prouse P, Gumpel JM. Near fatal eosinophilic gastroenteritis responding to oral sodium chromoglycate. *Gut* 1988; **29**: 1282-1285 [PMID: 3143628]
72 **Di Gioacchino M**, Pizzicannella G, Fini N, Falasca F, Antinucci R, Masci S, Mezzetti A, Marzio L, Cuccurullo F. Sodium cromoglycate in the treatment of eosinophilic gastroenteritis. *Allergy* 1990; **45**: 161-166 [PMID: 2109547]
73 **Beishuizen A**, van Bodegraven AA, Bronsveld W, Sindram JW. Eosinophilic gastroenteritis--a disease with a wide clinical spectrum. *Neth J Med* 1993; **42**: 212-217 [PMID: 8377880]
74 **Van Dellen RG**, Lewis JC. Oral administration of cromolyn in a patient with protein-losing enteropathy, food allergy, and eosinophilic gastroenteritis. *Mayo Clin Proc* 1994; **69**: 441-444 [PMID: 8170195 DOI: 10.1016/S0025-6196(12)61640-1]
75 **Pérez-Millán A**, Martín-Lorente JL, López-Morante A, Yuguero L, Sáez-Royuela F. Subserosal eosinophilic gastroenteritis treated efficaciously with sodium cromoglycate. *Dig Dis Sci* 1997; **42**: 342-344 [PMID: 9052516]
76 **Suzuki J**, Kawasaki Y, Nozawa R, Isome M, Suzuki S, Takahashi A, Suzuki H. Oral disodium cromoglycate and ketotifen for a patient with eosinophilic gastroenteritis, food allergy and protein-losing enteropathy. *Asian Pac J Allergy Immunol* 2003; **21**: 193-197 [PMID: 15032404]
77 **Sheikh RA**, Prindiville TP, Pecha RE, Ruebner BH. Unusual presentations of eosinophilic gastroenteritis: case series and review of literature. *World J Gastroenterol* 2009; **15**: 2156-2161 [PMID: 19418590 DOI: 10.3748/wjg.15.2156]
78 **Melamed I**, Feanny SJ, Sherman PM, Roifman CM. Benefit of ketotifen in patients with eosinophilic gastroenteritis. *Am J Med* 1991; **90**: 310-314 [PMID: 2003512 DOI: 10.1016/0002-9343(91)80010-J]
79 **Freeman HJ**. Longstanding eosinophilic gastroenteritis of more

than 20 years. *Can J Gastroenterol* 2009; **23**: 632-634 [PMID: 19816628]
80 **Prussin C**, James SP, Huber MM, Klion AD, Metcalfe DD. Pilot study of anti-IL-5 in eosinophilic gastroenteritis. *J Allergy Clin Immunol* 2003; **111**: S275 [DOI: 10.1016/S0091-6749(03)80986-7]
81 **Kim YJ**, Prussin C, Martin B, Law MA, Haverty TP, Nutman TB, Klion AD. Rebound eosinophilia after treatment of hypereosinophilic syndrome and eosinophilic gastroenteritis with monoclonal anti-IL-5 antibody SCH55700. *J Allergy Clin Immunol* 2004; **114**: 1449-1455 [PMID: 15577851 DOI: 10.1016/j.jaci.2004.08.027]
82 **Foroughi S**, Foster B, Kim N, Bernardino LB, Scott LM, Hamilton RG, Metcalfe DD, Mannon PJ, Prussin C. Anti-IgE treatment of eosinophil-associated gastrointestinal disorders. *J Allergy Clin Immunol* 2007; **120**: 594-601 [PMID: 17765756 DOI: 10.1016/j.jaci.2007.06.015]
83 **Hochhaus G**, Brookman L, Fox H, Johnson C, Matthews J, Ren S, Deniz Y. Pharmacodynamics of omalizumab: implications for optimised dosing strategies and clinical efficacy in the treatment of allergic asthma. *Curr Med Res Opin* 2003; **19**: 491-498 [PMID: 14594521 DOI: 10.1185/030079903125002171]
84 **Turner D**, Wolters VM, Russell RK, Shakhnovich V, Muise AM, Ledder O, Ngan B, Friesen C. Anti-TNF, infliximab, and adalimumab can be effective in eosinophilic bowel disease. *J Pediatr Gastroenterol Nutr* 2013; **56**: 492-497 [PMID: 23221994]
85 **Naylor AR**. Eosinophilic gastroenteritis. *Scott Med J* 1990; **35**: 163-165 [PMID: 2077646]
86 **Shirai T**, Hashimoto D, Suzuki K, Osawa S, Aonahata M, Chida K, Nakamura H. Successful treatment of eosinophilic gastroenteritis with suplatast tosilate. *J Allergy Clin Immunol* 2001; **107**: 924-925 [PMID: 11344364]
87 **Dai YX**, Shi CB, Cui BT, Wang M, Ji GZ, Zhang FM. Fecal microbiota transplantation and prednisone for severe eosinophilic gastroenteritis. *World J Gastroenterol* 2014; **20**: 16368-16371 [PMID: 25473198 DOI: 10.3748/wjg.v20.i43.16368]
88 **Buljevac M**, Urek MC, Stoos-Veić T. Sonography in diagnosis and follow-up of serosal eosinophilic gastroenteritis treated with corticosteroid. *J Clin Ultrasound* 2005; **33**: 43-46 [PMID: 15690448]

P- Reviewer: Capasso R, Gupta N, Liu F **S- Editor**: Gong ZM
L- Editor: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i4.524

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 524-530
ISSN 2150-5349 (online)

MINIREVIEWS

How I treat my inflammatory bowel disease-patients with thiopurines?

Berrie Meijer, Chris JJ Mulder, Adriaan A van Bodegraven, Nanne KH de Boer

Berrie Meijer, Chris JJ Mulder, Nanne KH de Boer, Department of Gastroenterology and Hepatology, VU University Medical Center, 1007 MB, Amsterdam, The Netherlands

Adriaan A van Bodegraven, Department of Gastroenterology, Geriatrics, Internal and Intensive Care Medicine (Co-MIK), Zuyderland Medical Center, 6130 MB, Sittard-Geleen, The Netherlands

Author contributions: de Boer NKH was the guarantor of the article; Meijer B completed the first draft of the manuscript; Mulder CJJ, van Bodegraven AA and de Boer NKH critically revised the manuscript; Meijer B finalized the article.

Conflict-of-interest statement: There is no conflict of interest associated with any of the senior author or other coauthors contributed their efforts in this manuscript.

Manuscript source: Invited manuscript

Correspondence to: Nanne KH de Boer, MD, PhD, Department of Gastroenterology and Hepatology, VU University Medical Center, PO Box 7057, 1007 MB, Amsterdam, The Netherlands. khn.deboer@vumc.nl
Telephone: +31-20-4440613
Fax: +31-20-4440554

Received: July 21, 2016
Peer-review started: July 21, 2016
First decision: September 5, 2016
Revised: September 12, 2016
Accepted: October 5, 2016
Article in press: October 7, 2016
Published online: November 6, 2016

Abstract

Thiopurines are essential drugs to maintain remission in patients with inflammatory bowel disease (IBD). Thiopurines used in IBD are azathioprine (2.0-2.5 mg/kg), mercaptopurine (1.0-1.5 mg/kg) and thioguanine (0.2-0.3 mg/kg). However, mainly due to numerous adverse events associated with thiopurine use, almost 50% of the patients have to discontinue conventional thiopurine treatment. Extensive monitoring and the application of several treatment strategies, such as split-dose administration, co-administration with allopurinol or dose reduction/increase, may increase the chance of successful therapy. With this review, we provide practical information on how thiopurines are initiated and maintained in two thiopurine research centers in The Netherlands. We provide clinical information concerning safety issues, indications and management of therapy that may serve as a guide for the administration of thiopurines in IBD patients in daily practice.

Key words: Thiopurines; Azathioprine; Mercaptopurine; Thioguanine; Inflammatory bowel disease; Therapeutic drug monitoring; Pregnancy; Metabolites

Core tip: Conventional thiopurine therapy with azathioprine and mercaptopurine in inflammatory bowel disease is associated with several adverse events causing cessation of therapy in up to half of the patients. On the contrary, thiopurine therapy is often unnecessarily discontinued. In this practical review, we provide information on how thiopurine therapy is initiated and maintained using periodical laboratory tests and the application of various treatment strategies (including the administration of a third thiopurine; thioguanine), based on the experience in the two expert thiopurine centers in The Netherlands.

Meijer B, Mulder CJJ, van Bodegraven AA, de Boer NKH. How I treat my inflammatory bowel disease-patients with thiopurines? *World J Gastrointest Pharmacol Ther* 2016; 7(4): 524-530 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i4/524.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i4.524

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic condition of the gastrointestinal tract which is characterized by episodes of remission and relapses and encompasses both Crohn's disease (CD) and ulcerative colitis (UC). In the management of IBD, thiopurines [*i.e.*, azathioprine (AZA), mercaptopurine (MP) and thioguanine (TG)] play an important role in clinical practice, mainly in order to maintain remission[1-4]. Over the past decades, extensive research has been performed to elucidate the complex metabolism of thiopurine derivatives[5-7]. In this article, we demonstrate and discuss the way we use thiopurine therapy in the treatment of adult IBD patients in two referral centers in The Netherlands in daily practice. For information about thiopurine therapy in pediatric IBD, we refer to reviews focused on this patient group[8-10].

DISCOVERY OF THIOPURINES

Thiopurines were firstly described in the early 1950s by Gertrude Elion and George Hitchings, primarily as antimetabolite therapy[11]. Initially, thiopurines were used in the treatment of acute lymphatic leukemia in children and in the prevention of organ transplant rejection. The first IBD patient treated with thiopurines has been described by Dr. Bean[12] in 1962. At this moment, thiopurines are used in a variety of autoimmune disorders and hematologic malignancies[13,14].

PHARMACOLOGY OF THIOPURINES

The thiopurine derivatives AZA, MP and TG are all pro-drugs which are subsequently converted into the allegedly most important pharmacologically active end-metabolites, 6-thioguanine nucleotides (6-TGN)[5]. AZA is converted into MP by the enzyme glutathione S-transferase, after which MP is metabolized by three competing enzymatic systems. First, a part of the concentration MP is withdrawn from bioavailability by xanthine oxidase (XO) and thiopurine-S-methyltransferase (TPMT), converting MP into 6-thiouric acid (6-TUA) and 6-methylmercaptopurine (6-MMP), respectively. The remaining concentration of MP is metabolized *via* the purine salvage pathway into 6-TGN by a cascade of hypoxanthine-guanine phosphoribosyl transferase (HGPRT), inosine monophosphate dehydrogenase (IMPD) and guanosine monophosphate synthetase (GMPS). The 6-TGN can be incorporated in the DNA (thus achieving an anti-metabolic effect), but also account for inhibition of anti-apoptotic effects and down-regulation of pro-inflammatory cytokines. In daily practice, we measure 6-TGN and 6-MMP in red blood cells (RBC), which is mainly due to the fact that in patients with leukemia, the original indication for thiopurine therapy, successful treatment with thiopurines leads to the unavailability of leukocytes[11,15].

In contrast to AZA and MP, the metabolism of TG is less extensive as TG is directly converted into 6-TGN by HGPRT. Whether TG is also withdrawn from bioavailability by the effect of TPMT and XO, this effect is relatively smaller than in AZA and MP, leaving a larger portion of TG available for (direct) conversion into 6-TGN (Figure 1)[5,7,16,17].

INDICATIONS OF THIOPURINE THERAPY

When treating IBD patients in our centers, we apply the therapeutic approach known as accelerated step up care in both CD and UC[18]. Conventional thiopurines (*i.e.*, AZA and MP) do not play a standard role in the active phase of CD and UC as such, however it may be added to induction course therapy with corticosteroids in those patients who are suspected of having a more severe or prolonged disease course. In patients with only mildly active disease with good reaction on initial induction therapy, thiopurines do not have to be initiated straight away[19]. However, in those patients with a relapse of disease despite two induction courses of corticosteroids, thiopurines are required to maintain remission[20]. Furthermore, thiopurines are co-administered as a routine to treatment with anti-TNF therapy in our centers, in line with recent observations from the SONIC trial[2-4,21]. In those patients receiving vedolizumab (Entyvio®) evidence is scarce whether to (dis)continue simultaneous thiopurine therapy[22,23]. In our centers, we continue thiopurine therapy in the majority of patients, since patients receiving vedolizumab are likely to have highly complex disease in which vedolizumab is often initiated as rescue drug. Additionally, in line with the observations in the SONIC trial in patients receiving infliximab and adalimumab, we presume that thiopurines might have a protecting effect on the development of antibodies against vedolizumab[24,25]. Finally, thiopurines are administered in surgical CD patients to prevent post-surgical recurrence, especially in complex patients with fistulizing disease or multiple surgical interventions[3].

Thiopurine therapy is initiated in a dosage of 2.0-2.5 mg/kg for AZA or 1.0-1.5 mg/kg for MP, starting with 50 mg/d in the first week and increasing to full-dose when patients experience no adverse effects on low-dose therapy[1]. In those patients in whom thiopurines were co-administered next to induction corticosteroid therapy, the steroids are tapered down in 2-3 mo.

In our center, we prefer to initiate thiopurine therapy using MP, based on results of several rechallenge studies[26-31]. Furthermore, in those patients with mild adverse events (*i.e.*, no severe myelotoxicity or pancreatitis) on MP therapy, we rechallenge these patients with MP with low threshold.

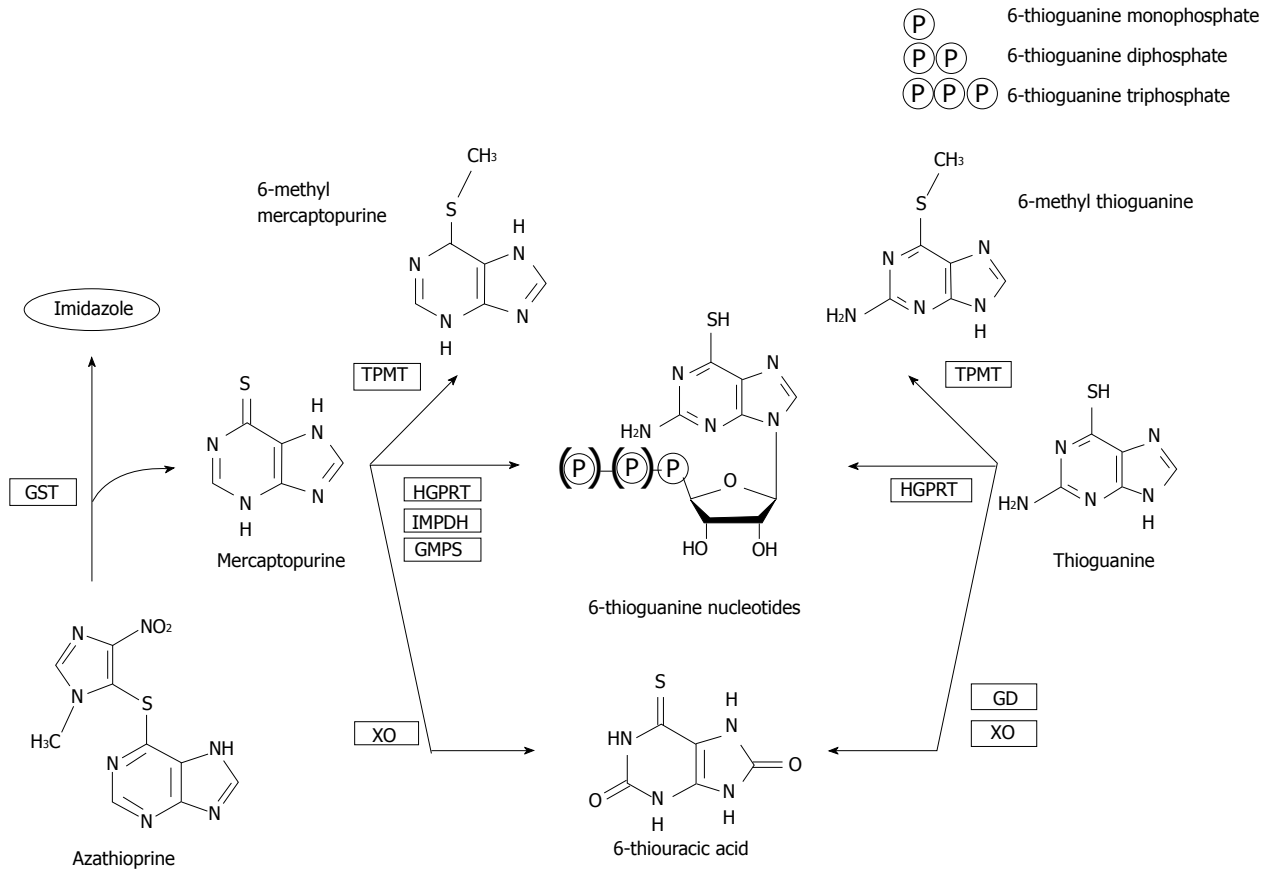

Figure 1 Simplified scheme of thiopurine metabolism. Azathioprine is converted to mercaptopurine by the enzyme glutathione S-transferase (GST), by separating the imidazole-group. 6-Mercaptopurine is enzymatically converted into 6-methylmercaptopurine (6-MMP) by thiopurine-S-methyltransferase (TPMT) and into 6-thiouracic acid (6-TUA) by xanthine oxidase (XO). The remaining portion of mercaptopurine is converted into the biochemically active end-metabolites 6-thioguaninenucleotides (6-TGN, consisting of 6-thioguanine monophosphate, 6-thioguanine diphosphate and 6-thioguanine triphosphate) by a pathway of hypoxanthine-guanine phosphoribosyl transferase (HGPRT), inosine monophosphate dehydrogenase (IMPDH) and guanosine monophosphate synthetase (GMPS). Thioguanine is metabolized by TPMT into 6-methylthioguanine (6-MTG) and into 6-TUA by guanine deaminase (GD) and XO. The remaining portion of thioguanine is directly converted into 6-TGN by HGPRT. Squared abbreviations display enzymatic conversions. Adapted from van Asseldonk *et al*[5].

TOXICITY OF THIOPURINE THERAPY

As thiopurines are associated with a broad spectrum of adverse events (*i.e.*, flu-like symptoms, arthralgia, gastrointestinal complaints, rash, pancreatitis, hepatotoxicity and myelotoxicity), one of the applied strategies to reduce the risk of developing adverse events is to measure TPMT activity before initiating thiopurine therapy, to identify patients at risk of developing adverse events based on an aberrant thiopurine metabolism[7,16,32,33]. Literature reports show that 1:300 (Caucasian) individuals have TPMT deficiency, making them at risk for developing (severe) myelosuppression due to preferential 6-TGN formation[34]. In our centers, however, TPMT activity is not determined as we initiate thiopurine therapy with a low-dose start up scheme. Furthermore, many patients with normal TPMT activity could still develop adverse events of thiopurine therapy[32,35,36]. For this reason amongst others, we choose to extensively monitor laboratory and clinical parameters in the first three months after initiation of thiopurine therapy. At week 0, 1, 2, 4, 8 and 12, hematologic and hepatic parameters are being measured, as well as creatinine and C-reactive protein (Table 1). After initiation, these parameters are determined each 3-4 mo during thiopurine maintenance therapy.

Table 1 Laboratory tests to determine risk of myelotoxicity and hepatotoxicity during initiation of thiopurine therapy

Hematologic parameters
Hemoglobin
White blood cell count
Platelet count
Hepatic parameters
Alkalic phosphatase
Gamma glutamyl transpeptidase
Alanine aminotransferase
Other parameters
Creatinine
C-reactive protein

Measuring of above mentioned parameters: Induction phase: week 0, 1, 2, 4, 8 and 12; Maintenance phase: Each 3-4 mo.

MEASURING METABOLITES

Measurement of thiopurine metabolites (6-TGN and 6-MMP) is not performed routinely in clinical practice at our centers. In those patients who experience adverse

Table 2 Interpretation of metabolite levels in patients with inflammatory bowel disease treated with azathioprine or mercaptopurine

6-TGN (pmol/8 × 10^8 RBC)	6-MMP (pmol/8 × 10^8 RBC)	Non-response	Adverse event (dose-dependent)	Recommendation
<< 230	<< 5700	Non-compliance	Not expected	Gain compliance
< 230	< 5700	Non-compliance/under dosing	Not expected	Gain compliance/increase dose[1]
230-400	< 5700	Possible resistance to thiopurine therapy	Not expected	Increase dose1 or change therapy[2]
> 400	< 5700	Therapy resistance	Myelotoxicity	Change therapy[2]
< 230	>> 5700	Shunting	Myelotoxicity	Consider allopurinol[3] or switch to TG[4]
< 230	> 5700	Shunting	Hepatotoxicity	Consider allopurinol, 5-ASA or switch to TG
230-400	> 5700	Possible resistance to thiopurine therapy	Hepatotoxicity	Consider allopurinol[3] or 5-ASA
> 400	> 5700	Therapy resistance	Hepatotoxicity	Change therapy[2]
			Myelotoxicity	Decrease dose[5]

Therapeutic target of therapy: 6-TGN: 230-400 pmol/8 × 10^8 RBC; 6-MMP: < 5700 pmol/8 × 10^8 RBC. [1]AZA: Increase with 50 mg, MP: Increase with 25 mg; [2]In case of thiopurine refractoriness, consider switch of therapy to non-thiopurine therapy; [3]Allopurinol co-treatment with 100 mg daily requires dose-adjustment of thiopurine therapy to approximately 25%-33% of original daily dose[38,60]; [4]Treatment with thioguanine in low dose (*i.e.*, 0.3 mg/kg) bypasses the formation of 6-MMP; [5]In case of adverse events. 6-TGN: 6-thioguanine nucleotides; 6-MMP: 6-methylmercaptopurine; RBC: Red blood cells; <: Lower than; <<: Much lower than; >: Higher than; >>: Much higher than; AZA: Azathioprine; MP: Mercaptopurine; TG: Thioguanine; 5-ASA: 5-aminosalicylic acid (mesalazine).

events or non-response to treatment, metabolite levels may explain why these patients are intolerant or resistant to therapy (Table 2). Based on these results, individual treatment strategies (*i.e.*, split-dose administration, dose reduction/increase, the addition of mesalazine or allopurinol to a 25%-33% dose of the original thiopurine in patients with an altered thiopurine metabolism, so-called "skewers") may be applied[37-39].

TG THERAPY

In those patients with either idiosyncratic (*e.g.*, pancreatitis) adverse events on conventional thiopurine therapy or adverse events based on elevated 6-MMP concentrations, these patients can be switched to TG. In some countries, TG is considered as rescue drug when conventional therapy fails, however, in The Netherlands this drug is officially registered as treatment option for IBD since March 2016. One of the feared complications of TG treatment is the development of nodular regenerative hyperplasia (NRH), a condition of the liver in which patients might develop non-cirrhotic portal hypertension. However, in contrary to earlier observations[40-42], the development of NRH is seldomly witnessed in those patients treated with low-dose TG therapy (*i.e.*, 0.3 mg/kg)[43-47] and furthermore not associated with clinically relevant liver disease[48]. In our patients treated with TG, liver biopsies are only performed in patients with symptoms of portal hypertension or persisting liver test abnormalities and no longer as a routine[48].

CANCER RISK

The use of thiopurines is associated with a three- to fivefold higher risk of the development of lymphoproliferative disorders, in particular non-Hodgkin lymphoma, as well as hepatosplenic T-cell lymphoma, especially in patients without prior Epstein-Barr virus (EBV) exposure[49]. We do not systematically test EBV seroprevalence in patients starting with thiopurines, since over 90% of Dutch inhabitants are exposed to EBV during childhood[50].

Furthermore, there is a clinically significant elevated risk of developing non-melanoma skin cancer, such as squamous cell carcinoma and basal cell carcinoma[49,51]. In our centers, we inform our patients of this higher risk and instruct them to, for example, apply sunscreen to unprotected skin and mention newly developed skin lesions directly to the treating physician or IBD-nurse. However, since the absolute incidence of these malignancies in thiopurine-using IBD patients is still low, we do not systematically screen our patients for the existence of these tumors.

PREGNANCY

According to recent literature reviews, conventional thiopurine use during pregnancy is not associated with a higher risk of preterm birth, congenital disorders or children with low birth weight[52-54]. For this reason amongst others, we do not cessate thiopurine therapy in patients that become pregnant, but we refer patients during pregnancy to a dedicated team of gynecologists with interest in IBD. Furthermore, after a successful pregnancy, there is insufficient evidence to discourage patients to give breastfeeding; however, this should always be adjusted to the individual patient wishes[55-57]. Evidence concerning the use of TG during pregnancy is scarce and further prospective trials are needed to confirm the safety of this thiopurine derivative in pregnant women[58].

WHEN TO STOP THIOPURINE THERAPY?

Whether patients achieving a deep remission may successfully stop thiopurines is not known[19,59,60]. In our centers, we continue thiopurine therapy with low threshold, especially in patients with a predicted complex

course (*i.e.*, severe or difficult to manage disease). An exception is the patient with deep prolonged (*i.e.*, ⩾ 2-3 years) remission on thiopurine therapy with no signs of active disease on clinical, biochemical, endoscopic, histological and radiologic evaluation. In these patients, thiopurine therapy could be ceased with a good probability of relapse-free disease.

CONCLUSION

Whereas treatment with thiopurine therapy in IBD patients is hampered by a high number of discontinuations, mostly due to adverse events, several treatment strategies may be applied to maximize effectiveness and optimize safety. With this article, we provided a practical overview on how thiopurine therapy is being prescribed in two of the thiopurine research expert centers in Europe. We provided information concerning pharmacotherapy, indications of thiopurine treatment, toxicity of thiopurines and how to optimize treatment in individual patients using different treatment strategies.

REFERENCES

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

2 **Dignass A**, Van Assche G, Lindsay JO, Lémann M, Söderholm J, Colombel JF, Danese S, D'Hoore A, Gassull M, Gomollón F, Hommes DW, Michetti P, O'Morain C, Oresland T, Windsor A, Stange EF, Travis SP. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Current management. *J Crohns Colitis* 2010; **4**: 28-62 [PMID: 21122489 DOI: 10.1016/j.crohns.2009.12.002]

3 **Chande N**, Patton PH, Tsoulis DJ, Thomas BS, MacDonald JK. Azathioprine or 6-mercaptopurine for maintenance of remission in Crohn's disease. *Cochrane Database Syst Rev* 2015; **10**: CD000067 [PMID: 26517527 DOI: 10.1002/14651858.CD000067.pub3]

4 **Chande N**, Tsoulis DJ, MacDonald JK. Azathioprine or 6-mercaptopurine for induction of remission in Crohn's disease. *Cochrane Database Syst Rev* 2013; **4**: CD000545 [PMID: 23633304 DOI: 10.1002/14651858.CD000545.pub4]

5 **Van Asseldonk DP**, de Boer NK, Peters GJ, Veldkamp AI, Mulder CJ, Van Bodegraven AA. On therapeutic drug monitoring of thiopurines in inflammatory bowel disease; pharmacology, pharmacogenomics, drug intolerance and clinical relevance. *Curr Drug Metab* 2009; **10**: 981-997 [PMID: 20214590]

6 **Al Hadithy AF**, de Boer NK, Derijks LJ, Escher JC, Mulder CJ, Brouwers JR. Thiopurines in inflammatory bowel disease: pharmacogenetics, therapeutic drug monitoring and clinical recommendations. *Dig Liver Dis* 2005; **37**: 282-297 [PMID: 15788214 DOI: 10.1016/j.dld.2004.09.029]

7 **de Boer NK**, van Bodegraven AA, Jharap B, de Graaf P, Mulder CJ. Drug Insight: pharmacology and toxicity of thiopurine therapy in patients with IBD. *Nat Clin Pract Gastroenterol Hepatol* 2007; **4**: 686-694 [PMID: 18043678 DOI: 10.1038/ncpgasthep1000]

8 **Ruemmele FM**, Turner D. Differences in the management of pediatric and adult onset ulcerative colitis--lessons from the joint ECCO and ESPGHAN consensus guidelines for the management of pediatric ulcerative colitis. *J Crohns Colitis* 2014; **8**: 1-4 [PMID: 24230969 DOI: 10.1016/j.crohns.2013.10.006]

9 **Ruemmele FM**, Veres G, Kolho KL, Griffiths A, Levine A, Escher JC, Amil Dias J, Barabino A, Braegger CP, Bronsky J, Buderus S, Martín-de-Carpi J, De Ridder L, Fagerberg UL, Hugot JP, Kierkus J, Kolacek S, Koletzko S, Lionetti P, Miele E, Navas López VM, Paerregaard A, Russell RK, Serban DE, Shaoul R, Van Rheenen P, Veereman G, Weiss B, Wilson D, Dignass A, Eliakim A, Winter H, Turner D. Consensus guidelines of ECCO/ESPGHAN on the medical management of pediatric Crohn's disease. *J Crohns Colitis* 2014; **8**: 1179-1207 [PMID: 24909831 DOI: 10.1016/j.crohns.2014.04.005]

10 **Rosen MJ**, Dhawan A, Saeed SA. Inflammatory Bowel Disease in Children and Adolescents. *JAMA Pediatr* 2015; **169**: 1053-1060 [PMID: 26414706 DOI: 10.1001/jamapediatrics.2015.1982]

11 **Elion GB**. The purine path to chemotherapy. *Science* 1989; **244**: 41-47 [PMID: 2649979]

12 **Bean RH**. The treatment of chronic ulcerative colitis with 6-mercaptopurine. *Med J Aust* 1962; **49**: 592-593 [PMID: 13969929]

13 **Karran P**, Attard N. Thiopurines in current medical practice: molecular mechanisms and contributions to therapy-related cancer. *Nat Rev Cancer* 2008; **8**: 24-36 [PMID: 18097462 DOI: 10.1038/nrc2292]

14 **Fong SC**, Blaker PA, Arenas-Hernandez M, Marinaki AM, Sanderson JD. Getting the best out of thiopurine therapy: thiopurine S-methyltransferase and beyond. *Biomark Med* 2015; **9**: 51-65 [PMID: 25605455 DOI: 10.2217/bmm.14.97]

15 **Dervieux T**, Chu Y, Su Y, Pui CH, Evans WE, Relling MV. HPLC determination of thiopurine nucleosides and nucleotides in vivo in lymphoblasts following mercaptopurine therapy. *Clin Chem* 2002; **48**: 61-68 [PMID: 11751539]

16 **Teml A**, Schaeffeler E, Herrlinger KR, Klotz U, Schwab M. Thiopurine treatment in inflammatory bowel disease: clinical pharmacology and implication of pharmacogenetically guided dosing. *Clin Pharmacokinet* 2007; **46**: 187-208 [PMID: 17328579 DOI: 10.2165/00003088-200746030-00001]

17 **Zaza G**, Cheok M, Krynetskaia N, Thorn C, Stocco G, Hebert JM, McLeod H, Weinshilboum RM, Relling MV, Evans WE, Klein TE, Altman RB. Thiopurine pathway. *Pharmacogenet Genomics* 2010; **20**: 573-574 [PMID: 19952870 DOI: 10.1097/FPC.0b013e328334338f]

18 **van Bodegraven AA**, van Everdingen JJ, Dijkstra G, de Jong DJ, Oldenburg B, Hommes DW. [Guideline 'Diagnosis and treatment of inflammatory bowel disease in adults'. I. Diagnosis and treatment]. *Ned Tijdschr Geneeskd* 2010; **154**: A1899 [PMID: 21029496]

19 **Frei P**, Biedermann L, Nielsen OH, Rogler G. Use of thiopurines in inflammatory bowel disease. *World J Gastroenterol* 2013; **19**: 1040-1048 [PMID: 23467510 DOI: 10.3748/wjg.v19.i7.1040]

20 **Goel RM**, Blaker P, Mentzer A, Fong SC, Marinaki AM, Sanderson JD. Optimizing the use of thiopurines in inflammatory bowel disease. *Ther Adv Chronic Dis* 2015; **6**: 138-146 [PMID: 25954498 DOI: 10.1177/2040622315579063]

21 **Colombel JF**, Reinisch W, Mantzaris GJ, Kornbluth A, Rutgeerts P, Tang KL, Oortwijn A, Bevelander GS, Cornillie FJ, Sandborn WJ. Randomised clinical trial: deep remission in biologic and immunomodulator naïve patients with Crohn's disease - a SONIC post hoc analysis. *Aliment Pharmacol Ther* 2015; **41**: 734-746 [PMID: 25728587 DOI: 10.1111/apt.13139]

22 **Bressler B**, Marshall JK, Bernstein CN, Bitton A, Jones J, Leontiadis GI, Panaccione R, Steinhart AH, Tse F, Feagan B. Clinical practice guidelines for the medical management of nonhospitalized ulcerative colitis: the Toronto consensus. *Gastroenterology* 2015; **148**: 1035-1058 .e3 [PMID: 25747596 DOI: 10.1053/j.gastro.2015.03.001]

23 **Côté-Daigneault J**, Bouin M, Lahaie R, Colombel JF, Poitras P. Biologics in inflammatory bowel disease: what are the data? *United European Gastroenterol J* 2015; **3**: 419-428 [PMID: 26535119 DOI: 10.1177/2050640615590302]

24 **Colombel JF**, Sandborn WJ, Reinisch W, Mantzaris GJ, Kornbluth A, Rachmilewitz D, Lichtiger S, D'Haens G, Diamond RH, Broussard DL, Tang KL, van der Woude CJ, Rutgeerts P. Infliximab, azathioprine, or combination therapy for Crohn's disease. *N Engl*

J Med 2010; **362**: 1383-1395 [PMID: 20393175 DOI: 10.1056/NEJMoa0904492]
25 **Lam MC**, Bressler B. Vedolizumab for ulcerative colitis and Crohn's disease: results and implications of GEMINI studies. *Immunotherapy* 2014; **6**: 963-971 [PMID: 25341118 DOI: 10.2217/imt.14.66]
26 **Meijer B**, Seinen ML, Leijte NN, Mulder CJ, van Bodegraven AA, de Boer NK. Clinical Value of Mercaptopurine After Failing Azathioprine Therapy in Patients With Inflammatory Bowel Disease. *Ther Drug Monit* 2016; **38**: 463-470 [PMID: 27158876 DOI: 10.1097/FTD.0000000000000312]
27 **Bowen DG**, Selby WS. Use of 6-mercaptopurine in patients with inflammatory bowel disease previously intolerant of azathioprine. *Dig Dis Sci* 2000; **45**: 1810-1813 [PMID: 11052324 DOI: 10.1023/A:1005569808947]
28 **Boulton-Jones JR**, Pritchard K, Mahmoud AA. The use of 6-mercaptopurine in patients with inflammatory bowel disease after failure of azathioprine therapy. *Aliment Pharmacol Ther* 2000; **14**: 1561-1565 [PMID: 11121903 DOI: 10.1046/j.1365-2036.2000.00872.x]
29 **Actis GC**, Pellicano R, Rosina F. 6-Mercaptopurine for Azathioprine Intolerant Inflammatory Bowel Disease: Literature Search and Reappraisal of Own Data. *Inflamm Allergy Drug Targets* 2015; **14**: 133-137 [PMID: 26728773 DOI: 10.2174/1871528114666160105112915]
30 **Domènech E**, Nos P, Papo M, López-San Román A, Garcia-Planella E, Gassull MA. 6-mercaptopurine in patients with inflammatory bowel disease and previous digestive intolerance of azathioprine. *Scand J Gastroenterol* 2005; **40**: 52-55 [PMID: 15841714 DOI: 10.1080/00365520410009492]
31 **Hindorf U**, Johansson M, Eriksson A, Kvifors E, Almer SH. Mercaptopurine treatment should be considered in azathioprine intolerant patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2009; **29**: 654-661 [PMID: 19183142 DOI: 10.1111/j.1365-2036.2008.03925.x]
32 **Chouchana L**, Narjoz C, Roche D, Golmard JL, Pineau B, Chatellier G, Beaune P, Loriot MA. Interindividual variability in TPMT enzyme activity: 10 years of experience with thiopurine pharmacogenetics and therapeutic drug monitoring. *Pharmacogenomics* 2014; **15**: 745-757 [PMID: 24897283 DOI: 10.2217/pgs.14.32]
33 **Gearry RB**, Barclay ML. Azathioprine and 6-mercaptopurine pharmacogenetics and metabolite monitoring in inflammatory bowel disease. *J Gastroenterol Hepatol* 2005; **20**: 1149-1157 [PMID: 16048561 DOI: 10.1111/j.1440-1746.2005.03832.x]
34 **Weinshilboum RM**, Sladek SL. Mercaptopurine pharmacogenetics: monogenic inheritance of erythrocyte thiopurine methyltransferase activity. *Am J Hum Genet* 1980; **32**: 651-662 [PMID: 7191632]
35 **Higgs JE**, Payne K, Roberts C, Newman WG. Are patients with intermediate TPMT activity at increased risk of myelosuppression when taking thiopurine medications? *Pharmacogenomics* 2010; **11**: 177-188 [PMID: 20136357 DOI: 10.2217/pgs.09.155]
36 **McGovern DP**, Travis SP, Duley J, Shobowale-Bakre el M, Dalton HR. Azathioprine intolerance in patients with IBD may be imidazole-related and is independent of TPMT activity. *Gastroenterology* 2002; **122**: 838-839 [PMID: 11878296]
37 **Seinen ML**, de Boer NK, Smid K, van Asseldonk DP, Bouma G, van Bodegraven AA, Peters GJ. Allopurinol enhances the activity of hypoxanthine-guanine phosphoribosyltransferase in inflammatory bowel disease patients during low-dose thiopurine therapy: preliminary data of an ongoing series. *Nucleosides Nucleotides Nucleic Acids* 2011; **30**: 1085-1090 [PMID: 22132961 DOI: 10.1080/15257770.2011.597371]
38 **Seinen ML**, van Asseldonk DP, de Boer NK, Losekoot N, Smid K, Mulder CJ, Bouma G, Peters GJ, van Bodegraven AA. The effect of allopurinol and low-dose thiopurine combination therapy on the activity of three pivotal thiopurine metabolizing enzymes: results from a prospective pharmacological study. *J Crohns Colitis* 2013; **7**: 812-819 [PMID: 23317929 DOI: 10.1016/j.crohns.2012.12.006]
39 **Meijer B**, Bouma G, de Boer NK. Optimize Thiopurine Therapy in Autoimmune Hepatitis. *Clin Gastroenterol Hepatol* 2016; **14**: 1062-1063 [PMID: 26872401 DOI: 10.1016/j.cgh.2016.02.006]
40 **Dubinsky MC**, Vasiliauskas EA, Singh H, Abreu MT, Papadakis KA, Tran T, Martin P, Vierling JM, Geller SA, Targan SR, Poordad FF. 6-thioguanine can cause serious liver injury in inflammatory bowel disease patients. *Gastroenterology* 2003; **125**: 298-303 [PMID: 12891528 DOI: 10.1016/S0016-5085(03)00938-7]
41 **Seiderer J**, Zech CJ, Reinisch W, Lukas M, Diebold J, Wrba F, Teml A, Chalupna P, Stritesky J, Schoenberg SO, Schima W, Göke B, Ochsenkühn T. A multicenter assessment of liver toxicity by MRI and biopsy in IBD patients on 6-thioguanine. *J Hepatol* 2005; **43**: 303-309 [PMID: 15964660 DOI: 10.1016/j.jhep.2005.02.051]
42 **Teml A**, Schwab M, Hommes DW, Almer S, Lukas M, Feichtenschlager T, Florin T, Seiderer J, Petritsch W, Bokemeyer B, Kreisel W, Herrlinger KR, Knoflach P, Bonaz B, Klugmann T, Herfarth H, Pedarnig N, Reinisch W. A systematic survey evaluating 6-thioguanine-related hepatotoxicity in patients with inflammatory bowel disease. *Wien Klin Wochenschr* 2007; **119**: 519-526 [PMID: 17943403 DOI: 10.1007/s00508-007-0841-0]
43 **Ansari A**, Elliott T, Fong F, Arenas-Hernandez M, Rottenberg G, Portmann B, Lucas S, Marinaki A, Sanderson J. Further experience with the use of 6-thioguanine in patients with Crohn's disease. *Inflamm Bowel Dis* 2008; **14**: 1399-1405 [PMID: 18521912 DOI: 10.1002/ibd.20492]
44 **van Asseldonk DP**, Jharap B, Kuik DJ, de Boer NK, Westerveld BD, Russel MG, Kubben FJ, van Bodegraven AA, Mulder CJ. Prolonged thioguanine therapy is well tolerated and safe in the treatment of ulcerative colitis. *Dig Liver Dis* 2011; **43**: 110-115 [PMID: 20739231 DOI: 10.1016/j.dld.2010.07.004]
45 **de Boer NK**, Zondervan PE, Gilissen LP, den Hartog G, Westerveld BD, Derijks LJ, Bloemena E, Engels LG, van Bodegraven AA, Mulder CJ. Absence of nodular regenerative hyperplasia after low-dose 6-thioguanine maintenance therapy in inflammatory bowel disease patients. *Dig Liver Dis* 2008; **40**: 108-113 [PMID: 18083079 DOI: 10.1016/j.dld.2007.10.013]
46 **Gilissen LP**, Derijks LJ, Driessen A, Bos LP, Hooymans PM, Stockbrügger RW, Engels LG. Toxicity of 6-thioguanine: no hepatotoxicity in a series of IBD patients treated with long-term, low dose 6-thioguanine. Some evidence for dose or metabolite level dependent effects? *Dig Liver Dis* 2007; **39**: 156-159 [PMID: 17188950 DOI: 10.1016/j.dld.2006.10.007]
47 **Seinen ML**, van Asseldonk DP, Mulder CJ, de Boer NK. Dosing 6-thioguanine in inflammatory bowel disease: expert-based guidelines for daily practice. *J Gastrointestin Liver Dis* 2010; **19**: 291-294 [PMID: 20922194]
48 **van Asseldonk DP**, Jharap B, Verheij J, den Hartog G, Westerveld DB, Becx MC, Russel MG, Engels LG, de Jong DJ, Witte BI, Mulder CJ, van Nieuwkerk CM, Bloemena E, de Boer NK, van Bodegraven AA. The Prevalence of Nodular Regenerative Hyperplasia in Inflammatory Bowel Disease Patients Treated with Thioguanine Is Not Associated with Clinically Significant Liver Disease. *Inflamm Bowel Dis* 2016; **22**: 2112-2120 [PMID: 27482972 DOI: 10.1097/MIB.0000000000000869]
49 **Magro F**, Peyrin-Biroulet L, Sokol H, Aldeger X, Costa A, Higgins PD, Joyce JC, Katsanos KH, Lopez A, de Xaxars TM, Toader E, Beaugerie L. Extra-intestinal malignancies in inflammatory bowel disease: results of the 3rd ECCO Pathogenesis Scientific Workshop (III). *J Crohns Colitis* 2014; **8**: 31-44 [PMID: 23721759 DOI: 10.1016/j.crohns.2013.04.006]
50 **National Institute for Public Health and the Environment (RIVM)**. LCI-guideline Pfeiffer's disease, 2011. [accessed 2016 Sept 12]. Available from: URL: http://www.rivm.nl/Documenten_en_publicaties/Professioneel_Praktisch/Richtlijnen/Infectieziekten/LCI_richtlijnen/LCI_richtlijn_Pfeiffer_ziekte_van
51 **Biancone L**, Onali S, Petruzziello C, Calabrese E, Pallone F. Cancer and immunomodulators in inflammatory bowel diseases. *Inflamm Bowel Dis* 2015; **21**: 674-698 [PMID: 25545375 DOI: 10.1097/MIB.0000000000000243]
52 **van der Woude CJ**, Ardizzone S, Bengtson MB, Fiorino G, Fraser G, Katsanos K, Kolacek S, Juillerat P, Mulders AG, Pedersen N, Selinger C, Sebastian S, Sturm A, Zelinkova Z, Magro F. The second European evidenced-based consensus on reproduction and

pregnancy in inflammatory bowel disease. *J Crohns Colitis* 2015; **9**: 107-124 [PMID: 25602023 DOI: 10.1093/ecco-jcc/jju006]

53 **Damas OM**, Deshpande AR, Avalos DJ, Abreu MT. Treating Inflammatory Bowel Disease in Pregnancy: The Issues We Face Today. *J Crohns Colitis* 2015; **9**: 928-936 [PMID: 26129693 DOI: 10.1093/ecco-jcc/jjv118]

54 **Jharap B**, de Boer NK, Stokkers P, Hommes DW, Oldenburg B, Dijkstra G, van der Woude CJ, de Jong DJ, Mulder CJ, van Elburg RM, van Bodegraven AA. Intrauterine exposure and pharmacology of conventional thiopurine therapy in pregnant patients with inflammatory bowel disease. *Gut* 2014; **63**: 451-457 [PMID: 23424097 DOI: 10.1136/gutjnl-2012-303615]

55 **de Meij TG**, Jharap B, Kneepkens CM, van Bodegraven AA, de Boer NK. Long-term follow-up of children exposed intrauterine to maternal thiopurine therapy during pregnancy in females with inflammatory bowel disease. *Aliment Pharmacol Ther* 2013; **38**: 38-43 [PMID: 23675854 DOI: 10.1111/apt.12334]

56 **Gardiner SJ**, Gearry RB, Roberts RL, Zhang M, Barclay ML, Begg EJ. Exposure to thiopurine drugs through breast milk is low based on metabolite concentrations in mother-infant pairs. *Br J Clin Pharmacol* 2006; **62**: 453-456 [PMID: 16995866 DOI: 10.1111/j.1365-2125.2006.02639.x]

57 **Van Assche G**, Dignass A, Reinisch W, van der Woude CJ, Sturm A, De Vos M, Guslandi M, Oldenburg B, Dotan I, Marteau P, Ardizzone A, Baumgart DC, D'Haens G, Gionchetti P, Portela F, Vucelic B, Söderholm J, Escher J, Koletzko S, Kolho KL, Lukas M, Mottet C, Tilg H, Vermeire S, Carbonnel F, Cole A, Novacek G, Reinshagen M, Tsianos E, Herrlinger K, Oldenburg B, Bouhnik Y, Kiesslich R, Stange E, Travis S, Lindsay J. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Special situations. *J Crohns Colitis* 2010; **4**: 63-101 [PMID: 21122490 DOI: 10.1016/j.crohns.2009.09.009]

58 **de Boer NK**, Van Elburg RM, Wilhelm AJ, Remmink AJ, Van Vugt JM, Mulder CJ, Van Bodegraven AA. 6-Thioguanine for Crohn's disease during pregnancy: thiopurine metabolite measurements in both mother and child. *Scand J Gastroenterol* 2005; **40**: 1374-1377 [PMID: 16334449]

59 **Clarke K**, Regueiro M. Stopping immunomodulators and biologics in inflammatory bowel disease patients in remission. *Inflamm Bowel Dis* 2012; **18**: 174-179 [PMID: 21674731 DOI: 10.1002/ibd.21792]

60 **Chocair P**, Duley J, Simmonds HA, Cameron JS, Ianhez L, Arap S, Sabbaga E. Low-dose allopurinol plus azathioprine/cyclosporin/prednisolone, a novel immunosuppressive regimen. *Lancet* 1993; **342**: 83-84 [PMID: 8100914]

P- Reviewer: Actis GC, Capasso R, Serban DE **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i4.531

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 531-539
ISSN 2150-5349 (online)

MINIREVIEWS

Widespread use of gastric acid inhibitors in infants: Are they needed? Are they safe?

Mark Safe, Wei H Chan, Steven T Leach, Lee Sutton, Kei Lui, Usha Krishnan

Mark Safe, Lee Sutton, Usha Krishnan, Department of Paediatric Gastroenterology, Sydney Children's Hospital, Randwick NSW 2031, Australia

Wei H Chan, Steven T Leach, Kei Lui, Usha Krishnan, School of Women's and Children's Health, University of New South Wales, Sydney NSW 2052, Australia

Lee Sutton, Department of Newborn Care, Royal Hospital for Women, Randwick NSW 2031, Australia

Author contributions: All authors contributed to the conception of the work, interpretation of data, and drafting and/or revision of final manuscript.

Conflict-of-interest statement: No potential conflicts of interest relevant to this article were reported.

Manuscript source: Invited manuscript

Correspondence to: Dr. Usha Krishnan, Department of Paediatric Gastroenterology, Sydney Children's Hospital, High Street, Randwick NSW 2031, Australia. usha.krishnan@sesiahs.health.nsw.gov.au
Telephone: +61-2-93821752
Fax: +61-2-93821787

Received: April 6, 2016
Peer-review started: April 7, 2016
First decision: June 6, 2016
Revised: July 16, 2016
Accepted: August 6, 2016
Article in press: August 8, 2016
Published online: November 6, 2016

Abstract

Gastroesophageal reflux is a common phenomenon in infants, but the differentiation between gastro-esophageal reflux and gastroesophageal reflux disease can be difficult. Symptoms are non-specific and there is increasing evidence that the majority of symptoms may not be acid-related. Despite this, gastric acid inhibitors such as proton pump inhibitors are widely and increasingly used, often without objective evidence or investigations to guide treatment. Several studies have shown that these medications are ineffective at treating symptoms associated with reflux in the absence of endoscopically proven oesophagitis. With a lack of evidence for efficacy, attention is now being turned to the potential risks of gastric acid suppression. Previously assumed safety of these medications is being challenged with evidence of potential side effects including GI and respiratory infections, bacterial overgrowth, adverse bone health, food allergy and drug interactions.

Key words: Gastroesophageal reflux; Infants; Proton pump inhibitors; Ranitidine; Safety; Adverse events

Core tip: Gastroesophageal reflux is a common phenomenon in infants, but the differentiation between gastroesophageal reflux and gastroesophageal reflux disease can be difficult. Symptoms are non-specific and there is increasing evidence that the majority of symptoms may not be acid-related. Despite this, gastric acid inhibitors such as proton pump inhibitors are widely and increasingly used, often without objective evidence or investigations to guide treatment. Several studies have shown that these medications are ineffective at treating symptoms associated with reflux in the absence of endoscopically proven oesophagitis. With a lack of evidence for efficacy, attention is now being turned to the potential risks of gastric acid suppression. Previously

assumed safety of these medications is being challenged with evidence of potential side effects including GI and respiratory infections, bacterial overgrowth, adverse bone health, food allergy and drug interactions.

Safe M, Chan WH, Leach ST, Sutton L, Lui K, Krishnan U. Widespread use of gastric acid inhibitors in infants: Are they needed? Are they safe? *World J Gastrointest Pharmacol Ther* 2016; 7(4): 531-539 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i4/531.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i4.531

INTRODUCTION

Gastro-oesophageal reflux (GOR) is the physiologic process involving the passage of gastric contents into the oesophagus which is often accompanied by postprandial regurgitation or vomiting[1]. The term gastro-oesophageal reflux disease (GORD) applies to persistent reflux that causes troublesome symptoms and/or complications, and is therefore, considered pathologic[1]. This distinction remains a challenge in infant care.

Infants are physiologically predisposed to GOR because of their shorter intra-abdominal oesophagus, frequent liquid feeds that distend the stomach, and supine position[2]. Infants with GOR have been found to have frequent transient lower oesophageal sphincter relaxations, which are thought to be the pathophysiological basis of the condition. Fifty-percent of infants reportedly experience daily regurgitation in the first 3 mo of life, which resolve by 12-14 mo in most healthy infants[3]. The pathogenic mechanism leading infant GOR to develop into GORD is unclear, although decreased neural protective reflexes and delayed gastric emptying are thought to play a role[1].

Since infant GORD has been linked to significant clinical morbidity in some patients, including worsening lung disease, aspiration and oesophagitis, medical intervention is frequently sought[4]. Common and non-specific symptoms attributed to GOR are often considered troublesome enough to justify treatment, especially in the neonatal intensive care setting[5]. This has led to the widespread usage of gastric acid inhibitors (GAI), in the form of proton pump inhibitors (PPIs) and/or histamine-2 receptor antagonists (H2RAs) in infants, despite uncertainty as to their efficacy and risks. This report will review recent evidence on the suitability of PPIs as an effective therapy for GORD in symptomatic infants and their potential for short- and long-term side effects.

GASTRIC ACID INHIBITOR USE IN INFANTS

GAI use for infants with symptoms attributed to GORD has risen dramatically despite only very limited approval for their use in this age group[6,7]. From 2000 to 2003, there was a 4-fold increase in off-label PPI prescriptions in this age-group, despite less than 10% of patients being investigated for GORD by diagnostic procedure[8]. There has also been a concerning rise in the frequency of GAI use in preterm infants, despite the lack of published evidence regarding pharmacological management of GOR or the safety and efficacy of GAI in preterm infants. According to a survey of neonatologists across 77 secondary and tertiary NICUs, GORD is perceived to affect more than one-fifth of infants born before 34 wk, and this perception may be leading to increased prescribing[9].

Symptoms described in infants with GORD include frequent regurgitation and vomiting, chronic cough, irritability, feeding resistance, failure to thrive, apnoea, bronchospasm and back-arching[2]. However, GORD diagnosis based on these symptoms is unreliable and non-specific. Regurgitation, irritability and vomiting thought to be secondary to GORD, are indistinguishable from the symptoms of food allergy, colic and other disorders[1]. Poor association between symptoms and pathologic acid exposure in oesophageal pH monitoring and histological scores, make symptoms unreliable in the diagnosis of GORD in infants[10]. GAI therapy in infants is largely extrapolated from studies of adults and older children, in whom symptoms are more reliably associated with acid exposure. In infants, significant recent data point to the possibility that the majority of symptoms are associated either with non-acid reflux or with no reflux at all[11]. In adults, there have been moves to even more potent acid suppression with the novel potassium competitive acid blockers such as vonoprazan. There is no safety data in children for this therapy, and considering that acid suppression has not been shown to affect symptoms in the majority of cases, there is likely to be very limited role for this drug.

Studies have also failed to find any association between GOR and cardiorespiratory events including apnoea, bradycardia, and oxygen desaturation in preterm infants[12,13]. Even so, two thirds of neonatologists have reported using GOR medications to treat apnoeas[14]. Overall, it has been widely recommended that GAI treatment in infants should be reserved for cases with evidence of pathological exposure to acid reflux episodes and/or oesophagitis[1]. Despite these recommendations, studies have found very poor adherence to guidelines and significant overtreatment with PPIs[15]. There is a concerning increase in the use of pharmacological intervention using acid suppression therapy using PPIs and H2RAs in preterm infants, with a presumed diagnosis of GORD based on symptoms alone in the absence of any objective measures for the diagnosis of GORD including pH and impedance monitoring or gastroscopy and biopsy[5]. Whilst there is no contemporary data outlining the relative frequency of H2RA and PPI use, the authors have observed a definite trend towards

PPI as the predominant medication prescribed or acid suppression.

Although, GAIs have previously been considered to be well tolerated by infants, emerging evidence suggests potential harmful associations between the use of GAIs and the development of infection and atopic disease in murine, adult and limited paediatric studies[16,17]. GAIs serve to protect the mucosa from excessive acid production, however giving such aggressive acid suppression at such a young age without evidence of oesophagitis remains controversial. Acid suppression is thought to interfere with natural defences against gastric bacterial colonization[18], and also protein digestion to trigger allergic sensitization of dietary peptides[19]. There is also mounting evidence that children are being exposed to unnecessarily high doses of PPI with doses of 1 mg/kg per day up to as high as 4 mg/kg per day used in clinical practice. Recent randomised trials have shown that although there is a dose-dependant reduction in acid production, for the treatment of erosive esophagitis there is no significant difference in healing between 5 mg/d and 10 mg/d for children < 20 kg[20,21].

ACTION AND EFFICACY OF PPI

PPIs bind irreversibly to the H^+-K^+-ATPase complex ("proton pump") of gastric parietal cells to prevent the reuptake of extracellular potassium in exchange with intracellular hydrogen, thus inhibiting acid secretion[22]. Their use in infants has been extrapolated from numerous adult studies, for whom PPIs are superior in healing erosive oesophagitis and providing symptom relief compared with H2RAs, which are more effective than placebo[1]. PPIs have been found to maintain intragastric pH > 4 for prolonged periods and to inhibit meal-induced acid secretion.

However, PPIs have consistently failed to show efficacy in reducing infant GORD symptoms compared with placebo. Chen *et al*[23] reviewed four randomised control trials (RCTs) of PPIs in treating symptomatic GORD infants < 12 mo, conducted by pharmaceutical companies under formal requests by the Food and Drug Administration. The results of independent studies such as Moore *et al*[24] have corroborated with their results, which are summarised in Table 1[23-28]. Notably, Moore *et al*[24] enrolled infants with endoscopically confirmed GORD and found omeprazole significantly reduced the reflux index (percentage of total duration pH < 4) in these infants compared with placebo, but irritability improved regardless of treatment. In the most recent randomised controlled trial of PPI (Esomeprazole) for the treatment of symptomatic GORD, without endoscopy, all children were initially treated with PPI and then randomised to continuation of PPI or placebo[25]. It found no statistically significant difference in apparent treatment failure between the PPI or placebo group.

SAFETY OF GASTRIC ACID INHIBITORS

With any pharmacological agent, there is potential for side effects. Headache, diarrhoea, constipation and nausea are idiosyncratic effects of PPIs that occur in 14% of children[1]. Acute interstitial nephritis, a rare, idiosyncratic hypersensitivity reaction to medications including PPIs, has also been reported in observational adult studies[29]. Increased risk of infection, for example, Clostridium Difficile, is increasingly being recognised[30]. Side effects related to the direct inhibition of gastric acid and reflex hypergastrinaemia, immunosuppression and drug metabolism have also been suggested (Table 2).

Bacterial overgrowth

The human stomach has a median pH of 1.4, and a pH < 4 has a powerful bactericidal effect on ingested acid-sensitive bacteria[18]. PPIs often cause a gastric environment with pH > 4, inducing a state of hypochlorhydria which allows the overgrowth of bacteria in the stomach[18]. Recently, Kanno *et al*[31] observed the effect of gastric acid inhibition in altering lower-intestinal microflora in PPI treated rats and asymptomatic humans with achlorhydria. The authors showed a significant dose-dependent increase in Lactobacillus and Veillonella populations (bacteria of oropharyngeal origin) in both rats and humans and in rats, potent gastric acid inhibition also led to a marked and significant increase of intestinal bacteria, including the Bacteroides fragilis group[31]. Modern genomic techniques have confirmed these PPI-related changes through 16S sequencing[32]. These microbial changes are thought to be due to the lack of the gastric acid barrier allowing bacteria to enter the intestine and also the effect of impaired protein digestion providing nutrients to facilitate bacterial growth[31]. Links have previously been made between these and similar changes to intestinal microbiome and the pathogenesis of inflammatory and malignant conditions of the bowel[33].

Risk of infections

The pathogenic mechanism that allows enteric bacteria to cause gastrointestinal infections is multi-factorial. Gastric acid inhibition reduces the gastric microbiocidal barrier, delays gastric emptying, reduces gastric mucus viscosity thereby increasing the risk of bacterial translocation in addition to increasing the risk of colonisation by bacterial agents. Gastric acid inhibition also has an adverse effect on leukocyte function by decreasing adhesion to endothelial cells, reducing chemotactic response to bacterial proteins and inhibiting neutrophil phagocytosis by phagosome acidification[16]. This is potentially important in neonates and infants, who have immature humoral immunity[16]. A study on the numbers and type of bacteria in nasogastric tubes of patients receiving GAI demonstrated increased numbers of bacteria including Streptococcus, a known cause of community acquired pneumonia[34]. It is possible that the risk of pneumonia is

Table 1 Summary randomised control trials examining proton pump inhibitors efficacy in reducing symptoms in infants with gastro-oesophageal reflux disease

Parameter	Esomeprazole	Lansoprazole	Pantoprazole	Omeprazole	Omeprazole (independent)	Esomeprazole
Control group	Placebo	Placebo	Placebo	Dosing range	Placebo	Placebo
Blinding	Double	Double	Double	Single	Double	Double
Trial of conservative measures	No	Yes	Yes	Yes	Yes[1]	No
Antacids allowed as rescue	Yes	No	Yes	Yes	No	Yes
Open-label phase to identify PPI responders	Yes (2 wk)	No	Yes (4 wk)	No	No	Yes (2 wk)
Randomised withdrawal from PPI	Yes	No	Yes	No	Yes	Yes
Length of randomised phase (wk)	4	4	4	8	4	4
Age in months	1-12	1-12	1-12	0-24[3]	3-12	1-11
n	40	81	50	35	30	80
GORD symptoms for clinical diagnosis	Vomiting; Regurgitation; Irritability; Supra-oesophageal disturbances; Respiratory Disturbance; Feeding difficulty	Crying; Fussiness; Irritability	Vomiting; Regurgitation; Spitting up; Irritability; Fussiness; Feeding Refusal; Choking; Gagging	Vomiting Regurgitation	[4]Frequent spilling Irritability/crying	Vomiting, regurgitation, irritability, cough, wheezing, stridor, labored breathing, resp symptoms triggered by feeding, food refusal, gagging, choking, hiccups for > 1 h/d
Primary endpoints	Time from randomisation to discontinuation because of symptom worsening perceived by parent or physician on symptom severity scale	Proportion with ≤ 50% reduction in PGA of symptoms	Proportion of infants who withdrew due to the "lack of efficacy" including worsening of symptoms, and/or antacid use for 7 consecutive days and/or oesophagitis and/or physician judgements	Change from baseline in daily symptoms based on PGA and parent perception	Reflux index from baseline	Time from randomization to discontinuation owing to symptom worsening in the double-blind phase
Primary end point efficacy result	Hazard ratio = 0.69 (PPI/Placebo); 95%CI: 0.35-1.35; $P = 0.275$	Responder rate: 54% (44/81) PPI *vs* 54% (44/81) Placebo; $P = 1.000$	Responder rate: 12% PPI *vs* 11% Placebo; $P = 1.000$	Mean daily vomiting/regurgitation episodes decreased by 4.34/d (0.5 mg/kg; 2.97/d – 1.0 mg/kg 4.35/d – 1.5 mg/kg; $P > 0.50$ in all group comparisons	Change from baseline of parent-recorded 24 h crying and fussing time and visual analogue scores of parental impression of the intensity of irritability Reflux index; -8.9% ± 5.6% PPI; -1.9% ± 2.0% Placebo $P < 0.001$ Cry/fuss times (min/24 h); 191 ± 120 (PPI); 201 ± 100 (Placebo) $P = 0.400$ Combined PPI and Placebo groups total cry fuss time[2] Baseline *vs* 2 wk $P = 0.040$ Baseline *vs* 4 wk $P = 0.008$ VA Score 5.0 ± 3.1 (PPI); 5.9 ± 2.1 (Placebo) $P = 0.214$	Discontinuation rates owing to symptom worsening were 48.8% (20/41) for placebo-treated *vs* 38.5% (15/39) for esomeprazole-treated patients (hazard ratio 0.69; $P = 0.28$)
Limitations of studies	Small sample size Symptom-based diagnosis Subjective assessment	Small sample size; Symptom-based diagnosis; Subjective assessment	Small sample size Symptom-based diagnosis Subjective assessment	Single blinded; Not placebo-controlled; Small sample size; Symptom-based diagnosis; Subjective assessment	Small sample size; Subjective assessment	Small sample size; Symptom-based diagnosis; Subjective assessment

[1]All infants were given empirical pharmacologic treatment (excluding PPIs) including cisapride (87%), H2 receptor antagonists (73%), antacid (67%) and thickening agent (20%); [2]Significant decrease in cry-fuss time independent of treatment; [3]Ninty percent of patients were younger than 12 mo; [4]Entry into study required a reflux index of > 5% or endoscopic biopsy evidence of oesophagitis. Data adapted from Chen *et al*[23]; Moore *et al*[24]; Orenstein *et al*[27]; Shakhnovich *et al*[28]. PPI: Proton pump inhibitor; GORD: Gastro-oesophageal reflux disease; PGA: Physician global assessment; VA: Visual analogue.

Table 2 Outline of the proposed side effects associated with proton pump inhibitors use, and the evidence supporting the association

Potential side effects	Level of evidence showing an association with PPI use
Acute Interstitial Nephritis	Level Ⅲ
Bacterial overgrowth in the stomach, small and large intestine	Murine models
Bacterial enteric infections	Level Ⅰ
Causative agents:	
Clostridium difficile	
Salmonella species	
Campylobacter species	
Pneumonia (Community-acquired)	Level Ⅰ
Necrotizing enterocolitis	Level Ⅲ[1]
Blood stream infections, including candidemia	Level Ⅲ[1]
Allergic sensitization in adults and in children with *in utero exposure*	Level Ⅲ Study and Murine Models
Parietal and Enterochromaffin-like cell hyperplasia	Level Ⅱ
Fundic gland polyps	Level Ⅲ
Vitamin B12 deficiency	Level Ⅲ
Fractures (osteoporotic and non-osteoporotic)	Level Ⅲ
Hypomagnesemia	Level Ⅳ and one level Ⅲ study
Reduced Antiplatelet effect of Clopidogrel	Level Ⅱ
Adverse Cardiovascular outcomes due to Clopidogrel interactions	Level Ⅲ[2]

[1]Only single reports showing an association with acid inhibition induced by H2RA treatment; [2]RCTs (level II) not shown an increase risk of adverse outcomes.

increased as result of reflux aspiration of gastrointestinal contents into the lungs. PPIs may also directly inhibit the H^+-K^+-ATPase present in the respiratory tract, altering the pH of its seromucinous secretions[35].

Adult studies

A meta-analysis of 26 observational studies found a significant association between PPI/H2RA use and Clostridium difficile infections (pooled OR = 1.95, 95%CI: 1.48-2.58), and "other" enteric infections (Salmonella or Campylobactor) (OR = 2.55, 95%CI: 1.53-4.26)[36]. Salmonella, Campylobacter and the vegetative form of C. difficile are acid-sensitive bacteria but are able to survive with PPI-induced acid suppression[36]. Experimental studies have shown that pretreatment with gastric acid inhibitors in a mouse model prior to *C. difficile* inoculation resulted in similar rates of infection, toxin production and colon injury compared with a group of mice pretreated with ampicillin[36]. Spore germination was also favoured by high pH levels and the presence of potassium chloride. Blockage of potassium pumps in the stomach could potentially lead to increased potassium as the proton pumps exchange potassium for hydrogen ions.

In a systematic review, Bavishi and Dupont[18] found that while it was difficult to establish causation in some studies due to other contributing factors such as advanced age and hospital exposure, patients on PPIs demonstrated a greater-than 4-fold risk for recurrent C. difficile infection[37].

A meta-analysis by Eom *et al*[35] also found significant association between PPIs and pneumonia (adjusted OR = 1.27, 95%CI: 1.11-1.46), with an even greater risk for community-acquired pneumonia (OR = 1.34, 95%CI: 1.14-1.57). This risk of pneumonia was markedly higher within the first week of PPI use (OR = 3.95, 95%CI: 2.86-5.45) suggesting that patients who were already susceptible to pneumonia would become ill soon after PPI treatment. With a small number of studies investigating the relationship between PPIs and hospital-acquired pneumonia, only an increased risk of hospital-acquired pneumonia was observed with H2RA therapy[35].

Paediatric studies

The few paediatric studies available have made similar conclusions. Notably, a prospective study of 93 paediatric patients (4-36 mo) with endoscopically diagnosed GORD, showed that children treated with either ranitidine or omeprazole for 8 wk were 3.58 and 6.39 times more likely to develop acute gastroenteritis and community-acquired pneumonia respectively, compared with healthy children during the 4 mo follow-up[17]. Comparing 4 mo before and after enrolment, a significant increase in the incidence of acute gastroenteritis and pneumonia was found only in the treatment group, demonstrating that infection susceptibility could continue even after therapy cessation[17].

The results of safety studies on the use of gastric acid inhibiting drugs in infants, particularly in intensive care, where hospital-acquired pathogens are responsible for significant morbidity and mortality are concerning[38]. A case-control study of very low birth weight infants showed H2RA use was associated with higher rates of necrotizing enterocolitis (OR = 1.71, 95%CI: 1.34-2.19)[39]. Stoll *et al*[40] also observed an increased risk of sepsis and meningitis with H2RAs given at 2 wk of age as a secondary outcome of their RCT comparing dexamethasone exposure. Beck-Sague *et al*[41] also reported H2RAs as a significant risk factor for bloodstream infections (RR = 4.2) in level Ⅲ neonatal intensive care, including Candida species; and the risk of candidemia

(OR = 2.44) was shown again by Saiman *et al*[42]. Very few studies have explored the risk of infections in the preterm infant population, but of these, Guillet *et al*[39] showed H2RA use was associated with higher rates of necrotising enterocolitis (NEC) (OR = 1.71) in large cohort study of 11072 very low birth weight infants. H2RAs have also been found to be a significant risk factor for blood stream infections in a level Ⅲ NICU[41], and candidemia[39]. The pathogenic mechanism of GAIs to cause infection is thought to be a result of reducing the gastric acid barrier against gastrointestinal tract colonisation with acid-sensitive bacteria such as Clostridium difficile[18]. Carrion and Egan[43] conducted a small prospective double-blind trial in 68 preterm infants (< 1250 g) supplemented with either HCl or water with feeds, and found that increased gastric bacterial colony counts were strongly correlated with gastric pH > 4 (P < 0.001), and acidification significantly reduced the incidences of NEC.

Allergic sensitization

Elevation of gastric pH also interferes with protein digestion, and it is hypothesised that normally digestible dietary peptides are preserved and recognised by the immune system as allergens[19]. Schöll *et al*[19] showed that omeprazole with hazelnut-extract treatment induced hazelnut-specific IgG1 in 3 of 5 mice (P = 0.754); and in the human study, 3.3% of patients receiving 3 mo of H2RA/PPI treatment also developed de novo allergic sensitization, which was higher than the reported prevalence of all tree nut allergies in the general US population (0.2%-0.7%). Schöll *et al*[44] also proposed that an allergic status induced in mothers had the potential to transfer (*via* placenta or breast milk) to the child. A study in pregnant mice demonstrated that increasing the gastric pH with sucralfate induced higher levels of codfish-specific IgG1 in mothers and offspring[44]. In offspring splenocytes, there was also a suppressed production of IFN-γ (Th1-cytokine), allowing the Th2-cytokine response to dominate (a phenotype predisposed to allergy); and T-regulatory cytokine IL-10, which regulates the allergic response[44]. A Swedish population register-based study found a significantly increased risk of developing childhood asthmas (51%), or any allergy (43%) in children exposed to PPIs/H2RAs in utero, irrespective of the drug type, trimester of exposure or maternal history of allergy[45].

HYPERGASTRINAEMIA AND MUCOSA CHANGES

Increasing gastric pH leads to hypergastrinemia, which has growth-promoting effects on several epithelial types[46]. Consequently, long-term PPI therapy is associated with parietal and enterochromaffin-like cell hyperplasia, as demonstrated by a RCT between esomeprazole treatment for 5 years compared with laparoscopic antireflux procedures for GORD[47]. Despite the proliferative drive of chronically elevated gastrin, no dysplastic changes were found.

Jalving *et al*[48] also found that PPI use > 1 year was associated with an increased risk of benign fundic gland polyps (OR = 2.8, 95%CI: 1.8-4.5), believed to arise from parietal cell protrusions and hyperplasia. One low-grade dysplastic polyp was found in a patient already predisposed with familial adenomatous polyposis, and did not appear to be PPI-related[48].

Vitamin and mineral deficiencies

By reducing gastric acidity, PPIs may interfere with the absorption of dietary protein-bound vitamin B12 and ionised calcium from dietary salts[22]. However, evidence of an effect of long-term PPI use in the elderly (over 65) on vitamin B12 has shown conflicting results. One case-control study (n = 53) found a 4.45 times increased risk for vitamin B12 deficiency in patients (> 12 mo of H2RAs/PPIs)[49]. However, a more recent cross-sectional study of 125 chronic (> 3 years) PPI users found no difference in serum vitamin B12 levels compared with controls[50].

PPIs have also been associated with an increased risk of fracture, as impaired calcium absorption is thought to cause a compensatory state of hyperparathyroidism to stimulate osteoclasts and bone resorption[51], but, there is also significant heterogeneity among these studies[52]. However, case-control studies have demonstrated significantly increased fracture risk in those with recent or current PPI use and at least one other risk factor for fracture[53,54].

During 2006-2012, there were 26 reported cases of hypomagnesaemia associated with PPIs in literature, with symptoms including electrocardiogram abnormalities and neuroexcitability, including tetanus and seizures, which resolved following withdrawal of PPI[52]. The mechanism of PPI-induced hypomagnesaemia is unknown, however, monitoring of serum magnesium levels has been recommended for susceptible patients, including patients using diuretics concurrently[55,56].

Drug interactions

In vitro studies have demonstrated a theoretical potential for PPIs and clopidogrel to interact through competitive binding at the cytochrome (CYP) 450 isoform CYP2C19, an enzyme involved in PPI metabolism[52]. Consequently, a significant reduction in the antiplatelet effect of clopidogrel has been reported. Although there have been no RCTs demonstrating increased cardiovascular risk, a recent propensity score analysis of a very large cohort showed an increased risk of myocardial infarction for adults taking PPI with an adjusted hazard ratio of 1.58[52].

CONCLUSION

This review highlights the issues regarding PPIs as treatment for infants with a presumed diagnosis of GORD based on symptomatology alone. For many clinicians, concern regarding the theoretical risk of tissue injury and

secondary morbidities, seem to outweigh any concern for the risks of PPI use. Currently, several RCTs of PPIs have shown a consistent lack of efficacy in relieving "distressed" GORD behaviours thought to be indicative of painful stimuli, suggesting they may have other underlying causes. Nonetheless, there is a need for more sizeable RCTs, standardised diagnostic procedures and better end-points in treatment in this population. Symptom assessments are clinically relevant but there is a lack of validated symptom-reported questionnaires for GORD in infants.

The safety of PPIs in infants also requires more prospective RCTs to remove the effect of confounders and bias. Irritable infants with uncomplicated GORD are hence recommended to continue lifestyle modifications, such as changing feeding techniques or formula composition, and avoid acid suppression. If PPIs are to be prescribed, only the minimal effective dose should be used, and should be weaned as soon as possible. There is no direct evidence to suggest increased safety of H2RA medication compared with PPI and in situations where acid suppression is indicated (*e.g.,* esophagitis) they have decreased potency. Attention should be paid to the substantial epidemiological evidence of increased infection risk with PPIs, especially in the vulnerable population group of preterm infants.

REFERENCES

1 **Vandenplas Y**, Rudolph CD, Di Lorenzo C, Hassall E, Liptak G, Mazur L, Sondheimer J, Staiano A, Thomson M, Veereman-Wauters G, Wenzl TG. Pediatric gastroesophageal reflux clinical practice guidelines: joint recommendations of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN). *J Pediatr Gastroenterol Nutr* 2009; **49**: 498-547 [PMID: 19745761 DOI: 10.1097/MPG.0b013e3181b7f563]

2 **Blanco FC**, Davenport KP, Kane TD. Pediatric gastroesophageal reflux disease. *Surg Clin North Am* 2012; **92**: 541-558, viii [PMID: 22595708 DOI: 10.1016/j.suc.2012.03.009]

3 **Nelson SP**, Chen EH, Syniar GM, Christoffel KK. Prevalence of symptoms of gastroesophageal reflux during infancy. A pediatric practice-based survey. Pediatric Practice Research Group. *Arch Pediatr Adolesc Med* 1997; **151**: 569-572 [PMID: 9193240 DOI: 10.1001/archpedi.1997.02170430035007]

4 **Birch JL**, Newell SJ. Gastrooesophageal reflux disease in preterm infants: current management and diagnostic dilemmas. *Arch Dis Child Fetal Neonatal Ed* 2009; **94**: F379-F383 [PMID: 19307225 DOI: 10.1136/adc.2008.149112]

5 **Malcolm WF**, Gantz M, Martin RJ, Goldstein RF, Goldberg RN, Cotten CM. Use of medications for gastroesophageal reflux at discharge among extremely low birth weight infants. *Pediatrics* 2008; **121**: 22-27 [PMID: 18166553 DOI: 10.1542/peds.2007-0381]

6 **Sandström M**, Davidson G, Tolia V, Sullivan JE, Långström G, Lundborg P, Brown K. Phase I, multicenter, randomized, open-label study evaluating the pharmacokinetics and safety profile of repeated once-daily doses of intravenous esomeprazole in children 0 to 17 years of age. *Clin Ther* 2012; **34**: 1828-1838 [PMID: 22832034 DOI: 10.1016/j.clinthera.2012.06.028]

7 **Illueca M**, Alemayehu B, Shoetan N, Yang H. Proton pump inhibitor prescribing patterns in newborns and infants. *J Pediatr Pharmacol Ther* 2014; **19**: 283-287 [PMID: 25762873 DOI: 10.5863/1551-6776-19.4.283]

8 **Barron JJ**, Tan H, Spalding J, Bakst AW, Singer J. Proton pump inhibitor utilization patterns in infants. *J Pediatr Gastroenterol Nutr* 2007; **45**: 421-427 [PMID: 18030207 DOI: 10.1097/MPG.0b013e31812e0149]

9 **Dhillon AS**, Ewer AK. Diagnosis and management of gastro-oesophageal reflux in preterm infants in neonatal intensive care units. *Acta Paediatr* 2004; **93**: 88-93 [PMID: 14989446 DOI: 10.1080/08035250310007934]

10 **Salvatore S**, Hauser B, Vandemaele K, Novario R, Vandenplas Y. Gastroesophageal reflux disease in infants: how much is predictable with questionnaires, pH-metry, endoscopy and histology? *J Pediatr Gastroenterol Nutr* 2005; **40**: 210-215 [PMID: 15699699 DOI: 10.1016/s8756-5005(08)70322-6]

11 **Orenstein SR**. Infant GERD: symptoms, reflux episodes & amp; reflux disease, acid & amp; non-acid reflllux--implications for treatment with PPIs. *Curr Gastroenterol Rep* 2013; **15**: 353 [PMID: 24197743 DOI: 10.1007/s11894-013-0353-1]

12 **Di Fiore J**, Arko M, Herynk B, Martin R, Hibbs AM. Characterization of cardiorespiratory events following gastroesophageal reflux in preterm infants. *J Perinatol* 2010; **30**: 683-687 [PMID: 20220760 DOI: 10.1038/jp.2010.27]

13 **Smits MJ**, van Wijk MP, Langendam MW, Benninga MA, Tabbers MM. Association between gastroesophageal reflux and pathologic apneas in infants: a systematic review. *Neurogastroenterol Motil* 2014; **26**: 1527-1538 [PMID: 25080836 DOI: 10.1111/nmo.12405]

14 **Abu Jawdeh EG**, Martin RJ. Neonatal apnea and gastroesophageal reflux (GER): is there a problem? *Early Hum Dev* 2013; **89** Suppl 1: S14-S16 [PMID: 23809340 DOI: 10.1016/S0378-3782(13)70005-7]

15 **Quitadamo P**, Papadopoulou A, Wenzl T, Urbonas V, Kneepkens CM, Roman E, Orel R, Pavkov DJ, Dias JA, Vandenplas Y, Kostovski A, Miele E, Villani A, Staiano A. European pediatricians' approach to children with GER symptoms: survey of the implementation of 2009 NASPGHAN-ESPGHAN guidelines. *J Pediatr Gastroenterol Nutr* 2014; **58**: 505-509 [PMID: 24663035 DOI: 0.1097/MPG.0b013e3182a69912]

16 **Canani RB**, Terrin G. Gastric acidity inhibitors and the risk of intestinal infections. *Curr Opin Gastroenterol* 2010; **26**: 31-35 [PMID: 19907324 DOI: 10.1097/MOG.0b013e328333d781]

17 **Canani RB**, Cirillo P, Roggero P, Romano C, Malamisura B, Terrin G, Passariello A, Manguso F, Morelli L, Guarino A. Therapy with gastric acidity inhibitors increases the risk of acute gastroenteritis and community-acquired pneumonia in children. *Pediatrics* 2006; **117**: e817-e820 [PMID: 16651285 DOI: 10.1542/peds.2005-1655]

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

19 **Schöll I**, Untersmayr E, Bakos N, Roth-Walter F, Gleiss A, Boltz-Nitulescu G, Scheiner O, Jensen-Jarolim E. Antiulcer drugs promote oral sensitization and hypersensitivity to hazelnut allergens in BALB/c mice and humans. *Am J Clin Nutr* 2005; **81**: 154-160 [PMID: 15640475]

20 **Omari T**, Davidson G, Bondarov P, Naucler E, Nilsson C, Lundborg P. Pharmacokinetics and Acid-suppressive Effects of Esomeprazole in Infants 1-24 Months Old With Symptoms of Gastroesophageal Reflux Disease. *J Pediatric Gastroenterol Nutr* 2015; **60**: S2-8 [DOI: 10.1097/01.mpg.0000469415.50085.f7]

21 **Tolia V,** Youssef NN, Gilger MA, Traxler B, Illueca M. Esomeprazole for the Treatment of Erosive Esophagitis in Children: An International, Multicenter, Randomized, Parallel-Group, Double-Blind (for Dose) Study. *J Pediatric Gastroenterol Nutr* 2015; **60**: S24-30 [DOI: 10.1097/01.mpg.0000469419.29000.94]

22 **Yang YX**, Metz DC. Safety of proton pump inhibitor exposure. *Gastroenterology* 2010; **139**: 1115-1127 [PMID: 20727892 DOI: 10.1053/j.gastro.2010.08.023]

23 **Chen IL**, Gao WY, Johnson AP, Niak A, Troiani J, Korvick J, Snow N, Estes K, Taylor A, Griebel D. Proton pump inhibitor

use in infants: FDA reviewer experience. *J Pediatr Gastroenterol Nutr* 2012; **54**: 8-14 [PMID: 21946832 DOI: 10.1097/MPG.0b013e31823890b4]
24 **Moore DJ**, Tao BS, Lines DR, Hirte C, Heddle ML, Davidson GP. Double-blind placebo-controlled trial of omeprazole in irritable infants with gastroesophageal reflux. *J Pediatr* 2003; **143**: 219-223 [PMID: 12970637 DOI: 10.1067/S0022-3476(03)00207-5]
25 **Winter H**, Gunasekaran T, Tolia V, Gottrand F, Barker PN, Illueca M. Esomeprazole for the Treatment of GERD in Infants Ages 1-11 Months. *J Pediatr Gastroenterol Nutr* 2015; **60** Suppl 1: S9-15 [PMID: 26422097 DOI: 10.1097/MPG.0b013e3182496b35]
26 **Omari T**, Davidson G, Bondarov P, Nauclér E, Nilsson C, Lundborg P. Pharmacokinetics and acid-suppressive effects of esomeprazole in infants 1-24 months old with symptoms of gastroesophageal reflux disease. *J Pediatr Gastroenterol Nutr* 2007; **45**: 530-537 [PMID: 18030229 DOI: 10.1097/MPG.0b013e31812e012f]
27 **Orenstein SR**, Hassall E, Furmaga-Jablonska W, Atkinson S, Raanan M. Multicenter, double-blind, randomized, placebo-controlled trial assessing the efficacy and safety of proton pump inhibitor lansoprazole in infants with symptoms of gastroesophageal reflux disease. *J Pediatr* 2009; **154**: 514-520.e4 [PMID: 19054529 DOI: 10.1016/j.jpeds.2008.09.054]
28 **Shakhnovich V**, Ward RM, Kearns GL. Failure of proton pump inhibitors to treat GERD in neonates and infants: a question of drug, diagnosis, or design. *Clin Pharmacol Ther* 2012; **92**: 388-392 [PMID: 22805424 DOI: 10.1038/clpt.2012.86]
29 **Sierra F,** Suarez M, Rey M, Vela MF. Systematic review: proton pump inhibitor-associated acute interstitial nephritis. *Alimentary Pharmacol Ther* 2007; **26**: 545-553 [PMID: 17661758 DOI: 10.1111/j.1365-2036.2007.03407.x]
30 **Turco R**, Martinelli M, Miele E, Roscetto E, Del Pezzo M, Greco L, Staiano A. Proton pump inhibitors as a risk factor for paediatric Clostridium difficile infection. *Aliment Pharmacol Ther* 2010; **31**: 754-759 [PMID: 20047577 DOI: 10.1111/j.1365-2036.2009.04229.x]
31 **Kanno T**, Matsuki T, Oka M, Utsunomiya H, Inada K, Magari H, Inoue I, Maekita T, Ueda K, Enomoto S, Iguchi M, Yanaoka K, Tamai H, Akimoto S, Nomoto K, Tanaka R, Ichinose M. Gastric acid reduction leads to an alteration in lower intestinal microflora. *Biochem Biophys Res Commun* 2009; **381**: 666-670 [PMID: 19248769 DOI: 10.1016/j.bbrc.2009.02.109]
32 **Rosen R**, Hu L, Amirault J, Khatwa U, Ward DV, Onderdonk A. 16S community profiling identifies proton pump inhibitor related differences in gastric, lung, and oropharyngeal microflora. *J Pediatr* 2015; **166**: 917-923 [PMID: 25661411 DOI: 10.1016/j.jpeds.2014.12.067]
33 **Gagnière J**, Raisch J, Veziant J, Barnich N, Bonnet R, Buc E, Bringer MA, Pezet D, Bonnet M. Gut microbiota imbalance and colorectal cancer. *World J Gastroenterol* 2016; **22**: 501-518 [PMID: 26811603 DOI: 10.3748/wjg.v22.i2.501]
34 **Vakil N**. Acid inhibition and infections outside the gastrointestinal tract. *Am J Gastroenterol* 2009; **104** Suppl 2: S17-S20 [PMID: 19262541 DOI: 10.1038/ajg.2009.47]
35 **Eom CS**, Jeon CY, Lim JW, Cho EG, Park SM, Lee KS. Use of acid-suppressive drugs and risk of pneumonia: a systematic review and meta-analysis. *CMAJ* 2011; **183**: 310-319 [PMID: 21173070 DOI: 10.1503/cmaj.092129]
36 **Leonard J**, Marshall JK, Moayyedi P. Systematic review of the risk of enteric infection in patients taking acid suppression. *Am J Gastroenterol* 2007; **102**: 2047-2056; quiz 2057 [PMID: 17509031 DOI: 10.1111/j.1572-0241.2007.01275.x]
37 **Kim JW**, Lee KL, Jeong JB, Kim BG, Shin S, Kim JS, Jung HC, Song IS. Proton pump inhibitors as a risk factor for recurrence of Clostridium-difficile-associated diarrhea. *World J Gastroenterol* 2010; **16**: 3573-3577 [PMID: 20653067 DOI: 10.3748/wjg.v16.i28.3573]
38 **Saiman L**, Ludington E, Dawson JD, Patterson JE, Rangel-Frausto S, Wiblin RT, Blumberg HM, Pfaller M, Rinaldi M, Edwards JE, Wenzel RP, Jarvis W. Risk factors for Candida species colonization of neonatal intensive care unit patients. *Pediatr Infect Dis J* 2001; **20**: 1119-1124 [PMID: 11740316 DOI: 10.1097/00006454-200112000-00005]
39 **Guillet R**, Stoll BJ, Cotten CM, Gantz M, McDonald S, Poole WK, Phelps DL. Association of H2-blocker therapy and higher incidence of necrotizing enterocolitis in very low birth weight infants. *Pediatrics* 2006; **117**: e137-e142 [PMID: 16390920 DOI: 10.1542/peds.2005-1543]
40 **Stoll BJ**, Temprosa M, Tyson JE, Papile LA, Wright LL, Bauer CR, Donovan EF, Korones SB, Lemons JA, Fanaroff AA, Stevenson DK, Oh W, Ehrenkranz RA, Shankaran S, Verter J. Dexamethasone therapy increases infection in very low birth weight infants. *Pediatrics* 1999; **104**: e63 [PMID: 10545589 DOI: 10.1542/peds.104.5.e63]
41 **Beck-Sague CM**, Azimi P, Fonseca SN, Baltimore RS, Powell DA, Bland LA, Arduino MJ, McAllister SK, Huberman RS, Sinkowitz RL. Bloodstream infections in neonatal intensive care unit patients: results of a multicenter study. *Pediatr Infect Dis J* 1994; **13**: 1110-1116 [PMID: 7892080 DOI: 10.1097/00006454-199412000-00008]
42 **Saiman L**, Ludington E, Pfaller M, Rangel-Frausto S, Wiblin RT, Dawson J, Blumberg HM, Patterson JE, Rinaldi M, Edwards JE, Wenzel RP, Jarvis W. Risk factors for candidemia in Neonatal Intensive Care Unit patients. The National Epidemiology of Mycosis Survey study group. *Pediatr Infect Dis J* 2000; **19**: 319-324 [PMID: 10783022 DOI: 10.1097/00006454-200004000-00011]
43 **Carrion V**, Egan EA. Prevention of neonatal necrotizing enterocolitis. *J Pediatr Gastroenterol Nutr* 1990; **11**: 317-323 [PMID: 2246712 DOI: 10.1097/00005176-199010000-00006]
44 **Schöll I**, Ackermann U, Ozdemir C, Blümer N, Dicke T, Sel S, Sel S, Wegmann M, Szalai K, Knittelfelder R, Untersmayr E, Scheiner O, Garn H, Jensen-Jarolim E, Renz H. Anti-ulcer treatment during pregnancy induces food allergy in mouse mothers and a Th2-bias in their offspring. *FASEB J* 2007; **21**: 1264-1270 [PMID: 17227952 DOI: 10.1096/fj.06-7223com]
45 **Dehlink E**, Yen E, Leichtner AM, Hait EJ, Fiebiger E. First evidence of a possible association between gastric acid suppression during pregnancy and childhood asthma: a population-based register study. *Clin Exp Allergy* 2009; **39**: 246-253 [PMID: 19134022 DOI: 10.1111/j.1365-2222.2008.03125.x]
46 **Heidelbaugh JJ**, Metz DC, Yang YX. Proton pump inhibitors: are they overutilised in clinical practice and do they pose significant risk? *Int J Clin Pract* 2012; **66**: 582-591 [PMID: 22607510 DOI: 10.1111/j.1742-1241.2012.02921.x]
47 **Fiocca R**, Mastracci L, Attwood SE, Ell C, Galmiche JP, Hatlebakk J, Bärthel A, Långström G, Lind T, Lundell L. Gastric exocrine and endocrine cell morphology under prolonged acid inhibition therapy: results of a 5-year follow-up in the LOTUS trial. *Aliment Pharmacol Ther* 2012; **36**: 959-971 [PMID: 22998687 DOI: 10.1111/apt.12052]
48 **Jalving M**, Koornstra JJ, Wesseling J, Boezen HM, DE Jong S, Kleibeuker JH. Increased risk of fundic gland polyps during long-term proton pump inhibitor therapy. *Aliment Pharmacol Ther* 2006; **24**: 1341-1348 [PMID: 17059515 DOI: 10.1111/j.1365-2036.2006.03127.x]
49 **Valuck RJ**, Ruscin JM. A case-control study on adverse effects: H2 blocker or proton pump inhibitor use and risk of vitamin B12 deficiency in older adults. *J Clin Epidemiol* 2004; **57**: 422-428 [PMID: 15135846 DOI: 10.1016/j.jclinepi.2003.08.015]
50 **den Elzen WP**, Groeneveld Y, de Ruijter W, Souverijn JH, le Cessie S, Assendelft WJ, Gussekloo J. Long-term use of proton pump inhibitors and vitamin B12 status in elderly individuals. *Aliment Pharmacol Ther* 2008; **27**: 491-497 [PMID: 18194503]
51 **Insogna KL**. The effect of proton pump-inhibiting drugs on mineral metabolism. *Am J Gastroenterol* 2009; **104** Suppl 2: S2-S4 [PMID: 19262542]
52 **Chen J**, Yuan YC, Leontiadis GI, Howden CW. Recent safety concerns with proton pump inhibitors. *J Clin Gastroenterol* 2012; **46**: 93-114 [PMID: 22227731]
53 **Kaye JA**, Jick H. Proton pump inhibitor use and risk of hip fractures in patients without major risk factors. *Pharmacotherapy* 2008; **28**: 951-959 [PMID: 18657011 DOI: 10.1592/phco.28.8.951]
54 **Corley DA**, Kubo A, Zhao W, Quesenberry C. Proton pump inhibitors and histamine-2 receptor antagonists are associated with

hip fractures among at-risk patients. *Gastroenterology* 2010; **139**: 93-101 [PMID: 20353792 DOI: 10.1053/j.gastro.2010.03.055]

55 **Danziger J**, William JH, Scott DJ, Lee J, Lehman LW, Mark RG, Howell MD, Celi LA, Mukamal KJ. Proton-pump inhibitor use is associated with low serum magnesium concentrations. *Kidney Int* 2013; **83**: 692-699 [PMID: 23325090]

56 **Shih CJ**, Chen YT, Ou SM, Li SY, Chen TJ, Wang SJ. Proton pump inhibitor use represents an independent risk factor for myocardial infarction. *Int J Cardiol* 2014; **177**: 292-297 [PMID: 25499395 DOI: 10.1016/j.ijcard.2014.09.036]

P- Reviewer: Hatta W **S- Editor**: Qi Y **L- Editor**: A
E- Editor: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i4.540

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 540-549
ISSN 2150-5349 (online)

ORIGINAL ARTICLE

Basic Study

A20 inhibits lipopolysaccharide-induced inflammation in enterocytes

Cui-Fang Zheng, Jie-Ru Shi, Ying Huang, Sheng-Nan Wang

Cui-Fang Zheng, Jie-Ru Shi, Ying Huang, Sheng-Nan Wang, Department of Gastroenterology, Children's Hospital of Fudan University, Shanghai 201102, China

Author contributions: Zheng CF and Huang Y designed the study; Zheng CF and Wang SN performed the research; Zheng CF and Shi JR analysed the data; Zheng CF, Shi JR and Huang Y wrote the paper.

Supported by The National Natural Science Foundation of China, No. 81300291.

Institutional review board statement: The publication of this manuscript has been reviewed and approved by the Children's Hospital of Fudan University Review Board.

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

Data sharing statement: The technical appendix, statistical code, and dataset are available from the corresponding author at yhuang815@163.com. The study participants provided their informed consent for data sharing. No additional data are available.

Manuscript source: Unsolicited manuscript

Correspondence to: Ying Huang, MD, PhD, Department of Gastroenterology, Children's Hospital of Fudan University, No. 399 Wan-Yuan Road, Minhang District, Shanghai 201102, China. yhuang815@163.com
Telephone: +86-21-64931727
Fax: +86-21-64931901

Received: May 1, 2016
Peer-review started: May 2, 2016
First decision: July 4, 2016
Revised: July 14, 2016
Accepted: August 15, 2016
Article in press: August 17, 2016
Published online: November 6, 2016

Abstract

AIM

To examine the role of A20 in the regulation of intestinal epithelial cells (IECs) inflammation.

METHODS

Using gene transfection, both stable overexpression and knockdown A20-expressed HT-29 cell lines were established. Accordingly, the cells were divided into the following groups: The control group, the A20 over-expression group, the A20 knockdown group and the respective controls. A20 was stimulated with lipo-polysaccharide (LPS) in a dose- and time-dependent manner and was detected using western blotting and real-time polymerase chain reaction (PCR) analyses. Immunofluorescence and western blotting analyses were performed to investigate the role of A20 in the regulation of nuclear factor (NF)-κB activation and translocation into the nucleus. ELISA and real-time PCR were performed to examine A20 in regulating the release of the following inflammatory cytokines: Tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6 and IL-8.

RESULTS

The expression of A20 in IECs was inducible. When intestinal epithelial cells were subjected to the stimulation of LPS, the expression of A20 was increased, and the expression of A20 was induced in a dose- and time-dependent manner. The expression of A20 was very low in HT-29 cells without LPS stimulation but rapidly increased and was maintained at a high level 2-4 h after stimulation

with LPS. These levels gradually declined with a change in time-course, and the expression of A20 increased with increasing LPS stimulation. Western blotting and immunofluorescence revealed that overexpression of A20 can inhibit NF-κB activation and its translocation to the nucleus. The overexpression of A20 can reduce the levels of proinflammatory cytokines involved in the pathophysiology of inflammatory bowel disease. There was no significant difference in the expression of IL-8 mRNA in the control group, A20 overexpression group or A20 knockdown group without LPS stimulation (P > 0.05); however, while after 2 h, 4 h and 8 h stimulation with LPS, the expression of IL-8 in the A20 overexpression group was lower than the control group and the A20 knockdown group (P < 0.05 or P < 0.01). The expression of TNF-α was different at different time points after 8 h of LPS stimulation (F = 31.33, DF = 5, P < 0.001), and the expression of TNF-α increased as the LPS stimulation time increased. Upon LPS stimulation, lower levels of TNF-α were detected in the A20 overexpression cell lines (P < 0.05). There were no significant differences in the induction of IL-6 and IL-1β among the control group, A20 overexpression group and A20 knockdown group (P > 0.05).

CONCLUSION

A20 plays an important role in limiting inflammation by inhibiting LPS-induced NF-κB responses in the gut luminal. A20 may be a potential therapeutic tool for inflammatory diseases.

Key words: A20 (TNFAIP3); Lipopolysaccharide; Nuclear factor-κB; Inflammatory bowel disease

Core tip: The use of A20-deficient mice and RNA interference technologies has revealed that mice or enterocytes lacking A20 showed hyper-responsiveness to stimulation. However, studies on whether the overexpression of A20 can extenuate enterocyte inflammation are limited. Our present results demonstrated that the expression of A20 was increased in a dose- and time-dependent manner upon lipopolysaccharide (LPS) stimulation in intestinal epithelial cells. More importantly, the overexpression of A20 suppressed the activation of nuclear factor-κB and the induction of pro-inflammatory molecules, such as Tumor necrosis factor-α and IL-8. Taken together, these findings indicate that A20 plays a critical role in limiting LPS-induced inflammation in the gut luminal and may be a potential therapeutic tool for immune and inflammatory diseases.

Zheng CF, Shi JR, Huang Y, Wang SN. A20 inhibits lipopolysaccharide-induced inflammation in enterocytes. *World J Gastrointest Pharmacol Ther* 2016; 7(4): 540-549 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i4/540.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i4.540

INTRODUCTION

Inflammatory bowel disease (IBD) has been proposed to be caused by an inappropriate inflammatory response to normal components of the intestinal micro-biota in genetically predisposed individuals[1-3]. Bacterial wall products play an important role in the activation of the immune and non-immune cells of the intestinal mucosa, and intestinal epithelial cells (IECs) represent a unique population of cells that exist in direct contact with a dense and complex milieu of commensal microorganisms. As a primary interface between pathogens and the intestinal tract, the epithelial cells lining the gut luminal play a key role in defence against microbial pathogens[4]. Rather than functioning as a passive barrier, IECs are an active participant in the mucosal immune response. The interaction between IECs and intestinal micro-biota results in the activation of multiple intracellular signalling events, including the activation of nuclear factor (NF)-κB. The inappropriate activation of NF-κB is central to the pathogenesis of IBD. On the one hand, NF-κB regulates the expression of various cytokines and other modulators of the inflammatory processes in IBD[5,6]. On the other hand, the inhibition of NF-κB activity has been suggested as a major component of the anti-inflammatory activity of glucocorticoids that are frequently used for treatment of IBD[7,8]. Thus, a tight regulation of the NF-κB signalling pathway and the genes induced is an absolute requirement. A20 (also known as Tumour Necrosis Factor Alpha-Induced Protein 3 or TNFAIP3) is a widely expressed and inducible cytoplasmic protein that plays a key role in the negative regulation of inflammation and immunity[9,10]. Several studies have shown that the ubiquitin-editing protein A20 is a key player in the negative feedback regulation of NF-κB signalling in response to multiple stimulants[11]. An essential role of A20 in the regulation of NF-κB signalling was clearly demonstrated with the generation of A20-deficient mice and RNA interference technologies. Mice deficient for A20 are extremely susceptible to sub-lethal doses of TNF and die prematurely due to severe multi-organ inflammation and cachexia[12].

More specifically related to IBD, a recent genome-wide association study identified A20 as a Crohn's disease (CD) susceptibility gene[13]. Specific deletion of A20 in enterocytes increased the susceptibility of mice to dextran sodium sulphate (DSS)-induced colitis and prevented the recovery from acute DSS-induced inflammation[14]. Finally, mucosal biopsies from 69 CD patients were analysed and confirmed a consistent down-regulation of mucosal A20 expression[15]. Our previous work found that there is an excessive inflammatory response but insufficient up-regulation of A20 expression in IBD patients[16]. These studies indicate that defective A20 expression or activity could be a risk factor for IBD.

The use of A20-deficient mice and RNA interference technologies has revealed that mice or enterocytes lacking A20 showed hyper-responsiveness to stimulation.

Based on the knowledge that A20 plays a central role in inflammation and immunity, this study aimed to determine whether A20 has a potential therapeutic value and whether overexpression of A20 could extenuate enterocyte inflammation. Previous studies have confirmed that the overexpression of A20 can attenuate allergic airway inflammation in mice[17] and protect kidneys from ischaemia/reperfusion injury[18]. However, studies on whether the overexpression of A20 may reduce intestinal inflammation are limited. Thus, we performed this study to examine the effectiveness of A20 in reducing IECs inflammatory reaction and NF-κB activation. In this study, we recapitulated the response of epithelial cells to LPS to mimic the *in vivo* response of intestinal epithelial cells to infection of pathogenic and/or commensal microbes, so as to explore the role of A20 in the regulation of intestinal epithelial cell inflammation.

MATERIALS AND METHODS

Reagents

The main reagents and antibodies used in our experiments are as follows: Unpurified LPS from *Escherichia coli* 0127:B8 (Sigma, St. Louis, MO, United States, L4516), anti-A20 polyclonal antibody (Abcam, Cambridge, United Kingdom, ab45366), anti-NF-κB p65 monoclonal antibody (Epitomics, California, United States, E379), anti-β-actin antibody (Sigma, St. Louis, MO, United States), TNF-α and IL-1β ELISA kit (Senxiong Technology Industrial Company, Shanghai, China), TRIzol (Invitrogen, Carlsbad, CA, United States, 15596-018), SYBR green PCR reagent kits (Toyobo Co, Osaka, Japan, QPK-201), and Lipofectamine 2000 (Invitrogen, Carlsbad, CA, United States).

Cells and cell culture

The human intestinal epithelial cell line HT-29 was purchased from the Chinese Academy of Sciences (Shang hai) and grown in Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen, C11965), supplemented with 10% foetal bovine serum (Invitrogen) and maintained at 37 ℃ in a humidified incubator under an atmosphere of 5% CO_2.

Plasmids and transfection

Human A20 cDNA was amplified with the following primers: F1: 5'-AGAGGTGTTGGAGAGCACAATGG-3' and R1: 5'-CACCTGTTTCCGGTTAGCCATACA-3', and HA-tagged A20 was cloned into the lentiviral vector pWPI. Lentiviral particles were produced by transfecting HEK-293T cells with pWPI.1-HA-A20, psPAX2 and pMD2.G. After incubation with HT-29 cells for 1 wk, the cells were screened using a flow cytometer.

To silence A20, we employed the lentiviral silencing system. ShRNA oligos against A20 were cloned into the lentiviral vector pLKO.1-TRC cloning vector (Addgene Plasmid 10878). The 21-bp target sequences of A20 were CACTGGAAGAAATACACATAT and GCACCGATACACACTGGAAAT. To produce lentiviral particles, the shRNA construct was co-transfected with psPAX2 and pMD2.G into HEK-293T cells. The media was harvested to obtain lentiviral particles. The target cells with lentiviral particles were infected for 24 h and selected with puromycin at a concentration of 2 μg/mL. The day before transfection, the cells were plated in growth medium at a density of approximately 70%. The shRNA was transfected at a concentration of 50 nmol/L using Lipofectamine 2000 according to the manufacturer's instructions.

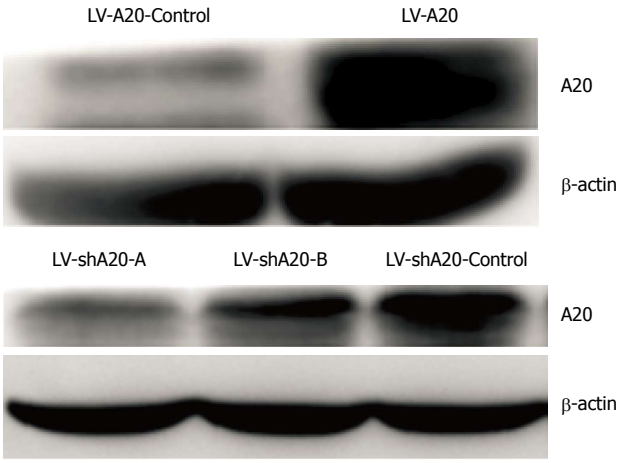

Figure 1 Expression of A20 in different groups. HT-29 cells were transduced with LV-A20, LV-shA20, or LV-controls. Cytosolic lysates were tested by Western blotting using anti-A20 antibodies or anti-β-actin antibodies to normalize protein levels. In A20 overexpression cell lines, the expression of A20 was much higher than that of the control. Two shRNA sequences, A and B, were obtained, and the expression of A20 in both of the A20 knockdown groups were much lower than the control. The effect of A was more pronounced than B. Thus, sequence A was selected for subsequent experiments.

HT-29 cells were transduced with LV-A20, LV-shA20, or LV-controls, respectively. Accordingly, the cells were divided into the following groups: The control group (HT-29 cells without transfection), A20 overexpression group, A20 knockdown group, A20 overexpression-control group and A20 knockdown-control group. Overexpression and silencing of A20 were validated by Western blotting analysis prior to the experimentation. The presence of the 90-kDa bands of extracts from transfected cell populations were significantly higher or lower than the controls (Figure 1). We have constructed two shRNA (A and B) sequences to silence A20, as shown in Figure 1. The silencing effect of A was more pronounced than B. Thus, sequence A was selected for subsequent experiments.

Real time-polymerase chain reaction

Total RNA was isolated from cells using TRIzol reagent, and equal amounts of RNA were reverse-transcribed into cDNA using a quantitative PCR cDNA kit (TaKaRa, Biotechnology, Dalian, China, DRR037Ab). Specific primers (Table 1) used in the real-time PCR studies were designed and generated by Takara Biotechnology Company (Dalian, China). Quantitative real-time PCR was performed using SYBR green PCR reagent kits according

Table 1 Primer sequences

Gene	Sequences(5′-3′)
A20	Forward: AAAGCCCTCATCGACAGAAA
	Reverse: CAGTTGCCAGCGGAATTTA
IL-6	Forward: AAGCCAGAGCTGTGCAGATGAGTA
	Reverse: TGTCCTGCAGCCACTGGTTC
IL-8	Forward: ACACTGCGCCAACACAGAAATTA
	Reverse: TTTGCTTGAAGTTTCACTGGCATC
GAPDH	Forward: GCACCGTCAAGGCTGAGAAC
	Reverse: TGGTGAAGACGCCAGTGGA

to the manufacturer's instructions. Data were recorded and analysed using the real-time PCR analysis software Bio-Rad iQ5. The A20, IL-6 and IL-8 mRNA levels were normalized against GAPDH levels in the cells. The relative gene expression was calculated by comparing the number of thermal cycles that were necessary to generate the threshold amounts of product (CT).

Western blotting analysis and enzyme-linked immunosorbent assay

HT-29 cells were homogenized with protease and phosphatase inhibitors and prepared in protein extraction solution (Thermo, 78835). Protein lysates, quantified using a BCA assay (Sangon Biotech Company, Ltd., Shanghai, China), were separated on reducing SDS-polyacrylamide gels and transferred onto polyvinyldifluoride membranes (PVDF, Millipore). The membranes were blocked with 5% non-fat milk TBS buffer for 2 h at room temperature and incubated with primary antibodies overnight at 4 ℃. β-actin levels were used to normalize loading. The first antibody exposure was followed by incubation with an anti-rabbit IgG, HRP-linked secondary antibody (Cell Signaling, Beverly, MA, United States). Antigen-antibody complexes were visualized using the enhanced chemiluminescence detection method (ECL kit, Thermo, Waltham, MA, United States).

The concentrations of TNF-α and IL-1β in the culture supernatants were measured using a commercially available enzyme-linked immunosorbent assay kit (Senxiong Technology Industrial Company, Shanghai, China), according to the manufacturer's instructions.

Immunofluorescence

The immunofluorescence of cultured cells was performed as recommended by the antibody manufacturers. Horseradish peroxidase-conjugated anti-rabbit IgG was used as a secondary antibody. Images were obtained on a BX51 microscope equipped with a colour camera using Picture Frame software (Olympus).

Statistical analysis

For all statistical analysis, data were expressed as the mean ± standard deviations (SD) or standard error of the mean. Student's *t*-test was used for comparisons between groups for continuous variables. One-way analysis of variance (ANOVA) was used to compare differences between groups. *P* values < 0.05 were accepted as significant.

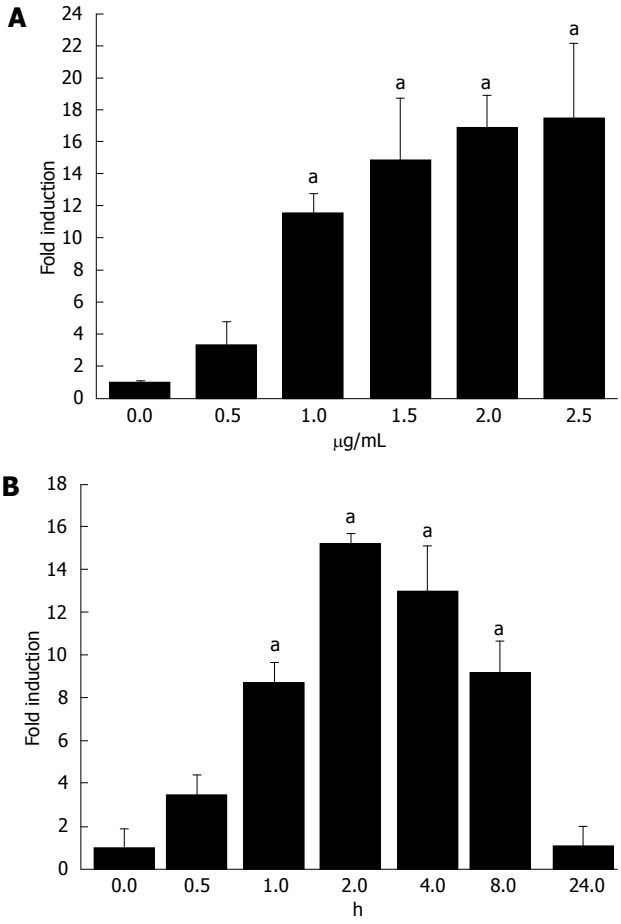

Figure 2 Relative A20 mRNA ratio in HT-29 cells after lipopolysaccharide stimulation with different doses or different time points. CT was calculated for the genes of interest and for the housekeeping gene GAPDH. For each cDNA sample, the CT for GAPDH was subtracted from the CT for each gene of interest to obtain the parameter $^{\Delta}$Ct, thereby normalizing the initial amount of RNA used. The amount of each target was calculated as $2^{\Delta\Delta}$Ct, where $^{\Delta\Delta}$CT is the difference between the $^{\Delta}$CT of the two cDNA samples to be compared. A: Cultured HT-29 cells were stimulated with LPS at different doses for 1 h, and the results showed that the expression of A20 was increased with increasing amounts of LPS stimulation. $^{a}P < 0.05$ *vs* non-stimulated cells; B: HT-29 cells were treated with 1 μg/mL LPS at various time points (0-8 h). The expression of A20 was very low in HT-29 cells without LPS stimulation but was rapidly increased and peaked at 2 h. $^{a}P < 0.05$ *vs* non-stimulated cells and cells stimulated for 24 h. LPS: Lipopolysaccharide.

RESULTS

A20 expression was rapidly increased upon stimulation with LPS in cultured enterocytes

Initially, we aimed to determine whether LPS could induce the expression of A20 in a dose-dependent manner. Cultured HT-29 cells were stimulated with LPS at different doses for 1 h and harvested for the analysis of A20 using real-time PCR and Western blotting analyses. As shown in Figures 2A and 3A, the expression of A20 increased with increasing LPS stimulation. Furthermore, to gain insight into the time-course changes of A20

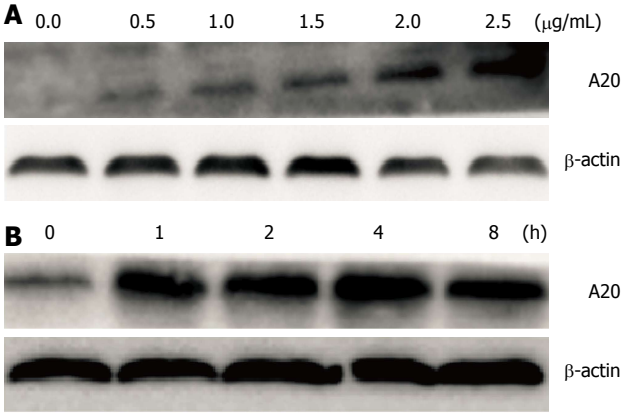

Figure 3 Expression of A20 in HT-29 cells after lipopolysaccharide stimulation with different doses or different time points. A: Cultured HT-29 cells were stimulated with lipopolysaccharide (LPS) at different doses for 1 h. Western blotting analysis showed that the expression of A20 was increased with increasing amounts of LPS stimulation; B: HT-29 cells were treated with 1 μg/mL LPS at various time points (0-8 h). Western blotting analysis showed that the expression of A20 was rapidly increased and maintained at a high level for 1-4 h after stimulation with LPS.

levels when enterocytes were subjected to infection, the expression of A20 protein and mRNA was detected at various time points after 1 μg/mL LPS stimulation using Western blotting and real-time PCR analyses. We found that the expression of A20 increased in a time-dependent manner after LPS stimulation. The expression of A20 was very low in HT-29 cells without LPS stimulation but rapidly increased and maintained a high level at 2-4 h after stimulation with LPS. These levels then declined gradually with a change in time-course (Figures 2B and 3B).

Overexpression of A20 inhibited NF-κB translocation to the nucleus

It is known that NF-κB is kept inactive by binding to the inhibitor of κB (IκB) proteins in resting cells. Upon stimulation with a wide variety of agonists, IκB is phosphorylated and subsequently polyubiquitinated and degraded by the proteasome, thereby releasing NF-κB, which then accumulates in the nucleus and activates the transcription of its target genes. As a negative regulator of NF-κB signalling pathway, we confirmed whether overexpression of A20 will alter the expression of NF-κB and its translocation to the nucleus in HT-29 cells. After a 2-h stimulation with LPS (1 μg/mL), Western blotting was performed to detect the expression of NF-κB p65 in the control group, A20 overexpression group and A20 knockdown group. As shown in Figure 4, the expression of NF-κB p65 in A20 overexpression cell lines was much lower than the control group and A20 knockdown group. In addition, immunofluorescence was performed to detect the location of NF-κB p65 in the different groups, and the results demonstrated that overexpression of A20 reduced NF-κB p65 translocation to the nucleus (Figure 5). Taken together, these data indicated that A20 inhibits the translocation of NF-κB to the nucleus in intestinal epithelial cells.

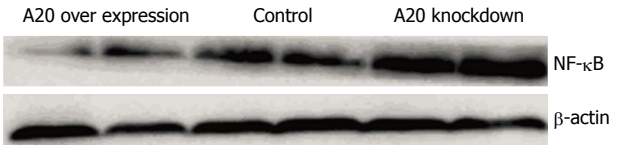

Figure 4 Overexpression of A20 decreased the level of nuclear factor-κB. Control group, A20 overexpression group and A20 knockdown group cells were treated with LPS (1 μg/mL) for 2 h. Western blotting showed that overexpression of A20 significantly decreased the level of NF-κB p65, whereas down-regulation of A20 increased NF-κ p65 expression. LPS: Lipopolysaccharide; NF: Nuclear factor.

Overexpression of A20 reduces the levels of proinflammatory cytokines involved in the pathophysiology of IBD

To determine whether the overexpression of A20 would decrease proinflammatory cytokines, such as TNF-α, IL-1β, IL-6 and IL-8, in IECs *via* modulation of NF-κB activation, the total RNA was isolated from IECs that were subjected to LPS. IL-6 and IL-8 expression were analysed using RT-PCR, while the expression of TNF-α and IL-1β in the culture supernatants were assayed by ELISA. The expression of TNF-α was different at different time points during an 8 h stimulation with LPS ($F = 31.33$, DF = 5, $P < 0.001$), and the expression of TNF-α was increased as the LPS stimulation time increased ($F = 111.435$, DF = 1, $P < 0.001$). At different time points during the 8 h stimulation with LPS, there was a significant difference in the expression of TNF-α between the A20 knockdown and the A20 overexpression ($P < 0.05$). In addition, there was a significant difference in the expression of TNF-α between the control group and the A20 knockdown group during the 8 h stimulation ($P < 0.05$, Figure 6A). Similar to the expression of TNF-α, the expression of IL-1β was different at different time points during the 8 h stimulation with LPS ($F = 9.216$, DF = 5, $P < 0.001$), and the expression of IL-1β increased with increasing LPS stimulation time ($F = 80.829$, DF = 1, $P < 0.001$). However, there was no significant difference in the expression of IL-1β in the control group, A20 knockdown group or A20 overexpression group during the 8 h stimulation of LPS ($F = 2.456$, DF = 2, $P = 0.166$, Figure 6B).

As shown in Figure 7, there was no significant difference in the expression of IL-8 mRNA in the control group, A20 overexpression-control group and A20 knockdown-control group. After 2 h, 4 h and 8 h stimulation with LPS, the expression of IL-8 mRNA in the A20 overexpression group was lower than the control group and A20 knockdown group, and the expression of IL-8 in the A20 knockdown group was higher than both the control group and the A20 overexpression group. However, there was no significant difference in the expression of IL-6 mRNA in the control group, A20 knockdown group or A20 overexpression group after 8 h of stimulation with LPS. These results demonstrated that

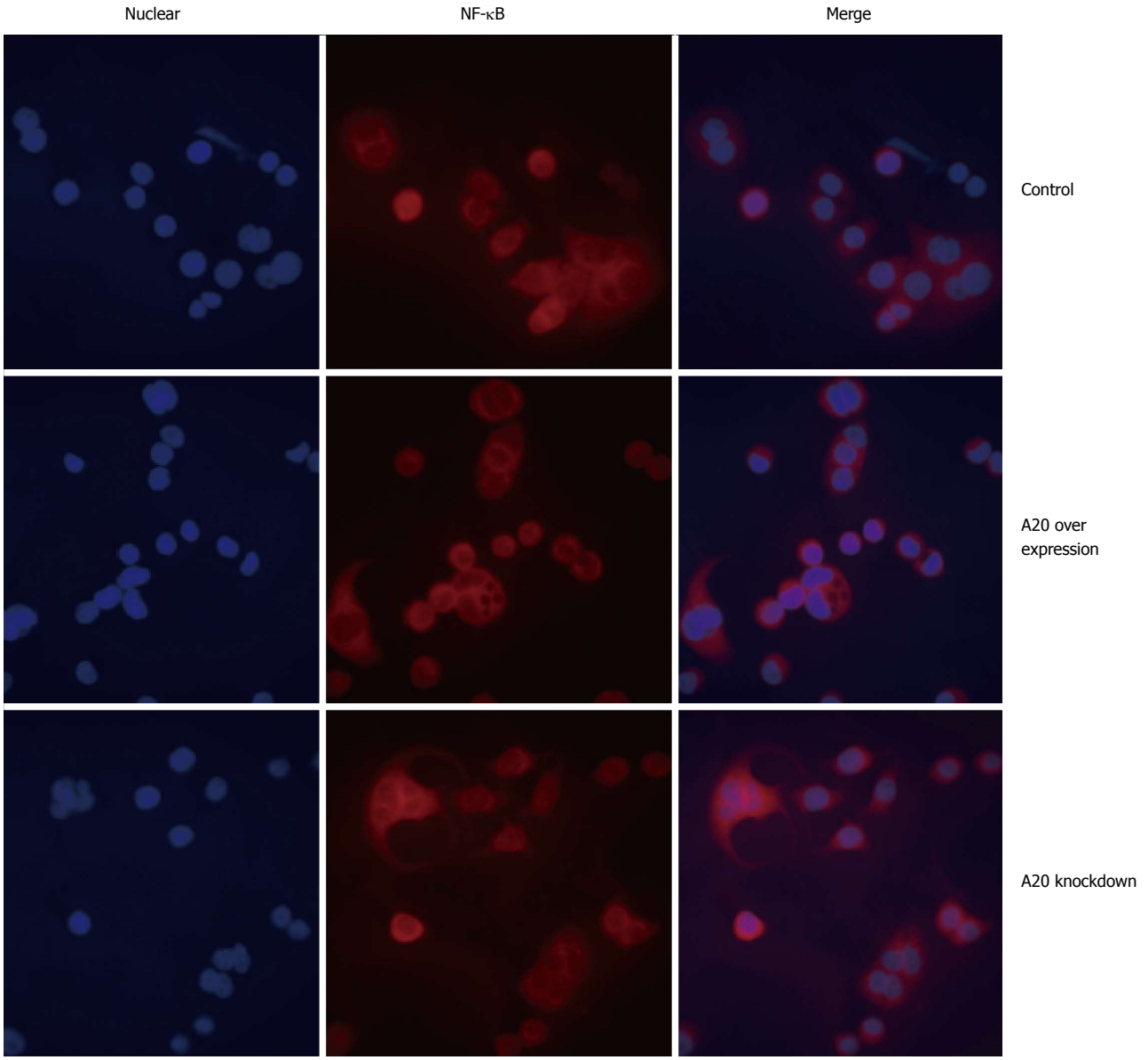

Figure 5 A20 inhibited nuclear factor-κB activation and translocation to the nucleus. Immunofluorescence was performed to detect the intracellular localization of NF-κB using anti-NF-κB p65 primary antibodies and fluorescent secondary antibodies (red) followed by confocal microscopy. DAPI-stained nuclei appear in blue. Weaker fluorescence intensity in the nucleus was detected in A20 overexpression cell lines.

the overexpression of A20 can significantly reduce the levels of pro-inflammatory cytokines.

DISCUSSION

TNF-α is regarded as a critical contributor to IBD. Its functions include the recruitment of circulating inflammatory cells to local tissue sites of inflammation, induction of oedema, the activation of the coagulation cascade, and the initiation of the formation of granuloma[19]. Clinical trials demonstrating symptomatic improvement and remission of IBD by suppressing TNF-α have provided additional evidence of the role of TNF-α in the pathogenesis of IBD[20,21]. Thus, TNF-α is considered an attractive target for the treatment of IBD. The introduction of anti-TNF-α agents (*e.g.*, Infliximab) has greatly advanced the therapeutic armamentarium of IBD, but the clinical application of infliximab is limited due to the occurrence of neutralizing antibodies[22,23], which is associated with allergic reactions and a loss of response. Thus, the development of novel therapeutic strategies for IBD is needed.

There is accumulating evidence to support the therapeutic potential of A20 in autoimmune diseases. The specific deletion of A20 in enterocytes increased the susceptibility of mice to dextran sodium sulphate (DSS)-induced colitis and prevented recovery from DSS-induced inflammation[14], whereas the expression of A20 by dendritic cells protects mice from LPS-induced mortality and DSS-induced colitis[24,25]. Not limited to IBD, A20 was also identified to be associated with numerous autoimmune diseases[11,26]. It was previously reported

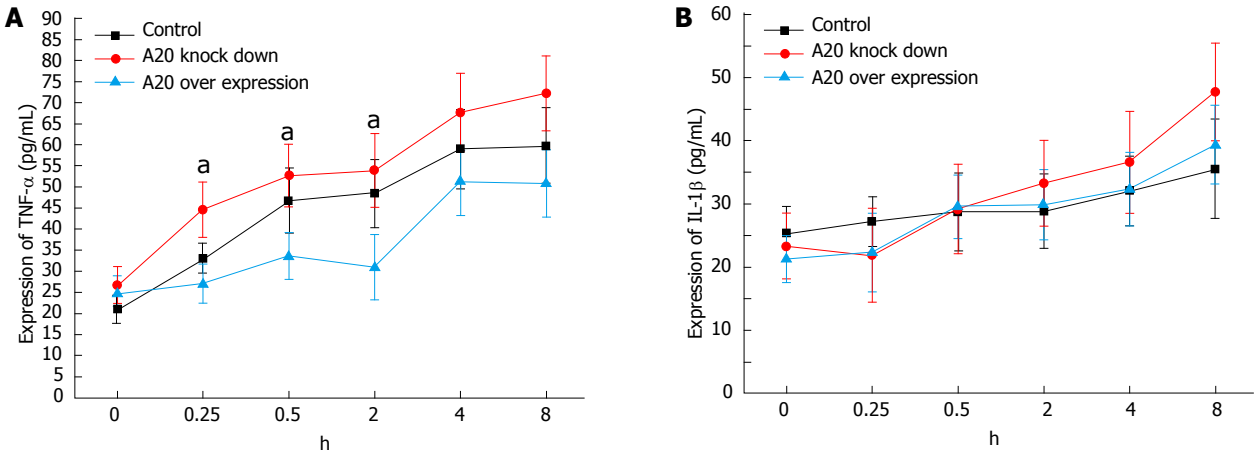

Figure 6 Expression of tumor necrosis factor-α and interleukin-1β in different groups. A: There was no significant difference in the expression of TNF-α among the control group, A20 knockdown-control group and A20 overexpression-control group. At 0.25 h, 0.5 h and 2 h, the expression of TNF-α in the A20 overexpression group was lower than the control group and A20 knockdown group, and the expression of TNF-α in the A20 knockdown group was higher than the control group; B: There was no significant difference in the expression of IL-1β in the control group, A20 knockdown group and A20 overexpression group after 8 h of LPS stimulation. $^{a}P < 0.05$. IL: Interleukin; TNF: Tumor necrosis factor; LPS: Lipopolysaccharide.

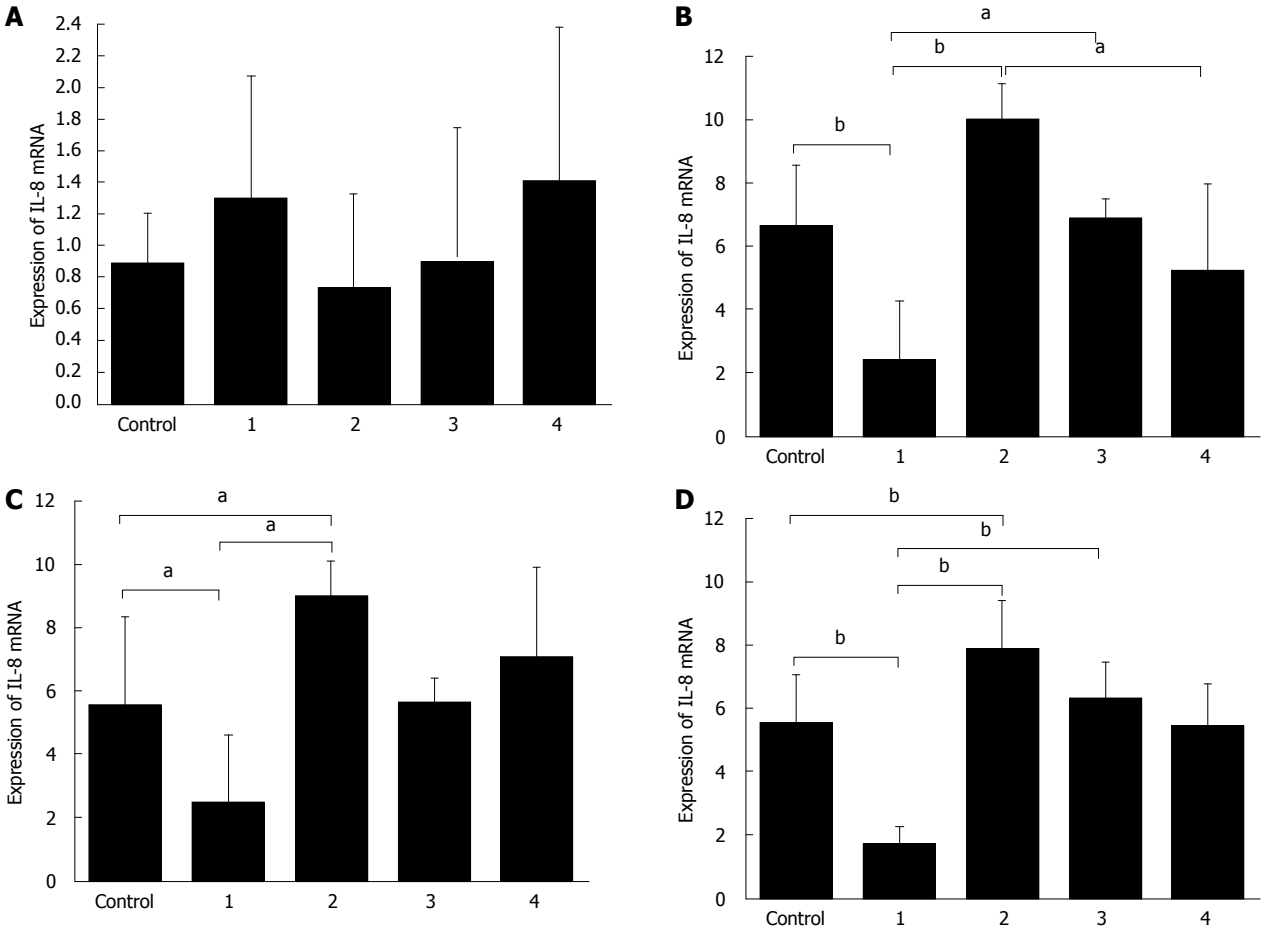

Figure 7 Interleukin-8 mRNA expression in different cell groups. 1: A20 overexpression group; 3: A20 overexpression-control group; 2: A20 knockdown group; 4: A20 knockdown-control group; A: Expression of IL-8 mRNA in different groups without LPS stimulation; B: Expression of IL-8 mRNA in different groups with LPS stimulation for 2 h; C: Expression of IL-8 mRNA in different groups with LPS stimulation for 4 h; D: Expression of IL-8 mRNA in different groups with LPS for 8 h. There were no significant differences in the IL-8 mRNA among the different groups without LPS stimulation. At some time points, the expression of IL-8 in the A20 overexpression group was lower than the control group and A20 knockdown group, and the expression of IL-8 in A20 knockdown group was higher than both the control group and A20 overexpression group. $^{a}P < 0.05$, $^{b}P < 0.01$. IL: Interleukin; TNF: Tumor necrosis factor; LPS: Lipopolysaccharide.

that prior injection with adenovirus containing A20 cDNA significantly diminished OVA challenge-mediated TNF-α production and attenuated allergic airway inflammation in mice[17]. In this study, we showed that the NF-κB responsive gene A20 is a major protective factor in intestinal epithelium cells, and the overexpression of A20 can significantly reduce the level of TNF-α in IECs. Taken together, these findings may provide some useful clues towards the development of novel anti- TNF-α strategies.

A20 has been described as a central gatekeeper in inflammation and immunity[9]. Emerging evidence indicates that LPS is a pathogenic factor that induces several inflammatory disorders, including necrotizing enterocolitis and IBD[27]. The tendency of A20-deficient mice to develop severe inflammation and their hyper-responsive nature to LPS[12] suggests that A20 may act as an endogenous regulatory system in controlling the inflammatory response to gram-negative bacteria. Oshima *et al*[28] showed that the gene expression of A20 is rapidly increased after ligand stimulation. In the present study, our results also confirmed that A20 expression was markedly up-regulated at both the mRNA and protein level upon stimulation with LPS in IECs. In addition, our experiments also found that LPS-induced A20 expression not only in a time dependent manner but also in a dose-dependent manner. The expression of A20 was very low in HT-29 cells without LPS stimulation but rapidly increased and was maintained at a high level 2-4 h after stimulation with LPS. This expression gradually declined with a change in time-course. Furthermore, the expression of A20 increased with increasing LPS stimulation.

Wang *et al*[29] found that A20 is necessary and sufficient for the development of LPS tolerance in enterocytes. Given that the human gut harbours a large collection of commensal bacteria, LPS released by gut microbes has a large effect on gut homeostasis, and the expression of A20 was rapidly increased in a dose- and time-dependent manner upon LPS stimulation. Taken together, these findings indicate the importance of A20 in the IEC response to inflammatory stimuli.

The transcription factor NF-κB has served as a standard for inducible transcription factors for more than 20 years. NF-κB can be found in the cytoplasm of most cells as an inactive complex with unprocessed precursor proteins (*e.g.*, p105) or IκB (*e.g.*, IκBa) proteins. Upon activation, NF-κB translocates into the nucleus and binds to DNA. The initiation of NF-κB signalling is tightly regulated because prolonged and excessive activation of NF-κB can lead to uncontrolled inflammation that is detrimental to the host, and thus, the inducible regulation of gene expression is a central element of normal physiology and is key to the ability of multicellular organisms to adapt to environmental, mechanical, chemical, and microbiological stresses[30]. In this study, RNA interference technology was utilized to down-regulate the expression of A20. Consistent with these findings, our results proved that the lack of A20 increased LPS-induced activation of NF-κB and its translocation to the nucleus. Furthermore, we also showed that overexpression of A20 resulted in a dramatic decrease in LPS-induced activation of NF-κB. Immunofluorescence results revealed that NF-κB translocation to the nucleus was reduced in the A20 overexpression cell group. These data indicated that the overexpression of A20 can inhibit LPS-induced NF-κB activation and translocation to the nucleus.

By far, the gastrointestinal tract is the most susceptible tissue to inflammatory responses due to its constant exposure to various antigenic, mutagenic and toxic factors. Deregulated cytokine production and signalling mechanisms by epithelial cells, mucosal lymphocytes and macrophages have been implicated in the pathogenesis of IBD. Numerous studies have identified altered proinflammatory cytokines in IBD[31]. A previous study showed that the overexpression of A20 significantly inhibited the activation of IL-8 in airway epithelial cells[32]. Furthermore, the present study demonstrates that overexpression of A20 can significantly reduce LPS-induced expression of TNF-α, IL-6 and IL-8, while the lack of A20 increased the level of TNF-α, IL-6 and IL-8 in IECs. These results demonstrated that A20 plays an important role in ameliorating the production of inflammatory cytokines.

Taken together, these findings underscore the importance of A20 in controlling inflammatory responses and indicate that A20 may be a potential therapeutic tool for the treatment of inflammatory diseases. However, its function remains poorly understood and additional investigations are necessary to elucidate the precise role of A20 in inflammatory diseases.

ACKNOWLEDGMENTS

We wish to express our deepest gratitude to the team of Professor Qing-Hai Ye, Zhongshan Hospital, the Liver Cancer Institute, Fudan University, Shanghai, for their generous help throughout the entire course of this project, without which it would not have been possible for us to complete this work.

COMMENTS

Background

A20 is regarded as the central gatekeeper in inflammation and immunity and is associated with numerous autoimmune diseases. The use of A20-deficient mice and RNA interference technologies has revealed that mice or enterocytes lacking A20 showed hyper-responsiveness to stimulations. Previous studies have confirmed that overexpression of A20 can attenuate allergic airway inflammation in mice and protects kidneys from ischaemia/reperfusion injury. However, studies on whether the overexpression of A20 extenuates enterocyte inflammation are rare.

Research frontiers

The inappropriate activation of nuclear factor (NF)-κB is central to the pathogenesis of inflammatory bowel disease (IBD). Thus, tight regulation of the NF-κB signalling pathway and the genes induced is an absolute requirement. Several studies have shown that the ubiquitin-editing protein A20 is a key player in the negative feedback regulation of NF-κB signalling in response to multiple stimulants.

Innovations and breakthroughs

A20 may be a potential therapeutic tool for immune and inflammatory diseases. Previous studies have identified that defective A20 expression or activity could be a risk factor for IBD. However, research studies related to the protective effect of A20 on intestinal epithelial cells are limited. These results demonstrated that the overexpression of A20 suppressed the activation of NF-κB and induction of proinflammatory molecules, such as TNF-α and IL-8. These data indicate that A20 plays an important role in limiting lipopolysaccharide (LPS)-induced inflammation in the gut luminal.

Applications

The introduction of infliximab has greatly advanced the therapeutic armamentarium of inflammatory bowel diseases, but the clinical application of infliximab is limited due to the occurrence of neutralizing antibodies. Thus, the development of novel therapeutic strategies for IBD is always needed. The experiments showed that A20 is critical for the inhibition of LPS-induced inflammation in enterocytes. These findings underscore the importance of A20 in controlling inflammatory responses and indicate that A20 may be a potential therapeutic tool for the treatment of inflammatory diseases.

Terminology

LPS is the major component of the outer membrane of gram-negative bacteria. The lentiviral vector is a type of retroviral vector that has become an ideal vector for target gene transfer due to its high efficiency of transfection, ability of transfection into dividing or non-dividing cells and its capacity for large target gene fragments. RNA interference technology is a type of technology that can be used to eliminate a specific gene or to suppress the expression of a specific gene.

Peer-review

This paper in interesting and clear way shows the negative regulation of inflammation under the influence of bacterial lipopolysaccharide. The experiments are well designed and the results demonstrate that A20 is able to limit the intestinal inflammation associated to NF-κB.

REFERENCES

1 **Xavier RJ**, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. *Nature* 2007; **448**: 427-434 [PMID: 17653185 DOI: 10.1038/nature06005]

2 **Artis D**. Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut. *Nat Rev Immunol* 2008; **8**: 411-420 [PMID: 18469830 DOI: 10.1038/nri2316]

3 **Round JL**, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol* 2009; **9**: 313-323 [PMID: 19343057 DOI: 10.1038/nri2515]

4 **Cario E**. Bacterial interactions with cells of the intestinal mucosa: Toll-like receptors and NOD2. *Gut* 2005; **54**: 1182-1193 [PMID: 15840688 DOI: 10.1136/gut.2004.062794]

5 **Atreya I**, Atreya R, Neurath MF. NF-kappaB in inflammatory bowel disease. *J Intern Med* 2008; **263**: 591-596 [PMID: 18479258 DOI: 10.1111/j.1365-2796.2008.01953.x]

6 **Kumar A**, Takada Y, Boriek AM, Aggarwal BB. Nuclear factor-kappaB: its role in health and disease. *J Mol Med* (Berl) 2004; **82**: 434-448 [PMID: 15175863 DOI: 10.1007/s00109-004-0555-y]

7 **Auphan N**, DiDonato JA, Rosette C, Helmberg A, Karin M. Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. *Science* 1995; **270**: 286-290 [PMID: 7569976 DOI: 10.1126/science.270.5234.286]

8 **Thiele K**, Bierhaus A, Autschbach F, Hofmann M, Stremmel W, Thiele H, Ziegler R, Nawroth PP. Cell specific effects of glucocorticoid treatment on the NF-kappaBp65/IkappaBalpha system in patients with Crohn's disease. *Gut* 1999; **45**: 693-704 [PMID: 10517905 DOI: 10.1136/gut.45.5.693]

9 **Coornaert B**, Carpentier I, Beyaert R. A20: central gatekeeper in inflammation and immunity. *J Biol Chem* 2009; **284**: 8217-8221 [PMID: 19008218 DOI: 10.1074/jbc.R800032200]

10 **Verstrepen L**, Verhelst K, van Loo G, Carpentier I, Ley SC, Beyaert R. Expression, biological activities and mechanisms of action of A20 (TNFAIP3). *Biochem Pharmacol* 2010; **80**: 2009-2020 [PMID: 20599425 DOI: 10.1016/j.bcp.2010.06.044]

11 **Vereecke L**, Beyaert R, van Loo G. The ubiquitin-editing enzyme A20 (TNFAIP3) is a central regulator of immunopathology. *Trends Immunol* 2009; **30**: 383-391 [PMID: 19643665 DOI: 10.1016/j.it.2009.05.007]

12 **Lee EG**, Boone DL, Chai S, Libby SL, Chien M, Lodolce JP, Ma A. Failure to regulate TNF-induced NF-kappaB and cell death responses in A20-deficient mice. *Science* 2000; **289**: 2350-2354 [PMID: 11009421 DOI: 10.1126/science.289.5488.2350]

13 **Wellcome Trust Case Control Consortium**. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007; **447**: 661-678 [PMID: 17554300 DOI: 10.1038/nature05911]

14 **Vereecke L**, Sze M, Mc Guire C, Rogiers B, Chu Y, Schmidt-Supprian M, Pasparakis M, Beyaert R, van Loo G. Enterocyte-specific A20 deficiency sensitizes to tumor necrosis factor-induced toxicity and experimental colitis. *J Exp Med* 2010; **207**: 1513-1523 [PMID: 20530205 DOI: 10.1084/jem.20092474]

15 **Arsenescu R**, Bruno ME, Rogier EW, Stefka AT, McMahan AE, Wright TB, Nasser MS, de Villiers WJ, Kaetzel CS. Signature biomarkers in Crohn's disease: toward a molecular classification. *Mucosal Immunol* 2008; **1**: 399-411 [PMID: 19079204 DOI: 10.1038/mi.2008.32]

16 **Zheng CF**, Huang Y. [Expression of zinc finger protein A20 in pediatric inflammatory bowel disease]. *Zhonghua Erke Zazhi* 2011; **49**: 261-265 [PMID: 21624200]

17 **Kang NI**, Yoon HY, Lee YR, Won M, Chung MJ, Park JW, Hur GM, Lee HK, Park BH. A20 attenuates allergic airway inflammation in mice. *J Immunol* 2009; **183**: 1488-1495 [PMID: 19553520 DOI: 10.4049/jimmunol.0900163]

18 **Lutz J**, Luong le A, Strobl M, Deng M, Huang H, Anton M, Zakkar M, Enesa K, Chaudhury H, Haskard DO, Baumann M, Boyle J, Harten S, Maxwell PH, Pusey C, Heemann U, Evans PC. The A20 gene protects kidneys from ischaemia/reperfusion injury by suppressing pro-inflammatory activation. *J Mol Med* (Berl) 2008; **86**: 1329-1339 [PMID: 18813897 DOI: 10.1007/s00109-008-0405-4]

19 **Van Deventer SJ**. Tumour necrosis factor and Crohn's disease. *Gut* 1997; **40**: 443-448 [PMID: 9176068 DOI: 10.1136/gut.40.4.443]

20 **Nakamura K**, Honda K, Mizutani T, Akiho H, Harada N. Novel strategies for the treatment of inflammatory bowel disease: Selective inhibition of cytokines and adhesion molecules. *World J Gastroenterol* 2006; **12**: 4628-4635 [PMID: 16937430 DOI: 10.3748/wjg.v12.i29.4628]

21 **Hyams J**, Crandall W, Kugathasan S, Griffiths A, Olson A, Johanns J, Liu G, Travers S, Heuschkel R, Markowitz J, Cohen S, Winter H, Veereman-Wauters G, Ferry G, Baldassano R. Induction and maintenance infliximab therapy for the treatment of moderate-to-severe Crohn's disease in children. *Gastroenterology* 2007; **132**: 863-873; quiz 1165-1166 [PMID: 17324398 DOI: 10.1053/j.gastro.2006.12.003]

22 **Vande Casteele N**, Khanna R, Levesque BG, Stitt L, Zou GY, Singh S, Lockton S, Hauenstein S, Ohrmund L, Greenberg GR, Rutgeerts PJ, Gils A, Sandborn WJ, Vermeire S, Feagan BG. The relationship between infliximab concentrations, antibodies to infliximab and disease activity in Crohn's disease. *Gut* 2015; **64**: 1539-1545 [PMID: 25336114 DOI: 10.1136/gutjnl-2014-307883]

23 **O'Meara S**, Nanda KS, Moss AC. Antibodies to infliximab and risk of infusion reactions in patients with inflammatory bowel disease: a systematic review and meta-analysis. *Inflamm Bowel Dis* 2014; **20**: 1-6 [PMID: 24280879 DOI: 10.1097/01.MIB.0000436951]

24 **Hammer GE**, Turer EE, Taylor KE, Fang CJ, Advincula R, Oshima S, Barrera J, Huang EJ, Hou B, Malynn BA, Reizis B, DeFranco A, Criswell LA, Nakamura MC, Ma A. Expression of A20 by dendritic cells preserves immune homeostasis and prevents colitis and spondyloarthritis. *Nat Immunol* 2011; **12**: 1184-1193 [PMID: 22019834 DOI: 10.1038/ni.2135]

25 **Xuan NT**, Wang X, Nishanth G, Waisman A, Borucki K, Isermann B, Naumann M, Deckert M, Schlüter D. A20 expression in dendritic cells protects mice from LPS-induced mortality. *Eur J Immunol*

2015; **45**: 818-828 [PMID: 25472594 DOI: 10.1002/eji.201444795]
26 **Zhang M**, Peng LL, Wang Y, Wang JS, Liu J, Liu MM, Hu J, Song B, Yang HB. Roles of A20 in autoimmune diseases. *Immunol Res* 2016; **64**: 337-344 [PMID: 26135958 DOI: 10.1007/s12026-015-8677-6]
27 **Rhee SH**. Lipopolysaccharide: basic biochemistry, intracellular signaling, and physiological impacts in the gut. *Intest Res* 2014; **12**: 90-95 [PMID: 25349574 DOI: 10.5217/ir.2014.12.2.90]
28 **Oshima N**, Ishihara S, Rumi MA, Aziz MM, Mishima Y, Kadota C, Moriyama I, Ishimura N, Amano Y, Kinoshita Y. A20 is an early responding negative regulator of Toll-like receptor 5 signalling in intestinal epithelial cells during inflammation. *Clin Exp Immunol* 2010; **159**: 185-198 [PMID: 19912257 DOI: 10.1111/j.1365-2249.2009.04048.x]
29 **Wang J**, Ouyang Y, Guner Y, Ford HR, Grishin AV. Ubiquitin-editing enzyme A20 promotes tolerance to lipopolysaccharide in enterocytes. *J Immunol* 2009; **183**: 1384-1392 [PMID: 19570823 DOI: 10.4049/jimmunol.0803987]
30 **Hayden MS**, Ghosh S. Shared principles in NF-kappaB signaling. *Cell* 2008; **132**: 344-362 [PMID: 18267068 DOI: 10.1016/j.cell.2008.01.020]
31 **Neurath MF**. Cytokines in inflammatory bowel disease. *Nat Rev Immunol* 2014; **14**: 329-342 [PMID: 24751956 DOI: 10.1038/nri3661]
32 **Gon Y**, Asai Y, Hashimoto S, Mizumura K, Jibiki I, Machino T, Ra C, Horie T. A20 inhibits toll-like receptor 2- and 4-mediated interleukin-8 synthesis in airway epithelial cells. *Am J Respir Cell Mol Biol* 2004; **31**: 330-336 [PMID: 15142865 DOI: 10.1165/rcmb.2003-0438OC]

P- Reviewer: Futoma-Koloch B, Kamada N, Vivas S
S- Editor: Gong ZM **L- Editor**: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i4.550

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 550-555
ISSN 2150-5349 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Usefulness of vonoprazan, a potassium ion-competitive acid blocker, for primary eradication of *Helicobacter pylori*

Shinya Yamada, Takumi Kawakami, Yoshikazu Nakatsugawa, Takahiro Suzuki, Hideki Fujii, Naoya Tomatsuri, Hideki Nakamura, Hideki Sato, Yusuke Okuyama, Hiroyuki Kimura, Norimasa Yoshida

Shinya Yamada, Takumi Kawakami, Yoshikazu Nakatsugawa, Takahiro Suzuki, Hideki Fujii, Naoya Tomatsuri, Hideki Nakamura, Hideki Sato, Yusuke Okuyama, Hiroyuki Kimura, Norimasa Yoshida, Department of Gastroenterology, Japanese Red Cross Kyoto Daiichi Hospital, Kyoto 605-0981, Japan

Author contributions: Yamada S and Yoshida N contributed to study conception and design; they also contributed to writing, editing and final approval of article; Kawakami T, Nakatsugawa Y, Suzuki T, Fujii H, Tomatsuri N, Nakamura H, Sato H, Okuyama Y and Kimura H performed the research.

Institutional review board statement: The study protocol was approved by the Ethics Committee of Japanese Red Cross Kyoto Daiichi Hospital.

Informed consent statement: All study participants, or their legal guardian, provided informed verbal consent prior to study enrollment.

Conflict-of-interest statement: There is no conflict of interest.

Data sharing statement: No additional data are available.

Manuscript source: Unsolicited manuscript

Correspondence to: Dr. Shinya Yamada, Department of Gastroenterology, Japanese Red Cross Kyoto Daiichi Hospital, Hon-machi 15-749, Kyoto 605-0981, Japan. syo335@koto.kpu-m.ac.jp
Telephone: +81-75-5611121
Fax: +81-75-5616308

Received: June 15, 2016
Peer-review started: June 17, 2016
First decision: July 20, 2016
Revised: July 28, 2016
Accepted: August 27, 2016
Article in press: August 29, 2016
Published online: November 6, 2016

Abstract

AIM

To investigate usefulness of triple therapy with vonoprazan, a potassium ion-competitive acid blocker and antibiotics, for *Helicobacter pylori* (*H. pylori*) eradication.

METHODS

The *H. pylori* eradication rate was examined in 2507 patients (2055 undergoing primary eradication and 452 undergoing secondary eradication, excluding patients with subtotal gastrectomy) at the Japanese Red Cross Kyoto Daiichi Hospital from March 2013 to September 2015. For patients treated from March 2013 to February 2015, a proton pump inhibitor (PPI) was used to reduce acid secretion, while vonoprazan was used after March 2015. The success rates of the 2 regimens (PPI + amoxicillin + clarithromycin/metronidazole, or vonoprazan + amoxicillin + clarithromycin/metronidazole) were compared.

RESULTS

The success rate of primary *H. pylori* eradication was significantly higher in the vonoprazan group. When stratified by the underlying disease, a significant increase of the *H. pylori* eradication rate was observed in patients with chronic gastritis. A significantly lower *H. pylori* eradication rate was observed in younger patients compared to older patients in the PPI group, but there was no difference according to age in the vonoprazan group. On the other

hand, the success rate of secondary eradication was similar at approximately 90% in both groups.

CONCLUSION

Vonoprazan is very useful for primary eradication of *H. pylori*, and may become a first-line acid secretion inhibitor instead of PPIs.

Key words: *Helicobacter pylori*; Eradication; Vonoprazan; Chronic gastritis; Potassium ion-competitive acid blocker

Core tip: Use of vonoprazan, a potassium ion-competitive acid blocker, is expected to achieve a higher eradication rate than conventional triple therapy. The success rates of the 2 regimens (use of proton pump inhibitor *vs* vonoprazan) were compared. The success rate of primary *Helicobacter pylori* (*H. pylori*) eradication was significantly higher in the vonoprazan group. When stratified by the underlying disease, a significant increase of the *H. pylori* eradication rate was observed in patients with chronic gastritis. Vonoprazan is very useful for primary eradication of *H. pylori*, and may become a first-line acid secretion inhibitor instead of proton pump inhibitors.

Yamada S, Kawakami T, Nakatsugawa Y, Suzuki T, Fujii H, Tomatsuri N, Nakamura H, Sato H, Okuyama Y, Kimura H, Yoshida N. Usefulness of vonoprazan, a potassium ion-competitive acid blocker, for primary eradication of *Helicobacter pylori*. *World J Gastrointest Pharmacol Ther* 2016; 7(4): 550-555 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i4/550.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i4.550

INTRODUCTION

Since *Helicobacter pylori* (*H. pylori*) eradication therapy for chronic gastritis was approved for insurance cover in February 2013 in Japan, the number of patients undergoing eradication of *H. pylori* has greatly increased. In September 2014, the International Agency for Research of Cancer (IARC) reported that 80% of stomach cancer is caused by *H. pylori* infection, and the incidence of stomach cancer can be reduced by 30%-40% through *H. pylori* eradication[1]. However, the success rate of bacterial eradication by conventional primary triple therapy, involving the administration of a proton pump inhibitor (PPI) + amoxicillin (AMPC) + clarithromycin (CAM) for 1 wk, has steadily declined due to an increase of CAM resistance[2]. On the other hand, it is reported that secondary eradication therapy using metronidazole (MNZ) has a success rate exceeding 90%[3-7]. Reports about carcinogenicity of MNZ have appeared (although the risk is low)[8], and there is the possibility of resistance increasing due to its increased use in the near future. Consequently, development of new primary eradication therapy is desired.

Vonoprazan, a potassium ion-competitive acid blocker (P-CAB), was launched in Japan in February 2015 before its release on the world market[9]. Use of vonoprazan is expected to achieve a higher eradication rate than conventional triple therapy due to its strong inhibitory effect on gastric acid secretion[10,11]. Against this background, the current study aimed at evaluating the usefulness of triple therapy containing P-CAB compared with 7-d PPI-based triple therapy.

MATERIALS AND METHODS

This study is a retrospective analysis of prospectively collected data comparing outcomes of patients received *H. pylori* eradiation therapy by vonoprazan from March to September 2015 against a historical cohort of patient by a proton pump inhibitor (PPI) carried out from March 2013 to February 2015.

The subjects were 2507 patients (2055 undergoing primary eradication and 452 undergoing secondary eradication, excluding patients with subtotal gastrectomy) who tested positive for *H. pylori* at the Gastroenterology Department of the Japanese Red Cross Kyoto Daiichi Hospital from March 2013 to September 2015. In patients treated from March 2013 to February 2015, a PPI was used to inhibit gastric acid secretion, while vonoprazan was used for patients treated after March 2015. Patients were received 7-d course of triple therapy with amoxicillin 1500 mg and clarithromycin 400 mg plus lansoprazole 60 mg, esomeprazole 40 mg, rabeprazole 20 mg, or vonoprazan 40 mg as first-line treatment, and 7-d course of triple therapy with amoxicillin 1500 mg and metronidazole 500 mg plus lansoprazole 60 mg, esomeprazole 40 mg, rabeprazole 20 mg, or vonoprazan 40 mg as second-line treatment. Success rate was compared between the 2 *H. pylori* eradication methods.

Before starting the eradication therapy, patients underwent a medical interview concerning their drug allergy. Adverse effect was evaluated after eradication therapy by a medical interview.

The presence of *H. pylori* infection was confirmed by a positive result in any of the following tests: Urea breath test (n = 52), rapid urease test (n = 668), serum *H. pylori* IgG antibody (n = 1074), fecal *H. pylori* antigen (n = 7), and microscopy (n = 254). Eradication effect was confirmed by performing the urea breath test at two months after treatment, using a cut-off of 2.5‰. We did not investigate the strains and levels of resistance of *H. pylori* to the antimicrobial drugs planned to administer.

Patients who received eradication therapy were divided into a group treated with a PPI (lansoprazole, omeprazole, rabeprazole, or esomeprazole) and a group treated with vonoprazan, and success rates were compared, including the success rates stratified according to gender, age, and underlying disease.

The study protocol was approved by the Ethics Committee of Japanese Red Cross Kyoto Daiichi Hospital and was conducted in compliance with the Helsinki Declaration.

Table 1 Patient profile of primary eradication therapy

	PPI group	Vonoprazan group
Average age	62.8 (18-94)	62.7 (22-89)
Gender (male:female)	902:818	170:165
PPI		
Lansoprazole	1206	
Omeprazole	0	
Rabeprazole	62	
Esomeprazole	452	
Underlying disease		
Chronic gastritis	1362	255
Peptic ulcer	248	55
After endoscopic therapy for early gastric cancer	107	25
Other	3	0

PPI: Proton pump inhibitor.

Table 2 Patient profile of secondary eradication therapy

	PPI group	Vonoprazan group
Average age	62.3 (19-96)	63.6 (39-89)
Gender(male:female)	182:204	28:38
PPI		
Lansoprazole	266	
Omeprazole	1	
Rabeprazole	32	
Esomeprazole	87	
Underlying disease		
Chronic gastritis	313	48
Peptic ulcer	44	8
After endoscopic therapy for early gastric cancer	28	10
Other	1	0

PPI: Proton pump inhibitor.

For statistical analysis, the χ^2 test and Fischer's exact probability test were used, and significance was accepted at $P < 0.05$. All analyses were performed using the program GraphPad Prism 4 (GraphPad Soft ware, San Diego, CA).

RESULTS

Patient profile

Among the 2055 patients receiving primary eradication therapy, a PPI was used in 1720 and vonoprazan was used in 335. Lansoprazole was the PPI most commonly used to inhibit acid secretion (1206 patients), followed by esomeprazole and rabeprazole. Omeprazole was not used. In both the PPI group and the vonoprazan group, chronic gastritis was the underlying disease in more than 75% of the patients, followed by peptic ulcer and endoscopic therapy (Table 1).

Among the 452 patients receiving secondary eradication therapy, a PPI was used in 386 and vonoprazan was used in 66. The trends for PPI use and underlying cause were similar to those in the primary eradication group (Table 2).

Table 3 Results of eradication therapy

	PPI group	Vonoprazan group	*P* value
Primary eradication therapy			
ITT analysis	73.2% (1259/1720)	85.7% (287/335)	< 0.0001
PP analysis	76.4% (1259/1647)	90.3% (287/318)	< 0.0001
Secondary eradication therapy			
ITT analysis	89.9% (347/386)	89.4% (59/66)	0.87
PP analysis	92.8% (347/374)	96.7% (59/61)	0.4

PPI: Proton pump inhibitor.

Success rate of H. pylori eradication therapy

Regarding the success rate of primary eradication therapy in the PPI group, it was 73.2% by ITT analysis and 76.4% by PP analysis, while it was 85.7% by ITT analysis and 90.3% by PP analysis in the vonoprazan group. The *H. pylori* eradication success rate was significantly higher in the vonoprazan group by both ITT analysis and PP analysis.

Regarding the success rate of secondary eradication therapy in the PPI group, it was 89.9% by ITT analysis and 92.8% by PP analysis, while it was 89.4% by ITT analysis and 96.7% by PP analysis in the vonoprazan group, with no significant difference between the 2 groups (Table 3).

Success rate and underlying disease

In patients with chronic gastritis undergoing primary eradication therapy, a significantly higher success rate was observed in the vonoprazan group than the PPI group by both ITT analysis (86.7%) and PP analysis (90.6%). In patients with peptic ulcer undergoing primary eradication, the success rate was also higher in the vonoprazan group, but no significant difference was observed compared to the PPI group. In patients undergoing primary eradication after endoscopic therapy for early gastric cancer, there was little difference of the success rate between the PPI group and the vonoprazan group (Table 4). In patients undergoing secondary eradication, the PPI group and the vonoprazan group showed no differences of the success rate in relation to underlying diseases (Table 5).

Success rate and gender or age

No difference of the success rate according to gender was observed in either the PPI group or the vonoprazan group. Patients younger than 50 years were defined as younger and those older than 50 years were defined as older, and the success rates in both age groups were examined for PPI and vonoprazan therapy. In the PPI group, the success rate was significantly lower in younger patients, but there was no difference of the success rate based on age in the vonoprazan group (Table 6).

Table 4 Success rate and underlying disease: Primary eradication therapy

		PPI group	Vonoprazan group	*P* value
Chronic gastritis	ITT analysis	72.5% (988/1362)	86.7% (221/255)	< 0.0001
	PP analysis	75.1% (988/1316)	90.6% (221/244)	< 0.0001
Peptic ulcer	ITT analysis	75.4% (187/248)	83.6% (46/55)	0.22
	PP analysis	82.7% (187/226)	93.9% (46/49)	0.051
After endoscopic therapy for early gastric cancer	ITT analysis	76.6% (82/107)	80% (20/25)	0.8
	PP analysis	78.1% (82/105)	80% (20/25)	1

PPI: Proton pump inhibitor.

Table 5 Success rate and underlying disease: Secondary eradication therapy

		PPI group	Vonoprazan group	*P* value
Chronic gastritis	ITT analysis	91.5% (292/319)	89.6% (43/48)	0.59
	PP analysis	93.9% (292/311)	100% (43/43)	0.15
Peptic ulcer	ITT analysis	86.4% (38/44)	100% (8/8)	0.57
	PP analysis	95.0% (38/40)	100% (8/8)	1
After endoscopic therapy for early gastric cancer	ITT analysis	78.6% (22/28)	80% (8/10)	1
	PP analysis	78.6% (22/28)	80% (8/10)	1

PPI: Proton pump inhibitor.

Table 6 Success rate and age: Primary eradication therapy

		Younger than 50 yr	Older than 50 yr	*P* value
PPI group	ITT analysis	67.8% (185/273)	74.3% (1074/1446)	0.03
	PP analysis	72.3% (185/256)	77.2% (1074/1391)	0.09
Vonoprazan group	ITT analysis	84.8% (50/59)	86.2% (238/276)	0.84
	PP analysis	92.6% (50/54)	90.2% (238/264)	0.8

PPI: Proton pump inhibitor.

Adverse events

In the PPI group, 7 patients (0.4%) discontinued treatment due to adverse events during primary eradication therapy, including 2 cases of diarrhea, 4 cases of rash, and 1 other event. Two patients (0.5%) from the PPI group discontinued secondary eradication therapy, including 1 case of diarrhea and 1 other event. No cases of discontinuation of treatment due to adverse events were observed in the vonoprazan group. The incidence of major adverse effect such as diarrhea, dysgeusia and skin rash showed no difference between the two groups. No specific adverse effect was observed in the vonoprazan group.

DISCUSSION

In this investigation, the success rate of primary *H. pylori* eradication therapy was significantly higher in the vonoprazan group, and vonoprazan treatment achieved a significantly higher success rate in patients with chronic gastritis. In the PPI group, the success rate of *H. pylori* eradication therapy was significantly lower for younger patients than for older patients, but no difference related to age was observed in the vonoprazan group. On the other hand, the success rate of secondary *H. pylori* eradication therapy was similar (Approximately 90%) in the PPI group and the vonoprazan group.

Vonoprazan is a new potassium ion-competitive acid blocker, which is stable in an acid environment, and shows rapid and potent inhibition of gastric acid secretion[9]. Vonoprazan is instantly protonated in an acidic and even in a neutral environment, and is suggested to bind to and inhibit H^+,K^+-ATPase in the

protonated form[9]. Insufficient inhibition of gastric acid secretion has previously been reported to cause failure of *H. pylori* eradication[12]. Vonoprazan has been reported to rapidly increase the gastric pH for an extended period from the initial day of administration[10,11]. In this study, the success rate for young patients was much higher than that for older patients in the vonoprazan group. From these results, it can be inferred that the success rate of primary *H. pylori* eradication therapy was increased in the vonoprazan group due to vonoprazan improving the antibacterial activity of the antibiotics used in combination. In addition, the enzyme involved in metabolism of vonoprazan is another possible reason. The gene polymorphism of the liver enzyme cytochrome P450 (CYP) 2C19 affects the metabolic rate and the acid inhibitory effect of PPIs. However, vonoprazan is mainly metabolized in the liver by CYP3A4[12]. Therefore, vonoprazan exerts a potent inhibitory activity regardless of CYP2C19 polymorphism. CAM, which is used in primary *H. pylori* eradication therapy, is also metabolized by CYP3A4[13], and the $AUC_{0\text{-}12}$ and Cmax of vonoprazan are reported to be respectively increased 1.5 times and 1.6 times during combined administration of AMPC, CAM, and vonoprazan compared with single-agent administration[14]. In the present study, the success rate showed a significant increase with primary *H. pylori* eradication therapy, suggesting that the interaction of vonoprazan and CAM promoted the action of both agents. However, a smaller population was examined for secondary eradication therapy, so collection of more cases is needed in the future.

Moreover, regarding the *H. pylori* eradication rate in relation to the underlying disease in the vonoprazan group, a significantly higher success rate was seen in patients with chronic gastritis. While a similar trend to that for chronic gastritis was also observed for peptic ulcer, an almost equal *H. pylori* eradication rate was observed in the vonoprazan and PPI groups among patients treated after endoscopic therapy. Further investigation is also needed to determine if the difference in the eradication rate by underlying disease was due to the small number of subjects, host factors, or bacterial factors. In this study, we demonstrated that vonoprazan was very useful for primary eradication of *H. pylori.* However, the success rate of secondary *H. pylori* eradication therapy had no difference in the PPI group and the vonoprazan group. MNZ based conventional triple therapy has sufficiently high eradication success rate in Japan[3-7]. It has been reported that sufficient acid inhibition during eradication was more important in CAM based regimen than MNZ[15]. Therefore, we could not show the difference in secondary eradication therapy between the PPI group and the vonoprazan group.

Our study has some limitations. Although we used historical controls in the same institute, this was a retrospective study at a single center. Second, adverse effect was not precisely evaluated because we could not track patients who were not confirmed the effect of eradication therapy. Therefore, important events during the eradication therapy might have been lost. Although Murakami *et al*[16] reported that no marked differences were observed in adverse effects between the vonoprazan and the PPI group, Suzuki *et al*[17] indicated that the incidence of skin rash was significantly higher with vonoprazan therapy than with PPI therapy. Further investigation will be needed to clarify the adverse effect of vonoparazan in *H. pylori* eradication therapy. Third, factors which may affect success rate of *H. pylori* eradication therapy, such as alcohol, smoking, or the use of other medications were not recorded in the patients.

Despite these limitations, the results obtained were comparable to the *H. pylori* eradication rate at the time when vonoprazan was approved for patients with healed gastroduodenal ulcers[16], and the incidence of adverse events was similar to that with conventional eradication therapy using PPIs. In the future, *H. pylori* eradication therapy using the three-agent combination of AMPC, CAM, and vonoprazan may possibly become a first-line option.

In conclusion, vonoprazan is considered to be useful for triple therapy aimed at primary *H. pylori* eradication, and the possibility of using vonoprazan as first-line treatment to inhibit acid secretion instead of PPIs was suggested. For secondary eradication therapy, further investigation is needed to determine if the success rate is higher than that achieved with PPIs.

COMMENTS

Background

The International Agency for Research of Cancer reported that 80% of stomach cancer is caused by *Helicobacter pylori* (*H. pylori*) infection, and the incidence of stomach cancer can be reduced by 30%-40% through *H. pylori* eradication. Consequently, development of new primary eradication therapy is desired.

Research frontiers

The authors' group pioneered a novel primary eradication therapy for *H. pylori*, using vonoprazan. The number of patients undergoing eradication of *H. pylori* has greatly increased. However, the success rate of bacterial eradication by conventional primary triple therapy has steadily declined due to an increase of clarithromycin (CAM) resistance.

Innovations and breakthroughs

The success rate of bacterial eradication by conventional primary triple therapy, involving the administration of a proton pump inhibitor (PPI) + amoxicillin (AMPC) + CAM for 1 wk, has steadily declined due to an increase of CAM resistance. However, use of vonoprazan achieved a higher eradication rate than conventional triple therapy due to its strong inhibitory effect on gastric acid secretion.

Applications

Vonoprazan is considered to be useful for triple therapy aimed at primary *H. pylori* eradication, and the possibility of using vonoprazan as first-line treatment to inhibit acid secretion instead of PPIs was suggested.

Terminology

Vonoprazan is a novel oral potassium-competitive acid blocker, which is stable in an acid environment, and shows rapid and potent inhibition of gastric acid secretion.

Peer-review

The authors concluded that vonoprazan therapy for *H. pylori* eradication may be advantageous over those utilizing PPIs. The paper is well written, and the possible advantageous mechanism of vonoprazan action is adequately explored in the Discussion. Therefore, the paper should be of interest to the readership of the Journal.

REFERENCES

1 **Herrero R**, Park JY, Forman D. The fight against gastric cancer - the IARC Working Group report. *Best Pract Res Clin Gastroenterol* 2014; **28**: 1107-1114 [PMID: 25439075 DOI: 10.1016/j.bpg.2014.10.003]

2 **Kobayashi I**, Murakami K, Kato M, Kato S, Azuma T, Takahashi S, Uemura N, Katsuyama T, Fukuda Y, Haruma K, Nasu M, Fujioka T. Changing antimicrobial susceptibility epidemiology of Helicobacter pylori strains in Japan between 2002 and 2005. *J Clin Microbiol* 2007; **45**: 4006-4010 [PMID: 17942652 DOI: 10.1128/JCM.00740-07]

3 **Nagahara A**, Miwa H, Ohkura R, Yamada T, Sato K, Hojo M, Sato N. Strategy for retreatment of therapeutic failure of eradication of Helicobacter pylori infection. *J Gastroenterol Hepatol* 2001; **16**: 613-618 [PMID: 11422612 DOI: 10.1046/j.1440-1746.2001.02491]

4 **Isomoto H**, Inoue K, Furusu H, Enjoji A, Fujimoto C, Yamakawa M, Hirakata Y, Omagari K, Mizuta Y, Murase K, Shimada S, Murata I, Kohno S. High-dose rabeprazole-amoxicillin versus rabeprazole-amoxicillin-metronidazole as second-line treatment after failure of the Japanese standard regimen for Helicobacter pylori infection. *Aliment Pharmacol Ther* 2003; **18**: 101-107 [PMID: 12848631 DOI: 10.1046/j.0269-2813.2003.01659.x]

5 **Murakami K**, Sato R, Okimoto T, Nasu M, Fujioka T, Kodama M, Kagawa J. Efficacy of triple therapy comprising rabeprazole, amoxicillin and metronidazole for second-line Helicobacter pylori eradication in Japan, and the influence of metronidazole resistance. *Aliment Pharmacol Ther* 2003; **17**: 119-123 [PMID: 12492740 DOI: 10.1046/j.0269-2813.2003.01401.x]

6 **Miwa H**, Nagahara A, Kurosawa A, Ohkusa T, Ohkura R, Hojo M, Enomoto N, Sato N. Is antimicrobial susceptibility testing necessary before second-line treatment for Helicobacter pylori infection? *Aliment Pharmacol Ther* 2003; **17**: 1545-1551 [PMID: 12823158 DOI: 10.1046/j.1365-2036.2003.01541.x]

7 **Shimoyama T**, Fukuda S, Mikami T, Fukushi M, Munakata A. Efficacy of metronidazole for the treatment of clarithromycin-resistant Helicobacter pylori infection in a Japanese population. *J Gastroenterol* 2004; **39**: 927-930 [PMID: 15549444]

8 **Beard CM**, Noller KL, O'Fallon WM, Kurland LT, Dahlin DC. Cancer after exposure to metronidazole. *Mayo Clin Proc* 1988; **63**: 147-153 [PMID: 3339906 DOI: 10.1016/S0025-6196(12)64947-7]

9 **Hori Y**, Imanishi A, Matsukawa J, Tsukimi Y, Nishida H, Arikawa Y, Hirase K, Kajino M, Inatomi N. 1-[5-(2-Fluorophenyl)-1-(pyridin-3-ylsulfonyl)-1H-pyrrol-3-yl]-N-methylmethanamine monofumarate (TAK-438), a novel and potent potassium-competitive acid blocker for the treatment of acid-related diseases. *J Pharmacol Exp Ther* 2010; **335**: 231-238 [PMID: 20624992 DOI: 10.1124/jpet.110.170274]

10 **Jenkins H**, Sakurai Y, Nishimura A, Okamoto H, Hibberd M, Jenkins R, Yoneyama T, Ashida K, Ogama Y, Warrington S. Randomised clinical trial: safety, tolerability, pharmacokinetics and pharmacodynamics of repeated doses of TAK-438 (vonoprazan), a novel potassium-competitive acid blocker, in healthy male subjects. *Aliment Pharmacol Ther* 2015; **41**: 636-648 [PMID: 25707624 DOI: 10.1111/apt.13121]

11 **Sakurai Y**, Mori Y, Okamoto H, Nishimura A, Komura E, Araki T, Shiramoto M. Acid-inhibitory effects of vonoprazan 20 mg compared with esomeprazole 20 mg or rabeprazole 10 mg in healthy adult male subjects--a randomised open-label cross-over study. *Aliment Pharmacol Ther* 2015; **42**: 719-730 [PMID: 26193978 DOI: 10.1111/apt.13325]

12 **Cederbrant G**, Kahlmeter G, Schalén C, Kamme C. Additive effect of clarithromycin combined with 14-hydroxy clarithromycin, erythromycin, amoxycillin, metronidazole or omeprazole against Helicobacter pylori. *J Antimicrob Chemother* 1994; **34**: 1025-1029 [PMID: 7730216 DOI: 10.1093/jac/34.6.1025]

13 The interview form for vonoprazan fumarate (Takecab® tablets 10 mg and 20 mg) ver. 6, (in Japanese). Takeda Pharmaceutical Company Limited. 2015. Available from: URL: http://www.takeda.co.jp/

14 **Rodrigues AD**, Roberts EM, Mulford DJ, Yao Y, Ouellet D. Oxidative metabolism of clarithromycin in the presence of human liver microsomes. Major role for the cytochrome P4503A (CYP3A) subfamily. *Drug Metab Dispos* 1997; **25**: 623-630 [PMID: 9152603]

15 **Sugimoto M**, Uotani T, Sahara S, Ichikawa H, Yamade M, Sugimoto K, Furuta T. Efficacy of tailored Helicobacter pylori eradication treatment based on clarithromycin susceptibility and maintenance of acid secretion. *Helicobacter* 2014; **19**: 312-318 [PMID: 24690010 DOI: 10.1111/hel.12128]

16 **Murakami K**, Sakurai Y, Shiino M, Funao N, Nishimura A, Asaka M. Vonoprazan, a novel potassium-competitive acid blocker, as a component of first-line and second-line triple therapy for Helicobacter pylori eradication: a phase III, randomised, double-blind study. *Gut* 2016; 65: 1439-1446 [PMID: 26935876 DOI: 10.1136/gutjnl-2015-311304]

17 **Suzuki S**, Gotoda T, Kusano C, Iwatsuka K, Moriyama M. The Efficacy and Tolerability of a Triple Therapy Containing a Potassium-Competitive Acid Blocker Compared With a 7-Day PPI-Based Low-Dose Clarithromycin Triple Therapy. *Am J Gastroenterol* 2016; **111**: 949-956 [PMID: 27185079 DOI: 10.1038/ajg.2016.182]

P- Reviewer: Abadi ATB, Engin AB, Kadayifci A, Murakami K, Slomiany BL, Siavoshi F **S- Editor**: Qi Y **L- Editor**: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i4.556

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 556-563
ISSN 2150-5349 (online)

ORIGINAL ARTICLE

Observational Study

Family history and disease outcomes in patients with Crohn's disease: A comparison between China and the United States

Pei-Qi Wang, Jun Hu, Elie S Al Kazzi, Eboselume Akhuemonkhan, Min Zhi, Xiang Gao, Raquel Holand de Paula Pessoa, Sami Ghazaleh, Tuhina Cornelius, Suhel Abbas Sabunwala, Shadi Ghadermarzi, Kartikeya Tripathi, Mark Lazarev, Pin-Jin Hu, Susan Hutfless

Pei-Qi Wang, Eboselume Akhuemonkhan, Shadi Ghadermarzi, Mark Lazarev, Susan Hutfless, Department of Medicine, Division of Gastroenterology and Hepatology, Johns Hopkins University, Baltimore, MD 21287, United States

Jun Hu, Guangdong provincial Key Laboratory of Colorectal and Pelvic Floor Diseases, the Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou 510655, Guangdong Province, China

Jun Hu, Min Zhi, Xiang Gao, Pin-Jin Hu, Department of Gastroenterology, the Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou 510655, Guangdong Province, China

Elie S Al Kazzi, Department of Medicine, MedStar Health Internal Medicine, Baltimore, MD 21287, United States

Raquel Holand de Paula Pessoa, Department of Health and Sport Sciences, Federal University of Acre, Rio Branco, Acre 69915-900, Brazil

Sami Ghazaleh, Department of Medicine, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus 00972, Palestine

Tuhina Cornelius, Suhel Abbas Sabunwala, Ravindra Nath Tagore Medical College, Udaipur, Rajasthan 313001, India

Kartikeya Tripathi, St Vincent Hospital, Worcester, MA 01608, United States

Susan Hutfless, Department of Epidemiology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD 21205, United States

Author contributions: Wang PQ participated in study design, analyzed and interpreted the data, and drafted the initial manuscript; Hutfless S as the principle investigator, supervised and advised the study; Hu J, Al Kazzi ES, Akhuemonkhan E, Zhi M, Gao X, de Paula Pessoa RH, Ghazaleh S, Cornelius T, Sabunwala SA, Ghadermarzi S and Tripathi K participated in recruitment of study participants; all authors participated in reviewing and revising the manuscript.

Supported by (in part) Johns Hopkins Institute for Clinical and Translational Research, No. UL1TR001079.

Institutional review board statement: The study was reviewed and approved by the Institutional Review Boards at Johns Hopkins Medicine and the Institutional Review Boards at the Sixth Affiliated Hospital of Sun Yat-sen University.

Informed consent statement: All study participants provided consent.

Conflict-of-interest statement: There are no conflicts of interest to report.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

Correspondence to: Susan Hutfless, PhD, Assistant Professor, Director, Department of Medicine, Division of Gastroenterology and Hepatology, Johns Hopkins University, 600 N Wolfe Street, Blalock 449, Baltimore, MD 21287, United States. shutfle1@jhmi.edu
Telephone: +1-410-5020194

Received: June 6, 2016
Peer-review started: June 12, 2016
First decision: July 20, 2016
Revised: August 5, 2016
Accepted: September 13, 2016

Article in press: September 15, 2016
Published online: November 6, 2016

Abstract

AIM

To investigate the differences in family history of inflammatory bowel disease (IBD) and clinical outcomes among individuals with Crohn's disease (CD) residing in China and the United States.

METHODS

We performed a survey-based cross-sectional study of participants with CD recruited from China and the United States. We compared the prevalence of IBD family history and history of ileal involvement, CD-related surgeries and IBD medications in China and the United States, adjusting for potential confounders.

RESULTS

We recruited 49 participants from China and 145 from the United States. The prevalence of family history of IBD was significantly lower in China compared with the United States (China: 4.1%, United States: 39.3%). The three most commonly affected types of relatives were cousin, sibling, and parent in the United States compared with child and sibling in China. Ileal involvement (China: 63.3%, United States: 63.5%) and surgery for CD (China: 51.0%, United States: 49.7%) were nearly equivalent in the two countries.

CONCLUSION

The lower prevalence of familial clustering of IBD in China may suggest that the etiology of CD is less attributed to genetic background or a family-shared environment compared with the United States. Despite the potential difference in etiology, surgery and ileal involvement were similar in the two countries. Examining the changes in family history during the continuing rise in IBD may provide further insight into the etiology of CD.

Key words: Crohn's disease; Family history; Disease outcome; Inflammatory bowel disease; Epidemiology; genetics; Environment; Medication; Surgery

Core tip: Crohn's disease (CD) diagnoses are increasing in Asia. While family history of inflammatory bowel disease (IBD) is recognized as the strongest independent risk factor for CD in western populations, it is unknown if family history plays a role in Asians. This study compares the prevalence of IBD family history, the relationships of affected relatives, and CD-related outcomes such as ileal involvement, surgery, and medication use between China and the United States.

Wang PQ, Hu J, Al Kazzi ES, Akhuemonkhan E, Zhi M, Gao X, de Paula Pessoa RH, Ghazaleh S, Cornelius T, Sabunwala SA, Ghadermarzi S, Tripathi K, Lazarev M, Hu PJ, Hutfless S. Family history and disease outcomes in patients with Crohn's disease: A comparison between China and the United States. *World J Gastrointest Pharmacol Ther* 2016; 7(4): 556-563 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i4/556.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i4.556

INTRODUCTION

Crohn's disease (CD) is a form of inflammatory bowel disease (IBD), featured by chronic inflammation, most frequently affecting the terminal ileum and colon, but can also involve other portions of the gastrointestinal (GI) tract from mouth to anus[1]. The first mention of CD appeared in the literature in 1932 in a publication by Burrill Crohn reporting on a subacute or chronic necrotizing and cicatrizing inflammatory disease of the terminal ileum affecting mainly young adults in New York city[2]. Incidence rates of CD were first published in 1965, reporting an estimated 1.3 cases per 100000 person-years during 1955 to 1963 in Scotland[3]. Since then, the incidence rate of CD in western countries has shown a significant increasing trend, and the annual incidence rates between 1991 to 2008 were estimated to range from 10.6 to 29.3 per 100000 in North America, Europe, and Australia[4]. In contrast, the CD incidence rate in Asia was first published in 1974, reporting 0.04 cases per 100000 person-years in Singapore during 1965-1970[5]. Similar to western countries, the incidence of CD has increased over time. The estimated annual CD incidence rate (per 100000 person-years) based on population-based longitudinal cohorts in Hong Kong[6], Japan[7], and South Korea[8] has increased from 0.1-0.6 in 1986-1992 to 1.0-1.3 in 1998-2005.

In China, CD was first documented in the literature in 1960s and the incidence is assumed to be increasing based on single hospital-based studies. The current estimated incidence is 0.1 to 1.3 cases per 100000 person-years[9]. The proposed increase in incidence has been attributed to environmental exposures, such as industrialization and urbanization of the society, westernization of lifestyle and dietary habits, because of the parallelism of their occurrence[4,10,11].

The lower incidence of CD may be due in part to differences in genetic risk factors. Polymorphisms of the *NOD2/CARD15* gene have been found to account for up to 20% of CD in Caucasian[12] populations[13], but there have not been consistent findings of such association in China[14-17].

Family history is a window to both the genetic background and shared environment. Having a relative with IBD is the strongest known risk factor for developing CD in western countries[18]. However, family history of IBD among Chinese CD patients has not been studied in detail. Thirteen hospital-based studies reported the prevalence of family history of IBD (any affected family

member) among Chinese CD patients to be 0% to 4.5%, although the details on the relations of family members are not known[6,19-29]. In contrast, 8% to 25% of CD patients in western countries have a blood relative with IBD[30,31], and 8% to 14.5% of CD patients have a first-degree relative with IBD[18,31].

Clinical characteristics and complication rates differ among Asians with CD. A systematic review of 151 studies suggested Asian CD patients are more likely to be male, with a higher prevalence of ileocolonic involvement and lower surgical rates than Western estimates[29,32].

We hypothesized that there would be differences in family history of IBD and clinical outcomes between CD patients in China and the United States. We targeted three aims to address our hypothesis. The first was to investigate and compare the prevalence of family history of IBD among CD patients in China and the United States. The second was to examine the distribution of relationships of the affected relatives. Third, we aimed to study the difference in prevalence of CD outcomes including ileal involvement, CD-related surgeries and use of medications.

MATERIALS AND METHODS

Study population

Patients were recruited from one source in China and two sources in the United States. In China, patients with CD seen by gastroenterologists at the IBD clinic of the 6th Affiliated Hospital of Sun Yat-sen University (SYSU), Guangdong, China, during a clinic visit from May 2014 to December 2015. This hospital is a referral center for IBD patients in the Guangdong area. In the United States, patients were recruited at Johns Hopkins University-affiliated clinics or through the participant recruitment website ResearchMatch (RM). RM is a disease-neutral, institution-neutral, online volunteer recruitment platform designed to match volunteers with researchers[33]. The Johns Hopkins participants were recruited by email, letter, flyer, or during a clinic visit between May 2015 and February 2016. Individuals who indicated that they had IBD on their ResearchMatch profiles were recruited *via* email from May 2015 to February 2016.

In China, participants were administered a survey by face-to-face interview with a healthcare professional. In the United States participants completed the survey online (bit.ly/IBD-MIMAS). The survey was created and administered using the REDCap[34] project hosted at Johns Hopkins University. The survey included questions about demographics, timing of CD diagnosis, disease location and treatment history, family history of IBD and relationship, age at diagnosis of IBD type of the affected family members (Appendix tables). Participants were included in the analysis only if they completed the family history and disease outcome modules of the survey and were aged 18 years or older at the time of survey completion. Individuals with unknown family history were excluded (1 individual from the United States who was adopted).

IBD family history assessment

Family history of IBD was classified using self-reported information on the survey. Questions were asked regarding knowledge of any family history, details on the number of relatives affected, as well as the relationship to the participant (Appendix Table 2). Any family history of IBD was defined as having one or more blood relatives diagnosed with any type of IBD. For participants who had any family history of IBD, they were further subdivided as having first-degree relative family history if they had at least one parent, sibling or child with IBD.

Crohn's disease related outcomes assessment

The primary clinical outcome was ileal involvement. History of ileal involvement was determined by self-report (Appendix Table 3). Secondary outcomes included self-reported history of CD-related surgery, and ever use of IBD-related medications including steroids, immunomodulators, and biologics (Appendix Table 4).

Potential confounding factors

Participants self-reported sex, date of birth, date of CD diagnosis, number of siblings, number of children, and smoking status at CD diagnosis (Appendix Tables 1 and 2). Duration of disease was defined as the period of time from the date of clinical diagnosis to the date of survey completion. Smoking status at the time of diagnosis was categorized as current, former, and never smoker. Participants with more family members and longer disease duration are more likely to have a family member with IBD and the family size and duration of disease differed between countries making these factors potential confounders. Similarly, individuals with longer disease duration had greater potential to have ileal disease detected or medication changes.

Statistical analysis

We compared the demographic differences between China and the United States using frequencies for categorical variables and median and range for continuous variables. Frequency distributions were compared using Fisher's exact tests and medians were compared with Mood's median tests. We created a figure similar to a pedigree but for the entire population to examine the familial relationships between countries. We used multivariable logistic regression models to compare the prevalence of family history between China and the United States, adjusting for the total number of siblings and children and the duration of disease at the time of survey. We used separate multivariable logistic regression models to compare the difference in the outcomes between countries adjusting for sex, smoking status at CD diagnosis, age at diagnosis older than 40 years *vs* less, and disease duration. We conducted all the statistical analysis in SAS® 9.4 (SAS Institute Inc., Cary, NC, United States).

Table 1 Demographics of Crohn's disease patients in China and the United States recruited 2014-2016

	China (n = 49)	United States (n = 145)	P value
Age at clinical diagnosis (yr)	26.6 (13.1-46.7)	25.9 (5.1-73.1)	0.87
Age at survey completion (yr)	29.5 (19.2-49.9)	43.4 (18.3-82.7)	< 0.0001
Duration of disease at time of survey (yr)	2.2 (0.0-12.7)	12.4 (0.0-55.4)	< 0.0001
Calendar year of diagnosis	2013 (2003-2015)	2003 (1960-2015)	< 0.0001
Before 1969	0	1.4%	
1970-1979	0	6.2%	
1980-1989	0	9.7%	
1990-1999	0	25.5%	
2000-2009	12.2%	30.3%	
2010-2015	87.8%	26.9%	
Female (%)	42.9%	58.6%	0.06
Smoking status at diagnosis (%)			0.5
Current smoker	12.2%	16.5%[1]	
Former smoker	6.1% (n = 3)	10.80%	
Never smoker	81.6%	72.7%	
Number of siblings	2 (0-7)	2 (0-9)	0.2
0	8.2% (n = 4)	6.9%	
1	12.2%	29.7%	
2	44.9%	30.3%	
3	12.2%	18.6%	
4+	22.5%	14.5%	
Number of children	0 (0-3)	0 (0-7)	0.81
0	53.1%	51.0%	
1	26.5%	15.9%	
2	18.4%	22.8%	
3	2.0% (n = 1)	9.0%	
4+	0	1.3%	

[1]Six participants recruited in the United States did not report smoking status at diagnosis.

RESULTS

Patient demographics

We included 194 individuals with CD, 49 from China and 145 from the United States (Table 1). In the United States, 111 were recruited from the academic center and 34 from ResearchMatch. Similarities between the two countries included a median age of approximately 26 years at diagnosis and a similar median number of siblings and children. Differences between the two countries included those from China were more likely to be male; had a younger age at diagnosis and age at the time of survey completion; and were diagnosed more recently.

Family history of IBD

After adjusting for duration of disease at the time of survey and the total number of relatives, the difference in family history was statistically significant. The prevalence of any family history of IBD was markedly lower in China than the United States (China: 4.1% *vs* United States: 39.3%, adjusted P = 0.0008, Table 2). Family history of IBD in first-degree relatives was also less prevalent in China than in the United States (China: 4.1% *vs* United States: 23.5%, adjusted P = 0.01). Only two participants in China had a family history of IBD. Both had a first-degree relative affected (Figure 1).

For participants who reported any family history of IBD, the distribution of relationships of affected relatives was significantly different (Figure 1). For the two patients who had family history of IBD in China, one had a sibling affected and the other a child. In the United States, the mean number of affected relatives was 1.6 (median 1, maximum 5). The most commonly affected types of relatives were cousin (41.1%), sibling (37.5%), and parent (28.6%).

Disease outcomes

Participants from the two countries had equally high prevalence of ileal involvement (China: 63.3% *vs* United States: 63.5%, adjusted P = 0.74) and surgery (China: 51.0% *vs* United States: 49.7%, adjusted P = 0.19; Table 3). Compared with the United States, the percentage of participants that had ever used steroids or a biologic was significantly lower in China. The difference in the use of immunomodulators was of borderline statistical significance with China having greater use than the United States (China: 73.5%, United States: 61.4%, adjusted P = 0.08).

DISCUSSION

The prevalence of family history of IBD and CD related clinical outcomes were different in China and the United States. The probability that CD patients from China had any blood relative affected with IBD was only 1/10 that of the United States CD patients, despite both patient populations coming from academic medical centers with dedicated IBD clinics. The percentage of CD patients in China who had first-degree relative affected with

Table 2 Prevalence and odds ratio of having family history of inflammatory bowel disease in China *vs* the United States

	China (n = 49)	United States (n = 145)	Unadjusted OR (95%CI) P value (Reference = US)	Adjusted[1] OR (95% CI) P value (Reference = US)
Any family history of IBD (%)	4.1% (n = 2)	39.3%	0.07 (0.02-0.28) P = 0.0002	0.08 (0.02-0.34) P = 0.0008
First-degree family history of IBD (%)	4.1% (n = 2)	23.5%	0.14 (0.03-0.60) P = 0.008	0.14 (0.03-0.65) P = 0.01

[1]Adjusted for the total number of siblings and children, and duration of disease at survey completion. IBD: Inflammatory bowel disease; OR: Odds ratio.

Table 3 Prevalence and odds ratio of Crohn's disease outcomes in China *vs* the United States

Outcome	China (n = 49)	United States (n = 145)	Unadjusted OR (95%CI), P value (Reference = US)	Adjusted[1] OR (95%CI), P value (Reference = US)
Ileal involvement	63.3%	63.5%	0.99 (0.50-1.94) P = 0.98	1.14 (0.51-2.55) P = 0.74
Surgery for IBD	51.0%	49.7%	1.06 (0.55-2.02) P = 0.87	1.70 (0.77-3.75) P = 0.19
Ever steroids use	46.9%	91.0%	0.09 (0.04-0.19) P < 0.0001	0.19 (0.07-0.50) P = 0.0007
Steroids use within 3 mo of diagnosis	24.5%	46.2%	0.38 (0.18-0.78) P = 0.009	0.53 (0.22-1.25) P = 0.15
Ever immunomodulators[2]	73.5% (n = 36)	61.4% (n = 89)	1.74 (0.85-3.57) P = 0.13	2.13 (0.92-4.91) P = 0.08
6-MP/Azathioprine	88.9%	89.9%		
Methotrexate	19.4%	23.6%		
Cyclosporine	0%	6.7%		
Tacrolimus	0%	3.4%		
Ever biologics use	34.7%	73.8%	0.19 (0.09-0.38) P < 0.0001	0.09 (0.04-0.24) P < 0.0001
Ever TPN use	8.2%	21.4%	0.33 (0.11-0.98) P = 0.05	0.67 (0.19-2.38) P = 0.54
Antibiotics use within 30 d before time of survey	18.8%	15.9%	1.22 (0.52-2.87) P = 0.64	1.29 (0.47-3.55) P = 0.62

[1]Adjusted for sex, smoking status at diagnosis, age at diagnosis older than 40 years or less, and duration of disease at survey completion; [2]For ever users of immunomodulators, the percentage of use of each type of immunomodulators was calculated. IBD: Inflammatory bowel disease; 6-MP: Mercaptopurine; TPN: Total parenteral nutrition; OR: Odds ratio.

IBD was 1/6 that of the United States. Despite the differences in family history, the two countries shared high prevalence of ileal involvement and surgical history for IBD. However, CD patients in China were less likely to have ever used steroids and biologics but more likely to use immunomodulators, which might reflect differences in access to medications or practice variation.

Both the Chinese and the US estimates of family history prevalence are high compared with other studies. One possible reason is that the recruitment sites have dedicated IBD clinics with large numbers of patients referred by outside providers often because of greater disease severity, which might be associated with genetic predisposition. The higher estimates could also be due to the difference in ascertainment methods of family history. We ascertained family history through self-report on a questionnaire instead of obtaining information from physicians' notes in medical records. We believe that self-report might be able to capture more accurate information on detailed family history than medical records.

There has been much interest in investigating the characteristics of CD in Asian and western countries, but CD patients in China and the US have rarely been compared. Luo *et al*[29] compared family history of 85 Chinese and 68 American patients based on information obtained from medical records. They concluded Chinese CD patients had lower prevalence of CD family history than Americans (China 1% *vs* United States 12%, P = 0.016). One strength of our study was that we used the same questionnaire in both settings (available in English and Mandarin after back-translation confirmation) to ascertain family history during the same time period. In the comparison of family history prevalence, we adjusted for confounders such as patient's disease duration and family size.

We analyzed the distribution of relationships of affected relatives, which was rarely reported in previous studies in China. Although no individuals from China reported a cousin with IBD, this was the most commonly affected relative type in the United States. This finding

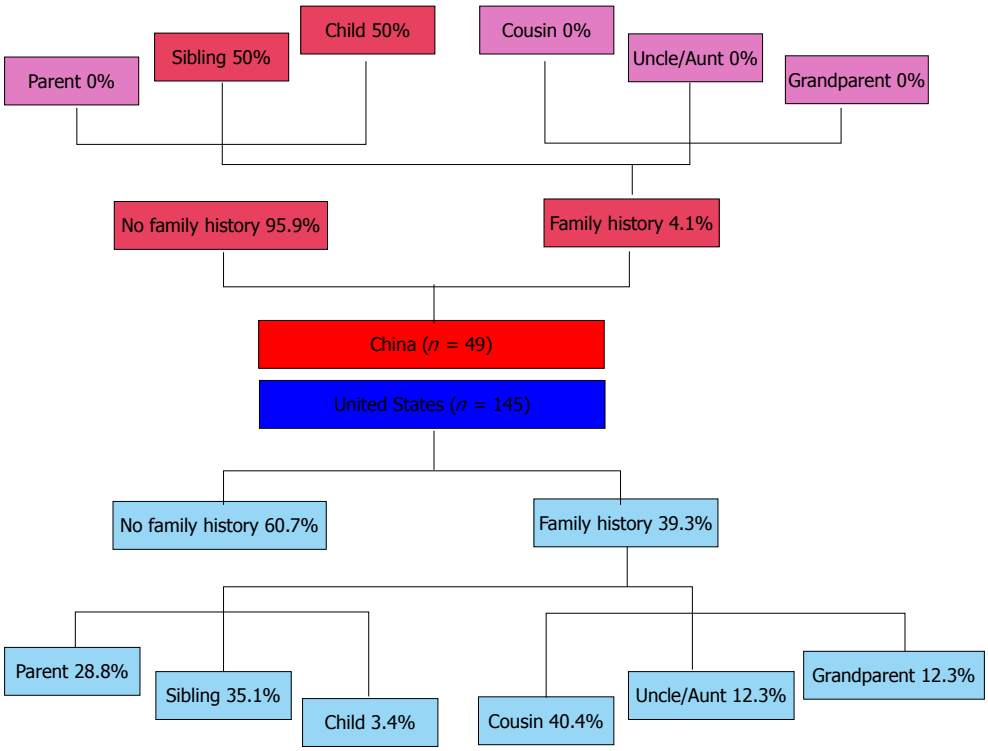

Figure 1 Relationship map of relatives with inflammatory bowel disease to patients with Crohn's disease in China and the United States.

could point to shared environmental risk between cousins and cases that could be explored further, especially among the Chinese CD participants as diagnoses of IBD are expected to increase in the coming years.

More than half of CD patients in both China and the United States had history of ileal involvement and about half had undergone surgeries to treat CD. This implies that CD patients of the two countries had similar severity of disease despite differences in family history and duration of disease. In contrast, Luo *et al*[29] found Chinese CD patients had significantly lower odds of having ileocaecum involvement compared with Americans. We were not able to examine the association of IBD family history and CD outcome by country in the present study, because the small number of individuals with IBD family history in China (n = 2) limited this analysis.

CD patients in China were less likely to have used steroids or biologics than those in the United States. Prideaux *et al*[23] had a similar finding when comparing steroid use at diagnosis and ever use of biologics between CD patients in Melbourne *vs* those in Hong Kong (steroids: Hong Kong 37.0% *vs* Melbourne 61.2%, $P < 0.001$; anti-tumor necrosis factor therapy: Hong Kong 11.0% *vs* Melbourne 39.9%, $P < 0.001$). This could be due to either real difference in the needs for steroids treatment or difference in physicians' practicing patterns. These same reasons could explain the differences in biologic use with the additional facts that biologics were introduced into China market later than the United States (2007 *vs* 1999), that only infliximab is currently available, and that most Chinese patients pay out of pocket at the United States equivalent rates limiting the population that has access to these drugs.

In conclusion, the lower prevalence of familial clustering of IBD among CD patients in China may suggest that the etiology of CD in Chinese population is to a lesser extent attributed to genetic background or family-shared environment compared with the US population. Despite the potential difference in etiology, CD patients from China were as likely to have a history of ileal involvement or have a history of surgery for IBD.

ACKNOWLEDGMENTS

We acknowledge Ludwig-Bayless Science Award for overall support of the study. We also acknowledge ResearchMatch.org. Part of the recruitment for the study included was done *via* ResearchMatch, a national health volunteer registry that was created by several academic institutions and supported by the United States National Institutes of Health as part of the Clinical Translational Science Award (CTSA) program. ResearchMatch has a large population of volunteers who have consented to be contacted by researchers about health studies for which they may be eligible.

COMMENTS

Background

Family history of inflammatory bowel disease (IBD) is the strongest risk factor for developing Crohn's disease (CD) among Western population, and it is also potentially associated with higher probability of having ileal involvement and demand for surgical treatment for CD. However, the prevalence of IBD family

history and its role in CD among Asian population has not been well studied.

Research frontiers

CD has a complex and multifactorial etiology. The roles of and interactions among genetic background, gut microbiota, diet, and autoimmunity are at the frontiers of research. Although the incidence rate of CD in Asian countries is relatively low compared with Western countries, CD incidence is increasing. This epidemiological change gives us a unique opportunity to explore the driving factors of the disease, which could increase the authors understanding of CD and ultimately lead to novel therapeutics.

Innovations and breakthroughs

Few studies compare family history and disease outcomes of Western and Asian CD patients. The few available studies are mostly based on chart review of patients' medical records from different countries and in different settings, which may be biased by the level of detail recorded within the different medical systems. The current study is innovative in that CD patients from China and United States were investigated with the same survey during the same time period Patients reported information on family history and smoking history themselves, rather than relying on medical notes.

Applications

The results of this study suggest lower prevalence of IBD familial clustering among CD patients in China as compared with the United States. This may suggest that the etiology of CD in Chinese population is to a lesser extent attributed to genetic background or family-shared environment compared with the United States population. Despite the potential difference in etiology, CD patients from China were just as likely to have a history of ileal involvement or have a history of IBD-related surgery as those from United States.

Terminology

CD is a form of IBD, featured by chronic inflammation, most frequently affecting the terminal ileum and colon, but can also involve other portions of the gastrointestinal tract from mouth to anus.

Peer-review

In the presented study the differences in family history and clinical outcomes among individuals residing in China and the United States were investigated with a survey-based cross-sectional study. The prevalence of IBD family history was significantly lower in China. It will be interesting to see if the results change as the Chinese study population is studied for a longer period of time, including both longer follow-up of the Chinese population and a larger sample size.

REFERENCES

1 **Abraham C**, Cho JH. Inflammatory bowel disease. *N Engl J Med* 2009; **361**: 2066-2078 [PMID: 19923578 DOI: 10.1056/NEJMra0804647]

2 **Crohn BB**, Ginzburg L, Oppenheimer GD. Regional ileitis: a pathologic and clinical entity. 1932. *Mt Sinai J Med* 2000; **67**: 263-268 [PMID: 10828911 DOI: 10.1001/jama.1932.02740680019005]

3 **Kyle J**, Blair DW. Epidemiology of regional enteritis in North-East Scotland. *Br J Surg* 1965; **52**: 215-217 [PMID: 14261132 DOI: 10.1002/bjs.1800520318]

4 **Molodecky NA**, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, Benchimol EI, Panaccione R, Ghosh S, Barkema HW, Kaplan GG. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology* 2012; **142**: 46-54.e42; quiz e30 [PMID: 22001864 DOI: 10.1053/j.gastro.2011.10.001]

5 **Lee SK**. Crohn's disease in Singapore. *Med J Aust* 1974; **1**: 266-269 [PMID: 4821537 DOI: 10.1016/s0016-5085(98)80100-5]

6 **Leong RW**, Lau JY, Sung JJ. The epidemiology and phenotype of Crohn's disease in the Chinese population. *Inflamm Bowel Dis* 2004; **10**: 646-651 [PMID: 15472528 DOI: 10.1097/00054725-200409000-00022]

7 **Morita N**, Toki S, Hirohashi T, Minoda T, Ogawa K, Kono S, Tamakoshi A, Ohno Y, Sawada T, Muto T. Incidence and prevalence of inflammatory bowel disease in Japan: nationwide epidemiological survey during the year 1991. *J Gastroenterol* 1995; **30** Suppl 8: 1-4 [PMID: 8563866]

8 **Yang SK**, Yun S, Kim JH, Park JY, Kim HY, Kim YH, Chang DK, Kim JS, Song IS, Park JB, Park ER, Kim KJ, Moon G, Yang SH. Epidemiology of inflammatory bowel disease in the Songpa-Kangdong district, Seoul, Korea, 1986-2005: a KASID study. *Inflamm Bowel Dis* 2008; **14**: 542-549 [PMID: 17941073 DOI: 10.1002/ibd.20310]

9 **Ye L**, Cao Q, Cheng J. Review of inflammatory bowel disease in China. *ScientificWorldJournal* 2013; **2013**: 296470 [PMID: 24348149 DOI: 10.1155/2013/296470]

10 **Ng SC**, Bernstein CN, Vatn MH, Lakatos PL, Loftus EV, Tysk C, O'Morain C, Moum B, Colombel JF. Geographical variability and environmental risk factors in inflammatory bowel disease. *Gut* 2013; **62**: 630-649 [PMID: 23335431 DOI: 10.1007/s11894-013-0313-9]

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

12 **Hugot JP**, Chamaillard M, Zouali H, Lesage S, Cézard JP, Belaiche J, Almer S, Tysk C, O'Morain CA, Gassull M, Binder V, Finkel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel JF, Sahbatou M, Thomas G. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001; **411**: 599-603 [PMID: 11385576 DOI: 10.1038/35079107]

13 **Ogura Y**, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr RH, Achkar JP, Brant SR, Bayless TM, Kirschner BS, Hanauer SB, Nuñez G, Cho JH. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 2001; **411**: 603-606 [PMID: 11385577 DOI: 10.1038/35079114]

14 **Guo QS**, Xia B, Jiang Y, Qu Y, Li J. NOD2 3020insC frameshift mutation is not associated with inflammatory bowel disease in Chinese patients of Han nationality. *World J Gastroenterol* 2004; **10**: 1069-1071 [PMID: 15052696 DOI: 10.3748/wjg.v10.i7.1069]

15 **Lv C**, Yang X, Zhang Y, Zhao X, Chen Z, Long J, Zhang Y, Zhong C, Zhi J, Yao G, Jiang B, Zhi F. Confirmation of three inflammatory bowel disease susceptibility loci in a Chinese cohort. *Int J Colorectal Dis* 2012; **27**: 1465-1472 [PMID: 22426692 DOI: 10.1007/s00384-012-1450-6]

16 **Wang ZW**, Ji F, Teng WJ, Yuan XG, Ye XM. Risk factors and gene polymorphisms of inflammatory bowel disease in population of Zhejiang, China. *World J Gastroenterol* 2011; **17**: 118-122 [PMID: 21218092 DOI: 10.3748/wjg.v17.i1.118]

17 **Li M**, Gao X, Guo CC, Wu KC, Zhang X, Hu PJ. OCTN and CARD15 gene polymorphism in Chinese patients with inflammatory bowel disease. *World J Gastroenterol* 2008; **14**: 4923-4927 [PMID: 18756601 DOI: 10.3748/wjg.14.4923]

18 **Peeters M**, Nevens H, Baert F, Hiele M, de Meyer AM, Vlietinck R, Rutgeerts P. Familial aggregation in Crohn's disease: increased age-adjusted risk and concordance in clinical characteristics. *Gastroenterology* 1996; **111**: 597-603 [PMID: 8780562 DOI: 10.1053/gast.1996.v111.pm8780562]

19 **Li Y**, Ren J, Wang G, Gu G, Wu X, Ren H, Hong Z, Hu D, Wu Q, Li G, Liu S, Anjum N, Li J. Diagnostic delay in Crohn's disease is associated with increased rate of abdominal surgery: A retrospective study in Chinese patients. *Dig Liver Dis* 2015; **47**: 544-548 [PMID: 25840874 DOI: 10.1016/j.dld.2015.03.004]

20 **Wang XQ**, Zhang Y, Xu CD, Jiang LR, Huang Y, Du HM, Wang XJ. Inflammatory bowel disease in Chinese children: a multicenter analysis over a decade from Shanghai. *Inflamm Bowel Dis* 2013; **19**: 423-428 [PMID: 23340680 DOI: 10.1097/MIB.0b013e318286f9f2]

21 **Yi F**, Chen M, Huang M, Li J, Zhao J, Li L, Xia B. The trend in newly diagnosed Crohn's disease and extraintestinal manifestations of Crohn's disease in central China: a retrospective study of a single center. *Eur J Gastroenterol Hepatol* 2012; **24**: 1424-1429 [PMID: 22895389 DOI: 10.1097/MEG.0b013e3283583e5c]

22 **Li Y**, Zhu W, Zuo L, Zhang W, Gong J, Gu L, Cao L, Li N, Li J.

Frequency and risk factors of postoperative recurrence of Crohn's disease after intestinal resection in the Chinese population. *J Gastrointest Surg* 2012; **16**: 1539-1547 [PMID: 22555673 DOI: 10.1007/s11605-012-1902-x]

23 **Prideaux L**, Kamm MA, De Cruz P, Williams J, Bell SJ, Connell WR, Brown SJ, Lust M, Desmond PV, Chan H, Chow DK, Wu JC, Leong RW, Sung JJ, Chan FK, Ng SC. Comparison of clinical characteristics and management of inflammatory bowel disease in Hong Kong versus Melbourne. *J Gastroenterol Hepatol* 2012; **27**: 919-927 [PMID: 22098103 DOI: 10.1111/j.1440-1746.2011.06984.x]

24 **Song XM**, Gao X, Li MZ, Chen ZH, Chen SC, Hu PJ, He YL, Zhan WH, Chen MH. Clinical features and risk factors for primary surgery in 205 patients with Crohn's disease: analysis of a South China cohort. *Dis Colon Rectum* 2011; **54**: 1147-1154 [PMID: 21825896 DOI: 10.1097/DCR.0b013e318222ddc3]

25 **Chow DK**, Sung JJ, Tsoi KK, Wong VW, Wu JC, Leong RW, Chan FK. Predictors of corticosteroid-dependent and corticosteroid-refractory inflammatory bowel disease: analysis of a Chinese cohort study. *Aliment Pharmacol Ther* 2009; **29**: 843-854 [PMID: 19154567 DOI: 10.1111/j.1365-2036.2009.03944.x]

26 **Chow DK**, Sung JJ, Wu JC, Tsoi KK, Leong RW, Chan FK. Upper gastrointestinal tract phenotype of Crohn's disease is associated with early surgery and further hospitalization. *Inflamm Bowel Dis* 2009; **15**: 551-557 [PMID: 19067420 DOI: 10.1002/ibd.20804]

27 **Leong RW**, Lawrance IC, Chow DK, To KF, Lau JY, Wu J, Leung WK, Chan FK, Sung JJ. Association of intestinal granulomas with smoking, phenotype, and serology in Chinese patients with Crohn's disease. *Am J Gastroenterol* 2006; **101**: 1024-1029 [PMID: 16573779 DOI: 10.1111/j.1572-0241.2006.00503.x]

28 **Leong RW**, Armuzzi A, Ahmad T, Wong ML, Tse P, Jewell DP, Sung JJ. NOD2/CARD15 gene polymorphisms and Crohn's disease in the Chinese population. *Aliment Pharmacol Ther* 2003; **17**: 1465-1470 [PMID: 12823148 DOI: 10.1046/j.1365-2036.2003.01607.x]

29 **Luo CH**, Wexner SD, Liu QS, Li L, Weiss E, Zhao RH. The differences between American and Chinese patients with Crohn's disease. *Colorectal Dis* 2011; **13**: 166-170 [PMID: 19878519 DOI: 10.1111/j.1463-1318.2009.02094.x]

30 **Roth MP**, Petersen GM, McElree C, Vadheim CM, Panish JF, Rotter JI. Familial empiric risk estimates of inflammatory bowel disease in Ashkenazi Jews. *Gastroenterology* 1989; **96**: 1016-1020 [PMID: 2925048 DOI: 10.1016/0016-5085(89)91618-1]

31 **Weterman IT**, Peña AS. Familial incidence of Crohn's disease in The Netherlands and a review of the literature. *Gastroenterology* 1984; **86**: 449-452 [PMID: 6693011]

32 **Thia KT**, Loftus EV, Sandborn WJ, Yang SK. An update on the epidemiology of inflammatory bowel disease in Asia. *Am J Gastroenterol* 2008; **103**: 3167-3182 [PMID: 19086963 DOI: 10.1111/j.1572-0241.2008.02158.x]

33 **Harris PA**, Scott KW, Lebo L, Hassan N, Lightner C, Pulley J. ResearchMatch: a national registry to recruit volunteers for clinical research. *Acad Med* 2012; **87**: 66-73 [PMID: 22104055 DOI: 10.1097/ACM.0b013e31823ab7d2]

34 **Harris PA**, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009; **42**: 377-381 [PMID: 18929686 DOI: 10.1016/j.jbi.2008.08.010]

P- Reviewer: Ozen H, Vradelis S **S- Editor**: Gong ZM **L- Editor**: A
E- Editor: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i4.564

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 564-571
ISSN 2150-5349 (online)

ORIGINAL ARTICLE

Observational Study

Good adherence to mediterranean diet can prevent gastrointestinal symptoms: A survey from Southern Italy

Francesco Paolo Zito, Barbara Polese, Letizia Vozzella, Antonietta Gala, Daniela Genovese, Viviana Verlezza, Fabiana Medugno, Antonello Santini, Luigi Barrea, Martina Cargiolli, Paolo Andreozzi, Giovanni Sarnelli, Rosario Cuomo

Francesco Paolo Zito, Barbara Polese, Letizia Vozzella, Antonietta Gala, Daniela Genovese, Viviana Verlezza, Fabiana Medugno, Martina Cargiolli, Paolo Andreozzi, Giovanni Sarnelli, Rosario Cuomo, Department of Surgery, Gastroenterology and Endocrinology, University of Naples "Federico II", Naples, 80131 Campania, Italy

Antonello Santini, Department of Pharmacy, University of Naples "Federico II", Naples, 80131 Campania, Italy

Luigi Barrea, IOS s.r.l and COLEMAN S.p.A., Naples, 80131 Campania, Italy

Author contributions: Sarnelli G and Cuomo R ideated the project; Polese B, Gala A, Genovese D, Verlezza V and Medugno F collected the data; Zito FP and Vozzella L wrote the first draft of the manuscript; Santini A, Barrea L, Cargiolli M and Andreozzi P critically reviewed the manuscript; all authors approved the final version of the manuscript.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: There are no conflicts of interest to report.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

Correspondence to: Rosario Cuomo, MD, Associate Professor, Department of Surgery, Gastroenterology and Endocrinology, University of Naples "Federico II", Via S. Pansini 5, Building 6, Naples, 80131 Campania, Italy. rcuomo@unina.it
Telephone: +39-81-7463892
Fax: +39-339-7221830

Received: June 1, 2016
Peer-review started: June 2, 2016
First decision: July 4, 2016
Revised: July 19, 2016
Accepted: September 13, 2016
Article in press: September 15, 2016
Published online: November 6, 2016

Abstract

AIM

To evaluate how different levels of adherence to a mediterranean diet (MD) correlate with the onset of functional gastrointestinal disorders.

METHODS

As many as 1134 subjects (598 M and 536 F; age range 17-83 years) were prospectively investigated in relation to their dietary habits and the presence of functional gastrointestinal symptoms. Patients with relevant chronic organic disease were excluded from the study. The Mediterranean Diet Quality index for children and adolescents (KIDMED) and the Short Mediterranean Diet Questionnaire were administered. All subjects were grouped into five categories according to their ages: 14-24 years; 25-34; 35-49; 50-64; above 64.

RESULTS

On the basis of the Rome Ⅲ criteria, our population consisted of 719 (63.4%) individuals who did not meet the criteria for any functional disorder and were classified as controls (CNT), 172 (13.3%) patients meeting

criteria for prevalent irritable bowel syndrome (IBS), and 243 (23.3%) meeting criteria for prevalent functional dyspepsia (FD). A significantly lower adherence score in IBS (0.57 ± 0.23, $P < 0.001$) and FD (0.56 ± 0.24, $P < 0.05$) was found compared to CNT (0.62 ± 0.21). Females with FD and IBS exhibited significantly lower adherence scores (respectively 0.58 ± 0.24, $P < 0.05$ and 0.56 ± 0.22, $P < 0.05$) whereas males were significantly lower only for FD (0.53 ± 0.25, $P < 0.05$). Age cluster analyses showed a significantly lower score in the 17-24 years and 25-34 year categories for FD (17-24 years: 0.44 ± 0.21, $P < 0.001$; 25-34 years: 0.48 ± 0.22, $P < 0.05$) and IBS (17-24 years: 0.45 ± 0.20, $P < 0.05$; 24-34 years: 0.44 ± 0.21, $P < 0.001$) compared to CNT (17-24 years: 0.56 ± 0.21; 25-34 years: 0.69 ± 0.20).

CONCLUSION

Low adherence to MD may trigger functional gastrointestinal symptoms, mainly in younger subjects. Moreover, with increasing age, patients tend to adopt dietary regimens closer to MD.

Key words: Mediterranean diet; Irritable bowel syndrome; Dietary regimen; Functional gastro-intestinal disorders; Functional dyspepsia

Core tip: Diet seems to be one of the most important triggering factor for functional gastrointestinal disorders (FGID). In fact, patients suffering from irritable bowel syndrome or functional dyspepsia frequently report the onset of symptoms after a meal or the consumption of certain types of foods. The mediterranean (MD) diet is universally considered a health-promoting dietary regimen, since populations adopting this type of diet exhibit a lower rate of major cardiovascular, neoplastic, metabolic morbidity and mortality. Emerging evidence supports a beneficial effect of MD on the gastrointestinal tract, although the association between a high adherence to MD and FGID symptoms is still unclear.

Zito FP, Polese B, Vozzella L, Gala A, Genovese D, Verlezza V, Medugno F, Santini A, Barrea L, Cargiolli M, Andreozzi P, Sarnelli G, Cuomo R. Good adherence to mediterranean diet can prevent gastrointestinal symptoms: A survey from Southern Italy. *World J Gastrointest Pharmacol Ther* 2016; 7(4): 564-571 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i4/564.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i4.564

INTRODUCTION

The Mediterranean diet (MD) is considered as a complex set of eating habits adopted by peoples in countries bordering the Mediterranean Sea. It includes high consumption of olive oil, fiber-rich foods, milk or dairy products and low consumption of meat or meat-based products[1]. In the last few years, this dietary regimen has been universally proposed as a health-protective diet since populations who have adopted it show a remarkable reduction in all-cause mortality[2], especially from cardiovascular diseases and cancer, compared to the United States or Northern European countries[3]. The beneficial effects of MD may be attributed to the large consumption of antioxidants contained in raw fruit and vegetables or to a reduced consumption of saturated fats.

A healthy diet is also important to preserve gastrointestinal balance. Indeed, some alimentary regimens or even some meals are able to trigger symptoms in individuals with functional gastrointestinal disorders (FGIDs), such as functional dyspepsia (FD) or irritable bowel syndrome (IBS). FGIDs are highly prevalent chronic disorders occurring in the absence of any organic etiology[4]. They are often associated with psychological co-morbidities, such as depression or anxiety, which negatively influence the quality of life and cause absence from work or school with a consequently relevant economic burden. The etiology of FGID is thought to be multifactorial, and includes altered brain-gut interactions, genetic predispositions, and/or environmental factors, such as diet[5,6]. Actually, food - especially some types of foods - seems to be the most important triggering factor. Up to 75% of adults with IBS report that diets high in carbohydrates, fatty foods, coffee, alcohol, and hot spices worsen their GI symptoms[7]. Dyspeptic patients usually report that meal size, eating patterns, caloric intake as well as nutrient composition-lipid content in particular-strongly influence the onset of dyspeptic symptoms. Several mechanisms have been hypothesized to account for the association between food and gastrointestinal symptoms, *i.e.,* influence of food on microbiota composition; luminal distension related to gas production from bacterial fermentation; direct effects of some nutrients on GI sensitivity and motility[8,9].

Emerging evidence[4,10] supports the hypothesis that MD may be beneficial also for functional gastrointestinal disorders. Against this background, the aim of our study was to evaluate how different levels of adherence to MD correlate with the onset of functional gastrointestinal disorders, such as irritable bowel syndrome or functional dyspepsia.

MATERIALS AND METHODS

Population features

Our study was performed in Campania, a region of Southern Italy between May 2011 and April 2012. As many as 1134 subjects (598 M and 536 F; age range 17-83 years) without a prior abdominal surgery or relevant organic chronic disease, on the basis of an accurate history taking, were investigated in relation to their dietary habits; of these, 114 were outpatients of the "Federico Ⅱ" University Hospital (Naples), 401 had been surveyed during an open event for health prevention

Table 1 KidMed test to assess adherence to the Mediterranean diet

Scoring	
+1	Has one fruit or fruit juice every day
+1	Has a second fruit every day
+1	Has fresh or cooked vegetables regularly once a day
+1	Has fresh or cooked vegetables more than once a day
+1	Consumes fish regularly (at least 2-3 times per week)
-1	Goes more than once a week to a fast-food (hamburger) restaurant
+1	Likes pulses and eats them more than once a week
+1	Consumes pasta or rice almost every day (5 or more times per week)
+1	Has cereals or grains for breakfast
+1	Consumes nuts regularly (at least 2-3 times per week)
+1	Uses olive oil at home
-1	Skips breakfast
+1	Has a dairy product for breakfast (yoghurt, milk, *etc.*)
-1	Has commercially baked goods or pastries for breakfast
+1	Has two yoghurt and/or some cheese (40 g) daily
-1	Has sweets and candy several times every day

held in the city of Caserta; 619 were evaluated during a health care program in a secondary school in Naples.

Clinical questionnaire

All subjects were administered the Rome Ⅲ questionnaire to assess the presence of upper or lower gastrointestinal symptoms: 719 patients did not report any relevant gastrointestinal symptoms (controls), 172 met the criteria for prevalent irritable bowel syndrome and 243 for prevalent functional dyspepsia. Controls, IBS and FD patients were classified according to age: 14-24 years old, 25 to 34 years old, 35 to 49 years old, 50 to 64 years old and, finally, above 64 years of age.

Adherence to MD questionnaire

In addition, all subjects completed a standardized food frequency questionnaire (FFQ) evaluating their adherence to the Mediterranean Diet model. In order to explore the adherence to MD in different age categories, we used two different questionnaire: The Mediterranean Diet Quality index for children and adolescents (KIDMED) was administered to participants whose ages ranged from 17 to 24 years, and the Short Mediterranean Diet Questionnaire to those older than 24 years. Both questionnaires were developed according to the principles behind Mediterranean dietary patterns.

The KIDMED index is based on a 16-question test with scores ranging from 0 to 12; questions denoting a negative connotation compared to the Mediterranean diet model were assigned a value of -1, those with positive aspects were assigned +1 (Table 1). The score obtained was classified into three levels of adherence to the Mediterranean dietary model: > 8 optimal; 4-7 intermediate, ≤ 3 very low adherence[11,12].

The Short Mediterranean Diet Questionnaire derives from a larger validated FFQ including 136 items; it is based on a 9-question test assessing the frequency of consumption for nine typical food categories. A specific frequency score was assigned to each food to attribute +1 only when food consumption satisfied the criteria (Table 2). The final composite score ranged from 0 to 9 and, as for the KIDMED index, it was classified into three levels of adherence to the Mediterranean diet: > 7 optimal; 4-6 intermediate, ≤ 3 very low adherence[13].

Table 2 Short mediterranean diet questionnaire

Scoring	
+1	Olive Oil (> 1 spoon/d)
+1	Fruit (≥ 1 serving/d)
+1	Vegetables or Salads (≥ 1 serving/d)
+1	Fruit (≥ 1 serving/d) and vegetable (≥ 1 serving/d)
+1	Legumes (≥ 2 serving/d)
+1	Fish (≥ 3 serving/d)
+1	Wine (≥ 1 glass/d)
+1	Meat (≤ 1 serving/d)
+1	White bread (≤ 1 serving/d) and rice (≤ 1 serving/wk) or whole-grain bread (> 5/wk)

Since two different types of questionnaires were used, the final index ranged from 0 to 9 or from 0 to 12; for this reason, to equalize the score obtained for each patient, final scores were divided by the maximum result achievable depending on the questionnaire employed.

Statistical analysis

Data were evaluated using SPSS for Windows version 13 (SPSS Inc., Chicago, IL, United States). Results were analyzed using *t*-test and ANOVA. Differences were considered significant when the *P* value was below 0.05. A multinomial regression model was used to analyze whether the level of adherence to MD was associated with functional gastrointestinal disorders, age category, sex or BMI. Results are reported as adjusted odds ratio with 95%CI; *P*-values below 0.05 were considered as significant. The output of the multinomial logistic regression is presented as a set of two dichotomous logistic regressions that provide a pairwise comparison of the phenotypes, as follows: High *vs* low adherence to MD and high *vs* intermediate adherence to MD. The oldest group, controls, individuals presenting with third grade obesity, and females were used as reference category in the multinomial regression model.

RESULTS

Overall, 1134 subjects were investigated as to presence of both upper and lower gastrointestinal symptoms and were thus classified into the following groups: Controls, functional dyspepsia or irritable bowel syndrome. Thereafter, they were all stratified by level of adherence to the Mediterranean diet (low, intermediate and high adherence) and age category (17-24; 25-34; 35-49; 50-65; > 65 years) (Table 3).

Table 3 Distribution by level of adherence to the Mediterranean diet (low, intermediate and high adherence) and age category (17-24; 25-34; 35-49; 50-65; > 65 years) *n* (%)

		Level of adherence to MD		
	Age category (yr)	Low	Intermediate	High
CNT	17-24	61 (20.5)	158 (53.0)	79 (26.5)
	25-34	11 (11.8)	55 (59.1)	27 (29)
	35-49	14 (9.9)	91 (64.5)	36 (25.5)
	50-65	22 (16.4)	89 (66.4)	23 (17.2)
	> 65	3 (5.7)	37 (69.8)	13 (21.9)
IBS	17-24	10 (34.5)	17 (56.8)	2 (6.9)
	25-34	6 (25)	18 (75)	0
	35-49	13 (28.3)	27 (58.7)	6 (13)
	50-65	4 (9.8)	29 (70.7)	8 (19.5)
	> 65	5 (15.6)	20 (62.5)	7 (21.9)
FD	17-24	38 (39.2)	43 (44.3)	16 (16.5)
	25-34	7 (20.6)	25 (73.5)	2 (5.9)
	35-49	7 (14)	34 (68)	9 (18)
	50-65	7 (15.2)	25 (54.3)	14 (30.4)
	> 65	4 (25)	6 (37.5)	6 (37.5)

MD: Mediterranean diet; IBS: Irritable bowel syndrome; FD: Functional dyspepsia.

Univariate analyses

A significantly lower adherence score among FD (0.56 ± 0.24, $P < 0.001$) and IBS (0.57 ± 0.23, $P < 0.05$) was found compared to CNT (0.62 ± 0.21) (Figure 1A). When the data were stratified by gender, females with FD and IBS exhibited significantly lower adherence scores (respectively 0.58 ± 0.24, $P < 0.05$ and 0.56 ± 0.22, $P < 0.05$) as compared to CNT (0.64 ± 0.22), whereas in males they were significantly lower only for FD (0.53 ± 0.25, $P < 0.05$) compared to CNT (0.61 ± 0.21) (Figure 1B and C). Age cluster adherence scores were significantly lower in the 17-24 years and 25-34 years categories for FD (17-24 years: 0.44 ± 0.21, $P < 0.001$; 25-34 years 0.58 ± 0.22, $P < 0.05$) and IBS (17-24 years: 0.45 ± 0.20, $P < 0.05$; 25-34 years: 0.49 ± 0.21, $P < 0.001$) compared to CNT (17-24 years: 0.56 ± 0.21; 25-34 years: 0.69 ± 0.20). In the 35-49 year category, the adherence score was significantly lower only in the IBS group (0.56 ± 0.24, $P < 0.001$) compared to CNT (0.69 ± 0.19). No differences were observed between the other age clusters (Figure 2). However, when stratified by gender, in the 17-24 year category, a lower adherence score was confirmed for females with FD (0.48 ± 0.22, $P < 0.05$) and IBS (0.44 ± 0.17, $P < 0.05$) *vs* CNT (0.57 ± 0.22), whereas the male group presented a significantly lower adherence score only for FD (0.39 ± 0.19, $P < 0.001$) compared to CNT (0.55 ± 0.20). At the same time, stratifying the 25-34 year category by gender, only IBS males (0.46 ± 0.20, $P < 0.05$) and females (0.50 ± 0.21, $P < 0.05$) had a significantly lower mean adherence score compared to controls (0.70 ± 0.18 and 0.68 ± 0.21). Finally, stratifying other age categories by gender, only IBS females in the 35-49 year group (0.55 ± 0.24, $P < 0.05$) exhibited a significantly lower mean adherence score compared to controls (0.69 ± 0.19). No differences were observed for other gender groups (Table 4).

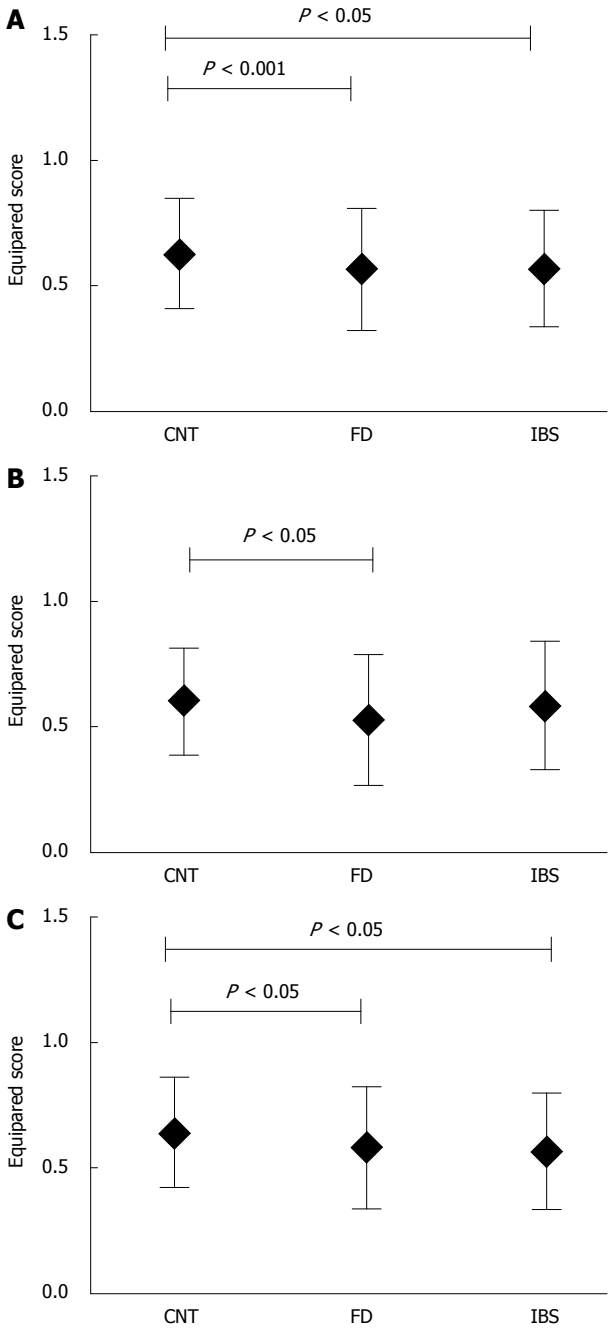

Figure 1 Equalized mean score to mediterranean diet in CNT, functional dyspepsia and irritable bowel syndrome patients. A: All subjects show a significantly lower adherence score in FD and irritable bowel syndrome (IBS) compared to CNT; B: In the male group only FD exhibited significantly lower adherence scores compared to CNT; C: In females a significant difference for FD and IBS compared to CNT was confirmed.

Multivariate analyses

Both low (OR = 3.24, 95%CI: 1.73-6.08, $P < 0.0001$) and intermediate (OR = 1.91, 95%CI: 1.14-3.22, $P < 0.05$) levels of adherence to MD were independently associated with the presence of IBS. However only FD

Table 4 Mean adherence score sorted by gender and cluster

Age range (yr)	Sex	CNT	FD	IBS
		Mean ± SD	Mean ± SD	Mean ± SD
17-24	Tot	0.56 ± 0.21	0.44[b] ± 0.21	0.45[a] ± 0.20
	M	0.55 ± 0.20	0.39[b] ± 0.19	0.46 ± 0.26
	F	0.57 ± 0.22	0.48[a] ± 0.22	0.44[a] ± 0.17
25-34	Tot	0.69 ± 0.20	0.58[a] ± 0.22	0.49[b] ± 0.21
	M	0.70 ± 0.18	0.60 ± 0.24	0.46[a] ± 0.20
	F	0.68 ± 0.21	0.56 ± 0.17	0.50[a] ± 0.21
35-49	Tot	0.69 ± 0.19	0.64 ± 0.20	0.56 ± 0.24
	M	0.70 ± 0.20	0.74 ± 0.15	0.65 ± 0.21
	F	0.69 ± 0.19	0.61 ± 0.21	0.55[a] ± 0.24
50-64	Tot	0.62 ± 0.20	0.69 ± 0.24	0.66 ± 0.18
	M	0.60 ± 0.19	0.65 ± 0.21	0.75 ± 0.26
	F	0.63 ± 0.20	0.70 ± 0.25	0.64 ± 0.19
> 64	Tot	0.71 ± 0.18	0.67 ± 0.28	0.64 ± 0.24
	M	0.74 ± 0.12	0.70 ± 0.32	0.69 ± 0.26
	F	0.69 ± 0.21	0.64 ± 0.25	0.62 ± 0.23

[a]$P < 0.05$ *vs* CNT; [b]$P < 0.001$ *vs* CNT. FD: Functional dyspepsia; IBS: Irritable bowel syndrome.

Table 5 Multivariate analyses

	Low adherence			Intermediate adherence		
	OR	95%CI	*P* value	OR	95%CI	*P* value
FD	2.42	1.47-3.99	< 0.0001	1.34	0.88-2.03	NS
IBS	3.24	1.73-6.08	< 0.0001	1.91	1.13-3.22	< 0.05
17-24 yr	4.65	2.00-10.81	< 0.0001	1.78	0.13-24.96	NS

IBS: Irritable bowel syndrome; FD: Functional dyspepsia.

(OR = 2.42, 95%CI: 1.47-3.99, $P < 0.0001$) and the youngest age category (OR = 4.65, 95%CI: 2.00-10.81, $P < 0.0001$) were associated with a low level of adherence to MD (Table 5, Figure 3A and B).

DISCUSSION

The present study provides evidence that the MD adherence score is significantly lower in subjects with GI symptoms than in asymptomatic subjects, showing an inverse relationship between adherence to MD and prevalence of gastrointestinal symptoms. Moreover, among FGID subjects, only younger age categories (17-24 and 25-34 years.) were associated with lower adherence to MD compared to controls, whereas for older people, no differences were observed between symptomatic and asymptomatic subjects.

Many factors, such as age, gender, nationality, socio-economic condition, may influence dietary adherence to MD[14]. Our results confirm the data from several studies exploring dietary habits in different European countries, widely demonstrating that young people exhibit the lowest level of adherence to MD[12,15,16]. In Italy, a lower adherence to MD has also been confirmed among young people, particularly for those coming from northern region. As shown by Noale *et al*[17] of the Italian adolescents examined in their study, only

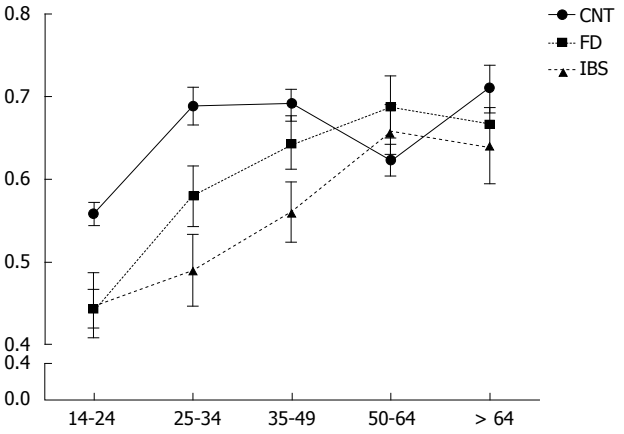

Figure 2 Distribution of equalized mean adherence score to mediterranean diet in CNT, functional dyspepsia and irritable bowel syndrome stratifying for age clusters. In the 17-24 years and 25-34 years groups both FD and IBS exhibited significantly lower mean adherence score compared to CNT. In the 35-49 years group only IBS patients exhibited a significantly lower mean score compared to CNT. No differences were observed in other age categories (data ± SEM are shown). FD: Functional dyspepsia; IBS: Irritable bowel syndrome.

14% exhibited a high adherence to the Mediterranean diet, and 47% a moderate adherence. In fact, not only did the mean daily calorie intake exceed the dietary requirements recommended by "Nutrient Intake Goal for the Italian population" or LARN tables, but they also observed an increase in the intake of saturated fats and proteins as compared to carbohydrates, fruit or vegetables. This may be a consequence of the frequent use of easy-to-prepare and ready-to-use products or fast foods, which are characterized by a lower nutritional quality due to the addition of sugar and saturated fats[18].

Unbalanced diets adopted by young people may contribute to the onset of gastrointestinal symptoms in patients suffering from functional dyspepsia or irritable bowel syndrome[6,8]. Patients with FGID exhibit gastrointestinal hypersensitivity and exaggerated reflex after ingestion of lipids. Moreover, fats influence gastric activity, by delaying gastric emptying and promoting relaxation of the fundus in healthy subjects as well as in dyspeptic patients[7,19,20]. However, fundus relaxation appears impaired in functional dyspepsia since, following lipid infusion, the discomfort threshold observed in FD patients appears to be lower than in controls[21]. In clinical practice, all these disturbances following lipid intake occur much more frequently in dyspeptic patients than in healthy individuals.

Similarly to FD, patients with IBS report abdominal bloating following the intake of foods rich in fatty acids. Fatty meals are also able to alter intestinal motility, increasing whole intestinal transit time and, in some cases, inducing a reflex stimulation of colonic motor activity (gastrocolonic reflex) which may explain the post-prandial defecation observed in IBS-D patients[7].

On the basis of such evidence, it has been hypothesized that since the Mediterranean diet is based only minimally on foods or eating habits able to trigger

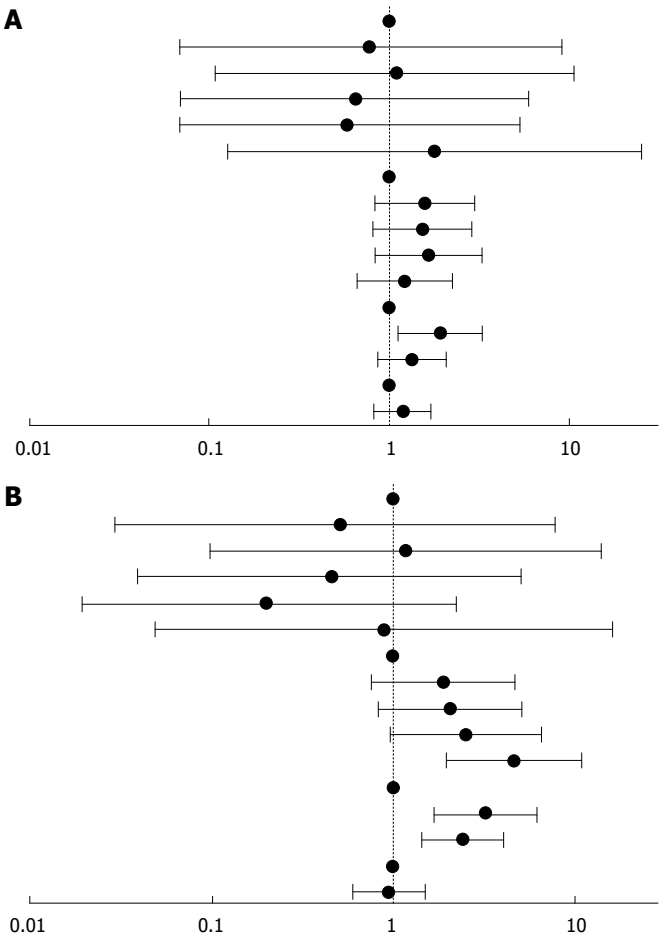

Figure 3 Multivariate analysis shows a pairwise comparison of the phenotypes (body mass index category, age category, clinical pattern and sex) as follows: A: Comparison between high *vs* intermediate adherence to MD: only IBS was significantly associated with Intermediate adherence to MD; B: Comparison between high *vs* low adherence to MD: both IBS and FD as well as the youngest age category were significantly associated with low adherence to MD. The oldest group, controls, individuals presenting with third grade obesity and females are used as reference category in the multinomial regression model. MD: Mediterranean diet; IBS: Irritable bowel syndrome; FD: Functional dyspepsia.

gastrointestinal symptoms, it represents a therapeutic dietary regimen for FGID patients. Indeed, MD is a restricted-calorie dietary model with fats providing less than 35% of total calories[2,22,23]. In addition, MD is characterized by small meals that may exert beneficial effects in both FD patients - by favoring easy gastric emptying, and in IBS-D patients - since it stimulates impaired gastro-colonic reflex only to a limited extent[4,7]. The use of olive oil, as main source of fatty acids, largely preferred to meat and meat-based foods, provides a high intake of mono-unsaturated and omega-3 fatty acids, which may alter gastric emptying less than saturated fats. At the same time, several studies have shown that these kinds of fatty acids may even reduce the microscopic inflammation of colonic mucosa occurring in IBS patients, providing long-term beneficial effects[24].

Noteworthy, our study has shown that subjects in older age categories show greater adherence to MD but nonetheless have persistent functional gastrointestinal symptoms; several factors have been taken into account to explain such evidence. Ageing is characterized by cellular senescence - with consequent reduction in cellular proliferation capability, enteric neuronal loss and low-grade systemic inflammation status called "inflammaging". Consequently, ageing itself may strongly influence gastrointestinal motility, sensitivity, nutrient absorption or gut microbiota composition. Moreover, the presence of several co-morbidities, both mild or severe, is associated with an increased use of medications that deeply influence gastrointestinal activity and its environment (pH, temperature or motility), with secondary effects on bacterial colonization[25,26]. Several research studies have shown that, in the elderly, the stability and variety of microbiota tend to diminish[27]. In fact, there is evidence that Bacteroides and Bifidobacteria become less abundant, mylolytic activity and the availability of short chain fatty acid (SCFA) decrease, with a concomitant increase in the presence of facultative anaerobes, fusobacteria and clostridia[28]. For these reasons, differently from younger individuals, many factors other than diet predispose elderly people to the onset of gastrointestinal problems, which explains the persistence of GI symptoms despite a higher adherence to MD.

The use of questionnaire, both KIDMED and Short Mediterranean one, to evaluate the adherence to a specific diet needs caution because a short score focuses just to a few foods, representative for that alimentary behavior, instead of the whole diet. Moreover, it would be very useful to expand the dietary analysis considering even the amount of food intake in order to evaluate the calories consumed in association with the adherence to MD. This study was performed on subjects living in the same region, therefore, since MD is a dietary regimen which involves several populations, a multicenter prospective study would clarify better the beneficial effect of this diet on functional gastrointestinal symptoms.

In conclusion, the association between food intake and FGID seems to be very complex since each food item may exert a specific effect on the gastrointestinal tract. As several studies have reported that food intake is associated with the onset of symptoms in these disorders, dietary intervention is strongly recommended in the management of FGID. Nutritional therapeutic measures may include different aspects of food intake such as meal size, calorie intake as well as nutrient composition or even meal viscosity. A wide range of dietary regimens, including the Mediterranean Diet, have been proposed for FGID, however the efficacy of such "therapeutic diets" on gastrointestinal symptoms needs further evaluation.

COMMENTS

Background

The mediterranean diet (MD) is universally considered as a health protecting dietary regimen since people who adopt it exhibit a lower incidence of cardiovascular, metabolic and neoplastic disease. However, this dietary pattern may have beneficial effects even on gastrointestinal functional disorders, such as functional dyspepsia or irritable bowel syndrome.

Research frontiers

As the beneficial effects of the MD are widely accepted, the author's aim is to evaluate how different levels of adherence to this type of dietary regimen may influence the onset of functional gastrointestinal symptoms.

Innovations and breakthroughs

The data show that low adherence to MD may trigger functional gastrointestinal symptoms, mainly in younger subjects. Moreover, with increasing age, patients attribute greater importance to their diet and, for this reason, tend to adopt eating habits closer to MD.

Applications

Dietary interventions are fundamental for a correct therapeutic approach of functional gastro-intestinal disorders, such as irritable bowel syndrome or functional dyspepsia. The data support the notion that the adoption of a diet very close to MD - instead of extremely restricted dietary regimens, should be proposed in individuals presenting these disorders.

Peer-review

The topic was interesting and this study was well conducted. The results could help to identify a new dietary habit to prevent gastrointestinal disorders.

REFERENCES

1 **Trichopoulou A**, Martínez-González MA, Tong TY, Forouhi NG, Khandelwal S, Prabhakaran D, Mozaffarian D, de Lorgeril M. Definitions and potential health benefits of the Mediterranean diet: views from experts around the world. *BMC Med* 2014; **12**: 112 [PMID: 25055810 DOI: 10.1186/1741-7015-12-112]

2 **Castro-Quezada I**, Román-Viñas B, Serra-Majem L. The Mediterranean diet and nutritional adequacy: a review. *Nutrients* 2014; **6**: 231-248 [PMID: 24394536 DOI: 10.3390/nu6010231]

3 **Lopez-Garcia E**, Rodriguez-Artalejo F, Li TY, Fung TT, Li S, Willett WC, Rimm EB, Hu FB. The Mediterranean-style dietary pattern and mortality among men and women with cardiovascular disease. *Am J Clin Nutr* 2014; **99**: 172-180 [PMID: 24172306 DOI: 10.3945/ajcn.113.068106]

4 **Azpiroz F**, Hernandez C, Guyonnet D, Accarino A, Santos J, Malagelada JR, Guarner F. Effect of a low-flatulogenic diet in patients with flatulence and functional digestive symptoms. *Neurogastroenterol Motil* 2014; **26**: 779-785 [PMID: 24548289 DOI: 10.1111/nmo.12324]

5 **Vanuytsel T**, Tack JF, Boeckxstaens GE. Treatment of abdominal pain in irritable bowel syndrome. *J Gastroenterol* 2014; **49**: 1193-1205 [PMID: 24845149 DOI: 10.1007/s00535-014-0966-7]

6 **Tack J**, Talley NJ. Functional dyspepsia--symptoms, definitions and validity of the Rome III criteria. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 134-141 [PMID: 23399526 DOI: 10.1038/nrgastro.2013.14]

7 **Gibson PR**, Shepherd SJ. Food choice as a key management strategy for functional gastrointestinal symptoms. *Am J Gastroenterol* 2012; **107**: 657-666; quiz 667 [PMID: 22488077 DOI: 10.1038/ajg.2012.49]

8 **Feinle-Bisset C**, Azpiroz F. Dietary and lifestyle factors in functional dyspepsia. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 150-157 [PMID: 23296252 DOI: 10.1038/nrgastro.2012.246]

9 **Lacy BE**, Talley NJ, Locke GR, Bouras EP, DiBaise JK, El-Serag HB, Abraham BP, Howden CW, Moayyedi P, Prather C. Review article: current treatment options and management of functional dyspepsia. *Aliment Pharmacol Ther* 2012; **36**: 3-15 [PMID: 22591037 DOI: 10.1111/j.1365-2036.2012.05128.x]

10 **Halmos EP**, Power VA, Shepherd SJ, Gibson PR, Muir JG. A diet low in FODMAPs reduces symptoms of irritable bowel syndrome. *Gastroenterology* 2014; **146**: 67-75.e5 [PMID: 24076059 DOI: 10.1053/j.gastro.2013.09.046]

11 **Sahingoz SA**, Sanlier N. Compliance with Mediterranean Diet Quality Index (KIDMED) and nutrition knowledge levels in adolescents. A case study from Turkey. *Appetite* 2011; **57**: 272-277 [PMID: 21624407 DOI: 10.1016/j.appet.2011.05.307]

12 **Antonogeorgos G**, Panagiotakos DB, Grigoropoulou D, Papadimitriou A, Anthracopoulos M, Nicolaidou P, Priftis KN. The mediating effect of parents' educational status on the association between adherence to the Mediterranean diet and childhood obesity: the PANACEA study. *Int J Public Health* 2013; **58**: 401-408 [PMID: 23128782 DOI: 10.1007/s00038-012-0424-3]

13 **Martínez-González MA**, Fernández-Jarne E, Serrano-Martínez M, Wright M, Gomez-Gracia E. Development of a short dietary intake questionnaire for the quantitative estimation of adherence to a cardioprotective Mediterranean diet. *Eur J Clin Nutr* 2004; **58**: 1550-1552 [PMID: 15162136 DOI: 10.1038/sj.ejcn.1602004]

14 **Arriscado D**, Muros JJ, Zabala M, Dalmau JM. Factors associated with low adherence to a Mediterranean diet in healthy children in northern Spain. *Appetite* 2014; **80**: 28-34 [PMID: 24798762 DOI: 10.1016/j.appet.2014.04.027]

15 **Tognon G**, Moreno LA, Mouratidou T, Veidebaum T, Molnár D, Russo P, Siani A, Akhandaf Y, Krogh V, Tornaritis M, Börnhorst C, Hebestreit A, Pigeot I, Lissner L. Adherence to a Mediterranean-like dietary pattern in children from eight European countries. The IDEFICS study. *Int J Obes* (Lond) 2014; **38** Suppl 2: S108-S114 [PMID: 25219407 DOI: 10.1038/ijo.2014.141]

16 **Santomauro F**, Lorini C, Tanini T, Indiani L, Lastrucci V, Comodo N, Bonaccorsi G. Adherence to Mediterranean diet in a sample of Tuscan adolescents. *Nutrition* 2014; **30**: 1379-1383 [PMID: 25280416 DOI: 10.1016/j.nut.2014.04.008]

17 **Noale M**, Nardi M, Limongi F, Siviero P, Caregaro L, Crepaldi G, Maggi S. Adolescents in southern regions of Italy adhere to the Mediterranean diet more than those in the northern regions. *Nutr Res* 2014; **34**: 771-779 [PMID: 25213381 DOI: 10.1016/j.nutres.2014.08.001]

18 **Serra-Majem L**, Ribas L, García A, Pérez-Rodrigo C, Aranceta J. Nutrient adequacy and Mediterranean Diet in Spanish school children and adolescents. *Eur J Clin Nutr* 2003; **57** Suppl 1: S35-S39 [PMID: 12947450]

19 **Tack J**, Caenepeel P, Piessevaux H, Cuomo R, Janssens J. Assessment of meal induced gastric accommodation by a satiety drinking test in health and in severe functional dyspepsia. *Gut* 2003; **52**: 1271-1277 [PMID: 12912857]

20 **Cuomo R**, Vandaele P, Coulie B, Peeters T, Depoortere I, Janssens J, Tack J. Influence of motilin on gastric fundus tone and on meal-induced satiety in man: role of cholinergic pathways. *Am J Gastroenterol* 2006; **101**: 804-811 [PMID: 16635226]

21 **Feinle C**, Meier O, Otto B, D'Amato M, Fried M. Role of duodenal lipid and cholecystokinin A receptors in the pathophysiology of functional dyspepsia. *Gut* 2001; **48**: 347-355 [PMID: 11171824]

22 **Sirtori CR**, Tremoli E, Gatti E, Montanari G, Sirtori M, Colli S, Gianfranceschi G, Maderna P, Dentone CZ, Testolin G. Controlled evaluation of fat intake in the Mediterranean diet: comparative activities of olive oil and corn oil on plasma lipids and platelets in high-risk patients. *Am J Clin Nutr* 1986; **44**: 635-642 [PMID: 3094360]

23 **Shai I**, Spence JD, Schwarzfuchs D, Henkin Y, Parraga G, Rudich A, Fenster A, Mallett C, Liel-Cohen N, Tirosh A, Bolotin A, Thiery J, Fiedler GM, Blüher M, Stumvoll M, Stampfer MJ. Dietary intervention to reverse carotid atherosclerosis. *Circulation* 2010; **121**: 1200-1208 [PMID: 20194883 DOI: 10.1161/CIRCULATIONAHA.109.879254]

24 **Santoro A**, Pini E, Scurti M, Palmas G, Berendsen A, Brzozowska A, Pietruszka B, Szczecinska A, Cano N, Meunier N, de Groot CP, Feskens E, Fairweather-Tait S, Salvioli S, Capri M, Brigidi P, Franceschi C. Combating inflammaging through a Mediterranean whole diet approach: the NU-AGE project's conceptual framework and design. *Mech Ageing Dev* 2014; **136-137**: 3-13 [PMID: 24342354 DOI: 10.1016/j.mad.2013.12.001]

25 **Biagi E**, Nylund L, Candela M, Ostan R, Bucci L, Pini E, Nikkïla J, Monti D, Satokari R, Franceschi C, Brigidi P, De Vos W. Through ageing, and beyond: gut microbiota and inflammatory status in seniors and centenarians. *PLoS One* 2010; **5**: e10667 [PMID: 20498852 DOI: 10.1371/journal.pone.0010667]

26 **Saffrey MJ**. Cellular changes in the enteric nervous system during ageing. *Dev Biol* 2013; **382**: 344-355 [PMID: 23537898 DOI: 10.1016/j.ydbio.2013.03.015]

27 **Claesson MJ**, Jeffery IB, Conde S, Power SE, O'Connor EM, Cusack S, Harris HM, Coakley M, Lakshminarayanan B, O'Sullivan O, Fitzgerald GF, Deane J, O'Connor M, Harnedy N, O'Connor K, O'Mahony D, van Sinderen D, Wallace M, Brennan L, Stanton C, Marchesi JR, Fitzgerald AP, Shanahan F, Hill C, Ross RP, O'Toole PW. Gut microbiota composition correlates with diet and health in the elderly. *Nature* 2012; **488**: 178-184 [PMID: 22797518 DOI: 10.1038/nature11319]

28 **Del Chierico F**, Vernocchi P, Dallapiccola B, Putignani L. Mediterranean diet and health: food effects on gut microbiota and disease control. *Int J Mol Sci* 2014; **15**: 11678-11699 [PMID: 24987952 DOI: 10.3390/ijms150711678]

P- Reviewer: Chiang TA, Sun XT, Tantau A **S- Editor**: Qi Y
L- Editor: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i4.572

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 572-578
ISSN 2150-5349 (online)

ORIGINAL ARTICLE

Randomized Clinical Trial

Efficacy of small-volume simethicone given at least 30 min before gastroscopy

Mingjun Song, Andrew Boon Eu Kwek, Ngai Moh Law, Jeannie Peng Lan Ong, Jessica Yi-Lyn Tan, Prem Harichander Thurairajah, Daphne Shih Wen Ang, Tiing Leong Ang

Mingjun Song, Andrew Boon Eu Kwek, Ngai Moh Law, Jeannie Peng Lan Ong, Jessica Yi-Lyn Tan, Prem Harichander Thurairajah, Daphne Shih Wen Ang, Tiing Leong Ang, Department of Gastroenterology and Hepatology, Changi General Hospital, Singapore 529889, Singapore

Author contributions: Song M, Kwek ABE and Ang TL were responsible for the study conception and design; Song M was responsible for data analysis and interpretation, and manuscript drafting; Kwek ABE and Ang TL critically revised the article for important intellectual content; all the authors reviewed and approved the final version to be published.

Supported by Changi General Hospital Research Grant, No. 2015[CHF2015.02-S].

Institutional review board statement: The study was reviewed and approved by the SingHealth Centralized Institutional Review Board (Ref: 2015/2519).

Clinical trial registration statement: The study is registered under clinicaltrials.gov (NCT02555228).

Informed consent statement: All study participants provided informed written consent prior to study enrollment.

Conflict-of-interest statement: All authors declare no potential conflicting interests related to this paper.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

Correspondence to: Tiing Leong Ang, Chief, Department of Gastroenterology and Hepatology, Changi General Hospital, 2 Simei Street 3, Singapore 529889, Singapore. tiing_leong_ang@cgh.com.sg
Telephone: +65-68503974
Fax: +65-67816202

Received: June 27, 2016
Peer-review started: June 28, 2016
First decision: August 10, 2016
Revised: August 16, 2016
Accepted: October 17, 2016
Article in press: October 19, 2016
Published online: November 6, 2016

Abstract

AIM

To evaluate the efficacy of 5 mL simethicone solution in decreasing gastric foam if given at least 30 min before gastroscopy.

METHODS

This was a randomized, placebo controlled, endoscopist blinded study performed at Changi General Hospital. Patients were at least 21 years old, had no prior surgical resection of the upper gastrointestinal tract, and scheduled for elective diagnostic gastroscopies. The primary outcome was the total mucosal visibility score (TMVS) which was evaluated using McNally score. The sample size was calculated to be 24 per group (SD 2.4, 80% power, $P < 0.05$, 2-sample t test).

RESULTS

Fifty-four patients were randomised to receive either simethicone [1 mL liquid simethicone (100 mg) in 5 mL of water] or placebo (5 mL of water) at least 30 min before their gastroscopy. Six accredited consultants conducted

the gastroscopy, and the interobserver agreement of scoring TMVS was good with a Kappa statistic of 0.73. The simethicone group had significantly better mean TMVS compared to placebo (5.78 ± SD 1.65 *vs* 8.89 ± SD 1.97, $P < 0.001$). The improvement was statistically significant for the duodenum and the gastric antrum, angularis, body, and fundus. Percent 51.9 of patients in the simethicone group had a TMVS of 4 (no bubbles at all) to 5 (only 1 area with minimal bubbles), while in the placebo group 3.7% of patients had TMVS of 4 or 5. The number needed to treat was 2.1 to avoid a TMVS of 6 and more. The simethicone group also had a significantly shorter procedure time with less volume of additional flushes required during gastroscopy to clear away obscuring gastric foam.

CONCLUSION

With a premedication time of at least 30 min, 5 mL simethicone can significantly decrease gastric foam, decrease the volume of additional flushes, and shorten gastroscopy time.

Key words: Simethicone; Premedication; Gastroscopy; Gastric foam

Core tip: This is the first study to evaluate the efficacy of a low volume (5 mL) simethicone solution compared to a placebo using the McNally score to calculate the total mucosal visibility for gastroscopy. Our study showed that although earlier studies had favored higher volumes (typically 100 mL), a low volume is still effective as long as adequate premedication time of at least 30 min is allowed. Such a small volume is more suitable for patients with swallowing difficulties and the formulation had excellent patient compliance with no adverse effects.

Song M, Kwek ABE, Law NM, Ong JPL, Tan JYL, Harichander Thurairajah P, Ang DSW, Ang TL. Efficacy of small-volume simethicone given at least 30 min before gastroscopy. *World J Gastrointest Pharmacol Ther* 2016; 7(4): 572-578 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i4/572.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i4.572

INTRODUCTION

Image-enhanced gastroscopy, such as narrow-band imaging and magnifying endoscopy, can detect subtle early gastric cancer or precancerous lesions and this technology is widely available in Singapore[1]. However, the presence of foam, bubbles and mucus can preclude the benefits of enhanced endoscopy, as subtle mucosal lesions could be covered. In the latest Singapore Cancer Registry, more than 50% of gastric cancers were diagnosed at stage Ⅳ of disease[2]. This suggests that improvement is required for endoscopic detection of early gastric cancer.

Many studies have proven that premedication before gastroscopy will improve the total mucosal visibility scores. However, there is significant heterogeneity between these studies in terms of premedication time, mucosal scoring systems, primary outcome measurements and the type of medications used. Pronase, N-acetylcysteine, dimethicone, dimethylpolysiloxane and simethicone have all been demonstrated by multiple studies to be effective mucolytic and anti-foaming agents[3-11]. All the studies suggested that the best premedication regime is a combination of premedication (a mucolytic and an anti-foaming agent) delivered at large volumes (typically with 100 mL of water). Only one study had a treatment arm looking at small volume premedication (100 mg simethicone in 5 mL water) but this was not compared against a placebo group and the study used a unique 3-grade scoring system[11].

In Singapore, pronase, dimethicone and dimethylpolysiloxane are not available. N-acetylcysteine is a prescription drug and simethicone is an over-the-counter drug for infant colic used off-label for flushes during endoscopy. Premedication before gastroscopy is not routinely given to patients at all endoscopy centers due to several reasons; the perception that premedication may slow down the endoscopy schedule; the worry of adverse reactions from the medications (such as allergic reactions to N-acetylcysteine); and the worry of aspiration from drinking premedication shortly before gastroscopy. As Singapore has an aging population[12], it is common now to perform endoscopy on elderly patients with swallowing dysfunction. However 100 mL premedication solution before gastroscopy puts these patients at risk for aspiration, especially in the setting of moderate sedation during the procedure[13].

We hypothesized that if the premedication time is extended to at least 30 min, 100 mg of simethicone added to 5 mL of water will be able to mix with gastric secretions and swallowed saliva to coat a larger surface area of the gastric mucosa, and significantly improve mucosal visibility compared to placebo.

MATERIALS AND METHODS

Patient selection

This study was conducted in Changi General Hospital in Singapore, from 14th August 2015 to 19th November 2015, at the outpatient gastroenterology clinics. All patients who were planned for gastroscopy as part of their management plan were asked by their respective clinic attending if they would permit a research coordinator to speak to them. If they agreed, the research coordinator would find the patient at the endoscopy listing room to obtain informed consent from the patient to participate in the study. Patients who were at least 21 years old, mentally competent to give informed consent, and scheduled for outpatient elective diagnostic gastroscopy were enrolled. Patients who were incarcerated; had prior history of surgical resection of the esophagus, stomach, or

duodenum; had known hypersensitivity to simethicone; or required gastroscopy for urgent indications such as suspected gastrointestinal bleeding were all excluded from the study.

Study design

This was a randomized, placebo-controlled, endoscopist-blinded study which was approved by the SingHealth Centralized Institutional Review Board (Ref: 2015/2519) and registered under clinicaltrials.gov (NCT02555228). The randomisation sequence (in blocks of 6) was computer generated by a statistician at Changi General Hospital's Clinical Trials and Research Unit (CTRU). The allocation sequence was written on separate cards as number codes and each card was placed inside a sealed opaque envelope. After a study participant registered for the elective gastroscopy, the research coordinator would open an opaque envelope outside the endoscopy suites and the patient would be allocated to either the simethicone group (100 mg of liquid simethicone added to 5 mL of water) or the placebo group (5 mL of water) based on the number written on a card.

Study participants underwent gastroscopy by 1 of the 6 accredited consultant endoscopists who were blinded to the premedication as well as the premedication time. The premedication was prepared by a research coordinator at a separate location, out of sight from the endoscopist or the endoscopy nurses inside the procedure suites. The patient was informed not to disclose the nature of the premedication to the endoscopy suite nurses or endoscopists. The research coordinator worked together with the scheduling nurse at the endoscopy center to ensure that the premedication was taken at least 30 min before the gastroscopy commenced. To confirm that the patient did not tell the endoscopist about the premedication's nature, the research coordinator followed the patient into the endoscopy suite and stood beside the patient until the procedure was over and the data collection form had been completed by the endoscopist. Premedication time was defined from the administration of the solution to the insertion of the tip of the gastroscope into the patient's mouth. All patients received topical analgesic xylocaine 10% spray to the back of their throat and intravenous midazolam with fentanyl to achieve moderate sedation during gastroscopy.

During the procedure, the endoscopist was allowed to flush additional diluted simethicone solution (1 to 3 drops of simethicone added to about 100 mL of water) down the gastroscope channel if there was obscuring gastric foam preventing a satisfactory view. The total volume of additional flushes was recorded by the endoscopy nurse assisting the procedure. After the endoscopist completed an adequate inspection of the mucosal surfaces, the endoscopist withdrew the tip of the gastroscopy up to the gastroesophageal junction and the research coordinator noted the time. The procedure time was defined as the period of time from insertion of the gastroscope to the withdrawal of the gastroscopy back to the gastroesophageal junction. After this, the endoscopist advanced the gastroscope back into the stomach and proceeded to do any interventions deemed necessary such as biopsies of detected lesions. This ensured that the procedure time measured was standardized and not confounded by the number of additional endoscopic interventions due to detection of more lesions.

Endoscopic scoring system of mucosal visibility

Before initiation of the study, all the endoscopists were instructed on the endoscopic scoring system which was based on the McNally scoring system (Figure 1)[14]. Prior to any additional flushes with diluted simethicone solution, the endoscopists evaluated and noted the McNally score for the esophagus, the gastric fundus and body, the gastric antrum and angularis, and the duodenum. The scoring per area was from the range of 1 to 4; 1 if there was no bubble at all, 2 if there were minimal bubbles which the endoscopist had to actively look out for, 3 if the bubbles were obvious but not totally obscuring the view and 4 if the bubbles were so severe that vision is obscured. The total mucosal visibility score (TMVS) was calculated by the sum of scores in all the areas and ranged from 4 to 16.

Outcomes measured

The primary end-point measured was the mean TMVS in the simethicone premedication group and the placebo group respectively. The secondary end-points measured were the mean visibility scores per area, the mean procedure time per group, the mean volume of additional flushes required per group, adverse events reported by the patient or monitoring endoscopy nurses, and the number of gastric lesions reported by the endoscopist.

Statistical analysis

We felt that a difference of 2 points in mean TMVS between the two groups would be clinically significant. An earlier study conducted in Thailand had used a similar scoring system and we adopted the standard deviation in their study results for our estimation[15]. The calculated sample size for each group was 24 patients (SD 2.4, 2 sample *t* test, $P < 0.05$, 80% power). Assuming that the drop-out rate could be around 10%, we aimed to recruit 27 patients per group.

All categorical variables were analysed with Pearson Chi-square test and all continuous variables were analysed with 2-sample *t* test, using SPSS V.19.0 software for Windows (SPSS Inc, Chicago, Illinois, United States). $P < 0.05$ was deemed statistically significant.

RESULTS

A total of 56 patients were enrolled in the study. One patient withdrew consent on the day of the gastroscopy. Another patient subsequently called to cancel the gastroscopy. The remaining 54 patients completed the study with no adverse events (Figure 2) Baseline characteristics of the patients in the 2 groups were similar in terms of mean age, gender, and premedication

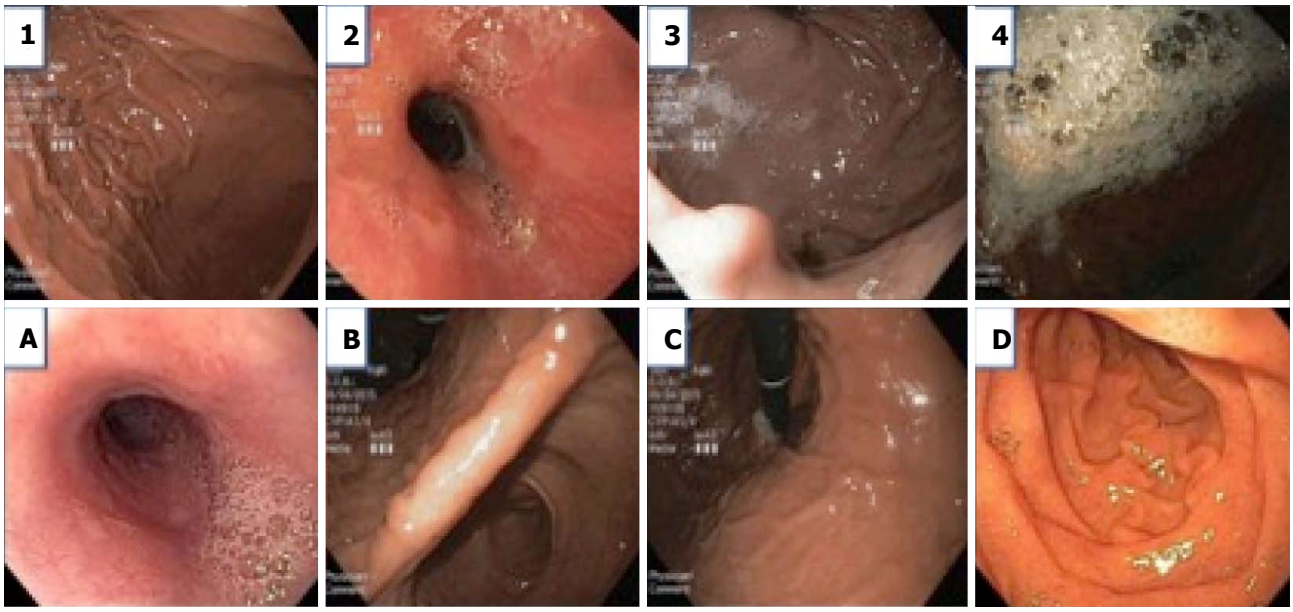

Figure 1 Endoscopic scoring system. Score of 1: No bubbles; Score of 2: Minimal bubbles which the endoscopist must actively look for; Score of 3: Foam is obviously present but not severe; Score of 4: Severe foam obscuring vision; Area A: Esophagus; Area B: The antrum and angularis of the stomach; Area C: The body and fundus of the stomach; Area D: Duodenum. TMVS is the sum of the scores of areas A, B, C and D added together. TMVS: Total mucosal visibility score.

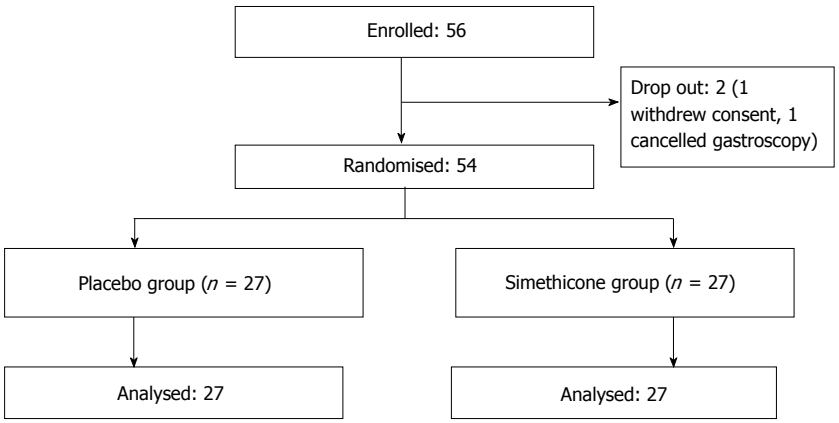

Figure 2 Workflow of patient enrollment.

time (Table 1). Six experienced endoscopists conducted the gastroscopy for all the patients, 4 of the endoscopists were involved in both groups (Figure 3). Before the study, all endoscopists separately scored the TMVS for the endoscopic images of four anonymous participants. They were blinded to the treatment allocation and patient particulars. The scores were tallied by an independent investigator to determine the interobserver agreement which was found satisfactory with a kappa statistic of 0.73.

The mean TMVS was significantly lower in the simethicone group compared to the placebo group (5.78 ± 1.65 for simethicone group, 8.89 ± 1.97 for placebo group, $P < 0.001$). This improvement in mucosal visibility score was significant for the areas of the stomach (body, fundus, antrum, and angularis) and the duodenum. However, simethicone premedication did not significantly improve mucosal visibility score of the esophagus. The simethicone group also had a shorter mean procedure time ($P = 0.049$) as well as lower mean volume of additional flushes required during gastroscopy ($P < 0.001$) (Table 2).

In the simethicone group, 51.9% of the patients had TMVS of 4 (no bubbles in all areas inspected) to 5 (one area had minimal bubbles), whereas in the placebo group only 3.7% of the patients achieved this ($P < 0.001$) (Figure 4). The number needed to treat (NNT) was 2.1 to avoid a TMVS of 6 or more.

DISCUSSION

To our knowledge, this is the first study conducted which evaluated the benefit of a very low volume of simethicone monotherapy for gastroscopy preparation, which was compared against a placebo using the McNally scoring method. Prior studies had shown that larger volumes produced better results and a 100 mL solution was generally accepted as the best. Bertoni *et al*[10] showed in their study that 90 mL solutions were superior to 30 mL solutions when ingested 5 min before the start of

Table 1 Baseline characteristics of the patients *n* (%)

	Placebo group (*n* = 27)	Simethicone group (*n* = 27)	*P* vaule
Mean age (yr ± SD)	52.9 ± 15.5	57.7 ± 12.5	0.215
Male gender (%)	9 (33.3)	15 (55.6)	0.1
Mean premedication time (min:s ± SD)	41:08 ± 9:56	44:46 ± 12:36	0.245
Indication for gastroscopy			
Dyspepsia	19 (70.4)	10 (37.0)	0.014
Reflux symptoms	3 (11.1)	6 (22.2)	0.467
Positive *H. pylori* serology	0	1 (3.7)	1
Variceal screen	1 (3.7)	2 (7.4)	1
Anemia	4 (14.8)	3 (11.1)	1
Dysphagia	0	1 (3.7)	1
Intestinal metaplasia surveillance	0	3 (11.1)	0.236
Cancer surveillance after endoscopic mucosal resection	0	1	1

H. pylori: *Helicobacter pylori*.

Table 2 Study results

	Placebo group	Simethicone group	*P* value
TMVS ± SD	8.89 ± 1.97	5.78 ± 1.65	< 0.001
Mean esophagus score ± SD	1.59 ± 0.57	1.48 ± 0.57	0.482
Mean duodenum score ± SD	2.26 ± 0.81	1.26 ± 0.53	< 0.001
Mean antrum and angularis score ± SD	2.56 ± 1.05	1.30 ± 0.54	< 0.001
Mean body and fundus score ± SD	2.44 ± 0.97	1.74 ± 0.81	0.006
Mean volume of additional water flushes required ± SD (mL)	84.81 ± 110.18	3.89 ± 11.46	< 0.001
Procedure time ± SD (s)	193.67 ± 87.04	154.85 ± 49.07	0.049

TMVS: Total mucosal visibility score.

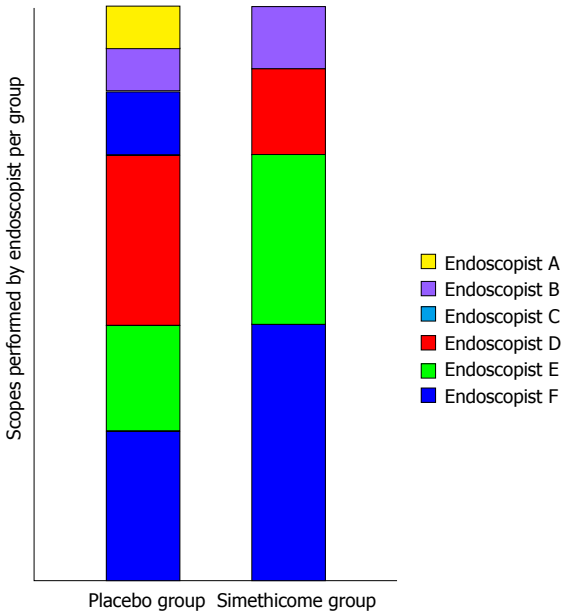

Figure 3 Endoscopist contribution per group.

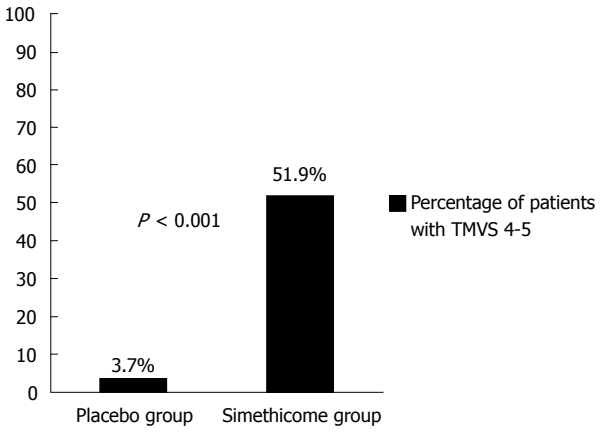

Figure 4 Percentage of patients with total mucosal visibility score of 4-5 during gastroscopy. TMVS: Total mucosal visibility score.

gastroscopy. Chang *et al*[6] showed in their recent study that when ingested within 30 min before gastroscopy, their 100 mL solution consisting of mucolytic and anti-foaming agent resulted in the best mucosal visibility scores. In this study, the premedication time was increased significantly (mean premedication times were 41:08 ± 9:56 min for placebo group and 44:46 ± 12:36 min for simethicone group), which allowed mixing of the simethicone with gastric secretion and swallowed saliva to coat the mucosal surface. This resulted in significant improvement of TMVS compared to placebo (Figures 5 and 6). Although the improvement in mucosal visibility scores was not significant for the esophageal area, the mean scores for the esophageal area were already very low to begin with (1.48 ± 0.57 in the simethicone group and 1.59 ± 0.57 in the placebo group). We postulated that this was because of the tubular structure of the esophagus as well as the peristaltic movements of the esophagus allowing mucus and secretions to flow down into the stomach. In additional, our study population is made up of healthy patients who were predominantly undergoing gastroscopy for dyspepsia; only 1 patient had dysphagia and 9 patients had reflux symptoms. This may result in the study population having a better mucosal visibility score in the esophageal area at baseline and explain why low volume simethicone solution did not make much of a difference. There was also significantly lower volume of additional flushes required during gastroscopy if simethicone was given. This, in turn, resulted in a significantly shorter procedure time for mucosal inspection (Figure 7). The ideal TMVS was 4 with absolutely no bubbles in all areas. In our study, the NNT to achieve a TMVS of 4 to 5 during gastroscopy was just 2.1.

Our study had two main limitations. Firstly, the study was not powered to investigate for the improvement of gastric lesion detection with enhanced endoscopy techniques. Our study participants were all recruited from the clinics for elective gastroscopies and the findings

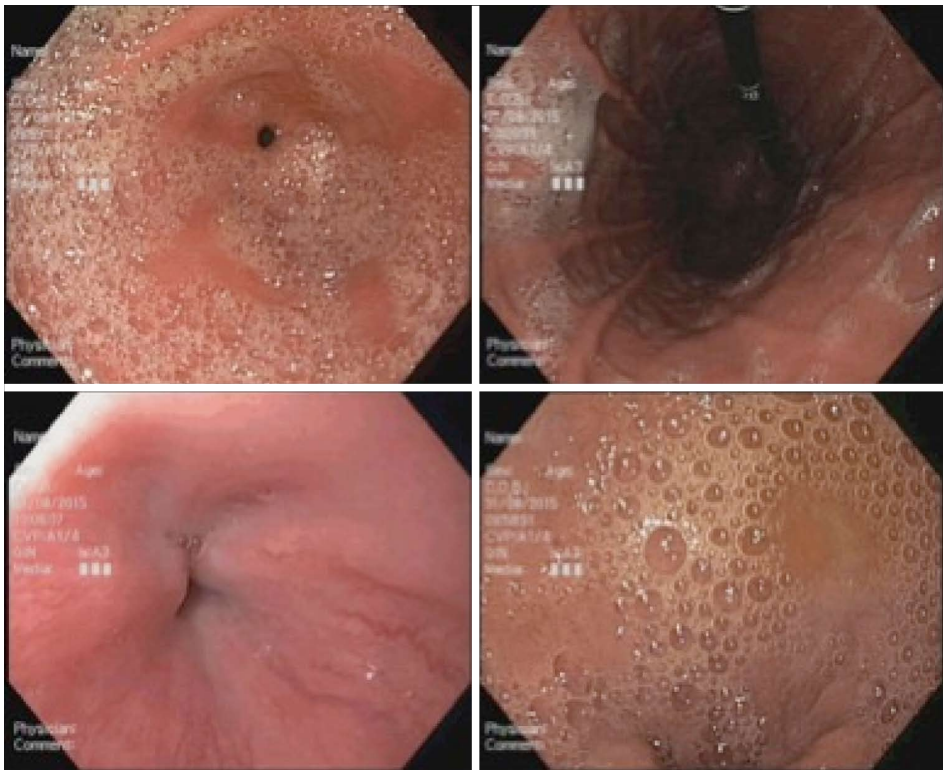

Figure 5 Endoscopic images of a patient in the placebo group (total mucosal visibility score 13).

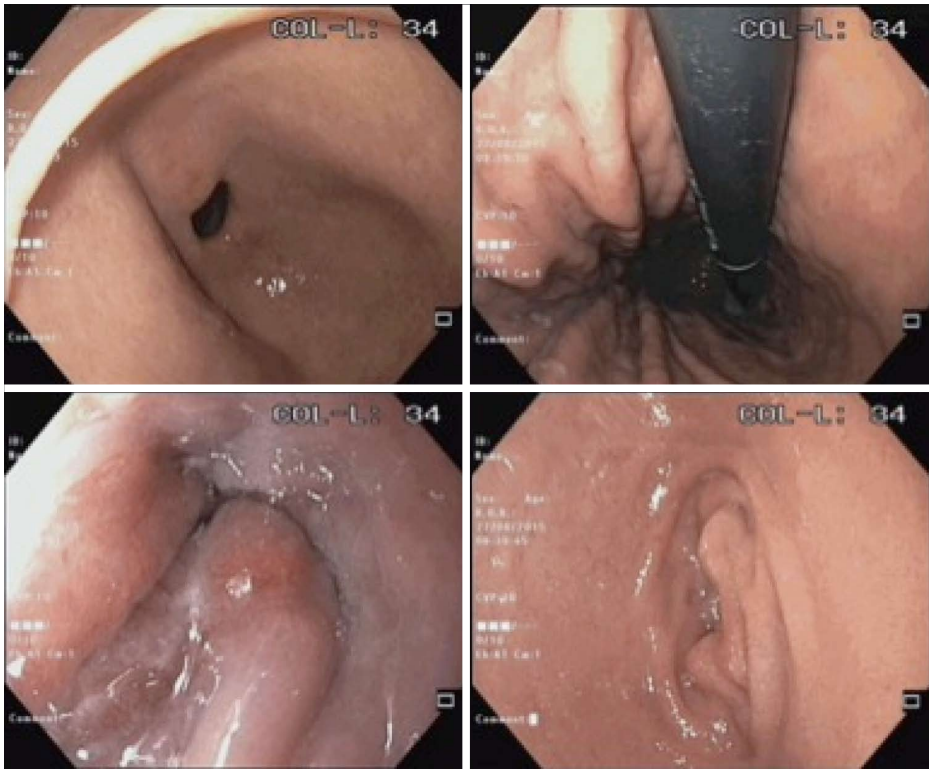

Figure 6 Endoscopic images of a patient in the simethicone group (total mucosal visibility score 4).

were predominantly benign functional dyspepsia or non-erosive gastritis which did not require further enhanced imaging as determined by the endoscopist. In order to investigate this aspect, we would have required a much larger study population which will likely require a multi-center collaboration. Secondly, we did not investigate the additional benefit of adding mucolytics to the regime which many other studies had done. This is because N-acetylcysteine is not readily available (it requires prior prescription and collection from the pharmacy by the patient) whereas simethicone is stored at all endoscopy suites and we wanted to find a convenient premedication regime that our endoscopists will be comfortable using.

None of the patients had any adverse event. As the volume of premedication required is only 5 to 6 mL in total, this is likely suitable for use in patients with

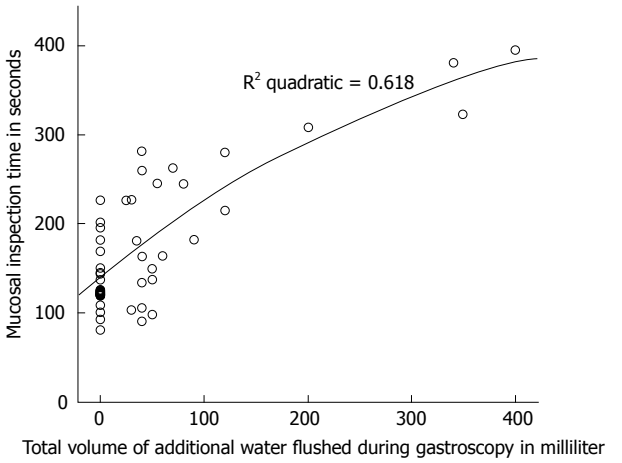

Figure 7 Correlation between volume of additional water flushes during gastroscopy (mL) and total mucosal inspection time (s).

swallowing dysfunction as such volumes are routinely used as modified water swallowing tests swallowing test[13].

ACKNOWLEDGMENTS

Ms Nway Nway Aye, department research coordinator.

COMMENTS

Background

Numerous studies have used various formulations before gastroscopy to decrease gastric foam or gastric mucus, with the overall conclusion that a larger volume with combined therapy is more effective.

Research frontiers

The potential benefit of extending premedication time has been evaluated in earlier studies with varying results.

Innovations and breakthroughs

This is the first randomized controlled study to evaluate the efficacy of a small volume simethicone solution (as compared to placebo with water) in the context of a premedication time of at least 30 min. It shows that despite earlier studies favoring combination therapy with larger volumes, a longer premedication time with a small volume monotherapy can significantly improve mucosal visibility scores. The study sample was calculated using the standard deviation from the results of a study with the same McNally scoring system. The study results offer a convenient and effective premedication that requires no additional prescription in Singapore and will likely be tolerated by patients with swallowing dysfunction.

Applications

This study's finding has resulted in the standardised use of simethicone premedication at Changi General Hospital endoscopy center prior to elective gastroscopies in the low volume formulation.

Peer-review

It is a good practical idea. They think the importance of search could be more applicable if the study done for enteroscopy not upper endoscopy. The paper is well written, well organised.

REFERENCES

1 **Song M,** Ang TL. Early detection of early gastric cancer using image-enhanced endoscopy: Current trends. *Gastrointest Inter* 2014; **3**: 1-7 [DOI: 10.1016/j.gii.2014.02.005]
2 **Singapore Cancer Registry 2015.** Cancer Survival in Singapore 1973-2012. USA: National Registry of Diseases Office, 2015
3 **Cha JM**, Won KY, Chung IK, Kim GH, Lee SY, Cho YK. Effect of pronase premedication on narrow-band imaging endoscopy in patients with precancerous conditions of stomach. *Dig Dis Sci* 2014; **59**: 2735-2741 [PMID: 24861034 DOI: 10.1007/s10620-014-3218-z]
4 **Kuo CH**, Sheu BS, Kao AW, Wu CH, Chuang CH. A defoaming agent should be used with pronase premedication to improve visibility in upper gastrointestinal endoscopy. *Endoscopy* 2002; **34**: 531-534 [PMID: 12170403 DOI: 10.1055/s-2002-33220]
5 **Lee GJ**, Park SJ, Kim SJ, Kim HH, Park MI, Moon W. Effectiveness of Premedication with Pronase for Visualization of the Mucosa during Endoscopy: A Randomized, Controlled Trial. *Clin Endosc* 2012; **45**: 161-164 [PMID: 22866258 DOI: 10.5946/ce.2012.45.2.161]
6 **Chang CC**, Chen SH, Lin CP, Hsieh CR, Lou HY, Suk FM, Pan S, Wu MS, Chen JN, Chen YF. Premedication with pronase or N-acetylcysteine improves visibility during gastroendoscopy: an endoscopist-blinded, prospective, randomized study. *World J Gastroenterol* 2007; **13**: 444-447 [PMID: 17230616 DOI: 10.3748/wjg.v13.i3.444]
7 **Neale JR**, James S, Callaghan J, Patel P. Premedication with N-acetylcysteine and simethicone improves mucosal visualization during gastroscopy: a randomized, controlled, endoscopist-blinded study. *Eur J Gastroenterol Hepatol* 2013; **25**: 778-783 [PMID: 23739279 DOI: 10.1097/MEG.0b013e32836076b2]
8 **Chen MJ**, Wang HY, Chang CW, Hu KC, Hung CY, Chen CJ, Shih SC.. The add-on N-acetylcysteine is more effective than dimethicone alone to eliminate mucus during narrow-band imaging endoscopy: a double-blind, randomized controlled trial. *Scand J Gastroenterol* 2013; **48**: 241-245 [PMID: 23268593 DOI: 10.3109/00365521.2012.749509]
9 **Asl SM**, Sivandzadeh GR. Efficacy of premedication with activated Dimethicone or N-acetylcysteine in improving visibility during upper endoscopy. *World J Gastroenterol* 2011; **17**: 4213-4217 [PMID: 22072853 DOI: 10.3748/wjg.v17.i37.4213]
10 **Bertoni G**, Gumina C, Conigliaro R, Ricci E, Staffetti J, Mortilla MG, Pacchione D. Randomized placebo-controlled trial of oral liquid simethicone prior to upper gastrointestinal endoscopy. *Endoscopy* 1992; **24**: 268-270 [PMID: 1612040 DOI: 10.1055/s-2007-1010479]
11 **Chang WK**, Yeh MK, Hsu HC, Chen HW, Hu MK. Efficacy of simethicone and N-acetylcysteine as premedication in improving visibility during upper endoscopy. *J Gastroenterol Hepatol* 2014; **29**: 769-774 [PMID: 24325147 DOI: 10.1111/jgh.12487]
12 **National Population and Talent Division**, Prime Minister's Office, Singapore Department of Statistics, Ministry of Home Affairs, Immigration and Checkpoints Authority. Population in Brief 2015. Singapore: National Population and Talent Division, Prime Minister's Office, Singapore Department of Statistics, Ministry of Home Affairs, Immigration and Checkpoints Authority, 2015
13 **Osawa A**, Maeshima S, Tanahashi N. Water-swallowing test: screening for aspiration in stroke patients. *Cerebrovasc Dis* 2013; **35**: 276-281 [PMID: 23548854 DOI: 10.1159/000348683]
14 **McNally PR**, Maydonovitch CL, Wong RK. The effectiveness of simethicone in improving visibility during colonoscopy: a double-blind randomized study. *Gastrointest Endosc* 1988; **34**: 255-258 [PMID: 3292345 DOI: 10.1016/S0016-5107(88)71324-3]
15 **Keeratichananont S**, Sobhonslidsuk A, Kitiyakara T, Achalanan N, Soonthornpun S. The role of liquid simethicone in enhancing endoscopic visibility prior to esophagogastroduodenoscopy (EGD): A prospective, randomized, double-blinded, placebo-controlled trial. *J Med Assoc Thai* 2010; **93**: 892-897 [PMID: 20718163]

P- Reviewer: De Silva AP, Shehata MMM, Yeoh SW, Zuo XL
S- Editor: Qi Y **L- Editor**: A **E- Editor**: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i4.579

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 579-583
ISSN 2150-5349 (online)

CASE REPORT

Osteonecrosis of both knees in a woman with Crohn's disease

Mara Barbosa, José Cotter

Mara Barbosa, José Cotter, Department of Gastroenterology, Hospital da Senhora da Oliveira, Rua dos Cutileiros, Creixomil, 4835-044 Guimarães, Portugal

Author contributions: The authors made equal contributions to the study; Barbosa M drafted the manuscript; Cotter J critically reviewed the manuscript.

Institutional review board statement: This study was reviewed and approved by Hospital da Senhora da Oliveira Institutional Review Board.

Informed consent statement: Written informed consent was obtained from the patient described in this case report.

Conflict-of-interest statement: Mara Barbosa and José Cotter certify that they have no conflit-of-interest.

Manuscript source: Unsolicited manuscript

Correspondence to: Mara Barbosa, MD, Department of Gastroenterology, Hospital da Senhora da Oliveira, Creixomil, 4835-044 Guimarães, Portugal. maraisabelbarbosa@net.sapo.pt
Telephone: +351-933-112632
Fax: +351-253-513592

Received: April 11, 2016
Peer-review started: April 13, 2016
First decision: July 20, 2016
Revised: August 7, 2016
Accepted: September 13, 2016
Article in press: September 15, 2016
Published online: November 6, 2016

Abstract

Osteonecrosis is a very rare complication of Crohn's disease (CD). It is not clear if it is related to corticosteroid therapy or if it occurs as an extraintestinal manifestation of inflammatory bowel disease. We present the case of a patient with CD who presented with osteonecrosis of both knees. A 22 years old woman was diagnosed with CD in April 2012 (Montreal Classification A2L1 + L4B3p). She was started on prednisolone (40 mg/d), azathioprine (100 mg/d) and messalazine (3 g/d). In July 2012, due to active perianal disease, infliximab therapy was initiated. In September 2012, she had a pelvic abscess complicated by peritonitis and an ileal segmental resection and right hemicolectomy were performed. In December 2012 she was diagnosed with bilateral septic arthritis of both knees with walking impairment. She was treated with amoxicillin-clavulanic acid, started a physical rehabilitation program and progressively improved. However, then, bilateral knee pain exacerbated by movement developed. Magnetic resonance imaging showed multiple osseous medullary infarcts in the distal extremity of the femurs, proximal extremity of the tibiae and patellas and no signs of subchondral collapse, which is consistent with osteonecrosis. The patient recovered completely and maintains therapy with azathioprine and messalazine. A review of the literature is also done.

Key words: Osteonecrosis; Knee; Inflammatory bowel disease; Crohn's disease; Magnetic resonance imaging

Core tip: Although very rare, osteonecrosis is a devasting event that can occur in Crohn's disease (CD). We present the case of a 22 years old woman with CD who was diagnosed with osteonecrosis of both knees. As we demonstrate with this report, awareness of risk factors, such as corticosteroid therapy and inflammatory bowel

disease activity, is crucial to establish the diagnosis of this inflammatory bowel disease rheumatological complication. Prompt treatment is recommended. A review of the literature is also presented.

Barbosa M, Cotter J. Osteonecrosis of both knees in a woman with Crohn's disease. *World J Gastrointest Pharmacol Ther* 2016; 7(4): 579-583 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i4/579.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i4.579

INTRODUCTION

Osteonecrosis or avascular necrosis is defined as cellular death of bone components due to interruption of blood supply. Consequently, there is a collapse and destruction of articular surfaces, pain and disability[1]. Epiphysis of long bones (femoral and humeral heads and femoral condyles) are primarily involved, with the hip being the most commonly affected joint. Several clinical entities (connective tissue disorders, hemoglobinopathies, coagulation disorders, pregnancy, alcohol abuse, inflammatory bowel diseases (IBD) and corticosteroid use) have been associated with osteonecrosis, but its pathophysiology is not completely understood[2]. The true incidence of this rare manifestation in IBD is not known[1]. It has been reported to range from 0.5% to 4.3%[3]. We present the case of a 22 years old woman with Crohn's disease (CD) who was diagnosed with osteonecrosis of both knees.

CASE REPORT

A 22 years old woman was diagnosed with CD in April 2012 (Montreal Classification A2L1 + L4B3p - diagnosis at 22 years-old; ileal plus jejunal involvement; penetrant behavior and perianal disease - rectovulvar fistulae). She was initially treated with prednisolone (40 mg/d), azathioprine (100 mg corresponding to 2 mg/kg per day) and messalazine (3 g/d). In July 2012, due to fistulae non-healing, a seton was placed and infliximab therapy was started (three infusions - 0, 2 and 6 wk - 5 mg/kg). Complete closure of the rectovulvar fistulae was then confirmed. In September 2012, she had had a pelvic abscess complicated by peritonitis and she was operated. Drainage of the abscess, ileal segmental resection and right hemicolectomy was performed. From April 2012 to December 2012 a gradual weaning of corticosteroid therapy was done. In December 2002 she presented with fever, intense pain, swelling and stiffness of both knees and impaired range of motion for six weeks. Bilateral articular effusions were observed. She got bedridden. There was no history of arthritis. Laboratory studies revealed a leucocytosis with neutrophilia (17.000/mm^3 per 89%) and an elevated erythrocyte sedimentation rate (28 mm^3 per hour). Bilateral arthrocentesis was performed with diagnostic and drainage intent. Synovial fluid was purulent. Culture of the synovial fluid was positive for *S. pneumoniae.* Amoxicillin plus clavulanic acid and analgesia (acetaminophen and tramadol) was begun. Bilateral arthrotomy of knees with biopsy of the synovium was performed. The histological examination of the synovial tissue revealed synoviocyte hyperplasia, inflammatory infiltrate, mainly composed by polymorphonuclear neutrophils, and purulent exudates; these findings were consistent with the diagnosis of bilateral septic arthritis. There was no exacerbation of intestinal symptoms of CD. After an initial period of immobilization, she was started on a physical rehabilitation program and progressively improved: Inflammatory signs of knees disappeared and she started to walk with crutches. However, bilateral knee pain developed, exacerbated by movement, mainly at climbing stairs. Plain film radiographies of the knees demonstrated multiple bilateral hypotransparent areas in the distal extremity of the femurs, in the proximal extremity of the tibiae and in the patellas and also absence of signs of subchondral collapse (Figure 1). Computed tomography (CT) revealed multiple lacunar areas in the same localizations (Figure 2). Magnetic resonance imaging (MRI) showed a "geographic" pattern resulting from multiple osseous medullary infarcts in the distal 15 cm of the femurs, in the proximal 10 cm of the tibiae and in the patellas; there were also no signs of subchondral collapse (Figures 3-5). These imagiologic findings were consistent with the diagnosis of osteonecrosis. The total body radionuclide bone scan (methylene biphosphonate labeled with technetium^{-99m}) revealed an increased uptake of the agent in the distal ephiphysis of the femurs, in the proximal epiphysis of the tibiae and in the patellas; it also excluded other focus of the disease. A stage 2 of Association Research Circulation Osseous (ARCO) was established. The peripheral blood smear was normal. Lipid levels (cholesterol and triglycerides) were within normal range. Antinuclear antibody, rheumatoid factor, antismooth muscle antibody and antiphospholipid antibodies were negative. Procoagulant factors (C and S proteins, antithrombina Ⅲ and Ⅴ Leiden factor) were

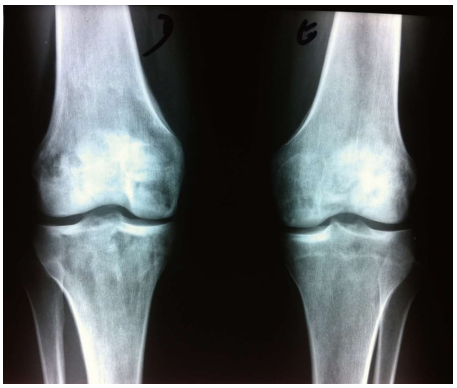

Figure 1 Plain film radiographs (bilateral knees) showing multiple bilateral hypotransparent areas.

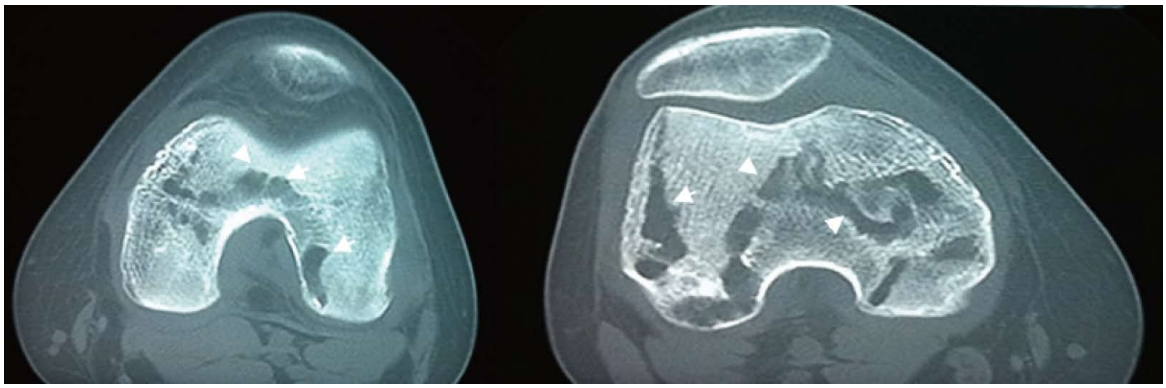

Figure 2 Computed tomography showing multiple lacunar areas in the femurs and patellas.

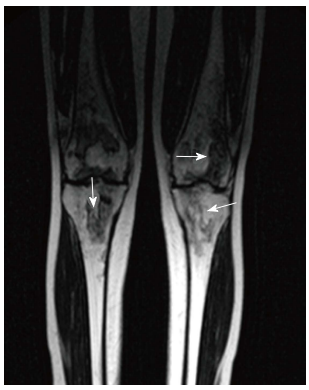

Figure 3 Magnetic resonance imaging (bilateral knees, T1-weighted images, coronal view) showing areas and serpiginous rims of low signal intensity in the femurs, tibiae and patellas, characteristic of osteonecrosis.

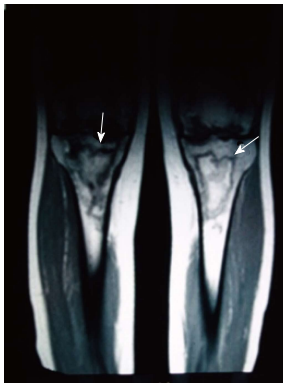

Figure 4 Magnetic resonance imaging (bilateral knees, T2-weighted images, coronal view) showing prominent medullary infarcts.

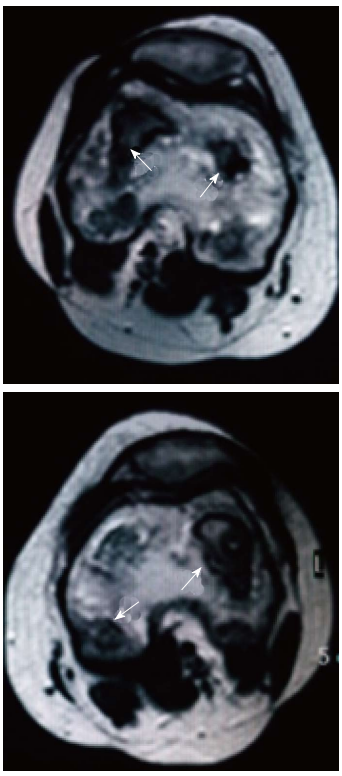

Figure 5 Magnetic resonance imaging (T2-weighted images, axial view) showing low signal serpiginous rims.

normal. The patient recovered completely and maintains therapy with azathioprine and messalazine.

DISCUSSION

The etiology and pathogenesis of osteonecrosis in IBD remain to be elucidated[3]. Some risk factors have been implicated, such as corticosteroid therapy[1,3,4] (systemic and topic) and disease activity so it can be considered an extra-intestinal manifestation of IBD[5,6]. Several studies report the occurrence of osteonecrosis in IBD patients either during or after corticosteroids use. Although initial data described a six to eight months period after initiating therapy for steroid-associated osteonecrosis to occur, this temporal relationship was not confirmed afterwards, with some other reports describing an erratic pattern of development[6]. No clear dose-response is established[1,3] but, corticosteroids doses are significantly higher in patients with osteonecrosis than in those without it[1,4,7]. Moreover, this complication may present with lower dosage of corticosteroids, comparing with other conditions[1]. All in all, a more precise association between corticosteroid use in IBD and osteonecrosis is still needed[3]. IBD patients can develop osteonecrosis unrelated to corticosteroid therapy. CD is associated with a hipercoagulable state, mainly during periods of active disease[5]; the fibrin microclots may lead to osteonecrosis

by occluding epiphyseal capillaries and limiting the blood supply to the bone. This predisposition to thrombosis supports the hypothesis that osteonecrosis might be a rheumatological condition associated with IBD, rather than a complication of its treatment. Several risk factors can be simultaneously present. Our patient needed a relatively prolonged course of corticosteroid therapy and CD was still not in complete remission before the development of osteonecrosis. In this particular case, the event of bilateral septic arthritis, almost certainly secondary to the immunossupression therapy (corticosteroids, azathioprine and infliximab), might also have contributed to its occurrence.

Pain in the affected joint, usually exacerbated by weight-bearing, is typically the presenting symptom, although patients can remain entirely asymptomatic. Despite being initially mild, pain progressively worsens over time, being present at rest, and a decrease range of motion results[1,8]. Early diagnosis is important as treatment might avert disease progression[2,9]. However, it may be challenging, as 4% to 17% of patients with CD have type 1 - pauci-articular arthropathy, in which large bearing weight joints (ankles, knees, hips, wrists, elbows and shoulders) are affected[10]. A high index of suspicion in patients with risk factors is therefore mandatory[1]. This patient had well established risk factors for osteonecrosis, the persistence of bilateral knee pain despite the disappearance of the other inflammatory signs led us to investigate the possibility of this bone complication.

Diagnosis can be made by plain film radiographs, radionuclide bone scan, CT, MRI or invasive techniques (bone marrow pressure, stress test with injection of saline, intramedullary venography, superselective angiography and bone biopsy), the later being reserved for selected cases. At present, the goldstandard for the diagnosis of osteonecrosis is MRI, as it can depict the earliest imagiologic changes of the disease, with the best sensitivity and the best accuracy (75%-100%) compared with the other methods[1,3]. MRI shows a decreased signal intensity in both T1- and T2-weighted images[1,8,11]. Plain film radiographs are usually initially unremarkable[1,8]; afterwards they can demonstrate cystic or sclerotic changes, subchondral fractures (the "crescent sign") and eventually secondary osteoarthritic changes[8]. Radionuclide bone scan using a bone-imaging agent (labeled with technetium-99m) is another helpful diagnostic imaging study in early stages of osteonecrosis[1,8], when plain films radiographs are normal or nearly normal. It is of utmost usefulness at screening, because bone scan can detect asymptomatic joint involvement[12]. However, it has the disadvantage of being non-specific, except when it shows a central area of decreased uptake surrounded by an area of increased uptake[8]. CT is a good technique at evaluating disease extension[8]. IBD patients tend to present with multifocal osteonecrosis[1,4]. Histology is the definitive method for the diagnosis of osteonecrosis, although it is usually unnecessary. Histological changes are encountered in both cortical bone and bone marrow. Necrosis of bone tissue (disappearance of the osteocytes) is followed by a regenerative process in surrounding tissues. Bone marrow lesions include edema, hemorrhage, fibrilloreticulosis, hipocellularity, necrosis of hematopoietic cells and replacement of adipocytes by eosinophilic debris[8]. The most commonly accepted classification system to stage osteonecrosis was devised by the ARCO. It encompasses 4 stages. The first one, stage 0, is defined as the presence of histological changes without any associated clinical signs or symptoms. In the last one, stage 4, there is evidence of progression to osteoarthritis (joint space narrowing and complete joint destruction)[13]. In our case, diagnosis was made by plain film radiographs, CT and MRI. A stage 2 of ARCO was established. In order to screen other localizations for osteonecrosis, a radionuclide bone scan was undertaken, which did not reveal other foci of the disease. No underlying analytical risk factor of any type (including any thrombophilic disorders) was found.

Management depends on the location and severity of joint involvement[1,8]. Conservative treatment includes restriction of weight-bearing on the affected joint or even immobilization, strengthening of the muscles surrounding the affected bone and analgesia[1,8]. No drug treatment has proven effective in averting disease progression, although bisphosphonates have shown some promise[14]. Surgical approaches include arthroplasty, core decompression, osteotomies and non-vascularized and vascularized bone grafting. In advanced cases, following subchondral collapse, total arthroplasty is the main surgical solution, although failure rates in patients with osteonecrosis are significantly higher in comparison with other conditions[1,8].

Conservative management was successful in our patient. She resumed walking without crutches and normal daily activities a few months later.

Regarding prevention, whenever possible, steroid-sparing agents should be the first option[8]. With this in mind, our patient was maintained on azathioprine and on anti-TNF therapy. If corticosteroid treatment is deemed necessary, it should be kept to the minimum effective dosage and patients may be offered a statin, as there is some evidence that it decreases the incidence of osteonecrosis in patients receiving high-dose steroids[15]. Moreover, hyperlipidemia and diabetes should be treated and alcohol ingestion avoided.

In conclusion, although very rare, osteonecrosis is a devasting event that can occur in CD. As we demonstrate with this report, awareness of risk factors, such as corticosteroid therapy and inflammatory bowel disease activity, is crucial to make the diagnosis of this rheumatological IBD complication. Prompt treatment is recommended.

COMMENTS

Case characteristics

A 22 years old woman with active Crohn's disease (CD) treated with prednisolone, messalazine, azathioprine and infliximab presented with bilateral

knee pain exacerbated by movement, after an episode of bilateral septic arthritis of both knees.

Clinical diagnosis
On clinical examination, bilateral knee pain aggravated by movement and weight-bearing was observed.

Differential diagnosis
Another causes of osteonecrosis were excluded, such as: Systemic lupus erythematosus (with or without antiphospholipid syndrome), as well as other connective-tissue diseases, hematological diseases (sickle cell disease, hemoglobinopathies), hyperlipidemia.

Laboratory diagnosis
The peripheral blood smear was normal. Lipid levels (cholesterol and triglycerides) were within normal range. Antinuclear antibody, rheumatoid factor, antismooth muscle antibody and antiphospholipid antibodies were negative. Procoagulant factors (C and S proteins, antithrombina Ⅲ and Ⅴ Leiden factor) were normal.

Imaging diagnosis
Plain film radiographies of the knees demonstrated multiple bilateral hypotransparent areas in the distal extremity of the femurs, in the proximal extremity of the tibiae and in the patellas and also absence of signs of subchondral collapse. Computed tomography revealed multiple lacunar areas in the same localizations. Magnetic resonance imaging showed a "geographic" pattern resulting from multiple osseous medullary infarcts in the distal 15 cm of the femurs, in the proximal 10 cm of the tibiae and in the patellas; there were also no signs of subchondral collapse. These imagiologic findings were consistent with the diagnosis of osteonecrosis.

Pathological diagnosis
Histological examination of both cortical bone and bone marrow was not performed because imagiological findings showed typical findings of osteonecrosis.

Treatment
A conservative strategy was adopted. After an initial period of immobilization and restriction of weight-bearing with the use of crutches, the patient was started on a rehabilitation programme. The patient recovered completely and maintains therapy with azathioprine and messalazine.

Related reports
There are few case reports in the literature of osteonecrosis in inflammatory bowel disease (IBD). The description of involvement of both knees is exceedingly rare.

Term explanation
All terms in this case report are standard and used in the field of gastroenterology.

Experiences and lessons
Although very rare, osteonecrosis is a devasting event that can occur in CD. As they demonstrate with this report, awareness of risk factors, such as corticosteroid therapy and IBD activity, is crucial to make the diagnosis of this rheumatological IBD complication. Prompt treatment is recommended.

Peer-review
This case report demonstrates very well the occurrence of osteonecrosis in the setting of inflammatory.

REFERENCES

1 **Klingenstein G**, Levy RN, Kornbluth A, Shah AK, Present DH. Inflammatory bowel disease related osteonecrosis: report of a large series with a review of the literature. *Aliment Pharmacol Ther* 2005; **21**: 243-249 [PMID: 15691298 DOI: 10.1111/j.1365-2036.2005.02231.x]

2 **Assouline-Dayan Y**, Chang C, Greenspan A, Shoenfeld Y, Gershwin ME. Pathogenesis and natural history of osteonecrosis. *Semin Arthritis Rheum* 2002; **32**: 94-124 [PMID: 12430099 DOI: 10.1053/sarh.2002.33724b]

3 **Freeman HJ**, Freeman KJ. Prevalence rates and an evaluation of reported risk factors for osteonecrosis (avascular necrosis) in Crohn's disease. *Can J Gastroenterol* 2000; **14**: 138-143 [PMID: 10694287 DOI: 10.1155/2000/958086]

4 **Hauzeur JP**, Malaise M, Gangji V. Osteonecrosis in inflammatory bowel diseases: a review of the literature. *Acta Gastroenterol Belg* 2009; **72**: 327-334 [PMID: 19902866]

5 **Khan A**, Illiffe G, Houston DS, Bernstein CN. Osteonecrosis in a patient with Crohn's disease unrelated to corticosteroid use. *Can J Gastroenterol* 2001; **15**: 765-768 [PMID: 11727007 DOI: 10.1155/2001/293059]

6 **Lanyi B**, Dienes HP, Kruis W. [Recurrent aseptic osteonecrosis in Crohn's disease - extraintestinal manifestation or steroid related complication?]. *Dtsch Med Wochenschr* 2005; **130**: 1944-1947 [PMID: 16123897 DOI: 10.1055/s-2005-872607]

7 **Kamata N**, Oshitani N, Sogawa M, Yamagami H, Watanabe K, Fujiwara Y, Arakawa T. Usefulness of magnetic resonance imaging for detection of asymptomatic osteonecrosis of the femoral head in patients with inflammatory bowel disease on long-term corticosteroid treatment. *Scand J Gastroenterol* 2008; **43**: 308-313 [PMID: 18938768 DOI: 10.1080/00365520701676773]

8 **Hochberg M**. Rheumatology. 4th edition. Londres. USA: Mosby Elsevier, 2008

9 **Madsen PV**, Andersen G. Multifocal osteonecrosis related to steroid treatment in a patient with ulcerative colitis. *Gut* 1994; **35**: 132-134 [PMID: 8307435 DOI: 10.1136/gut.35.1.132]

10 **Van Assche G**, Dignass A, Reinisch W, van der Woude CJ, Sturm A, De Vos M, Guslandi M, Oldenburg B, Dotan I, Marteau P, Ardizzone A, Baumgart DC, D'Haens G, Gionchetti P, Portela F, Vucelic B, Söderholm J, Escher J, Koletzko S, Kolho KL, Lukas M, Mottet C, Tilg H, Vermeire S, Carbonnel F, Cole A, Novacek G, Reinshagen M, Tsianos E, Herrlinger K, Oldenburg B, Bouhnik Y, Kiesslich R, Stange E, Travis S, Lindsay J. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Special situations. *J Crohns Colitis* 2010; **4**: 63-101 [PMID: 21122490 DOI: 10.1016/j.crohns.2009.09.009]

11 **Malizos KN**, Karantanas AH, Varitimidis SE, Dailiana ZH, Bargiotas K, Maris T. Osteonecrosis of the femoral head: etiology, imaging and treatment. *Eur J Radiol* 2007; **63**: 16-28 [PMID: 17555906 DOI: 10.1016/j.ejrad.2007.03.019]

12 **Sakai T**, Sugano N, Nishii T, Haraguchi K, Yoshikawa H, Ohzono K. Bone scintigraphy for osteonecrosis of the knee in patients with non-traumatic osteonecrosis of the femoral head: comparison with magnetic resonance imaging. *Ann Rheum Dis* 2001; **60**: 14-20 [PMID: 11114276 DOI: 10.1136/ard.60.1.14]

13 **Gardeniers JM**. ARCO international classification of osteonecrosis. *ARCO Newsletter* 1993; **5**: 79-82

14 **Lai KA**, Shen WJ, Yang CY, Shao CJ, Hsu JT, Lin RM. The use of alendronate to prevent early collapse of the femoral head in patients with nontraumatic osteonecrosis. A randomized clinical study. *J Bone Joint Surg Am* 2005; **87**: 2155-2159 [PMID: 16203877 DOI: 10.2106/JBJS.D.02959]

15 **Pritchett JW**. Statin therapy decreases the risk of osteonecrosis in patients receiving steroids. *Clin Orthop Relat Res* 2001; **(386)**: 173-178 [PMID: 11347831 DOI: 10.1097/00003086-200105000-00022]

P- Reviewer: Maric I **S- Editor**: Gong ZM **L- Editor**: A
E- Editor: Lu YJ

World Journal of Gastrointestinal Pharmacology and Therapeutics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4292/wjgpt.v7.i4.584

World J Gastrointest Pharmacol Ther 2016 November 6; 7(4): 584-586
ISSN 2150-5349 (online)

LETTERS TO THE EDITOR

Gastroenterology, hepatology and movies: A holistic insight

Guilherme Macedo, Marco Silva

Guilherme Macedo, Marco Silva, Gastroenterology Department, Centro Hospitalar São João, Faculty of Medicine of the University of Porto, 4200-319 Porto, Portugal

Author contributions: Macedo G contributed to study concept and design and drafting of the manuscript; Silva M contributed to drafting of the manuscript.

Conflict-of-interest statement: Nothing to declare.

Manuscript source: Unsolicited manuscript

Correspondence to: Guilherme Macedo, MD, PhD, WGO, FACG, AGAF, FAASLD, Former President of the Portuguese Association For the Study of the Liver, Gastroenterology Department, Centro Hospitalar São João, Faculty of Medicine of the University of Porto, Alameda Professor Hernâni Monteiro, 4200-319 Porto, Portugal. guilhermemacedo59@gmail.com
Telephone: +351-225-512100
Fax: +351-225-025766

Received: April 29, 2016
Peer-review started: April 29, 2016
First decision: July 5, 2016
Revised: September 5, 2016
Accepted: September 21, 2016
Article in press: September 23, 2016
Published online: November 6, 2016

Abstract

The Project "Movies and Health in Night talks" took place in Braga and Porto, northern Portugal, in the last 3 years. This Project demonstrated how medical knowledge may surround and integrate a cosmopolitan and holistic approach, so that we as doctors and the general public, are able to become much closer and much more prone to understand the vital cycles of our society.

Key words: Cinema; Liver; Gastroenterology; Movies; Public

Core tip: The Project "Movies and Health in Night talks", conceived and produced through the chisel of a Gastroenterologist, clearly demonstrated how medical knowledge may surround and integrate a cosmopolitan and holistic approach, so that we as doctors and the general public, are able to become much closer and much more prone to understand the vital cycles of our society. Throughout those lively nights, many brilliant remarks were brought up, unexpected comments, unengaged points of view largely discussed, almost in a libertarian atmosphere, addressing the main topics that different experts and public figures were invited to dissect, about some of the most emblematic movies from the last decades. It is our firm believe that one of Hepatologists still unexplored noble tasks is to promote an anthropologic way of addressing and solving gastrointestinal and liver diseases burden.

Macedo G, Silva M. Gastroenterology, hepatology and movies: A holistic insight. *World J Gastrointest Pharmacol Ther* 2016; 7(4): 584-586 Available from: URL: http://www.wjgnet.com/2150-5349/full/v7/i4/584.htm DOI: http://dx.doi.org/10.4292/wjgpt.v7.i4.584

TO THE EDITOR

A Cinema is many times defined as the art of synthesis and the art of dialectic composition of several other artistic expressions like literature, painting or music. It is

in fact the creative spring for dreams and fascination, an instrument of emotional and sometimes terror catharsis, and undoubtedly the source of social allegories. It mirrors the most extraordinary biographies, individual and collective paths, in a multitude of visions casted by the most intensive joys and sorrows. In its essence, it is capable of generating or amplify new myths and new tales, many of our civilization's poetic fancies. Essentially, it is able to enlighten new symbols, and elect new icons as models of behaviours, even promoting ritual gestures and worships; in this way, movies performers transfigure themselves into vectors of freedom or oppression, if they help to release our minds or if they condition our trails: In other words, they bear the responsibility of shaping Health and Disease in our civilization[1-3].

In fact, these are the characters that Movie Industry thrusted into our own behavioural genoma, imprinting new coordinates for individual and social references, interfering and moulding up our own values and projects.

In Politics, in Sports, in Music and Arts, among the Media, how much science and art closely live together, so that we are able to recreate our own idols, myths and symbols? What life examples do we intimately favour and how does it effect in our expectations of individual and collective Health[1,4]?

These were the inferred questions and the explicit philosophy underlying the Project "Movies and Health in Night talks" which took place in several weeks cycles, in the last 3 years in Braga and Porto, northern Portugal. This Project, conceived and produced through the chisel of a Gastroenterologist, clearly demonstrated how medical knowledge may surround and integrate a cosmopolitan and holistic approach, so that we as doctors and the general public, are able to become much closer and much more prone to understand the vital cycles of our society. Throughout those lively nights, many brilliant remarks were brought up, unexpected comments, unengaged points of view largely discussed, almost in a libertarian atmosphere, addressing the main topics that different experts and public figures were invited to dissect, about some of the most emblematic movies from the last decades.

Imagine the thrills: Before a live audience of around 100 people, in an auditorium prepared as if a movie was to be projected, but in a stage specifically arranged as if a cosy literary assembly would happen, a 2 h interview was anchored and lead by a Gastroenterologist, trying to set the pace of a most dynamic and at times provocative talk with well-known guests. Not in a medical meeting, or in a big specialty convention, but facing the lay public and even some media gurus! This was really a hard task and a brave new world to the Gastroenterologist, having to play a true pivotal task (away from his technological comfort zone…), in orienting and exploring the visions and perceptions of his 2 special guest stars. These guest stars changed every week, depending on the movie to be dissected, and consisted of widely known Portuguese artists, tv and radio public figures, sports people, and newsmen. On stage, in a TV like interview format, they expanded over the selected movie for that night. After presenting and viewing the initial trailer, the discussion was based on 4 takes of 6-8 min each; those takes were previously selected and edited by the anchor and really set the stage for a highly informal and free vivid discussion, changing points of view, also allowing handfuls of wise and bright references, sprinkled with personal experiences, funny, intimate, carefully and attentively followed by the audience. How did it work? The first part of the crosstalk, immediately after the trailer presentation, was very useful to place the topic, to put into context the subject to be addressed, focusing on the movie maker, actor's performance, interesting backgrounds and so on. Then, in a 15 min discussion after viewing the selected take, our guest stars were challenged to elaborate on their own thoughts and perspectives, and here again, the role of moderation was crucial, so that the scope has to be kept away from the initiatic and almost inexpugnable medical jargon, but at the same time health concerns should be brought under the spotlight, allowing everyone to understand and realize how extraordinarily common gastrointestinal and liver problems come along with personal decisions and behaviours or social changes.

Under the title "Cults, Vices and Fashions", which accomplished the first year's project, the selected movies were: Pollock (Ed Harris 2000), The People *vs* Harry Flynt (Milos Forman 1996), Pulp Fiction (Quentin Tarantino 1994) and 24 Hour Party People (Michael Winterbotton 2002). Those master pieces really showed how social behaviours and trends can truly influence health individually.

The second and third years projects addressed "Myths, Symbols and Idols", picking up Frida (Julie Taynor 2002), The Aviator (Martin Scorcese 2004), The Doors (Oliver Stone 2001), Ali (Michael Mann 2001), Eyes Wide Shut (Stanley Kubrick 1999), Easy Rider (Dennis Hopper 1969) and Maradona (Emir Kusturica 2008). This time the focus was on how individuals give their testimony and examples to become driving forces of our culture, again with health and disease being influenced by their own experience, in an environment loaded with alcohol, or sex, or drugs or even…sports.

Facts are that this cultural model made quite an impact in local social tissue. The auditorium was freely open to public, but a predominant fringe of university students and teachers was seen along with lay people and movie lovers (even some doubtful doctors from our Hospital and Medical School!). Many national newspapers and some radios gave echos about this Project. Again, this proved to be a new way of discovering new worlds in settled worlds, and a contribution to broaden the horizons where the skilled Gastroenterologist is generally moving, so many times being unaware of that. Just an irreverent attempts to stir up food for thought, awakening consciences and trying to shake the conventional borders of knowledge.

The truth is that, at the end of the day, we all won: Enlightened people now unexpectedly aware of how many trivial happenings might have influenced or still

may influence their own health history; media people that came to realize that doctors have much more to share than the traditional hermetic medical knowledge, and that it is written in their nature the drive for moving forward in cognitive skills about the surrounding world; and we doctors, specially we Gastroenterologists, much more conscious on that we should keep constantly in mind that diseases have larger boundaries than those anticipated in our challenging patient, and that those apparently clear cut patients have much larger landscapes than a given disease constraint. It is our firm believe that one of our still unexplored noble tasks is to promote an anthropologic way of addressing and solving gastrointestinal and liver diseases burden.

REFERENCES

1 **Díaz Membrives M**, Icart Isern MT, López Matheu MC. Literature review: Use of commercial films as a teaching resource for health sciences students. *Nurse Educ Today* 2016; **36**: 264-267 [PMID: 26490325 DOI: 10.1016/j.nedt.2015.10.002]

2 **Wahlert L**. "I Caught It at the Movies": Reflections on Medical History, Movie Theaters, and the Cinema of Contagion. *J Homosex* 2016; **63**: 323-328 [PMID: 26644058 DOI: 10.1080/00918369.2016.1124689]

3 **McKie A**. Using the arts and humanities to promote a liberal nursing education: strengths and weaknesses. *Nurse Educ Today* 2012; **32**: 803-810 [PMID: 22579881 DOI: 10.1016/j.nedt.2012.04.014]

4 **Lee A**. Health-promoting schools: evidence for a holistic approach to promoting health and improving health literacy. *Appl Health Econ Health Policy* 2009; **7**: 11-17 [PMID: 19558191 DOI: 10.1007/BF03256138]

P- Reviewer: Campo GM, Jaeschke H, Serrano-Luna J
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Lu YJ

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