ISSN 2150-5330 (online)

World Journal of *Gastrointestinal Pathophysiology*

World J Gastrointest Pathophysiol 2014 August 15; 5(3): 122-379

Published by Baishideng Publishing Group Inc

WJGP World Journal of Gastrointestinal Pathophysiology

A peer-reviewed, online, open-access journal of gastrointestinal pathophysiology

Editorial Board

2011-2015

The *World Journal of Gastrointestinal Pathophysiology* Editorial Board consists of 523 members, representing a team of worldwide experts in gastrointestinal pathophysiology. They are from 45 countries, including Argentina (2), Australia (14), Austria (3), Belgium (9), Brazil (10), Brunei Darussalam (1), Canada (20), China (30), Croatia (1), Czech Republic (2), Denmark (4), Egypt (1), Estonia (1), Finland (1), France (8), Germany (22), Greece (7), Hungary (5), India (10), Indonesia (1), Iran (2), Ireland (2), Israel (8), Italy (42), Japan (47), Lebanon (3), Malaysia (1), Mexico (2), Netherlands (8), Norway (1), Poland (4), Portugal (1), Romania (1), Russia (1), Singapore (4), South Korea (13), Spain (23), Sweden (11), Switzerland (4), Thailand (2), Turkey (6), Ukraine (1), United Kingdom (10), United States (173), and Venezuela (1).

Jian-Dong Huang, *Hong Kong*
Jia-Fu Ji, *Beijing*
Shi Liu, *Wuhan*
Zhan-Ju Liu, *Shanghai*
Xiao-Hong Wang, *Beijing*
Zhen-Ning Wang, *Shenyang*
Wei Wei, *Hefei*
Dong-Ping Xie, *Shanghai*
Wen-Xie Xu, *Shanghai*
Hua Yang, *Chongqing*
Xiao Yang, *Beijing*
Wei-Zhen Zhang, *Beijing*
Hua-Chuan Zheng, *Shenyang*
Da-Ling Zhu, *Harbin*
Jin-Xia Zhu, *Beijing*
Min-Sheng Zhu, *Nanjing*
Yong-Liang Zhu, *Hangzhou*

Croatia

Alen Protic, *Rijeka*

Czech Republic

Pavel Hladik, *Semily*
Martin Vokurka, *Prague*

Denmark

Lars Arendt-Nielsen, *Aalborg*
Frank Vinholt Schiodt, *Copenhagen*
Jonas Worsoe, *Aarhus*
Jing-Bo Zhao, *Aalborg*

Egypt

Mahmoud Aboelneen Khattab, *Minia*

Estonia

Enn Seppet, *Tartu*

Finland

Pauli Antero Puolakkainen, *Turku*

France

Bruno Bonaz, *Grenoble*
Pierre Marie Dechelotte, *Rouen*
Jean-Paul Lallès, *Saint-Gilles*
Charles-Henri Malbert, *Saint-Gilles*
Thierry Piche, *Nice*
Pascale Plaisancié, *Lyon*
Michelina Plateroti, *Lyon*
Veronique Vitton, *Marseille*

Germany

Hans Gunter Beger, *Ulm*
Carsten Bergmann, *Ingelheim*
Elke Cario, *Essen*
Arno J Dormann, *Koln*
Nikolaus Gassler, *Aachen*
Werner Hartwig, *Heidelberg*
Marion Hewicker-Trautwein, *Hannover*
Jens Hoeppner, *Freiburg*
Tobias Keck, *Freiburg*
Jorg Kleeff, *Munich*
Peter Malfertheiner, *Magdeburg*
Oliver Mann, *Hamburg*
Christoph Michalski, *Munich*
Andreas Klaus Nussler, *Munich*
Christian Pehl, *Vilsbiburg*
Peter Schemmer, *Heidelberg*
Marc Stemmler, *Freiburg*
Frank Tacke, *Aachen*
Sya Nomna Ukena, *Hannover*
Brigitte Vollmar, *Rostock*
Thomas Michael Wex, *Magdeburg*
Margot Zoller, *Heidelberg*

Greece

Stelios F Assimakopoulos, *Patras*
George N Dalekos, *Larissa*
Alkiviadis Efthymiou, *thessaloniki*
Maria Gazouli, *Athens*
Ioannis E Koutroubakis, *Heraklion*
Gerassimos J Mantzaris, *Athens*
George Papatheodoridis, *Athens*

Hungary

Mária Bagyánszki, *Szeged*
Mihály Boros, *Szeged*
Laszlo Czako, *Szeged*
Pal Miheller, *Budapest*
Zoltan Rakonczay, *Szeged*

India

Anil Kumar Agarwal, *Delhi*
Uday Bandyopadhyay, *Kolkata*
Sriparna Basu, *Varanasi*
Chandra Kanti Chakraborti, *Rourkela*
Rajeev Garg, *Punjab*
Chandra P Sharma, *Thiruvananthapuram*
Shailesh V Shrikhande, *Mumbai*
Virendra Singh, *Chandigarh*
Nicholas James Skill, *Indianapolis*
Prabhakar R Veerareddy, *Andhra Pradesh*

Indonesia

Laurentius A Lesmana, *Jakarta*

Iran

Gholamreza Roshandel, *Gorgan*
Shahram Shahabi, *Urmia*

Ireland

Billy Bourke, *Dublin*
Stephen Keely, *Dublin*

Israel

Yosefa Avraham, *Jerusalem*
Yaron Bar-Dayan, *Holon*
Shomron Ben-Horin, *Hashomer*
Boris Kirshtein, *Beer Sheva*
Stephen Malnick, *Rehovot*
Yaakov Maor, *Tel-Hashomer*
Rifaat Safadi, *Jerusalem*
Nachum Vaisman, *Tel Aviv*

Italy

Rosaria Acquaviva, *Catania*
Dario Acuna-Castroviejo, *Armilla*
Alessandro Antonelli, *Pisa*
Giacosa Attilio, *Genova*
Salvatore Auricchio, *Naples*
Guido Basilisco, *Milano*
Antonio Basoli, *Rome*
Claudio Bassi, *Verona*
Massimo Bellini, *Pisa*
Luigi Bonavina, *Milano*
Alfio Brogna, *Catania*
Giuseppe Calamita, *Bari*
Raffaele Capasso, *Naples*
Ignazio Castagliuolo, *Padova*
Enrico Stefano Corazziari, *Rome*
Francesco Cresi, *Torino*
Rosario Cuomo, *Napoli*
Salvatore Cuzzocrea, *Gazzi*
Mario M D'Elios, *Florence*
Cinzia Domeneghini, *Milan*
Luca Elli, *Milano*
Cresi Francesco, *Torino*
Walter Fries, *Messina*
Eugenio Gaudio, *Rome*
Marco Gobbetti, *Bari*
Fabio Grizzi, *Milan*
Enzo Grossi, *Milanese*
Enzo Ierardi, *Foggia*
Pietro Invernizzi, *Milan*
Angelo A Izzo, *Naples*
Anna Kohn, *Rome*
Giovanni Latella, *L'Aquila*
Massimo Marignani, *Rome*
Sergio Morini, *Rome*
Raffaele Pezzilli, *Bologna*
Cristiano Rumio, *Milan*
Giovanni Sarnelli, *Naples*
Edoardo Vincenzo Savarino, *Genoa*
Pierpaolo Sileri, *Rome*
Annamaria Staiano, *Naples*
Giacomo Carlo Sturniolo, *Padova*
Claudio Tiribelli, *Triest*

Japan

Akihiro Asakawa, *Kagoshima*
Hisashi Aso, *Sendai*
Yasu-Taka Azuma, *Osaka*
Shotaro Enomoto, *Wakayama*
Mikihiro Fujiya, *Hokkaido*
Takahisa Furuta, *Hamamatsu*
Akira Hokama, *Okinawa*
Ryota Hokari, *Saitama*
Yuichi Hori, *Kobe*

Hideki Iijima, *Osaka*
Masahiro Iizuka, *Akita*
Motohiro Imano, *Osaka*
Hajime Isomoto, *Nagasaki*
Tatehiro Kagawa, *Isehara*
Takumi Kawaguchi, *Kurume*
Haruki Kitazawa, *Sendai*
Xiao-Kang Li, *Tokyo*
Noriaki Manabe, *Okayama*
Atsushi Masamune, *Sendai*
Hiroyuki Matsubayashi, *Shizuoka*
Kazuyuki Matsushita, *Chuo-ku*
Reiko Miyazawa, *Gunma*
Kazunari Murakami, *Oita*
Hikaru Nagahara, *Tokyo*
Yuji Naito, *Kyoto*
Atsushi Nakajima, *Atsushi Nakajima*
Shoji Natsugoe, *Kagoshima*
Tsutomu Nishida, *Osaka*
Koji Nomoto, *Tokyo*
Naoaki Sakata, *Miyagi*
Shouji Shimoyama, *Tokyo*
Goshi Shiota, *Yonago*
Ikuo Shoji, *Hyogo*
Hidekazu Suzuki, *Tokyo*
Hitoshi Takagi, *Gunma*
Toru Takahashi, *Okayama*
Yoshihisa Takahashi, *Tokyo*
Kan Uchiyama, *Chiba*
Takato Ueno, *Kurume*
Yoshiyuki Ueno, *Sendai*
Hisayuki Uneyama, *Kwasaki*
Mitsunori Yamakawa, *Yamagata*
Takayuki Yamamoto, *Mie*
Yutaka Yata, *Gunma*
Naohisa Yoshida, *Kyoto*
Hitoshi Yoshiji, *Nara*

Lebanon

Costantine Fouad Daher, *Byblos*
Assaad M Soweid, *Beirut*
Julnar Usta, *Beirut*

Malaysia

Andrew Chua, *Perak*

Mexico

José María de la Roca-Chiapas, *Leon*
Maria Raquel Huerta Franco, *Guanajuato*

Netherland

Wouter J de Jonge, *Amsterdam*
Aldo Grefhorst, *Groningen*
Ruben Hummelen, *Rotterdam*
Daniel Keszthelyi, *Maastricht*
Cornelis F M Sier, *Leiden*
Pieter J Tanis, *Amsterdam*
Luc JW van der Laan, *Rotterdam*
Sander van der Marel, *Leiden*

Norway

Anne Marie Bakke, *Oslo*

Poland

Stanisław Hac, *Gdańsk*
Stanisław Jan Konturek, *Kraków*
Agata Mulak, *Wroclaw*
Napoleon Waszkiewicz, *Choroszcz*

Portugal

Ricardo Marcos, *Porto*

Romania

Mihai Ciocirlan, *Bucharest*

Russia

Ludmila Filaretova, *Petersburg*

Singapore

Madhav Bhatia, *Singapore*
Brian K P Goh, *Singapore*
Khek Yu Ho, *Singapore*
Cliff K S Ong, *Singapore*

South Korea

Jae Hee Cheon, *Seoul*
Myung Haing Cho, *Seoul*
Jae Bock Chung, *Seoul*
Ki-Baik Hahm, *Incheon*
Ho Jae Han, *Gwangju*
Chang Duk Jun, *Gwangju*
Hong Joo Kim, *Seoul*
Jin Kyung Kim, *Gyeongsan-Si*
Sang Geon Kim, *Seoul*
Won Jae Lee, *Seoul*
Kwan Kyu Park, *Daegu*
Seung Ha Park, *Busan*
Sung Joo Park, *Jeonbuk*

Spain

Raquel Abalo, *Alcorcón*
Juan G Abraldes, *Barcelona*
Agustin Albillos, *Madrid*
Maria-Angeles Aller, *Madrid*
Fernando Azpiroz, *Barcelona*
Ramon Bataller, *Barcelona*
Marco Bustamante, *Valencia*
Andres Cardenas, *Barcelona*
Dariao Acuna Castroviejo, *Armilla*
Joan Claria, *Barcelona*
Pere Clave, *Barcelona*
Manuel Giner, *Madrid*
Angel I Lanas, *Zaragoza*
Maite Martin, *Barcelona*
Maria Teresa Martin, *Barcelona*
Vicente Martinez, *Barcelona*
Jose M Matés, *Malaga*
Julio M Mayol, *Madrid*
Marçal Pastor-Anglada, *Barcelona*
María Eugenia Sáez, *Seville*
Yolanda Sanz, *Burjassot*
Carlos Taxonera, *Madrid*
Maria D Yago, *Granada*

Sweden

Marco Del Chiaro, *Stockholm*
Frida Fak, *Gothenburg*
Gunnar FA Flemstrom, *Uppsala*
Evangelos Kalaitzakis, *Gothenburg*
Kristina Lamas, *Umea*
Bob Roger Olsson, *Göteborg*
Sara Maria Regnér, *Malmö*
Peter thelin Schmidt, *Stockholm*
Xiao-Feng Sun, *Linkoping*
Henrik Thorlacius, *Malmö*
Curt Tysk, *Orebro*

Switzerland

Jyrki J Eloranta, *Zurich*
Andreas Geier, *Zurich*
Remy Meier, *Liestal*
Catherine Pastor, *Geneva*

Thailand

Thawatchai Akaraviputh, *Bangkok*
Weekitt Kittisupamongkol, *Bangkok*

Turkey

Mehmet Bektas, *Ankara*
Mukaddes Esrefoglu, *Malatya*
Ahmet Guven, *Ankara*
Muammer Karadeniz, *Manisa*
Elvan Ozbek, *Erzuru*
Ilhami Yuksel, *Ankara*

Ukraine

Oksana S Zayavhkivska, *Lviv*

United Kingdom

Geoffrey Burnstock, *London*
Janice E Drew, *Aberdeen*
Girish Gupte, *Birmingham*
David C Hay, *Edinburgh*
Nusrat Husain, *Cheshire*
Michael Leslie Lucas, *Glasgow*
Jamie Murphy, *London*
Vadim Sumbayev, *Kent*
Wing-Kin Syn, *Birmingham*

Andrea Varro, *Liverpool*

United States

Sami Rene Achem, *Jacksonville*
Tauseef Ali, *Oklahoma*
David H Alpers, *St Louis*
Gianfranco D Alpini, *Temple*
Shrikant Anant, *Oklahoma*
M Sawkat Anwer, *North Grafton*
Andrew Aronsohn, *Chicago*
Toms Augustin, *Sayre*
Gyorgy Baffy, *Boston*
Michael T Bailey, *Columbus*
Kim Elaine Barrett, *San Diego*
Marc D Basson, *Lansing*
Robert L Bell, *New Haven*
David H Berger, *Houston*
Urs A Boelsterli, *Storrs*
Richard G Boles, *Los Angeles*
Edward L Bradley III, *Sarasota*
Qiang Cai, *Atlanta*
Wei-Biao Cao, *Providence*
Subhash C Chauhan, *Sioux Falls*
Jian-De Chen, *Galveston*
Tao-Sheng Chen, *Memphis*
John Chiang, *Rootstown*
Mashkoor A Choudhry, *Maywood*
Parimal Chowdhury, *Little Rock*
Eric Cohen, *Boston*
Robert Cormier, *Duluth*
Srinivasan Dasarathy, *Cleveland*
Edwin A Deitch, *Newark*
Dan A Dixon, *Columbia*
James P Dolan, *Portland*
H Henry Dong, *Pittsburgh*
Hui Dong, *La Jolla*
Ashkan Farhadi, *Irvine*
Bin Feng, *Pittsburgh*
Jenifer Fenton, *East Lansing*
Alessandro Fichera, *Chicago*
Mitchell P Fink, *Pittsburgh*
P Marco Fisichella, *Maywood*
Leo R Fitzpatrick, *Hummelstown*
Robert Armour Forse, *Omaha*
Glenn Tsuyoshi Furuta, *Aurora*
Juan F Gallegos-Orozco, *Scottsdale*
Pandu R Gangula, *Nasvhille*
Timothy Gardner, *Lebanon*
Shannon Stroud Glaser, *Temple*
Francisco Gondim, *St. Louis*
John R Grider, *Richmond*
Yan-Fang Guan, *Cincinnati*
Gregory M Holmes, *Baton Rouge*
Ai-Xuan Le Holterman, *Chicago*
Richard Hu, *Los Angeles*
Hartmut Jaeschke, *Kansas*
Robert Thomas Jensen, *Los Angeles*
Sreenivasa S Jonnalagadda, *Louis*
Michel Kahaleh, *Charlottesville*
Andreas Martin Kaiser, *Los Angeles*
Randeep Singh Kashyap, *Rochester*
Laurie Keefer, *Chicago*
Richard Kellermayer, *Houston*
Chris Kevil, *Shreveport*
Sandeep Khurana, *Baltimore*
Pawel R Kiela, *Tucson*
Tammy Lyn Kindel, *Cincinnat*
Gordana Kosutic, *Durham*
David Kravetz, *San Diego*
Ashok Kumar, *Detroit*
John H Kwon, *Chicago*
Muriel Larauche, *Los Angeles*
I Michael Leitman, *New York*
Felix W Leung, *North Hills*
Suthat Liangpunsakul, *Indianapolis*
Feng-Xin Lu, *Boston*
Pauline Kay Lund, *Chapel Hill*
George Luo, *Lexington*
Guang-Xiang Luo, *Lexington*
Jay Luther, *Ann Arbor*
Ram I Mahato, *Memphis*
Akhil Maheshwari, *Birmingham*
Kenneth Maiese, *Newark*
Adhip P N Majumdar, *Detroit*
Jose E Manautou, *Storrs*
Craig J McClain, *Louisville*
Dermot McGovern, *Los Angeles*
B Greenwood-van Meerveld, *Oklahoma*
Douglas Scott Merrel, *Bethesda*
Murielle Mimeault, *Omaha*
Emiko Mizoguchi, *Boston*
Huan-Biao Mo, *Denton*
Adam Moeser, *Raleigh*
Ramzi M Mohammad, *Detroit*
Satdarshan Singh Monga, *Pittsburgh*
Roger Klein Moreira, *New York*
Sandeep Mukherjee, *Omaha*
Karnam S Murthy, *Richmond*
Michael J Nowicki, *Jackson*
Shuji Ogino, *Boston*
Mary Francis Otterson, *Wisconsin*
Chung Owyang, *Ann Arbor*
Helieh S Oz, *Lexington*
Marco G Patti, *Chicago*
Timothy Michael Pawlik, *Baltimore*
Sara Peleg, *Houston*
Nicholas C Popescu, *Bethesda*
Li-Ya Qiao, *Richmond*
Chao Qin, *Oklahoma*
Parvaneh Rafiee, *Milwaukee*
Sigrid A Rajasekaran, *Wilmington*
Vazhaikkurichi M Rajendran, *Morgantown*
Jean Pierre Raufman, *Baltimore*
Ramesh M Ray, *Memphis*
Arie Regev, *Indianapolis*
Douglas K Rex, *Carmel*
Yehuda Ringel, *Chapel Hill*
Richard A Rippe, *Rockville*
Chantal A Rivera, *Bossier*
Andrea Romani, *Cleveland*
Praveen K Roy, *Albuquerque*
Paul A Rufo, Boston
David B Sachar, *New York*
Bimaljit Singh Sandhu, *Richmond*
Sanjaya Kumar Satapathy, *New Hyde Park*
Anthony Senagore, *Los Angeles*
Muhammad Y Sheikh, *Fresno*
Bo Shen, *Cleveland*
Le Shen, *Chicago*
Frank A Simmen, *Little Rock*
Steven Mitchell Singer, *Washington*
Shailinder Jit Singh, *Washington*
Adam Jan Smolka, *Charleston*
Ned Snyder, *Houston*
Zhen-Yuan Song, *Chicago*
Gagan K Sood, *Houston*
Rhonda F Souza, *Dallas*
Stuart Jon Spechler, *Dallas*
Subbaramiah Sridha, *Augusta*
Catia Sternini, *Los Angeles*
Veedamali S Subramanian, *Long Beach*
Jun Sun, *Rochester*
Yvette Taché, *Los Angeles*
Xiao-Di Tan, *Chicago*
Paul Daniel Terry, *Atlanta*
Jennifer Tirnauer, *Farmington*
Andrea Todisco, *Ann Arbor*
George C Tsokos, *Boston*
Vic Velanovich, *Detroit*
Raj Vuppalanchi, *Indianapolis*
Estela Wajcberg, *Cranford*
Arnold Wald, *Madison*
Li-Xin Wang, *Los Angeles*
Horst Christian Weber, *Boston*
Steven D Wexner, *Weston*
Jackie D Wood, *Columbus*
Guo-Yao Wu, *College Station*
Christian Wunder, *Bethesda*
Zuo-Liang Xiao, *Cleveland*
Guang-Yin Xu, *Galveston*
Guo-Rong Xu, *East Orange*
Guang-Yu Yang, *Chicago*
Jay A Yelon, *Valhalla*
Yamaoka Yoshio, *Houston*
Shao-Yong Yu, *Hershey*
Yana Zavros, *Cincinnati*
Joerg Zehetner, *Los Angeles*
Jian X Zhang, *Charlotte*
Zhi Zhong, *Charleston*
Hui-Ping Zhou, *Richmond*
Zhan-Xiang Zhou, *Kannapolis*
Qing Zhu, *Bethesda*
Yao-Hui Zhu, *Stanford*

Venezuela

Fabian Michelangeli, *Caracas*

WJGP

World Journal of Gastrointestinal Pathophysiology

Contents

Quarterly Volume 5 Number 3 August 15, 2014

TOPIC HIGHLIGHT

Contents

World Journal of Gastrointestinal Pathophysiology
Volume 5 Number 3 August 15, 2014

Contents

World Journal of Gastrointestinal Pathophysiology
Volume 5 Number 3 August 15, 2014

AIM AND SCOPE

World Journal of Gastrointestinal Pathophysiology (*World J Gastrointest Pathophysiol*, *WJGP*, online ISSN 2150-5330, DOI: 10.4291), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJGP is to report rapidly the most recent results in basic and clinical research on gastrointestinal pathophysiology, including all aspects of normal or abnormal function of the gastrointestinal tract, hepatobiliary system, and pancreas. *WJGP* specifically covers growth and development, digestion, secretion, absorption, metabolism and motility relative to the gastrointestinal organs, as well as immune and inflammatory processes, and neural, endocrine and circulatory control mechanisms that affect these organs. This journal will also report new methods and techniques in gastrointestinal pathophysiological research.

We encourage authors to submit their manuscripts to *WJGP*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

INDEXING/ ABSTRACTING

World Journal of Gastrointestinal Pathophysiology is now indexed in PubMed Central, PubMed, Digital Object Identifier, and Directory of Open Access Journals.

NAME OF JOURNAL
World Journal of Gastrointestinal Pathophysiology

ISSN
ISSN 2150-5330 (online)

LAUNCH DATE
April 15, 2010

Frequency
Quarterly

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

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

PUBLICATION DATE
August 15, 2014

World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4291/wjgp.v5.i3.122

World J Gastrointest Pathophysiol 2014 August 15; 5(3): 122-132
ISSN 2150-5330 (online)

TOPIC HIGHLIGHT

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

Biofilms and *Helicobacter pylori*: Dissemination and persistence within the environment and host

Steven L Percival, Louise Suleman

Steven L Percival, Surface Science Research Centre and Institute of Ageing and Chronic Disease, University of Liverpool, Merseyside L69 3BX, United Kingdom
Louise Suleman, Musculoskeletal Biology, Institute of Ageing and Chronic Disease, University of Liverpool, Leahurst, Neston CH64 7TE, United Kingdom
Author contributions: Percival SL performed the literature search and prepared the original draft; Suleman L edited and supplemented the manuscript.
Correspondence to: Steven L Percival, PhD, Professor, Surface Science Research Centre and Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, Merseyside L69 3BX, United Kingdom. steven.percival@liverpool.ac.uk
Telephone: +44-161-3017560 Fax: +44-161-3017565
Received: January 10, 2014 Revised: April 17, 2014
Accepted: May 16, 2014
Published online: August 15, 2014

Key words: *Helicobacter pylori*; Biofilm; Coccoid forms; Virulence; Water

Core tip: The ability of *Helicobacter pylori* (*H. pylori*) to form biofilms is fundamental to its pathogenicity. Research into the mechanisms behind *H. pylori* resuscitation from coccoid to virulent spiral forms will aid a better understanding into infection recurrence in the host and the external environment.

Percival SL, Suleman L. Biofilms and *Helicobacter pylori:* Dissemination and persistence within the environment and host. *World J Gastrointest Pathophysiol* 2014; 5(3): 122-132 Available from: URL: http://www.wjgnet.com/2150-5330/full/v5/i3/122.htm DOI: http://dx.doi.org/10.4291/wjgp.v5.i3.122

Abstract

The presence of viable *Helicobacter pylori* (*H. pylori*) in the environment is considered to contribute to the levels of *H. pylori* found in the human population, which also aids to increase its genetic variability and its environment adaptability and persistence. *H. pylori* form biofilms both within the *in vitro* and *in vivo* environment. This represents an important attribute that assists the survival of this bacterium within environments that are both hostile and adverse to proliferation. It is the aim of this paper to review the ability of *H. pylori* to form biofilms *in vivo* and *in vitro* and to address the inherent mechanisms considered to significantly enhance its persistence within the host and in external environments. Furthermore, the dissemination of *H. pylori* in the external environment and within in the human body and its impact upon infection control shall be discussed.

INTRODUCTION

Helicobacter pylori (*H. pylori*) is an opportunistic pathogen that plays an important role in the aetiology of peptic and gastric ulcers. *H. pylori* primarily colonizes the antral part of the stomach whereby they either adhere to the walls of the stomach or simply remain in a planktonic, free-floating state. This bacterium has been reported to spread from the stomach to the intestine where it is then secreted in faeces[1]. Furthermore, *H. pylori* infection is known to be associated with nausea and vomiting which can lead to the spread of this pathogen to the oral cavity, leading to the colonisation of gingival and dental plaques[2].

H. pylori has been reported to colonise over half the world's population with clinical signs of infection only manifesting in less than 20% of these individuals[3]. Nevertheless, the majority of these individuals are colonised with *H. pylori* for life unless eradication using appropriate chemotherapeutic agents is successful. Lifelong colo-

nisation seems to be due to the ability of some strains of *H. pylori* to both adapt to the host's immunological responses and to also withstand the constantly changing gastric environment. In genetically predisposed individuals, colonisation with *H. pylori* is reported to heighten the risk of developing cancer[4].

H. pylori can be described as Gram negative, spiral- (S-shape) or cocci-shaped bacteria. It has been reported to exist in three forms, the viable and culturable spiral form, the viable but non-culturable (VBNC) coccoid form, which are less virulent, and the non-viable degenerative *H. pylori* form[5]. It is their spiral shape that is thought to enhance their colonisation of the gastric mucosa. Whilst generally considered microaerophilic, there is now evidence that *H. pylori* can grow in humidified aerobic conditions[6].

The colonisation of *H. pylori* and its effect on resident gastric microbiota is relatively unknown. A study by Bik *et al*[7] assessed the human gastric microbiota from 23 gastric biopsy samples using small subunit 16S rDNA clone library method and subsequently found that the presence of *H. pylori* had no effect on the microbial profile of the gut[7]. A recent study investigated the effects of *H. pylori* on the gastric microbiota in a Rhesus macaque model. The authors found no significant impact upon the non-Helicobacter taxa after *H. pylori* challenge[8]. However it appears that the microbial profile of the gut may have an effect on the degrees of pathogenicity of *H. pylori*. A germ-free gastric cancer mouse model showed less symptoms of disease and a later onset of neoplasia upon *H. pylori* infection when compared to those mice with a typical gastric microbiota profile[9].

As an avid coloniser of the gastric mucosa *H. pylori* must possess a number of characteristics that include flagella, adhesions, urease production, and biofilm forming ability[10,11]. The importance of the biofilm forming potential of *H. pylori* is fundamental to its pathogenicity. The formation of a biofilm is a virulence mechanism that aids in the enhancement and longevity of *H. pylori* in "unfriendly" and hostile environments, such as in the human stomach and the natural environment.

H. pylori was first found to demonstrate an ability to form *in vitro* biofilms in the early and late 1990s with solid evidence of this ability reported by Stark *et al*[12] in 1999. More recent reports on the ability of *H. pylori* to form biofilms within *in vitro*[13,14] and *in vivo* environments, specifically the gastric mucosa, have now been demonstrated[14-16]. In particular the *H. pylori* strain TK1402 isolated from a patient with duodenal and gastric ulcers has been shown to have very strong biofilm forming ability both inside and outside the host[14,15,17-20]. In this mode of growth it is likely that *H. pylori* is protected from external perturbations[18,21].

Biofilms can develop on both biotic and abiotic surfaces through the conversion of microorganisms in a free-floating or planktonic state, to a sessile state, where they become attached onto a surface. Once microorganisms attach onto a surface they proliferate, produce extracellular polymeric substance (EPS) and become firmly attached to that surface. The matrix of the biofilm is known to be composed of polysaccharides, extracellular DNA (eDNA), lipids and proteins that form the "house" of the biofilm[22,23]. It is the biofilm and the ability of microorganisms to form biofilms that form an essential element, aiding in their persistence, survivability, and recalcitrance to antimicrobial interventions and the hosts immune response. Furthermore the ability of pathogenic microbes to survive within diverse and hostile environments is enhanced significantly when growing within a biofilm. Growth within a biofilm is known to cause and exacerbate infections and is responsible for prolonging infection, leading to chronicity[24].

A biofilm is dynamically and structurally complex and is often referred to as a "living organism" due to its ability to adapt to external perturbations. Of particular concern with biofilms of public health significance is the fact that sections of biofilms can easily detach or shear off, enabling these sections or individual bacteria to recolonise other surfaces. Detachment or dissemination from the biofilm can be achieved by the dispersal of single cells or the detachment/shedding of large cellular aggregates. Both situations constitute a concern to public health particularly where fluid resides, as microbial dissemination is enhanced *e.g.*, catheters, blood stream, drinking water[24]. Further to this there is growing evidence that within a biofilm the horizontal transfer of genes can occur, leading to large variations in *H. pylori* strains, particularly in one host, enhancing their survival and immune evasion. Moreover, gene transfer *in situ* has an important role to play in immunological effectiveness and eradication of pathogens by the host[25]. In addition to this it is well documented that when microorganisms are growing within a biofilm they have increased tolerance to antimicrobial agents[26].

It is the aim of this paper to review the ability of *H. pylori* to form biofilms *in vivo* and *in vitro* and to address the inherent mechanisms considered to significantly enhance its persistence within the host and in external environments. Furthermore, the dissemination of *H. pylori* in the external environment and within in the human body and its impact upon infection control shall be discussed.

TRANSMISSION OF *H. PYLORI*

The routes of transmission of *H. pylori* are said to occur via an array of different pathways[27,28]. Although *H. pylori* are considered to be pathogens commonly associated with the human stomach, Brown proposed that *H. pylori* are able to survive in environments that are external to that of the human stomach[29]. Dental plaque has also been reported to contain *H. pylori;* however, in plaque, *H. pylori* are thought to only exist in a transient state[30-32]. Young *et al*[2] reported that both the spiral and viable coccoid form of *H. pylori* are present in the oral cavity. Souto and Colombo found *H. pylori* in a subgingival bio-

film in 11% of periodontally healthy patients compared to 50% of patients suffering from periodontitis[33]. The authors proposed that biofilm formation in the oral cavity should be considered as a potential reservoir for *H. pylori*.

There is building evidence to suggest that *H. pylori* may reside in potable water systems[11]. In general, waterborne bacteria can adhere to surfaces by aggregating matrix to form biofilms[26]. Information regarding the exact ecological niche where *H. pylori* reside and persist outside of the human host is limited. Despite this, there is growing evidence that external reservoirs of *H. pylori* may exist, potentially aiding transmission to the host. Furthermore there are also reports that *H. pylori* may have, as part of its life cycle, a zoonotic component. However, further scientific evidence of cultivability will be required to fully support this area.

The ability to form biofilms and the cell morphology and architecture formed depends greatly on the support material. To date however, in potable water supplies there is not enough substantial evidence that *H. pylori* within the viable state, plays a role in the development of a biofilm. Despite this, there is significant evidence that, in terms of epidemiological evidence, the risk of acquiring *H. pylori* increases in individuals who drink well water and river water or swim in pools, streams and rivers in particular[27,34-37]. Consequently, environmental water is considered a risk for the acquisition of *H. pylori* and therefore *H. pylori* biofilms in these environments should be a very important consideration when investigating reservoirs of source. The association of *H. pylori* with biofilms in water distribution systems can offer bacteria protection from disinfection and protozoan predation[38]. The challenge however, remains to determine the importance of waterborne *H. pylori*. It may be possible that a specifically adapted form of *H. pylori*, or simple *H. pylori* within a biofilm, may be required for persistence and transmission[39].

Although based on scientific logic, if *H. pylori* is able to survive and persist outside of the human host, its ability to develop a biofilm and survive within a biofilm may well help to answer fundamental questions regarding acquisition and potential eradication, particularly in the developing world.

THE DETECTION OF *H. PYLORI* IN THE ENVIRONMENT AND HOST

The ability of *H. pylori* to transform from a highly virulent, spiral shaped bacteria to a less virulent, non-culturable coccoid state, presents difficulties in the successful detection of this bacterium in both an environmental and clinical setting. In particular, the VBNC coccoid state is thought to arise under less favourable conditions, making the identification of *H. pylori* from water sources, particularly *H. pylori* within biofilms, unlikely using traditional culture methods.

Molecular methods such as real-time polymerase chain reaction (PCR) have been used to identify *H. pylori* in both spiral and coccoid states. Linke *et al*[40] used real-time PCR to target the ureA subunit of the *H. pylori* urea gene to identify *H. pylori* in drinking water biofilms. This *in vitro* study demonstrated successful identification of *H. pylori* from biofilms in silicone tubing, an imitation of drinking water systems. The study not only highlighted the capability of *H. pylori* to form biofilms in such a system but also emphasised the potential of using real-time PCR as a viable detection method. Although it is clear that more research into the identification of different strains of *H. pylori* using this method should be considered.

In terms of identification within the host, a very recent research paper by Fontenete *et al*[41] demonstrated the use of fluorescent *in situ* hybridisation (FISH) to identify *H. pylori* in culture and human gastric biopsies. This study mimicked *in vivo* conditions using gastric biopsies and modified the FISH method by replacing toxic chemicals, giving rise to the opportunity of using this method, given further development and trails, in *in vivo* situations.

THE ABILITY OF *H. PYLORI* TO FORM A BIOFILM

The ability of *H. pylori* to form a biofilm has been documented for over 15 years with biofilm growth heightened in environments which are composed of high carbon:nitrogen ratios[12,39]. The ability of *H. pylori* to develop biofilms has been reported in many *in vitro* studies[12-14,17,42,43]. The specific knowledge regarding the ability of *H. pylori* to form biofilms has been made possible by observations using microscopic techniques in particular scanning electron microscopy (SEM) specifically on glass but also on other materials[17].

Further to this, Yonezawa *et al*[18] reported that the *H. pylori* strain TK1402 (isolated from a Japanese patient with both duodenal and gastric ulcers) was able to form a biofilm but was dependent on the flagella, their ability to form cellular aggregates, and its ability to produce outer membrane vesicles. This ability to form biofilms has been shown to be modulated by quorum sensing molecules; in particular the LuxS proteins have been identified in *H. pylori*[44].

Quorum sensing within H. pylori biofilms

Quorum sensing is an intercellular method of communication between microorganisms using chemical signalling. Quorum sensing molecules can be enzymes or peptides depending upon the signalling system. The accumulation of these signalling molecules leads to an interaction with cytoplasmic DNA-binding receptor proteins such as the *lux* protein family, whereby quorum sensing genes are modulated. Quorum sensing molecules however do not always bind to receptor proteins intracellularly; peptide molecule binding can occur on cell membranes whereby signal transduction leads to gene

regulation[45].

In the case of *H. pylori*, this bacterium expresses a homolog of the luxS gene, a gene responsible for the production of the quorum sensing molecule, autoinducer 2 (AI-2)[45]. The *H. pylori luxS* homolog has been implicated in bacterial attachment. Cole *et al*[13] revealed a two-fold increase in the biofilm formation of *H. pylori luxS* mutants when compared to the wild-type control. The authors concluded that in some strains of *H. pylori*, a mutation in quorum sensing signalling actually increases biofilm formation[13]. Later work by Rader *et al*[46] demonstrated defective motility in *luxS* mutants and highlighted the importance of quorum sensing AI-2 molecules as a regulator of flagella-associated genes in *H. pylori*. Further work by Rader *et al*[47] revealed that the release of AI-2 molecules acts as a chemorepellent for *H. pylori*. At this stage, the authors hypothesised that this action may cause *H. pylori* to break away from the majority of the bacterial population, avoiding niche competition and encouraging *H. pylori* dispersal. In the context of both external environments and within the clinical setting of the host, quorum sensing within a *H. pylori* biofilm may encourage dispersal, a mechanism that may induce the likelihood of transmission to and from an external environment and the host, and dissemination within the host.

H. PYLORI GROWTH WITHIN BIOFILMS: THE IMPORTANCE OF COCCOID FORMS AND RESUSCITATION

Understanding the growth of H. pylori

In vitro studies are an important starting point in the understanding of the dynamics of *H. pylori* growth within a biofilm. In light of this, the study of *H. pylori* in biofilms present challenges in the laboratory; nevertheless, the growth of *H. pylori* has been documented to behave differently in different growth conditions.

Bessa *et al*[48] assessed the growth of *H. pylori* in four types of liquid culture medium to assess the physiological behaviour and growth standardisation of *H. pylori*. *H. pylori* in free-living and biofilm modes of growth were assessed in Brucella broth, brain heart infusion broth and Ham's F-12 medium supplemented with 2% fetal calf serum and Ham's F-12 without serum. Free-living growth was monitored for 72 h in each medium and characterised for bacterial density, cultivability, viability and morphology. Biofilm formation in the same medium was evaluated for biomass production, colony forming unit (CFU) counts and microscopic visualisation. Afterward, using Ham's F-12, the effect of amoxicillin and clarithromycin at sub-minimum inhibitory concentrations (sub-MICs) was evaluated on *H. pylori* biofilm formation and luxS gene expression. Differences in free-living growth were observed between the culture medium supplemented with serum and Ham's F-12 without serum. Biofilm formation was significantly dependent on the growth media used. Ham's F-12 appeared to be a good medium to support both growth phenotypes of *H. pylori*. Moreover, sub-MICs of antibiotics increased the biofilm formation and affected the luxS gene expression[48]. Optimising the growth conditions of *H. pylori*, especially in the biofilm mode, will be helpful to perform more accurate in-depth studies that will increase the knowledge about *H. pylori* biofilms, which should be a target to eradicate resistant infection. Humidified conditions with 5%-7% oxygen and 7%-10% CO_2 with some H_2 or 10% CO_2 are also reported to be ideal for the growth of *H. pylori*[49]. However, the expression of catalase and superoxide dismutase (SOD), allows *H. pylori* to persist in higher levels of oxygen[50,51].

H. pylori biofilms, VBNC coccoid phenotypes and resuscitation

The emergence of VBNC pathogens has been of much interest in recent years due to the notion that this state is a form of survival and protection.

The VBNC coccoid form of *H. pylori* is formed during stress and starvation[52]; therefore it is in this form in which *H. pylori* is thought to reside in biofilms.

It has been reported that atmospheric conditions enhance the formation of VBNC coccoid *H. pylori* which has been suggested to resemble the same characteristics of persister cells documented in biofilms[11,53]. Furthermore, these cells then have the ability to resuscitate and lead to infection recurrence[54,55]. Cellini *et al*[20] identified the presence of *H. pylori* in gastric mucosa biopsies of patients treated for *H. pylori* infection. In this study, patients were identified as harbouring *H. pylori* through culture methods or, if non-culturable, the molecular method, RT-PCR. Scanning electron microscopy (SEM) of biopsies from patients with culturable samples, revealed prevalent spiral forms, nonetheless, co-existant with coccoid forms embedded within a matrix. In non-culturable cases, SEM showed the presence of coccoid clusters in a matrix that was shown to be a biofilms, through the further identification of the *luxS* quorum sensing gene[20]. This study highlighted the importance of *H. pylori* biofilms, the presence of coccoid forms within the biofilm and resistance. Furthermore, it provided insight into the prevalence of coccoid forms in the gastric mucosa. With this is mind, it is important to focus research on the identification of these VBNC coccoid forms, and more importantly, understand the mechanisms behind recalcitrant coccoid states and how they can phenotypically shift into more virulent spiral forms.

The resuscitation of a pathogen in a VBNC state is of great clinical importance, given the extensive dormancy within the host for years before infection recurrence; thus the host is incorrectly diagnosed as infection-free. Therefore it is important to distinguish between viable and culturable pathogens and VBNC states in order understand the mechanism behind reactivation. Such detection methods can include Live/Dead assays and RT-PCR[40,56,57]. There have been several reported factors

that induce resuscitation in a number of pathogenic species of bacteria such as temperature shifts, peptidoglycan hydrolases and the release of human norepinephrine following tissue injury[58].

Earlier studies such as research by Cellini *et al*[59], stressed the importance of evaluating the survival potential of VBNC coccoid *H. pylori*. In this study, *H. pylori* ATCC 43504 was grown *in vitro* until a VBNC coccoid state was achieved, whereby "resuscitation" was then attempted using heat, pH and sonication shock methods. Unfortunately the authors were not confident in whether true resuscitation actually occurred, or whether it was simply a re-growth of undetected culturable cells. Richards *et al*[60] sought to create a modified resuscitation broth containing serum and lysed erythrocytes for *H. pylori* in the VBNC state. The resuscitation of *H. pylori* was recorded and the assessment of a gene involved in growth repression (*cdrA*) showed low expression in resuscitated *H. pylori*. These results show that although the *cdrA* gene is probably not responsible for loss of cultivability in *H. pylori*, the modified broth can be successfully used to resuscitate and therefore explore other possible mechanisms.

THE SURVIVAL AND PERSISTENCE OF *H. PYLORI* AND BIOFILMS

The ability to *H. pylori* to persist as a infectious entity and resist the armoury of antimicrobials employed to eradicate it, is considered to be due to both genetic variability but in addition, the ability of *H. pylori* to form biofilms which significantly aids its survival[15,16,18,61]. The formation of a biofilm by *H. pylori* has been shown to enable its protection from fluctuations in pH due to its ability to over produce EPS[12,62]. Siavoshi *et al*[6] set up a study to identify two mucoid strains of *H. pylori* and compare their growth under aerobic and microaerobic conditions with that of a control *H. pylori* strain. The authors found that the EPS produced by the two strains could serve as a physical barrier to reduce the oxygen diffusion and uptake of antibiotics into the bacterial cell. The EPS aimed to protect them against the increasing levels of oxygen, osmotic stress, acidic pH, host immune system, and antibiotics. The authors concluded that production of EPS by *H. pylori* could be an adaptation mechanism that facilitates bacterial survival and growth. This survival strategy would prevent bacterial removal by the host defence factors and antimicrobial therapy. Furthermore it would aid the persistent and long-term infection of *H. pylori* in the stomach and possibly the environment.

Survival and persistence in the environment

H. pylori in the viable and culturable form has been shown to survive > 10 d, whereas the VBNC coccoid form has been reported to survive for up to 1 year in fresh water[63]. Within distilled water West *et al*[64] reported that *H. pylori* can survive > 14 d, similar to that in saline, and > 7 d in sea water. More recent studies have shown that *H. pylori* can survive in deep ground water[65]. Interestingly numerous studies have reported that *H. pylori* are able to survive within a cultivable state for numerous weeks in water and other natural systems when compared to that of growth in nutrient rich conditions. The adaptation of *H. pylori* in different environments is reported to be intrinsic and consequently this may assist in the survival of the bacterium in the diverse environments outside of the human host. This potential persistence in the environment may not only be due to its ability to form biofilms but also its ability to survive within a community of other microorganisms within a polymicrobial ecosystem. This ability to survive hostile environments is made possible by a number of factors mentioned above but also by the ability of *H. pylori* to produce peptides[15].

An environment that has been reported to aid the survival of *H. pylori,* is that of water or more specifically in reference to public health, potable water - an oligotrophic environment that contrasts significantly to that of the gastric mucosa.

Mackay *et al*[66] and Park *et al*[67] colleagues first provided evidence that biofilms in water distribution systems may harbour *H. pylori*. Within this study *H. pylori* incorporated itself into a laboratory-scale biofilm and persisted for over 8 d. Further to this Bunn *et al*[68] utilised 16S rDNA sequences and provided further evidence that *H. pylori* can survive in biofilms within water. Azevedo *et al*[21] and Bragança *et al*[69], have also shown that *H. pylori* may be present on pipe samples in drinking water systems which remain adhered and grow as biofilms. However, in this study it was found that a lack of recovery using culturable techniques occurred quickly over time indicating that *H. pylori* quickly enters a non-culturable state in more "hostile" environments to that of the gastric mucosa. The survival of *H. pylori* in well water has also been documented, suggesting this is related to the ability of *H. pylori* to integrate into biofilms[69,70]. Substratum material used in conjunction with both domestic and distribution systems are known to be one of the factors affecting the growth of biofilms. Subsequently, Azevedo *et al*[21] showed that *H. pylori* was able to adhere to different plumbing materials. Watson *et al*[57] also demonstrated a close link between *Helicobacter* DNA in showerhead biofilm used in domestic households.

All the research findings above support the concept that water may provide a route for the transmission of *H. pylori* outside of the human host.

Survival and persistence within the host

H. pylori has been detected and isolated from different regions of the human body. These have included gastric biopsies, gastric juice, dental plaque, saliva, bile and faecal matter, indicating its ability to colonise surfaces either transiently or in the case of the gastric mucosa, permanently[2,15,16,71]. The viable spiral-shaped *H. pylori* are referred to as more virulent and therefore infectious whereas the less virulent coccoid form have a reduced

ability to colonise and induce inflammation and disease; an effect that has been observed in animal models[5].

Biofilms are reported to serve as population-level virulence factors. Consequently this will enable the resident bacteria to acquire virulence attributes[25]. Biofilms provide ideal areas for bacterial horizontal gene transfer, which will help the production and provide a source of related strains, but with different antigenic and virulence profiles. Ultimately this will help to confuse the host immune system providing the bacterial community with a means to confuse and overwhelm the host's immune system[72].

Grande *et al*[73] investigated that persistence of *H. pylori* might be associated with genetic variability and biofilm development. The researchers investigated the interaction between two clinical strains of *H. pylori* so they could understand the balance between strains that could co-exist in the same niche to be cooperative/competitive in their colonisation.

Interestingly *H. pylori* are a species that are very genetically diverse. To date it has not been possible to isolate two identical DNA patterns from different hosts[74]. This of course is significant in evading the immune response from the host and consequently will favour the survival of *H. pylori*. Such a difference may explain the long-term colonisation that occurs in some hosts. There is a high level of genetic recombination within biofilms[75]. It is within the biofilm that horizontal gene transfer can occur as evident by high levels of eDNA detected in *H. pylori* biofilms[76]. As the biofilm is highly tolerant to the host's immune response, the availability of eDNA which is evident in the biofilm matrix could then be acquired by other *H. pylori*. This may therefore lead to the development of highly virulent strains of *H. pylori* in the host leading to their persistence.

ROLE OF BIOFILMS IN DISSEMINATION AND DISPERSAL OF *H. PYLORI* IN THE NATURAL ENVIRONMENT AND THE HOST

The dissemination of *H. pylori* is thought to occur through person-to-person contact but it is now also evident as demonstrated above, that *H. pylori* may also reside in drinking water systems. Whether in planktonic or biofilm form, albeit in the human stomach or external water supplies; the spread of this bacterium in such adverse environments is inevitable. With *in vitro* evidence of *H. pylori* residing in these environments in biofilm form, it is important to contemplate another method of dissemination. Not only do biofilms demonstrate increased resistance towards antimicrobials; biofilms possess another mechanism that greatly impacts upon transmission and dissemination within the host. "Dispersal" is a mechanism whereby members of the microbial community within a biofilm, detach and attach to new surfaces, effectively colonising a new site[77]. It is highly possible that dispersal has great impact on the dissemination of *H. pylori* not only within the host but also in the external environment, increasing the likelihood of transmission.

Dispersal can be described in three stages; the first being the detachment of bacterial cells from the biofilm, followed by the translocation of cells to a new site and finally the attachment of these cells to the new surface[77]. Given the adverse and hostile environment both outside and within the host, the dispersal of *H. pylori* may seem like an unavoidable process. However, in many microbial biofilms, dispersal is thought to be a carefully controlled mechanism. Bacterial cells that reach the end of their biofilm life cycle become differentiated and highly motile. These dispersal cells are specialised in that they are regulated by the intracellular molecule cyclic-di-GMP (c-di-GMP). In general, it is thought that a reduction in c-di-GMP leads to dispersal. Furthermore, genes that are associated with motility such as the flagellum are up-regulated[78].

In terms of *H. pylori* dispersal within biofilms, research to support this mechanism in both the environment and in the human body is lacking. Evidence that does indicate that this is a likely occurrence in *H. pylori* biofilms relate to that of *H. pylori* motility within biofilms.

It has been known for over a decade now that motility is essential for the survival and successful colonisation of *H. pylori* within the host[79].

As mentioned earlier, research by Rader *et al*[47] showed that the presence of AI-2 quorum sensing molecules that can be synthesised by *H. pylori*, act as a chemorepellent, affecting motility. Therefore the formation of *H. pylori* biofilms within the host and in the environment, whereby quorum sensing is likely to occur, may encourage the dispersal of cells from the biofilm and thus new sites of infection.

H. PYLORI ERADICATION

Environmental eradication of H. pylori

Early research by Baker *et al*[80] has shown that *H. pylori* demonstrates resistance to low dosages of free chlorine that ordinarily kill the coliforms such as *Escherichia coli*. Consequently areas in water distribution systems may not prevent the entry and potential proliferation of *H. pylori* in water. Further studies by Mazari-Hiriart *et al*[81] and Moreno *et al*[82] have demonstrated that drinking water treatments employed to date may be ineffective particularly when *H. pylori* are present in the coccoid shape, which is a well known VBNC and a potentially infective state of *H. pylori*.

Baker *et al*[80] (2002) and Johnson *et al*[83] (1997) demonstrated that *H. pylori* is inactivated by chlorine. However, their studies and conclusions did not recover culturable cells but reported only on the VBNC state. A more recent study by Moreno *et al*[82] also demonstrated the survival of *H. pylori* but again only in the VBNC

state. Unfortunately all these studies did not take into account the survival and association of *H. pylori* in biofilms and the tolerance when grown as part of a biofilm[84]. A later study by Gião *et al*[85] demonstrated that viable *H. pylori* can survive in the viable state in biofilms. The efficacy of chlorine treatment on a biofilm that contained this bacterium was investigated further. In later studies, Gião *et al*[85] found that using a specific peptide nucleic acid (PNA) probe it could be demonstrated that *H. pylori* persist inside biofilms that had been exposed to chlorine at 0.2 and 1.2 mg/L. This occurred for at least 26 d. In this study, no culturable cells were recovered. However when viability stains were employed *H. pylori* was observed suggesting that it could survive within a biofilm at this concentration of chlorine[86].

If *H. pylori* are disseminated into the water cycle and allowing them to enter water distribution systems, it is possible that routinely used water treatment methods and disinfectants presently employed may not be as effective as once thought. This seems to be due to the ability of *H. pylori* to survive within a biofilm.

Eradication within the host

The first-line therapy for the eradication of *H. pylori* involves the combination of a proton pump inhibitor in conjunction with either clarithromycin (CLR) or metronidazole, and amoxicillin[87-89]. The antibiotic CLR is a macrolide antibiotic that is known to bind to the 50S subunit of the bacterial ribosome and thereby inhibiting the translation of peptides, leading to the inhibition of growth. However, of growing significance to *H. pylori* eradication is the increasing problem of CLR-resistance[88-92].

H. PYLORI RESISTANCE WITHIN BIOFILMS

There are growing reports regarding the resistance of *H. pylori* to clarithromycin, the common antibiotic which is used in its eradication in the human host[88]. The occurrence of CLR resistant *H. pylori* is very common with ranges being reported between 10% to 30%[93,94]. The basis of resistance is a point mutation in the domain V loop of the 23S rRNA gene (commonly an adenine-to-guanine transition at position 2142 or 2143)[88,90-96].

Furthermore Yonezawa *et al*[97] investigated the effects of *H. pylori* biofilm formation *in vitro* on clarithromycin (CLR) susceptibility. Within this study CLR susceptibility of intermediate (2-d) and mature (3-d) *H. pylori* biofilms on glass coverslips was determined. Concentrations of CLR applied to the biofilm ranged from 0.03 to 0.5 mg/mL. It was found that the biomass of the *H. pylori* biofilm increased after treatment with CLR at minimum inhibitory concentration levels by up to 4-fold (2-d biofilm) and 16-fold (3-d biofilm). In addition to this the minimum bactericidal concentrations of CLR against cells in a biofilm was higher (1.0 mg/mL) for the biofilm-grown cells when compared with the planktonic cells (0.25 mg/mL). Furthermore the expression of efflux pump genes significantly increased in the biofilm cells. Overall, this study demonstrated that *H. pylori* biofilm formation decreases the susceptibility to CLR. In addition it was found that *H. pylori* CLR resistance mutations were generated more frequently in biofilms than in planktonic cells. *H. pylori* has numerous constitutive genes which may help to rapidly neutralise oxidative antimicrobials. The rapid expression of constitutive enzymes may help to assist the survival of *H. pylori* in the environment. A survival strategy is the formation of coccoid phenotype.

CONCLUSION

The ability to grow and proliferate within a biofilm is significant to the longevity, survival and also dissemination of *H. pylori*. Growth within a biofilm is a significant risk factor in both its eradication and treatment and therefore its persistence both within the host and the environment. Within this state, its recalcitrance is enhanced and its ability to acquire genes enhancing virulence is evident. This adaptation is effective for its survival, genetic variability and persistence. The characteristics of *H. pylori* provide evidence of survival in the environment and therefore acquisition is heightened. It is well known that *H. pylori* in stressful environments convert from the virulent infectious spiral phenotype to that of the less virulent VBNC coccoid state. It is within this VBNC coccoid state that *H. pylori* is thought to reside within biofilms. Biofilms have been associated with persistent infections and increased resistance to antimicrobial action. Thus, the ability of *H. pylori* to resuscitate and revert from the coccoid to spiral form is a mechanism that requires attention in terms understanding the factors that may lead to infection recurrence both in the host and the external environment.

The dissemination of *H. pylori* is significant in its acquisition by the host. Person-to-person transmission is a strong risk factor. However, there is more evidence growing following an initial report in early 2000, that contaminated water may be an important conduit for dissemination and acquisition. However the lack of evidence relating to the presence of *H. pylori*, particularly in biofilm form, in the environment is apparent and may be due to the transformation of *H. pylori* from culturable spiral form to the VBNC coccoid form. The detection methods used to identify *H. pylori*, particularly in the VBNC coccoid state, need to be refined if successful identification of this microorganism is to be made.

The biofilm-forming potential of *H. pylori* means that eradication both within the host and the environment, is significantly reduced, which justifies the need to refine and develop treatment regimes and strategies that are more appropriate and effective than traditional therapies that have high failures rates in eradicating *H. pylori*. In the environment, present evidence suggests that traditionally used disinfectants are effective on planktonic *H.*

pylori but little evidence exists on the effectiveness of antimicrobials on *H. pylori* in environmental biofilms. This environment, be it potable water biofilms or biofilms in hot water systems in domestic houses, may be a possible reservoir for *H. pylori* and aid in its transmission and dissemination.

Appropriate anti-biofilm agents are therefore required to ensure that in the host, *H. pylori* can be eradicated fully and continuing dissemination does not occur.

REFERENCES

1 **Blaser MJ**, Kirschner D. Dynamics of Helicobacter pylori colonization in relation to the host response. *Proc Natl Acad Sci USA* 1999; **96**: 8359-8364 [PMID: 10411880 DOI: 10.1073/pnas.96.15.8359]

2 **Young KA**, Allaker RP, Hardie JM. Morphological analysis of Helicobacter pylori from gastric biopsies and dental plaque by scanning electron microscopy. *Oral Microbiol Immunol* 2001; **16**: 178-181 [PMID: 11358540]

3 **Kandulski A**, Selgrad M, Malfertheiner P. Helicobacter pylori infection: a clinical overview. *Dig Liver Dis* 2008; **40**: 619-626 [PMID: 18396114 DOI: 10.1016/j.dld.2008.02.026]

4 **Herrera V**, Parsonnet J. Helicobacter pylori and gastric adenocarcinoma. *Clin Microbiol Infect* 2009; **15**: 971-976 [PMID: 19874380 DOI: 10.1111/j.1469-0691.2009.03031.x]

5 **Andersen LP**, Rasmussen L. Helicobacter pylori-coccoid forms and biofilm formation. *FEMS Immunol Med Microbiol* 2009; **56**: 112-115 [PMID: 19453756 DOI: 10.1111/j.1574-695X.2009.00556.x]

6 **Siavoshi F**, Saniee P, Atabakhsh M, Pedramnia S, Tavakolian A, Mirzaei M. Mucoid Helicobacter pylori isolates with fast growth under microaerobic and aerobic conditions. *Helicobacter* 2012; **17**: 62-67 [PMID: 22221618]

7 **Bik EM**, Eckburg PB, Gill SR, Nelson KE, Purdom EA, Francois F, Perez-Perez G, Blaser MJ, Relman DA. Molecular analysis of the bacterial microbiota in the human stomach. *Proc Natl Acad Sci USA* 2006; **103**: 732-737 [PMID: 16407106]

8 **Martin ME**, Bhatnagar S, George MD, Paster BJ, Canfield DR, Eisen JA, Solnick JV. The impact of Helicobacter pylori infection on the gastric microbiota of the rhesus macaque. *PLoS One* 2013; **8**: e76375 [PMID: 24116104]

9 **Lofgren JL**, Whary MT, Ge Z, Muthupalani S, Taylor NS, Mobley M, Potter A, Varro A, Eibach D, Suerbaum S, Wang TC, Fox JG. Lack of commensal flora in Helicobacter pylori-infected INS-GAS mice reduces gastritis and delays intraepithelial neoplasia. *Gastroenterology* 2011; **140**: 210-220 [PMID: 20950613]

10 **Andersen LP**. Colonization and infection by Helicobacter pylori in humans. *Helicobacter* 2007; **12** Suppl 2: 12-15 [PMID: 17991171 DOI: 10.1111/j.1523-5378.2007.00574.x]

11 **Percival SL**, Thomas JG. Transmission of Helicobacter pylori and the role of water and biofilms. *J Water Health* 2009; **7**: 469-477 [PMID: 19491497]

12 **Stark RM**, Gerwig GJ, Pitman RS, Potts LF, Williams NA, Greenman J, Weinzweig IP, Hirst TR, Millar MR. Biofilm formation by Helicobacter pylori. *Lett Appl Microbiol* 1999; **28**: 121-126 [PMID: 10063642]

13 **Cole SP**, Harwood J, Lee R, She R, Guiney DG. Characterization of monospecies biofilm formation by Helicobacter pylori. *J Bacteriol* 2004; **186**: 3124-3132 [PMID: 15126474 DOI: 10.1128/JB.186.10.3124-3132.2004]

14 **Cellini L**, Grande R, Di Campli E, Di Bartolomeo S, Di Giulio M, Traini T, Trubiani O. Characterization of an Helicobacter pylori environmental strain. *J Appl Microbiol* 2008; **105**: 761-769 [PMID: 18410343 DOI: 10.1111/j.1365-2672.2008.03808.x]

15 **Carron MA**, Tran VR, Sugawa C, Coticchia JM. Identification of Helicobacter pylori biofilms in human gastric mucosa. *J Gastrointest Surg* 2006; **10**: 712-717 [PMID: 16713544 DOI: 10.1016/j.gassur.2005.10.019]

16 **Coticchia JM**, Sugawa C, Tran VR, Gurrola J, Kowalski E, Carron MA. Presence and density of Helicobacter pylori biofilms in human gastric mucosa in patients with peptic ulcer disease. *J Gastrointest Surg* 2006; **10**: 883-889 [PMID: 16769546 DOI: 10.1016/j.gassur.2005.12.009]

17 **Yonezawa H**, Osaki T, Kurata S, Fukuda M, Kawakami H, Ochiai K, Hanawa T, Kamiya S. Outer membrane vesicles of Helicobacter pylori TK1402 are involved in biofilm formation. *BMC Microbiol* 2009; **9**: 197 [PMID: 19751530]

18 **Yonezawa H**, Osaki T, Kurata S, Zaman C, Hanawa T, Kamiya S. Assessment of in vitro biofilm formation by Helicobacter pylori. *J Gastroenterol Hepatol* 2010; **25** Suppl 1: S90-S94 [PMID: 20586874]

19 **Yonezawa H**, Osaki T, Woo T, Kurata S, Zaman C, Hojo F, Hanawa T, Kato S, Kamiya S. Analysis of outer membrane vesicle protein involved in biofilm formation of Helicobacter pylori. *Anaerobe* 2011; **17**: 388-390 [PMID: 21515394]

20 **Cellini L**, Grande R, Di Campli E, Traini T, Giulio MD, Lannutti SN, Lattanzio R. Dynamic colonization of Helicobacter pylori in human gastric mucosa. *Scand J Gastroenterol* 2008; **43**: 178-185 [PMID: 17918004 DOI: 10.1080/00365520701675965]

21 **Azevedo NF**, Pinto AR, Reis NM, Vieira MJ, Keevil CW. Shear stress, temperature, and inoculation concentration influence the adhesion of water-stressed Helicobacter pylori to stainless steel 304 and polypropylene. *Appl Environ Microbiol* 2006; **72**: 2936-2941 [PMID: 16598000 DOI: 10.1128/AEM.72.4.2936-2941.2006]

22 **Whitchurch CB**, Tolker-Nielsen T, Ragas PC, Mattick JS. Extracellular DNA required for bacterial biofilm formation. *Science* 2002; **295**: 1487 [PMID: 11859186 DOI: 10.1126/science.295.5559.1487]

23 **Costerton JW**, Lewandowski Z, DeBeer D, Caldwell D, Korber D, James G. Biofilms, the customized microniche. *J Bacteriol* 1994; **176**: 2137-2142 [PMID: 8157581]

24 **Hall-Stoodley L**, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. *Nat Rev Microbiol* 2004; **2**: 95-108 [PMID: 15040259 DOI: 10.1038/nrmicro821]

25 **Hu FZ**, Ehrlich GD. Population-level virulence factors amongst pathogenic bacteria: relation to infection outcome. *Future Microbiol* 2008; **3**: 31-42 [PMID: 18230032 DOI: 10.2217/17460913.3.1.31]

26 **Costerton JW**, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science* 1999; **284**: 1318-1322 [PMID: 10334980 DOI: 10.1126/science.284.5418.1318]

27 **Goodman KJ**, Correa P, Tenganá Aux HJ, Ramírez H, DeLany JP, Guerrero Pepinosa O, López Quiñones M, Collazos Parra T. Helicobacter pylori infection in the Colombian Andes: a population-based study of transmission pathways. *Am J Epidemiol* 1996; **144**: 290-299 [PMID: 8686698 DOI: 10.1093/oxfordjournals.aje.a008924]

28 **Velázquez M**, Feirtag JM. Helicobacter pylori: characteristics, pathogenicity, detection methods and mode of transmission implicating foods and water. *Int J Food Microbiol* 1999; **53**: 95-104 [PMID: 10634701 DOI: 10.1016/S0168-1605(99)00160-9]

29 **Brown LM**. Helicobacter pylori: epidemiology and routes of transmission. *Epidemiol Rev* 2000; **22**: 283-297 [PMID: 11218379 DOI: 10.1093/oxfordjournals.epirev.a018040]

30 **Kamat AH**, Mehta PR, Natu AA, Phadke AY, Vora IM, Desai PD, Koppikar GV. Dental plaque: an unlikely reservoir of Helicobacter pylori. *Indian J Gastroenterol* 1998; **17**: 138-140 [PMID: 9795501]

31 **Oshowo A**, Gillam D, Botha A, Tunio M, Holton J, Boulos P, Hobsley M. Helicobacter pylori: the mouth, stomach, and gut axis. *Ann Periodontol* 1998; **3**: 276-280 [PMID: 9722711

DOI: 10.1902/annals.1998.3.1.276]
32 **Oshowo A**, Tunio M, Gillam D, Botha AJ, Holton J, Boulos P, Hobsley M. Oral colonization is unlikely to play an important role in Helicobacter pylori infection. *Br J Surg* 1998; **85**: 850-852 [PMID: 9667722 DOI: 10.1046/j.1365-2168.1998.00724.x]
33 **Souto R**, Colombo AP. Detection of Helicobacter pylori by polymerase chain reaction in the subgingival biofilm and saliva of non-dyspeptic periodontal patients. *J Periodontol* 2008; **79**: 97-103 [PMID: 18166098 DOI: 10.1902/jop.2008.070241]
34 **Karita M**, Teramukai S, Matsumoto S. Risk of Helicobacter pylori transmission from drinking well water is higher than that from infected intrafamilial members in Japan. *Dig Dis Sci* 2003; **48**: 1062-1067 [PMID: 12822863]
35 **Klein PD**, Graham DY, Gaillour A, Opekun AR, Smith EO. Water source as risk factor for Helicobacter pylori infection in Peruvian children. Gastrointestinal Physiology Working Group. *Lancet* 1991; **337**: 1503-1506 [PMID: 1675369 DOI: 10.1016/0140-6736(91)93196-G]
36 **Ahmed KS**, Khan AA, Ahmed I, Tiwari SK, Habeeb A, Ahi JD, Abid Z, Ahmed N, Habibullah CM. Impact of household hygiene and water source on the prevalence and transmission of Helicobacter pylori: a South Indian perspective. *Singapore Med J* 2007; **48**: 543-549 [PMID: 17538754]
37 **Nurgalieva ZZ**, Malaty HM, Graham DY, Almuchambetova R, Machmudova A, Kapsultanova D, Osato MS, Hollinger FB, Zhangabylov A. Helicobacter pylori infection in Kazakhstan: effect of water source and household hygiene. *Am J Trop Med Hyg* 2002; **67**: 201-206 [PMID: 12389948]
38 **Sibille I**, Sime-Ngando T, Mathieu L, Block JC. Protozoan bacterivory and Escherichia coli survival in drinking water distribution systems. *Appl Environ Microbiol* 1998; **64**: 197-202 [PMID: 9435076]
39 **Vincent P**. Transmission and acquisition of Helicobacter pylori infection: evidences and hypothesis. *Biomed Pharmacother* 1995; **49**: 11-18 [PMID: 7749074 DOI: 10.1016/0753-3322(96)82572-8]
40 **Linke S**, Lenz J, Gemein S, Exner M, Gebel J. Detection of Helicobacter pylori in biofilms by real-time PCR. *Int J Hyg Environ Health* 2010; **213**: 176-182 [PMID: 20427237 DOI: 10.1016/j.ijheh.2010.03.006]
41 **Fontenete S**, Guimarães N, Leite M, Figueiredo C, Wengel J, Filipe Azevedo N. Hybridization-based detection of Helicobacter pylori at human body temperature using advanced locked nucleic acid (LNA) probes. *PLoS One* 2013; **8**: e81230 [PMID: 24278398 DOI: 10.1371/journal.pone.0081230]
42 **Williams JC**, McInnis KA, Testerman TL. Adherence of Helicobacter pylori to abiotic surfaces is influenced by serum. *Appl Environ Microbiol* 2008; **74**: 1255-1258 [PMID: 18156334 DOI: 10.1128/AEM.01958-07]
43 **Di Campli E**, Di Bartolomeo S, Grande R, Di Giulio M, Cellini L. Effects of extremely low-frequency electromagnetic fields on Helicobacter pylori biofilm. *Curr Microbiol* 2010; **60**: 412-418 [PMID: 20033173 DOI: 10.1007/s00284-009-9558-9]
44 **Cellini L,** Grande R, Traini T, Di Campli E, Di Bartolomeo S, Di Iorio D, Caputi S. Biofilm formation and modulation of luxS and rpoD expression by Helicobacter pylori. *Biofilms* 2005; **2**: 119-127 [DOI: 10.1017/S1479050505001845]
45 **Parsek MR**, Greenberg EP. Sociomicrobiology: the connections between quorum sensing and biofilms. *Trends Microbiol* 2005; **13**: 27-33 [PMID: 15639629 DOI: 10.1016/j.tim.2004.11.007]
46 **Rader BA**, Campagna SR, Semmelhack MF, Bassler BL, Guillemin K. The quorum-sensing molecule autoinducer 2 regulates motility and flagellar morphogenesis in Helicobacter pylori. *J Bacteriol* 2007; **189**: 6109-6117 [PMID: 17586631 DOI: 10.1128/JB.00246-07]
47 **Rader BA**, Wreden C, Hicks KG, Sweeney EG, Ottemann KM, Guillemin K. Helicobacter pylori perceives the quorum-sensing molecule AI-2 as a chemorepellent via the chemoreceptor TlpB. *Microbiology* 2011; **157**: 2445-2455 [PMID: 21602215 DOI: 10.1099/mic.0.049353-0]
48 **Bessa LJ**, Grande R, Di Iorio D, Di Giulio M, Di Campli E, Cellini L. Helicobacter pylori free-living and biofilm modes of growth: behavior in response to different culture media. *APMIS* 2013; **121**: 549-560 [PMID: 23237527]
49 **Goodwin CS**, Armstrong JA. Microbiological aspects of Helicobacter pylori (Campylobacter pylori). *Eur J Clin Microbiol Infect Dis* 1990; **9**: 1-13 [PMID: 2406141 DOI: 10.1007/BF01969526]
50 **Park AM**, Li Q, Nagata K, Tamura T, Shimono K, Sato EF, Inoue M. Oxygen tension regulates reactive oxygen generation and mutation of Helicobacter pylori. *Free Radic Biol Med* 2004; **36**: 1126-1133 [PMID: 15082066 DOI: 10.1016/j.freeradbiomed.2004.02.001]
51 **Kangatharalingam N**, Amy PS. Helicobacter pylori comb. nov. Exhibits Facultative Acidophilism and Obligate Microaerophilism. *Appl Environ Microbiol* 1994; **60**: 2176-2179 [PMID: 16349304]
52 **Pérez-Rosas N**, Hazen TC. In situ survival of Vibrio cholerae and Escherichia coli in a tropical rain forest watershed. *Appl Environ Microbiol* 1989; **55**: 495-499 [PMID: 2655536]
53 **Lewis K**. Persister cells, dormancy and infectious disease. *Nat Rev Microbiol* 2007; **5**: 48-56 [PMID: 17143318 DOI: 10.1038/nrmicro1557]
54 **Cellini L**, Allocati N, Di Campli E, Dainelli B. Helicobacter pylori: a fickle germ. *Microbiol Immunol* 1994; **38**: 25-30 [PMID: 8052159 DOI: 10.1111/j.1348-0421.1994.tb01740.x]
55 **Lewis K.** Multidrug tolerance of biofilms and persister cells. Bacterial biofilms. Current Topics in Microbiology and Immunology: Springer, 2008: 107-131
56 **Pai SR**, Actor JK, Sepulveda E, Hunter RL, Jagannath C. Identification of viable and non-viable Mycobacterium tuberculosis in mouse organs by directed RT-PCR for antigen 85B mRNA. *Microb Pathog* 2000; **28**: 335-342 [PMID: 10839970 DOI: 10.1006/mpat.2000.0353]
57 **Watson CL**, Owen RJ, Said B, Lai S, Lee JV, Surman-Lee S, Nichols G. Detection of Helicobacter pylori by PCR but not culture in water and biofilm samples from drinking water distribution systems in England. *J Appl Microbiol* 2004; **97**: 690-698 [PMID: 15357718 DOI: 10.1111/j.1365-2672.2004.02360.x]
58 **Oliver JD**. Recent findings on the viable but nonculturable state in pathogenic bacteria. *FEMS Microbiol Rev* 2010; **34**: 415-425 [PMID: 20059548]
59 **Cellini L**, Robuffo I, Di Campli E, Di Bartolomeo S, Taraborelli T, Dainelli B. Recovery of Helicobacter pylori ATCC43504 from a viable but not culturable state: regrowth or resuscitation? *APMIS* 1998; **106**: 571-579 [PMID: 9674895 DOI: 10.1111/j.1699-0463.1998.tb01386.x]
60 **Richards CL**, Buchholz BJ, Ford TE, Broadaway SC, Pyle BH, Camper AK. Optimizing the growth of stressed Helicobacter pylori. *J Microbiol Methods* 2011; **84**: 174-182 [PMID: 21129415 DOI: 10.1016/j.mimet.2010.11.015]
61 **Cammarota G**, Branca G, Ardito F, Sanguinetti M, Ianiro G, Cianci R, Torelli R, Masala G, Gasbarrini A, Fadda G, Landolfi R, Gasbarrini G. Biofilm demolition and antibiotic treatment to eradicate resistant Helicobacter pylori: a clinical trial. *Clin Gastroenterol Hepatol* 2010; **8**: 817-820.e3 [PMID: 20478402 DOI: 10.1016/j.cgh.2010.05.006]
62 **Roberts IS**. The biochemistry and genetics of capsular polysaccharide production in bacteria. *Annu Rev Microbiol* 1996; **50**: 285-315 [PMID: 8905082 DOI: 10.1146/annurev.micro.50.1.285]
63 **Shahamat M**, Mai UE, Paszko-Kolva C, Yamamoto H, Colwell RR. Evaluation of liquid media for growth of Helicobacter pylori. *J Clin Microbiol* 1991; **29**: 2835-2837 [PMID: 1757556]
64 **West AP**, Millar MR, Tompkins DS. Effect of physical environment on survival of Helicobacter pylori. *J Clin Pathol* 1992; **45**: 228-231 [PMID: 1556231 DOI: 10.1136/jcp.45.3.228]

65 **Konishi K**, Saito N, Shoji E, Takeda H, Kato M, Asaka M, Ooi HK. Helicobacter pylori: longer survival in deep ground water and sea water than in a nutrient-rich environment. *APMIS* 2007; **115**: 1285-1291 [PMID: 18092962 DOI: 10.1111/j.1600-0643.2007.00594.x]

66 **Mackay WG**, Gribbon LT, Barer MR, Reid DC. Biofilms in drinking water systems: a possible reservoir for Helicobacter pylori. *J Appl Microbiol* 1998; **85** Suppl 1: 52S-59S [PMID: 21182693 DOI: 10.1111/j.1365-2672.1998.tb05283.x]

67 **Park SR**, Mackay WG, Reid DC. Helicobacter sp. recovered from drinking water biofilm sampled from a water distribution system. *Water Res* 2001; **35**: 1624-1626 [PMID: 11317912 DOI: 10.1016/S0043-1354(00)00582-0]

68 **Bunn JE**, MacKay WG, Thomas JE, Reid DC, Weaver LT. Detection of Helicobacter pylori DNA in drinking water biofilms: implications for transmission in early life. *Lett Appl Microbiol* 2002; **34**: 450-454 [PMID: 12028428 DOI: 10.1046/j.1472-765X.2002.01122.x]

69 **Braganra SM**, Azevedo NF, Simões LC, Keevil CW, Vieira MJ. Use of fluorescent in situ hybridisation for the visualisation of Helicobacter pylori in real drinking water biofilms. *Water Sci Technol* 2007; **55**: 387-393 [PMID: 17547009 DOI: 10.2166/wst.2007.282]

70 **Azevedo NF**, Pacheco AP, Keevil CW, Vieira MJ. Adhesion of water stressed Helicobacter pylori to abiotic surfaces. *J Appl Microbiol* 2006; **101**: 718-724 [PMID: 16907822 DOI: 10.1111/j.1365-2672.2006.03029.x]

71 **Thomas JE**, Gibson GR, Darboe MK, Dale A, Weaver LT. Isolation of Helicobacter pylori from human faeces. *Lancet* 1992; **340**: 1194-1195 [PMID: 1359263 DOI: 10.1016/0140-6736(92)92894-L]

72 **Ehrlich GD**, Ahmed A, Earl J, Hiller NL, Costerton JW, Stoodley P, Post JC, DeMeo P, Hu FZ. The distributed genome hypothesis as a rubric for understanding evolution in situ during chronic bacterial biofilm infectious processes. *FEMS Immunol Med Microbiol* 2010; **59**: 269-279 [PMID: 20618850 DOI: 10.1111/j.1574-695X.2010.00704.x]

73 **Grande R**, Di Campli E, Di Bartolomeo S, Verginelli F, Di Giulio M, Baffoni M, Bessa LJ, Cellini L. Helicobacter pylori biofilm: a protective environment for bacterial recombination. *J Appl Microbiol* 2012; **113**: 669-676 [PMID: 22639839 DOI: 10.1111/j.1365-2672.2012.05351.x]

74 **Cellini L**, Di Campli E, Di Candia M, Marzio L. Molecular fingerprinting of Helicobacter pylori strains from duodenal ulcer patients. *Lett Appl Microbiol* 2003; **36**: 222-226 [PMID: 12641715 DOI: 10.1046/j.1472-765X.2003.01295.x]

75 **Baltrus DA**, Amieva MR, Covacci A, Lowe TM, Merrell DS, Ottemann KM, Stein M, Salama NR, Guillemin K. The complete genome sequence of Helicobacter pylori strain G27. *J Bacteriol* 2009; **191**: 447-448 [PMID: 18952803 DOI: 10.1128/JB.01416-08]

76 **Grande R**, Di Giulio M, Bessa LJ, Di Campli E, Baffoni M, Guarnieri S, Cellini L. Extracellular DNA in Helicobacter pylori biofilm: a backstairs rumour. *J Appl Microbiol* 2011; **110**: 490-498 [PMID: 21143715 DOI: 10.1111/j.1365-2672.2010.04911.x]

77 **Kaplan JB**. Biofilm dispersal: mechanisms, clinical implications, and potential therapeutic uses. *J Dent Res* 2010; **89**: 205-218 [PMID: 20139339 DOI: 10.1177/0022034509359403]

78 **McDougald D**, Rice SA, Barraud N, Steinberg PD, Kjelleberg S. Should we stay or should we go: mechanisms and ecological consequences for biofilm dispersal. *Nat Rev Microbiol* 2012; **10**: 39-50 [PMID: 22120588 DOI: 10.1038/nrmicro2695]

79 **Eaton KA**, Morgan DR, Krakowka S. Motility as a factor in the colonisation of gnotobiotic piglets by Helicobacter pylori. *J Med Microbiol* 1992; **37**: 123-127 [PMID: 1629897 DOI: 10.1099/00222615-37-2-123]

80 **Baker KH**, Hegarty JP, Redmond B, Reed NA, Herson DS. Effect of oxidizing disinfectants (chlorine, monochloramine, and ozone) on Helicobacter pylori. *Appl Environ Microbiol* 2002; **68**: 981-984 [PMID: 11823249 DOI: 10.1128/AEM.68.2.981-984.2002]

81 **Mazari-Hiriart M**, Lopez-Vidal Y, Ponce de Leon S, Castillo-Rojas G, Hernandez-Eugenio C, Rojo F. Bacteria and disinfection byproducts in water from southern Mexico City. *Arch Environ Health* 2003; **58**: 233-237 [PMID: 14655904]

82 **Moreno Y**, Piqueres P, Alonso JL, Jiménez A, González A, Ferrús MA. Survival and viability of Helicobacter pylori after inoculation into chlorinated drinking water. *Water Res* 2007; **41**: 3490-3496 [PMID: 17585990 DOI: 10.1016/j.watres.2007.05.020]

83 **Johnson CH**, Rice EW, Reasoner DJ. Inactivation of Helicobacter pylori by chlorination. *Appl Environ Microbiol* 1997; **63**: 4969-4970 [PMID: 9406419]

84 **De Beer D**, Srinivasan R, Stewart PS. Direct measurement of chlorine penetration into biofilms during disinfection. *Appl Environ Microbiol* 1994; **60**: 4339-4344 [PMID: 7811074]

85 **Gião MS**, Azevedo NF, Wilks SA, Vieira MJ, Keevil CW. Persistence of Helicobacter pylori in heterotrophic drinking-water biofilms. *Appl Environ Microbiol* 2008; **74**: 5898-5904 [PMID: 18676697 DOI: 10.1128/AEM.00827-08]

86 **Gião MS**, Azevedo NF, Wilks SA, Vieira MJ, Keevil CW. Effect of chlorine on incorporation of Helicobacter pylori into drinking water biofilms. *Appl Environ Microbiol* 2010; **76**: 1669-1673 [PMID: 19966018 DOI: 10.1128/AEM.01378-09]

87 **Lind T**, Mégraud F, Unge P, Bayerdörffer E, O'morain C, Spiller R, Veldhuyzen Van Zanten S, Bardhan KD, Hellblom M, Wrangstadh M, Zeijlon L, Cederberg C. The MACH2 study: role of omeprazole in eradication of Helicobacter pylori with 1-week triple therapies. *Gastroenterology* 1999; **116**: 248-253 [PMID: 9922303 DOI: 10.1016/S0016-5085(99)70119-8]

88 **Asaka M**, Kato M, Takahashi S, Fukuda Y, Sugiyama T, Ota H, Uemura N, Murakami K, Satoh K, Sugano K. Guidelines for the management of Helicobacter pylori infection in Japan: 2009 revised edition. *Helicobacter* 2010; **15**: 1-20 [PMID: 20302585]

89 **Malfertheiner P**, Mégraud F, O'Morain C, Bell D, Bianchi Porro G, Deltenre M, Forman D, Gasbarrini G, Jaup B, Misiewicz JJ, Pajares J, Quina M, Rauws E. Current European concepts in the management of Helicobacter pylori infection--the Maastricht Consensus Report. The European Helicobacter Pylori Study Group (EHPSG). *Eur J Gastroenterol Hepatol* 1997; **9**: 1-2 [PMID: 9031888]

90 **Graham DY**, de Boer WA, Tytgat GN. Choosing the best anti-Helicobacter pylori therapy: effect of antimicrobial resistance. *Am J Gastroenterol* 1996; **91**: 1072-1076 [PMID: 8651150]

91 **Adamek RJ**, Suerbaum S, Pfaffenbach B, Opferkuch W. Primary and acquired Helicobacter pylori resistance to clarithromycin, metronidazole, and amoxicillin--influence on treatment outcome. *Am J Gastroenterol* 1998; **93**: 386-389 [PMID: 9517645 DOI: 10.1016/S0002-9270(97)00110-X]

92 **Mégraud F**, Doermann HP. Clinical relevance of resistant strains of Helicobacter pylori: a review of current data. *Gut* 1998; **43** Suppl 1: S61-S65 [PMID: 9764043]

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

94 **Horiki N**, Omata F, Uemura M, Suzuki S, Ishii N, Iizuka Y, Fukuda K, Fujita Y, Katsurahara M, Ito T, Cesar GE, Imoto I, Takei Y. Annual change of primary resistance to clarithromycin among Helicobacter pylori isolates from 1996 through 2008 in Japan. *Helicobacter* 2009; **14**: 86-90 [PMID: 19751432 DOI: 10.1111/j.1523-5378.2009.00714.x]

95 **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]

96 **García-Arata MI**, Baquero F, de Rafael L, Martín de Argila C, Gisbert JP, Bermejo F, Boixeda D, Cantón R. Mutations in 23S rRNA in Helicobacter pylori conferring resistance to erythromycin do not always confer resistance to clarithromycin. *Antimicrob Agents Chemother* 1999; **43**: 374-376 [PMID: 9925537]

97 **Yonezawa H**, Osaki T, Hanawa T, Kurata S, Ochiai K, Kamiya S. Impact of Helicobacter pylori biofilm formation on clarithromycin susceptibility and generation of resistance mutations. *PLoS One* 2013; **8**: e73301 [PMID: 24039906]

P- Reviewer: Blanco LP, Kim JM, Murakami K
S- Editor: Wen LL **L- Editor**: A **E- Editor:** Lu YJ

World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4291/wjgp.v5.i3.133

World J Gastrointest Pathophysiol 2014 August 15; 5(3): 133-146
ISSN 2150-5330 (online)

TOPIC HIGHLIGHT

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

Role of Toll-like receptors in *Helicobacter pylori* infection and immunity

Sinéad M Smith

Sinéad M Smith, Department of Clinical Medicine, Trinity Centre, Adelaide and Meath Hospital, Tallaght, Dublin 24, Ireland
Sinéad M Smith, School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Dublin 2, Ireland
Author contributions: Smith SM reviewed the literature, drafted and wrote the manuscript.
Correspondence to: Sinéad Smith, PhD, Assistant Professor in Applied and Translational Medicine, Department of Clinical Medicine, Trinity Centre, Adelaide and Meath Hospital, Room 1.44, Tallaght, Dublin 24, Ireland. smithsi@tcd.ie
Telephone: +353-1-8962998 Fax: +353-1-8962988
Received: January 21, 2014 Revised: February 25, 2014
Accepted: May 16, 2014
Published online: August 15, 2014

Abstract

The gram-negative bacterium *Helicobacter pylori* (*H. pylori*) infects the stomachs of approximately half of the world's population. Although infection induces an immune response that contributes to chronic gastric inflammation, the response is not sufficient to eliminate the bacterium. *H. pylori* infection causes peptic ulcers, gastric cancer and mucosa-associated lymphoid tissue lymphoma. Disease outcome is linked to the severity of the host inflammatory response. Gastric epithelial cells represent the first line of innate immune defence against *H. pylori*, and respond to infection by initiating numerous cell signalling cascades, resulting in cytokine induction and the subsequent recruitment of inflammatory cells to the gastric mucosa. Pathogen recognition receptors of the toll-like receptor (TLR) family mediate many of these cell signalling events. This review discusses recent findings on the role of various TLRs in the recognition of *H. pylori* in distinct cell types, describes the TLRs responsible for the recognition of individual *H. pylori* components and outlines the influence of innate immune activation on the subsequent development of the adaptive immune response. The mechanistic identification of host mediators of *H. pylori*-induced pathogenesis has the potential to reveal drug targets and opportunities for therapeutic intervention or prevention of *H. pylori*-associated disease by means of vaccines or immunomodulatory therapy.

Key words: *Helicobacter pylori*; Toll-like receptor; Gastric epithelium; Monocyte; Macrophage; Dendritic cell; Cytokine; Lipopolysaccharide

Core tip: Eradication rates for *Helicobacter pylori* (*H. pylori*) infection have fallen. The development of therapeutic alternatives to antibiotics, such as immunomodulatory therapy and vaccines requires a clearer understanding of host-pathogen interactions. As Toll-like receptors are intimately involved in the regulation of inflammation in response to *H. pylori* and represent key activators of adaptive immunity, they represent a target for therapeutic manipulation. Elucidating innate immune signals triggered by *H. pylori* will provide an understanding of how the balance between pro-inflammatory and anti-inflammatory signals fine-tunes the response to infection and insight into how the immune response may be manipulated therapeutically to successfully eradicate the bacterium.

Smith SM. Role of Toll-like receptors in *Helicobacter pylori* infection and immunity. *World J Gastrointest Pathophysiol* 2014; 5(3): 133-146 Available from: URL: http://www.wjgnet.com/2150-5330/full/v5/i3/133.htm DOI: http://dx.doi.org/10.4291/wjgp.v5.i3.133

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a gram-negative microaerophilic flagellated bacterium that specifically infects

the stomachs of approximately 50% of the world's population. Infection is thought to be acquired in early childhood and persists for life if left untreated, despite triggering vigorous innate and adaptive immune responses[1-4]. Prevalence of *H. pylori* infection varies throughout the world and is associated with lower socioeconomic conditions[5]. Most infected individuals are asymptomatic. However, infection may cause chronic gastritis and confers a 1%-10% risk of developing gastric or duodenal ulcers, a 0.1%-3% risk of developing gastric adenocarcinoma, and < 0.01% of developing mucosa-associated lymphoid tissue (MALT) lymphoma[2]. Disease risk varies in different populations and is associated with host genotype, strain-specific bacterial components and environmental factors. *H. pylori* colonization of the gastric mucosa is followed by infiltration of polymorphonuclear leukocytes, monocytes and lymphocytes[6]. Mucosal levels of pro-inflammatory cytokines and chemokines such as interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor α (TNFα) and interleukin-1β (IL-1β) are significantly higher in *H. pylori*-positive compared to *H. pylori*-negative gastric specimens[7-9]. Although, *H. pylori* infection induces an immune response that contributes to chronic gastric inflammation, the response is not sufficient to eliminate the bacterium[6,10]. Progression of disease from superficial gastritis to gastric cancer is linked to the severity of the host inflammatory response[11-13].

All consensus guidelines recommend eradication of *H. pylori* in symptomatic individuals using a standard first-line triple therapy consisting of a proton pump inhibitor together with the antibiotics clarithromycin and amoxicillin or metronidazole[4]. However, eradication rates have fallen in recent years in line with a rapid increase in antimicrobial resistance[14]. The most recent multicentre European assessment on *H. pylori* antimicrobial susceptibility has indicated that resistance rates for metronidazole and clarithromycin are 34.9% and 17.5% respectively[15]. Clarithromycin resistance has almost doubled in Europe in the last 10 years[15]. Furthermore, a high resistance rate of 14.1% has emerged for levofloxacin, which is used in rescue therapy for *H. pylori* infection[15]. This rapid emergence of antibiotic resistant strains of *H. pylori* is a cause for concern. The development of therapeutic alternatives to antibiotics, such as immunomodulatory therapy and vaccines requires a more lucid understanding of host-pathogen interactions. The mechanistic identification of host mediators of *H. pylori*-induced pathogenesis has the potential to reveal drug targets and opportunities for therapeutic intervention or prevention of *H. pylori*-associated disease.

The immune system consists of innate and adaptive immunity, that cooperate to efficiently control infections. The evolutionary conserved innate immune system provides the first line of defence against invading microbes, whereas the adaptive immune system is developed in later phases of infection and is highly specific, long lasting and possesses immunological memory[16]. Innate immune recognition of microbes is mediated by families of pathogen recognition receptors (PRRs), which recognize pathogen-associated molecular patterns (PAMPs) that are broadly shared by pathogens[17]. Upon PAMP recognition by a particular PRR, cell signalling cascades are triggered that are necessary for initiation of the host response. Additionally, PRR signalling induces the maturation of the major antigen presenting cells, dendritic cells (DCs), and the subsequent induction of adaptive immunity[17].

Gastric epithelial cells of the stomach mucosa represent the first line of innate immune defence against *H. pylori*, and respond to infection by initiating numerous cell signalling cascades[11]. PRRs of the Toll-like receptor (TLR) family have been shown to mediate many of these cell signalling events. In particular, a key role for TLR2 has been described in the response to *Helicobacter* in multiple cell contexts[11,18-21]. Recent data also suggest associations between TLR2 polymorphisms and the severity of intestinal metaplasia in *H. pylori*-positive patients[22] and with gastric cancer risk[23]. Additionally, polymorphisms in the TLR1 gene, which encodes a TLR2 co-receptor, are associated with *H. pylori* prevalence[24].

TLRS AND PATHOGEN RECOGNITION

TLRs are the most widely studied of the PRRs. Members of the TLR family are type I transmembrane proteins, consisting of a leucine-rich repeat-containing ectodomain involved in PAMP recognition, a transmembrane region and an intracellular portion that harbours a Toll-IL-1 receptor (TIR) domain involved in the activation of downstream signalling pathways. There are 10 TLR genes in humans[25]. TLRs are expressed on the cell surface or associated with intracellular vesicles, such as endosomes[16,17] (Figure 1). TLR1, TLR2, TLR4, TLR5 and TLR6 bind their respective ligands on the cell surface and recognize microbial membrane components such as lipids, lipoproteins and proteins[16,17]. TLR3, TLR7, TLR8, TLR9 are found in intracellular vesicles such as the endosome or lysosome and the endoplasmic reticulum, and are mainly involved in the recognition of microbial nucleic acids[16,17].

TLR4 was the first human TLR to be identified and recognizes bacterial lipopolysaccharide (LPS), which is a major constituent of the outer membrane of gram-negative bacteria[26]. LPS is a surface exposed glycolipid that consists of a hydrophobic membrane anchor portion, known as lipid A, and a non-repeating core oligosaccharide coupled to a distal polysaccharide (O-antigen) that extends from the bacterial surface[27,28]. The lipid A domain is responsible for the endotoxic properties associated with LPS. There is considerable LPS structural variability, due to diversity in both the chemical composition of the polysaccharide O-antigen and in lipid A variations, which contribute to the ability of some gram-negative bacteria to evade immune detection[27,28]. Smooth LPS is composed of a polysaccharide O-antigen side chain and has complete core oligosaccharides,

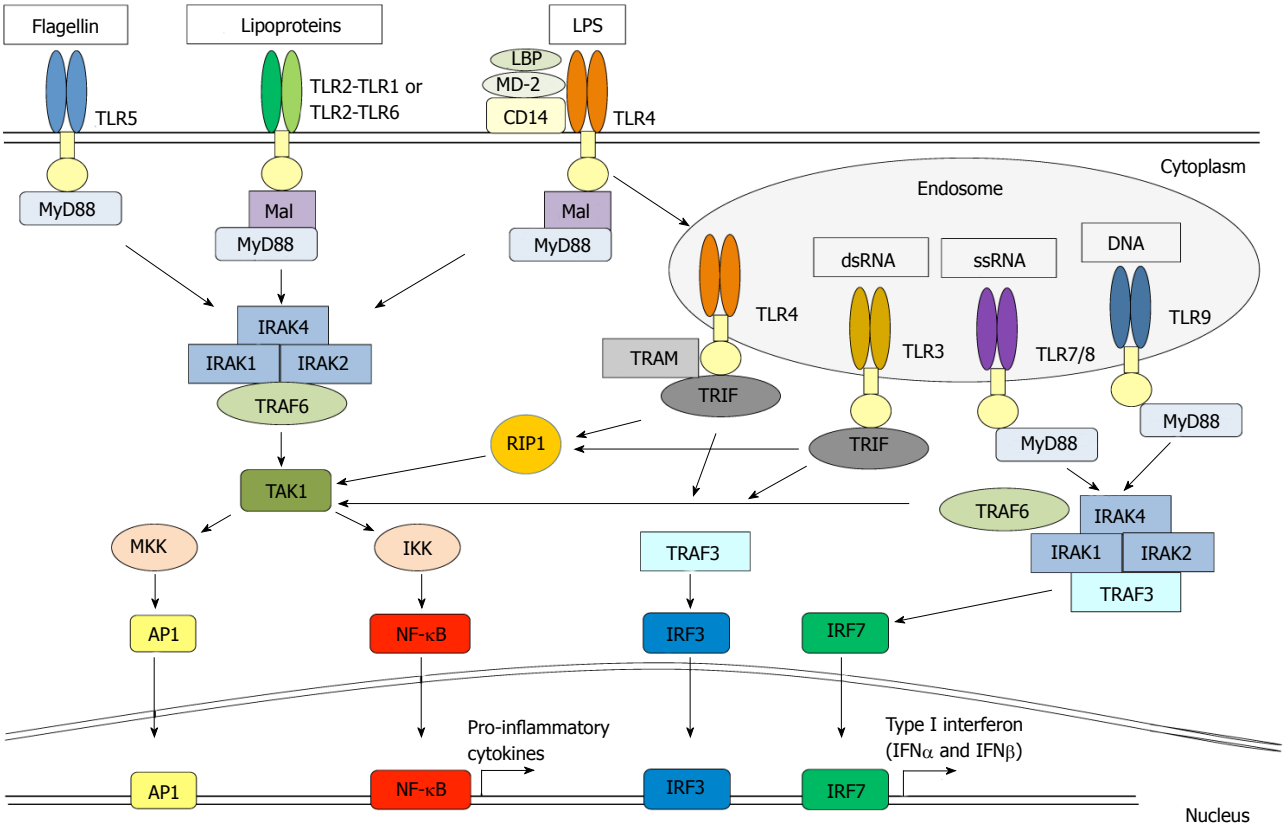

Figure 1 Toll-like receptor signalling. Toll-like receptors (TLRs) are type I transmembrane proteins, consisting of a leucine-rich repeat-containing ectodomain involved in pathogen-associated molecular pattern (PAMP) recognition, a transmembrane region and an intracellular portion that harbours a Toll-IL-1 receptor (TIR) domain involved in adapter protein recruitment and the activation of downstream signalling pathways. TLR1, TLR2, TLR4, TLR5 and TLR6 bind to their ligands on the cell surface and recognize microbial membrane components. TLR3, TLR7, TLR8, TLR9 are found in intracellular vesicles are mainly involved in the recognition of microbial nucleic acids. TLR signalling is initiated by ligand-induced receptor dimerization and TIR engagement with the adapter proteins MyD88 or TRIF. TLR4 localises from the cell membrane to endosomes to change signalling through MyD88 to TRIF. MyD88 is a central TLR adapter protein utilized by all TLRs, with the exception of TLR3, and transmits signals that result in the induction of inflammatory cytokines. The association between a TLR and MyD88 recruits members of the IRAK family. IRAK1 and IRAK4 are sequentially phosphorylated and dissociated from MyD88. This results in the activation of TRAF6, which in turn activates TAK1. TAK1 activates the IKK complex. In most resting cells, NF-κB is bound to the inhibitory IκB proteins (IκBα and IκBβ) in the cytoplasm. Upon activation of the IKK complex, IκB becomes phosphorylated and degraded, thus releasing NF-κB for translocation to the nucleus, where it interacts with promoters harboring κB binding elements. In addition, TAK1 stimulation results in the induction of MAP kinases kinases (MKKs) that activate p38, JNK and ERK, resulting in the subsequent activation of AP-1. In the case of TLR4, and to a lesser extent TLR2, the activation of this pathway involves the bridging adapter protein MAL, which links MyD88 to the TLR. The adapter protein TRIF is involved in the MyD88-independent TLR4 pathway, as well as the TLR3 signalling pathway. TRAM links TRIF to TLR4. Endosomal TLR-mediated signalling leads to the induction of type I interferon through the activation of the transcription factors IRF3 and IRF7.

whereas rough LPS lacks O-antigen and has shorter core oligosaccharides[16]. MD-2 is closely associated with TLR4 on the cell surface and is required for strong inflammatory cytokine induction in response to LPS. LPS-binding protein (LBP) and CD14 are also involved in the TLR4-mediated response to LPS[16]. Cells lacking CD14 are not responsive to smooth LPS but still respond to rough LPS or lipid A[16].

TLR2 recognizes a number of PAMPs on a variety of microorganisms, including zymosan from fungi, triacyl lipopeptides from bacteria and mycobacteria, diacyl lipopeptides from mycoplasma, and peptidoglycan and lipoteichoic acid from gram-positive bacteria[16,17]. TLR2 distinguishes between PRRs by hetero-dimerization with TLR1, TLR6, dectin-1 or CD14. TLR2 heterodimerizes with TLR1 to recognize triacylated lipopeptides from gram-positive bacteria[29,30] or with TLR6 to recognize diacylated lipopeptides, lipoteichoic acid and zymosan[31,32]. CD14 is involved in the recognition of diacylated lipopeptide, whereas the C-type lectin receptor dectin-1 collaborates with TLR2 in the recognition of β-glucan[16] found in the cell walls of fungi and yeasts. TLR2 has also been shown to recognize atypical forms of LPS[33-37]. TLR5 recognizes flagellin[38], a protein component of bacterial flagella. A role for TLR10 has not yet been shown, but the TLR10 sequence is most similar to TLR1 so TLR10 may heterodimerize with TLR2[25]. TLR3 recognizes double stranded RNA[39], which is a major component of many viruses. TLR9 is the receptor for CpG-rich hypomethylated DNA motifs[40], frequently found in bacterial DNA. TLR9 also responds to herpes virus DNA[41]. TLR7 and TLR8 sense single-stranded viral RNA[42-44].

TLR SIGNALLING

Upon PAMP recognition, TLRs trigger cell signalling pathways resulting in (1) the activation of the transcription factors nuclear factor-κB (NF-κB), activating protein-1 (AP-1) and interferon regulatory factors (IRFs); (2) expression of inflammatory cytokines, antimicrobial peptides and type I interferon (IFN); and (3) the subsequent recruitment of neutrophils, activation of macrophages and dendritic cells and the induction of IFN-stimulated genes. The specific response triggered by an individual TLR depends on the recruitment of a single or combination of TIR-domain containing adapter proteins[17]. MyD88 (myeloid differentiation primary response protein 88) is a key TLR adapter protein utilized by all TLRs, with the exception of TLR3, and transmits signals that result in the induction of inflammatory cytokines (Figure 1). The association between a TLR and MyD88 recruits members of the interleukin-1 receptor-associated kinase (IRAK) family. IRAK1 and IRAK4 are sequentially phosphorylated and dissociated from MyD88. This results in the activation of tumor necrosis factor receptor-associated factor 6 (TRAF6), which in turn activates transforming growth factor B-activated protein kinase 1 (TAK1). TAK1 activates the IKK [inhibitor of NF-κB (IκB) kinase] complex. In most resting cells, NF-κB is bound to the inhibitory IκB proteins (IκBα and IκBβ) in the cytoplasm. Upon activation of the IKK complex, IκB becomes phosphorylated and degraded, thus releasing NF-κB for translocation to the nucleus, where it interacts with promoters harboring κB binding elements to regulate gene transcription[45]. In addition, TAK1 stimulation results in activation of MAP kinase kinases (MKK) leading to the induction of the MAP kinases p38, JNK and ERK, resulting in the subsequent activation of AP-1[45] (Figure 1). In the case of TLR4, and to a lesser extent TLR2, the activation of this pathway involves the bridging adapter protein, MAL (MyD88 adapter-like, also known as TIR-domain-containing adapter protein, TIRAP)[46-49] , which links MyD88 to the TLR. The TIR-domain-containing adapter protein inducing IFNβ (TRIF, also known as TICAM1) is involved in the MyD88-independent TLR4 pathway, as well as the TLR3 signalling pathway[50-53] (Figure 1). TRIF-related adapter molecule (TRAM, also known as TICAM2) links TRIF to TLR4[54-56]. Endosomal TLR-mediated signalling leads to the induction of type I interferon through the activation of the transcription factors IRF3 and IRF7[25] (Figure 1).

THE ROLE OF TLRS IN *H. PYLORI* INFECTION

Epithelial cells

As gastric epithelial cells represent the first point of contact between *H. pylori* and the host, there has been a focus on the individual TLRs involved in the response to *H. pylori* infection in this cell context (Table 1). Expression of numerous TLRs has been confirmed in many gastric epithelial cells lines, including AGS, MKN28, MKN45, NUGC3 and KATOIII[19,57-60]. In addition TLR2 has been detected in epithelial cells from human gastric biopsy samples, with increased TLR2 expression reported in samples from *H. pylori*-infected patients[61,62]. Increased TLR4 expression has also been reported in the gastric mucosa of *H. pylori*-infected patients[58]. The Goldberg laboratory have reported a role for both TLR2 and TLR5 during *H. pylori* infection of MKN45 cells; inhibition of TLR2 or TLR5 (but not TLR4) function using dominant-negative mutant constructs decreased *H. pylori*-driven NF-κB activation[19]. In order to assess the contribution of individual TLRs during *H. pylori* infection, many investigators have utilised human embryonic kidney 293 (HEK293) cells stably expressing specific TLRs. HEK293 cells act as a suitable negative control as they do not express TLR2 or TLR4 endogenously[19,63,64]. Indeed additional studies from the Goldberg group have supported a role for TLRs during *H. pylori* infection by demonstrating that over-expression of TLR2 or TLR5 in HEK293 cells enhanced NF-κB activation and IL-8, macrophage inflammatory protein 3α (MIP-3α) and growth regulated protein α (GROα) mRNA expression in response to *H. pylori*[19]. Others have also confirmed that TLR2 expression in HEK293 cells results in enhanced IL-8 expression following *Helicobacter* infection[7,11]. Using HEK-TLR2 cells, the Goldberg group subsequently utilised microarray analysis to identify 28 TLR2-dependent genes whose expression was altered in response to *H. pylori* infection[20]. A number of these genes demonstrated distinct expression patterns between AGS cells (which do not express TLR2 endogenously[20,62]) and MKN45 cells (which express TLR2[19,57])[20].

Monocytes/macrophages

Following *H. pylori* infection, epithelial cells release a variety of cytokines and chemokines leading to the recruitment of monocytes/macrophages to the gastric mucosa. Mononuclear cell infiltration in the lamina propria is characteristic of *H. pylori*-induced chronic infection[65]. Human monocytes and macrophages express a wide repertoire of PRRs. *H. pylori* has been shown to induce secretion of inflammatory cytokines (IL-1β, IL-6, IL-8) from peripheral blood mononuclear cells and IL-8 from purified human monocytes and monocyte-derived macrophages[11]. Different studies have implicated alternative TLRs in the *H. pylori*-mediated response in monocytes/macrophages (Table 1). Maeda *et al*[57] (2001) demonstrated that peritoneal macrophages from C3H/HeJ mice carrying a point mutation in the TLR4 gene showed decreased NF-κB activation and TNFα secretion compared with C3H/HeN macrophages in response to *H. pylori* infection. On the other hand, Gobert *et al*[66] (2004) found no significant difference in terms of IL-6 mRNA induction between peritoneal macrophages isolated from wild-type mice, TLR2-, TLR4- and MyD88-deficient mice in response to *H. pylori* infection. Using bone-

Table 1 Toll-like receptor involvement in the response to *Helicobacter pylori* infection in different cell types

Cell type	Cell type	TLR involvement	Readout of TLR activation	Ref.
Epithelial	MKN45	TLR2	NF-κB-dependent reporter gene activity	Smith *et al*[19]
	HEK-TLR2	TLR5		
	HEK-TLR5			
	HEK-TLR2	TLR2	MIP-3α mRNA expression	Smith *et al*[19]
			IL-8 and	
			GROα mRNA expression	
	HEK-TLR2	TLR2	IL-8 production	Mandell *et al*[11]
	HEK-TLR2	TLR2	mRNA expression of multiple genes	Ding *et al*[20]
	AGS			
	MKN45			
	HEK-TLR2	TLR2	IL-8 mRNA expression	Smith *et al*[7]
Monocytes and macrophages	Mouse peritoneal macrophages	TLR4	NF-κB activation by electro mobility shift assay	Maeda *et al*[57]
			TNFα production	
	Mouse peritoneal macrophages	TLR2-, TLR4- and MyD88-independent	IL-6 mRNA expression	Gobert *et al*[66]
	Mouse BMDMs	TLR2	IL-6 production	Mandell *et al*[11]
	Mouse BMDMs	TLR2	IL-6 and IL-1β production	Obonyo *et al*[67]
		TLR4	IL-10 and IL-12 production	
	Mouse BMDMs	MyD88	IL-6 and IL-12 production	Rad *et al*[69]
Dendritic cells	Mouse BMDCs	MyD88	MHC II and co-stimulatory molecule induction	Rad *et al*[69]
			IL-6, IL-12 and TNFα production	
			mRNA expression of multiple genes	
	Mouse BMDCs	TLR2	mRNA expression of multiple genes	Rad *et al*[18]
		TLR4		
		TLR9	IL-6 and IL-12 production	
	Mouse BMDCs	TLR2	IL-1β production	Kim *et al*[70]
	Mouse BMDCs	TLR2	IL-12, TNFα, IL-6 and IL-23 production	Sun *et al*[73]
B cells	Mouse B cells	MyD88	IL-6 and IL-12 production	Rad *et al*[69]
	Mouse B cells	MyD88	IL-10, IL-6 and TNFα production	Sayi *et al*[21]
		TLR2	CD80 and CD86 expression	
			Secretion of antibodies	

TLR: Toll-like receptor.

marrow derived macrophages (BMDMs) from knockout mice, Mandell *et al*[11] (2004) reported that the cytokine (IL-6) response to *H. pylori* was mediated by TLR2. *H. pylori*-infected BMDMs from wild-type or TLR4-deficient mice produced a robust cytokine response, whereas macrophages form TLR2-deficient mice were unresponsive. It is possible that alternative TLRs are involved in the *H. pylori*-mediated induction of individual cytokines within a particular cell context. Indeed, Obonyo *et al*[67] (2007) demonstrated that *H. pylori* induced IL-12 and IL-10 through TLR4/MyD88 signalling and IL-6 and IL-1β through TLR2/MyD88 signalling using BMDMs from knockout mice. As such, this study would suggest that *H. pylori* infection activates both TLR2 and TLR4 signalling in BMDMs leading to the secretion of distinct cytokines. This hypothesis is possible, given that individual *H. pylori* components have been suggested to trigger TLR2 or TLR4 signalling (Table 2).

Dendritic cells

In recent years, there has been an increasing interest in the mechanisms by which *H. pylori* initiates adaptive immunity and instructs the phenotype of the T cell response. During the activation of adaptive immunity, different T-helper (Th) cell subsets arise that exhibit characteristic patterns of cytokine secretion. As the major antigen presenting cells, DCs play a key role in the induction of the adaptive immune response. DCs express a wide range of PRRs[68] and possess the unique ability to capture antigen from the periphery and activate naïve T cells to direct T cell differentiation by producing three types of signals; antigen presentation, co-stimulation and cytokine secretion[18,69]. Rad *et al*[69] have shown that *H. pylori* activates DCs in a MyD88-dependent manner (Table 1). Production of pro-inflammatory cytokines (IL-6, IL12 and TNFα), and induction of major histocompatibility complex class Ⅱ (MHC Ⅱ) and co-stimulatory molecules in MyD88-deficient DCs was impaired compared to wild-type cells following *H. pylori* stimulation. Further analysis of the *H. pylori*-controlled DC transcriptome by microarray analysis indicated that MyD88 was involved in the regulation of numerous genes involved in DC maturation, antigen uptake and presentation, as well as effector cell recruitment and activation[69]. *H. pylori*-mediated cytokine stimulation was also impaired in B cells and macrophages from the MyD88-deficient mice (Table 1). The *in vitro* findings were reflected *in vivo* in the form of reduced gastric inflammation and increased bacterial colonization following 4 mo *H. pylori* infection in MyD88-deficient mice, suggesting that the impaired immune response in MyD88-deficient mice enables better bacterial survival[69]. *Helicobacter*-specific

IgG2c/IgG1 ratios were reduced in MyD88-deficient mice, implying the involvement of the MyD88 pathway in the instruction of a Th1 phenotype[69]. Subsequent research by Rad *et al*[18] (2009) further characterized TLR-mediated signalling in DCs during *H. pylori* infection. They identified a MyD88-dependent component of the DC activation program that was induced by TLR2 and to a minor extent TLR4. Microarray analysis of *H. pylori*-stimulated DCs showed complementary, redundant and synergistic interactions between TLRs. Using TLR2-deficient cells the anti-inflammatory cytokine IL-10 was identified as a TLR2-dependent *H. pylori* responsive gene in DCs[18]. In addition, they demonstrated that IL-6 and IL-12 production was inhibited by approximately 50% in TLR2/TLR4/TLR9-deficient BMDCs compared to TLR2/TLR4-deficient cells in response to *H. pylori* infection, implying that TLR9-dependent recognition of *H. pylori* in DCs contributes to the cytokine response[18]. More recently Kim *et al*[70] (2013) have also implicated TLR signalling in response to *H. pylori* by demonstrating a role for TLR2 in *H. pylori*-induced IL-1β production in mouse BMDCs.

Although *H. pylori*-infected individuals generate a strong immune response, they fail to eradicate the bacterium. Emerging evidence suggests that failure to eliminate *H. pylori* may be due to its ability to induce a regulatory T cell (Treg) response, as expression of the Treg marker Foxp3 is increased in *H. pylori*-infected gastric tissue compared to that of uninfected individuals[71,72]. Sun *et al*[73] (2013) have recently investigated the functional role of TLR2 signalling in BMDCs in response to *H. pylori* and the subsequent effects on T cell responses. Firstly, they demonstrated that *H. pylori*-infected BMDCs from TLR2-deficient mice exhibited impaired production of the pro-inflammatory cytokines that promote both Th1 responses (IL-12 and TNFα) and Th17 responses (IL-6 and IL-23) compared to wild-type cells. Additionally, this report suggests that *H. pylori* may skew differentiation of naïve T cells towards Th17 and Treg responses as opposed to Th1 responses, as *H. pylori*-stimulated BMDCs from TLR2 knock-out mice induced a higher splenocyte production of IFNγ (Th1 response) and lower production of IL-17 (Th17 response) and IL-10 (Treg response)[73]. *In vivo* analyses following *H. pylori* infection for 2 mo showed a lower degree of gastric *H. pylori* colonization in TLR2 knock-out mice and more severe gastritis, implying that the TLR2-mediated response to *H. pylori* promotes a bacterial survival advantage. Sun *et al*[73] also demonstrated that the gastric mucosa of the infected TLR2 knock-out mice had lower Foxp3, IL-10 and IL-17A expression, but higher expression of IFNγ compared to wild-type mice. The *H. pylori*-specific Th1 response was higher and the Treg and Th17 responses were lower in the spleens of infected TLR2 knock-out mice, suggesting that *H. pylori* mediates immune tolerance through TLR2-derived signals and inhibits Th1 immunity, thus evading the host defence[73]. It is noteworthy that the Rad *et al*[69] (2007) study suggested MyD88-dependent TLR signalling promotes a Th1 response in *H. pylori*-infected mice and is protective against *H. pylori* colonization, while the Sun *et al*[73] (2013) study implies that TLR2 signalling inhibits Th1 immunity, supports a Treg/Th17 response and promotes *H. pylori* colonization. It is possible that different TLR ligands from the same pathogen induce distinct but opposing signals. Furthermore, it is likely that the complementary, redundant and synergistic interactions between TLRs in DCs subsequently reported by Rad *et al*[18] (2009) contribute to the observations from studies involving MyD88-deficient mice.

B cells

Evidence suggests that B cells contribute to the immunepathogenesis of *H. pylori* infection[74]. Sayi *et al*[21] (2011) have demonstrated that B cells play a role in regulating T cell responses and gastric immunopathology in response to *Helicobacter*. Building on the finding by Rad *et al*[69] (2007) that TLR2 is required for *Helicobacter*- mediated IL-6 and IL-12 induction in B cells, Sayi *et al*[21] showed that cytokine production (IL-10, IL-6 and TNFα), surface expression of the activation markers CD80 and CD86 and induction of antibody secretion was impaired in *Helicobacter*-stimulated B cells from both MyD88- and TLR2-deficient mice compared to wild type control cells (Table 1). The *Helicobacter*-stimulated B cells induced IL-10-producing $CD4^+CD25^+$ T regulatory-1 (Tr-1)-like cells in a TLR2- and MyD88-dependent manner[21]. The Tr-1 cells acquired suppressive activity *in vitro* and suppressed excessive gastric *Helicobacter*-associated immunopathology *in vivo*, suggesting that TLR2-mediated signalling in B cells plays a role in regulating the balance of *Helicobacter*-specific T cell responses to prevent excessive Th1-driven immunopathology and promote mucosal homeostasis, but enabling bacterial persistence[21].

RECOGNITION OF DISTINCT *H. PYLORI* COMPONENTS BY SPECIFIC TLRS

LPS

Based on the involvement of TLRs in regulating immunopathology in the context of *H. pylori* infection, many investigators have set out to elucidate the contribution of individual *H. pylori* components to the control of TLR-driven innate immune responses. *H. pylori* LPS has a lower endotoxicity than other gram-negative bacteria such as *Escherichia coli* or *Salmonella enterica*[75-78]. Although there has been substantial investigation into the innate immune response to *H. pylori* LPS, there have been conflicting findings with regard to the TLR responsible for its recognition (Table 2). Some studies have implicated the classic gram-negative bacterial LPS receptor TLR4[11,28,58,60,79,80], while others have suggested a role for TLR2[7,12,19,37,63]. Initial evidence for TLR4-mediated recognition of *H. pylori* LPS was provided by Kawahara *et al*[79] (2001) who demonstrated that LPS from clinical isolates of *H. pylori* induced increased superoxide anion (O_2^-) production in guinea pig gastric pit cells that ex-

Table 2 Toll-like receptors involved in the response to distinct *Helicobacter pylori* components

H. pylori component	TLR	Ref.
LPS	TLR4	Kawahara *et al*[79]
		Su *et al*[80]
		Ishihara *et al*[58]
		Mandell *et al*[11]
		Chochi *et al*[60]
		Cullen *et al*[28]
	TLR2	Smith *et al*[19]
		Lepper *et al*[63]
		Yokota *et al*[12]
		Triantafilou *et al*[37]
		Smith *et al*[7]
Flagellin	TLR5	Smith *et al*[19]
	TLR5 evasion	Lee *et al*[84]
		Gewirtz *et al*[83]
HSP60	TLR2	Takenaka *et al*[59]
		Zhao *et al*[85]
	TLR2-independent	Gobert *et al*[66]
HP0175	TLR4	Basak *et al*[87]
		Pathak *et al*[65]
		Basu *et al*[86]
NAP	TLR2	Amedei *et al*[6]
H. pylori DNA	TLR9	Rad *et al*[18]
H. pylori RNA	TLR7/TLR8	Rad *et al*[18]

TLR: Toll-like receptor; *H. pylori*: *Helicobacter pylori*.

press endogenous TLR4, but not TLR2. Subsequently, TLR4 antibodies were shown to inhibit LPS-mediated IL-8 secretion from phorbol myristate acetate (PMA)-stimulated THP1 macrophages and *H. pylori* demonstrated increased adherence to Chinese Hamster Ovary (CHO) cells transfected with TLR4 compared with that of CHO-TLR2 or untransfected CHOs[80]. Using reporter gene assays, Ishihara *et al*[58] (2004) described *H. pylori* LPS-mediated NF-κB activation and transcription from the IL-8 promoter in AGS gastric epithelial cells over-expressing TLR4 and MD2[58]. In addition, Mandell *et al*[11] (2004) demonstrated that although TLR2 plays a key role in the response to intact *H. pylori*, TLR4-deficient murine BMDMs were unresponsive to LPS isolated from clinical strains of *H. pylori* with regard to cytokine (IL-6) production. More recently, Chochi *et al*[60] (2008) demonstrated that a clinical isolate of *H. pylori* LPS augmented proliferation using a panel of gastric cancer cell lines (MKN28, MKN45, NUGC3 and KATOIII) in a TLR4-dependent manner. Lastly, while investigating the role of lipid A modifications in *H. pylori* pathogenesis, Cullen *et al*[28] (2011) reported that modification of *H. pylori* LPS in terms of lipid A dephosphorylation leads to decreased LPS-mediated NF-κB activation in HEK-TLR4 cells, providing a mechanism whereby *H. pylori* evades innate immune recognition.

In support of TLR2 as the *H. pylori* LPS receptor, Smith *et al*[19] (2003) demonstrated that LPS isolated from *H. pylori* NCTC 26695 induced NF-κB-dependent reporter gene activity in HEK293 cells transfected with TLR2, but not with TLR4. In addition, LPS from *H. pylori* strain LC11 and two clinical isolates activated NF-κB in HEK-TLR2 cells but not HEK-TLR4 cells. Also using HEK cell lines transfected with TLRs, studies from the Triantafilou laboratory indicated that *H. pylori* LPS induced TNFα production in TLR2-expressing cells, but not TLR4-expressing cells[37,63]. TLR2 was responsible for *H. pylori* LPS-mediated NF-κB-driven reporter gene activity in CHO fibroblasts and HEK cells over-expressing TLR2[37,63]. Inhibition of endogenous TLR2 expression in vascular endothelial cells by RNA interference resulted in a reduction of TNFα production[37]. Using fluorescence resonance energy transfer analysis, they also demonstrated that TLR2 is recruited to lipid rafts and associates with TLR1 in cells following LPS stimulation in vascular endothelial cells[37]. Further, Yokota *et al*[12] demonstrated that clinical preparations of *H. pylori* LPS-mediated induction of IL-8 secretion from T24 uroepithelial cells was suppressed by expression of a dominant negative TLR2 mutant, but not with a TLR4 mutant. NF-κB-dependent luciferase reporter assays indicated that over-expression of TLR2 and TLR1 or TLR2 and TLR6 conferred LPS responsiveness in HEK293 cells. The combination of TLR2 and TLR1 expression resulted in higher responsiveness to *H. pylori* LPS than TLR2 and TLR6 expression[12].

Studies by Smith *et al*[7] (2011) have also supported a role for TLR2 in the innate immune recognition of *H. pylori* LPS. LPS prepared from 3 reference strains (NCTC 11637, NCTC 26695 and CCUG 17874) and 4 clinical isolates of *H. pylori* induced IL-8 mRNA expression in HEK293 cells over-expressing TLR2 but not TLR4. IL-8 induction in HEK-TLR2 cells was found to be dose-dependent with a significant level of induction observed at the lowest LPS concentration tested (250 ng/mL). The effect was shown to be LPS specific, as pre-incubation of the *H. pylori* LPS preparations with the antibiotic polymyxin B, a well-known inhibitor of the activating properties of LPS, resulted in a dose-dependent decrease in IL-8 induction in HEK-TLR2 cells[7]. It was also found that *H. pylori* LPS did not induce IL-8 expression in AGS cells, which do not express TLR2 endogenously[20,62], whereas IL-8 was induced in MKN45 cells and T84 colorectal carcinoma cells which have been shown to express endogenous TLR2[19,57,81]. In order to delineate LPS-mediated signalling downstream of TLR engagement, co-transfection using dominant negative constructs and small-interfering RNA demonstrated that *H. pylori* LPS functioned as a classic TLR2 ligand by signalling through pathways involving MyD88, MAL, IRAK1, IRAK4, TRAF6, IKKβ and IκBα to activate NF-κB and transcription form the IL-8 promoter[7]. Through a combination of microarrays, quantitative PCR and ELISAs, it was demonstrated that *H. pylori* LPS induced expression of ICAM1 and the chemokines CXCL1, CXCL2, CXCL3 and CCL20 in TLR2-expressing HEK cells and MKN45 gastric epithelial cells but not HEK293, HEK-TLR4 or AGS cells. Increased expression of these genes was confirmed in gastric tissue biopsy samples from *H. pylori*-infected patients when

compared to uninfected controls[7].

The reasons for the conflicting results between the studies are unclear. Possible explanations include differences in experimental systems involving alternative read-outs for TLR activation and various cell lines from different species. In addition, contamination of the LPS preparation with other components, such as protein, nucleic acids or other bacterial LPS molecules could account for conflicting findings. However, results from Smith *et al*[7] (2011) demonstrating that polymyxin B inhibited TLR2-mediated IL-8 induction in HEK-TLR2 cells would imply that the TLR2-mediated response observed was LPS-specific and not due to the presence of other contaminating TLR ligands, at least in this cell context. Contrasting findings may also have arisen due to heterogeneity of the structures of *H. pylori* LPS molecules resulting from strain differences and/or culturing conditions. Tran *et al*[75] (2005) reported that the lipid A portion of *H. pylori* LPS undergoes several structural modifications through the action of specific modifying enzymes. There is also considerable LPS structural variability due to diversity in the chemical composition of the polysaccharide O-antigen[27,28]. The study by Yokota *et al*[12] (2007) reported similar TLR2-dependent activities using LPS isolated from 6 different clinical isolates of *H. pylori* that demonstrated various characteristics, such as smooth/rough phenotypes and antigenicity of the polysaccharide portion[12]. Additionally, it has been shown that LPS isolated from other gram-negative bacteria that produce a mixture of lipid A species with modified forms of lipid A, such as *Porphyromonas gingivalis* and *Leptospira interrogans*, elicit immune responses through TLR2[33-37].

Flagellin

TLR5 has been identified as the receptor for bacterial flagellin[38], the protein subunit of the polymeric flagellar filament of different gram-positive and gram-negative bacteria. *H. pylori* flagella (5-7 per cell) confer motility and are composed of polymers of two protein subunits, the major flagellin FlaA and the minor flagellin FlaB[82,83], both of which are essential for the bacteria to survive in the stomach musica[84]. TLR5 is expressed on primary gastric epithelial cells and gastric epithelial cell lines, including AGS, HM02, MKN28 and MKN45[19,62,84]. Initial investigations into the innate immune recognition of *H. pylori* flagellin indicated that TLR5 expression in HEK293 cells conferred responsiveness to partially purified flagellin from *H. pylori* in terms of NF-κB-dependent reporter gene activity[19] (Table 2). In addition, transfection of MKN45 cells with a dominant negative TLR5 construct inhibited NF-κB activity in response to *H. pylori* flagellin[19]. However, other studies have since demonstrated that *H. pylori* flagellin is a significantly less potent stimulator of TLR5 signalling than flagellin from other gram-negative bacteria, such as *Salmonella typhimurium*[83,84]. Lee *et al*[84] (2003) demonstrated that although IL-8 release induced by *H. pylori* with mutations in one or both flagellins was delayed compared to wild type *H. pylori*, purified native or recombinant flagellins did not significantly stimulate IL-8 secretion from gastric epithelial cells despite the presence of TLR5, suggesting that the delayed effect with the mutant strains may have been a result of decreased bacterial motility or adherence. Gewirtz *et al*[83] (2004) found no impairment in the IL-8 inducing ability of *H. pylori* in AGS cells as a result of FlaA mutations compared to the wild type strain. In keeping with the findings of Lee *et al*[84] (2003), purified *H. pylori* flagellin failed to induce significant innate immune responses in gastric epithelial cells as assessed by p38 MAPK induction and IL-8 secretion. The low innate immune response to *H. pylori* flagellin in the stomach *in vivo* may provide another mechanism that contributes to the ability of *H. pylori* to evade host responses and to promote long term bacterial persistence.

Heat shock protein 60

The 60 kDa heat-shock protein (HSP60) of *H. pylori* plays a role in the adherence and attachment of *H. pylori* to the gastric epithelium and is a potent immune antigen that stimulates IL-8 induction in gastric epithelial cells[59]. HSP60-induced immune responses are associated with gastric inflammation and the pathogenesis of MALT[59]. Takenaka *et al*[59] (2004) have suggested that *H. pylori* HSP60 is a TLR2 ligand as HSP60-mediated NF-κB activation and IL-8 production in KATO Ⅲ human gastric epithelial cells was inhibited using a TLR2 blocking antibody (Table 2). *H. pylori* HSP60 has also been shown to induce IL-8 production in human monocytes. In support of TLR2 in the recognition of *H. pylori* HSP60, Zhao *et al*[85] (2007) reported that treatment of NOMO1 human monocytes with an anti-TLR2 blocking antibody or small interfering RNA for TLR2 inhibited NF-κB, ERK and p38 MAPK activation as well as IL-8 secretion in response to recombinant *H. pylori* HSP60 stimulation. In contrast to the findings in human cells, peritoneal macrophages from mice deficient in TLR2, TLR4, MyD88 or both TLR2 and TLR4 produced the same amount of IL-6 in response to *H. pylori* HSP60 as wild type macrophages, indicating TLR-independent IL-6 induction in this cell context[66].

H. pylori peptidyl prolyl cis-,trans-isomerase HP0175

H. pylori secretes the peptidyl prolyl *cis*-, *trans*-isomerase HP0175, which can induce apoptosis in gastric epithelial cells and is one of the highly and consistently reactive *H. pylori* antigens recognized in the sera of *H. pylori*-infected patients[65,86]. Studies from the Kundu laboratory have described a role for TLR4 in the recognition of HP0175 (Table 2). Initially, Basak *et al*[87] (2005) demonstrated interaction between TLR4 and HP0175 in AGS cells using pull-down immunoassays. Inhibition of TLR4 using a neutralizing antibody or a dominant negative construct inhibited HP0175-induced apoptosis in AGS cells[87]. Pathak *et al*[65] (2006) subsequently reported that HP0175 induced the release of IL-6 from PMA-differentiated

THP1 macrophages, whereas isogenic mutants of *H. pylori* 26695, in which the *Hp0175* gene was disrupted, elicited decreased IL-6 production. A role for TLR4 in this process was suggested because pre-treatment of cells with a TLR4 (but not TLR2) neutralising antibody or transfection with a dominant-negative TLR4 construct blocked HP0175-mediated IL-6 release. In addition, TLR4 expression (but not TLR2) in HEK293 cells conferred responsiveness to HP0175. Using ELISA-based binding assays, Pathak *et al*[65] also showed that HP0175 interacts with the extracellular domain of TLR4 in the absence of any accessory molecules. Finally, Basu *et al*[86] (2008) showed that HP0175 transactivates the epidermal growth factor receptor (EGFR) and stimulates EGFR-dependent vascular endothelial growth factor (VEGF) production in AGS cells in a TLR4-dependent manner.

NapA

The *H. pylori* neutrophil-activating protein (NAP) is a 150 kDa oligomeric virulence factor that is chemotactic for neutrophils, stimulates high production of oxygen radicals in neutrophils and their adhesion to endothelial cells. Amedei *et al*[6] (2006) have reported that the *H. pylori* NAP is a TLR2 agonist, because over-expression of TLR2 in HEK293 cells resulted in NAP-mediated NF-κB-dependent reporter gene activity (Table 2). NAP stimulation had no effect on untransfected HEK293 cells, or HEK293 cells over-expressing TLR3, TLR4, TLR5, TLR7, TLR8 or TLR9. In human monocytes and neutrophils, NAP stimulation induced the expression of IL-12[6], which is an important cytokine for the differentiation of naïve Th cells into the Th1 phenotype. NAP also induced monocytes to produce IL-23 and differentiate towards mature DCs[6]. Stimulation of antigen-induced T-cells with NAP resulted in increased numbers of IFN-γ-producing T cells and decreased numbers of IL-4-secreting cells, thus promoting a Th1 phenotype. In addition, using T cell clones generated from *in vivo*-activated T cells derived from the gastric mucosa of *H. pylori*-infected patients, NAP was shown to elicit Th1-polarizing capacity[6], implying that the TLR2: *H. pylori* NAP interactions promote the activation of innate immunity to drive IL-12 and IL-23 production and the subsequent promotion of Th1 immune responses.

Nucleic acids

TLR9 recognises unmethylated CpG DNA in bacteria[40] and also detects herpes virus DNA[41]. TLR7 and TLR8 have been shown to sense single-stranded viral RNA[42-44]. Rad *et al*[18] (2009) have demonstrated TLR9-mediated recognition of *H. pylori* DNA in DCs and the subsequent induction of pro-inflammatory cytokine secretion. They showed that IL-6 and IL-12 production was completely abrogated in TLR2/TLR4/TLR9-deficient BMDCs compared to TLR2/TLR4-deficient cells in response to purified *H. pylori* DNA following pre-treatment with ribonuclease. Expression of TLR9 is increased in mouse gastric tissue following *H. pylori* infection and is mainly localised to macrophages, DCs and $CD3^+$ cells in the gastric mucosa[88]. Although purified *H. pylori* DNA was reported to induce a TLR9-mediated increase in IL-6 and IL-12 expression in BMDCs[18], in a mouse model of *H. pylori* infection TLR9 signalling was shown to have an anti-inflammatory effect on the early phase of *H. pylori*-induced gastritis as genetic disruption of TLR9 resulted in an increase in *H. pylori*-induced gastric mucosal inflammation characterized by neutrophil infiltration and increased expression of TNFα and IFNγ[88]. In relation to TLR7 and TLR8-mediated recognition of *H. pylori*, Rad *et al*[18] (2009) showed that purified *H. pylori* RNA (pre-treated with deoxyribonuclease) induced pro-inflammatory cytokines in BMDCs in a MyD88-dependent manner involving the endosomal TLR8, possibly in collaboration with TLR7.

TARGETING TLR SIGNALLING THERAPEUTICALLY

As TLRs are intimately involved in the regulation of inflammation during innate immunity and represent key activators of adaptive immunity, they represent an attractive therapeutic target for treatment of inflammatory diseases. Indeed, oligonucleotide inhibitors of TLR7 and/or TLR9 have been shown to have therapeutic potential in animal models of systemic lupus erythematosus[89]. Additionally, an inhibitory TLR2 antibody was demonstrated to limit ischemia-reperfusion injury in the hearts of pigs[90] and kidneys of mice[91]. In the clinic, therapies involving the synthetic small molecule inhibitor of TLR4, Eritoran (also known as E5564), were used in trials for patients with sepsis, but only had marginal effects possibly as treatment was administered too late following disease onset[92,93]. TLR activation is also important for adjuvancy in vaccines and several TLR ligands have been shown to be efficacious as vaccine adjuvants[94,95]. For example, the vaccine adjuvant monophosphoryl lipid A, which is a less toxic version of LPS, promotes antibody responses via TLR4 activation[96]. Efficient preventative or therapeutic vaccination for *H. pylori* has not been achieved in humans to date[97]. Early signs of promise in animal models of *H. pylori* infection have been unsuccessful in humans. In a recent study to investigate the vaccine potential of *H. pylori* LPS, Altman *et al*[98] (2012) demonstrated enhanced antibody responses to a chemically modified LPS in mice and rabbits and partial protection against *H. pylori* challenge, warranting further investigation in this area. In terms of adjuvancy, immunization against *Helicobacter* using CpG and cholera toxin demonstrated synergism leading to sterile immunity in mice[99]. More recently, Mori *et al*[100] (2012) constructed a chimeric flagellin by replacing the N- and T-terminal segments of *H. pylori* flagellin with a TLR5-stimulating adjuvant component of *E. coli* flagellin in order to enhance innate and adaptive immunity. The resulting chimeric flagellin activated TLR5 signalling and elicited a strong antibody response in mice. Together

with alum, vaccination with the chimeric flagellin protected mice from *H. pylori* infection[100]. In other disease settings, local administration of the TLR2 ligand *H. pylori* NAP was demonstrated to decrease tumour growth by activating a cytotoxic Th1 response in a mouse model of bladder cancer[101]. Taken together, these studies indicate that defining *H. pylori*-derived molecules responsible for TLR activation and elucidating innate immune signals triggered by *H. pylori*, may provide insight into the design and development of novel human vaccine adjuvants and therapeutics.

H. PYLORI RECOGNITION BY OTHER PRRS

Microbial pathogens activate multiple PRRs and different PRRs may recognize the same PAMP within an organism[17]. Insight into the co-operation between TLRs and other PRRs during infection is necessary for a complete understanding of the innate immune response during infection. These PRRs include nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs) and C-type lectin receptors (CLRs). *H. pylori* peptidoglycan, delivered either by the type IV secretion system or through outer membrane vesicles secreted from the bacterium, is recognised by NOD1 in epithelial cells[102-107]. Moreover, studies by Kim *et al*[70] (2013) have described the cooperative interaction of TLR2 and NOD2 in the regulation of IL-1β in *H. pylori*-infected DCs. In addition to a role for TLR8 in the response to *H. pylori* RNA, findings by Rad *et al* (2009) indicated that *H. pylori* induces expression of type I interferon and interferon-stimulated genes in a MyD88- and TRIF-independent manner and demonstrated that the MyD88-independent type I IFN induction by *H. pylori* RNA was mediated by RIG-I[18]. In relation to *H. pylori*-mediated CLR signalling, Gringhuis *et al*[108] (2009) have reported that the fucose residues of *H. pylori* DC-SIGN ligands actively disrupt signalling down-stream of DC-SIGN to suppress pro-inflammatory cytokine induction.

CONCLUSION

Although, *H. pylori* induces a strong immune response, elimination of infection is not achieved. The pathogenesis of *H. pylori*-associated disease is linked to the severity of the host inflammatory response**.** Emerging evidence suggests that failure to eliminate *H. pylori* may be due to the ability of the bacterium to control T-cell responses. As TLRs are intimately involved in the regulation of inflammation during the innate immune response to *H. pylori* and represent key activators of adaptive immunity, a significant effort has been made to elucidate their role in the recognition of *H. pylori* and its components in multiple cell types. Much of the literature has focussed on the involvement of individual TLRs in the induction of pro-inflammatory cytokines in various *in vitro* cell culture models. There is substantial evidence to support the role of TLR2 in activating NF-κB or inducing cytokine induction in response to *H. pylori* infection in epithelial cells[7,11,19,20], monocytes/macrophages[11,67], dendritic cells[18,70,73] and B cells[21]. Numerous *H. pylori* ligands have been suggested to date that may contribute to these TLR2-dependent responses, including LPS[7,12,19,37,63], HSP60[59,85] and NAP[6]. TLR4 has also been implicated in the response to *H. pylori*[18,57,67], which may be mediated by LPS[11,28,58,60,79,80] and/or HP0175[65,86,87]. TLR9 has been identified as the receptor for *H. pylori* DNA[18]. Although *H. pylori* flagellin has been suggested as a TLR5 ligand[19], its activity as a TLR5 activator is low[83,84], providing a possible mechanism that contributes *H. pylori* persistence.

Recent studies using mouse models of infection have provided insight into the role of TLR signalling in regulating *H. pylori*-mediated T cell responses, gastric immunopathology and colonization *in vivo*. Interestingly, although a demonstrated role for TLR2 in the induction of pro-inflammatory cytokines has been described in distinct cell populations, the net effect of TLR2 signalling has been reported to mediate tolerance and promote bacterial persistence in mouse models of infection by skewing T cell responses[21,73]. Further *in vivo* studies elucidating innate immune signals triggered by *H. pylori*-mediated activation of TLRs, especially in cooperation with other PRRs, are necessary for a complete understanding of how the balance between pro-inflammatory and anti-inflammatory signals fine-tunes the immune response to *H. pylori* infection, and may provide insight into how the immune response may be manipulated therapeutically to successfully eradicate the bacterium.

REFERENCES

1 **Polk DB**, Peek RM. Helicobacter pylori: gastric cancer and beyond. *Nat Rev Cancer* 2010; **10**: 403-414 [PMID: 20495574 DOI: 10.1038/nrc2857]

2 **McColl KE**. Clinical practice. Helicobacter pylori infection. *N Engl J Med* 2010; **362**: 1597-1604 [PMID: 20427808 DOI: 10.1056/NEJMcp1001110]

3 **Salama NR**, Hartung ML, Müller A. Life in the human stomach: persistence strategies of the bacterial pathogen Helicobacter pylori. *Nat Rev Microbiol* 2013; **11**: 385-399 [PMID: 23652324 DOI: 10.1038/nrmicro3016]

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

5 **Queiroz DM**, Harris PR, Sanderson IR, Windle HJ, Walker MM, Rocha AM, Rocha GA, Carvalho SD, Bittencourt PF, de Castro LP, Villagrán A, Serrano C, Kelleher D, Crabtree JE. Iron status and Helicobacter pylori infection in symptomatic children: an international multi-centered study. *PLoS One* 2013; **8**: e68833 [PMID: 23861946 DOI: 10.1371/journal.pone.0068833]

6 **Amedei A**, Cappon A, Codolo G, Cabrelle A, Polenghi A, Benagiano M, Tasca E, Azzurri A, D'Elios MM, Del Prete G, de Bernard M. The neutrophil-activating protein of Helicobacter pylori promotes Th1 immune responses. *J Clin*

Invest 2006; **116**: 1092-1101 [PMID: 16543949 DOI: 10.1172/JCI27177]
7 **Smith SM**, Moran AP, Duggan SP, Ahmed SE, Mohamed AS, Windle HJ, O'Neill LA, Kelleher DP. Tribbles 3: a novel regulator of TLR2-mediated signaling in response to Helicobacter pylori lipopolysaccharide. *J Immunol* 2011; **186**: 2462-2471 [PMID: 21220698 DOI: 10.4049/jimmunol.1000864]
8 **Yamaoka Y**, Kita M, Kodama T, Sawai N, Kashima K, Imanishi J. Expression of cytokine mRNA in gastric mucosa with Helicobacter pylori infection. *Scand J Gastroenterol* 1995; **30**: 1153-1159 [PMID: 9053967]
9 **Moss SF**, Legon S, Davies J, Calam J. Cytokine gene expression in Helicobacter pylori associated antral gastritis. *Gut* 1994; **35**: 1567-1570 [PMID: 7828974]
10 **Windle HJ**, Ang YS, Athie-Morales V, McManus R, Kelleher D. Human peripheral and gastric lymphocyte responses to Helicobacter pylori NapA and AphC differ in infected and uninfected individuals. *Gut* 2005; **54**: 25-32 [PMID: 15591500 DOI: 10.1136/gut.2003.025494]
11 **Mandell L**, Moran AP, Cocchiarella A, Houghton J, Taylor N, Fox JG, Wang TC, Kurt-Jones EA. Intact gram-negative Helicobacter pylori, Helicobacter felis, and Helicobacter hepaticus bacteria activate innate immunity via toll-like receptor 2 but not toll-like receptor 4. *Infect Immun* 2004; **72**: 6446-6454 [PMID: 15501775 DOI: 10.1128/IAI.72.11.6446-6454.2004]
12 **Yokota S**, Ohnishi T, Muroi M, Tanamoto K, Fujii N, Amano K. Highly-purified Helicobacter pylori LPS preparations induce weak inflammatory reactions and utilize Toll-like receptor 2 complex but not Toll-like receptor 4 complex. *FEMS Immunol Med Microbiol* 2007; **51**: 140-148 [PMID: 17645528 DOI: 10.1111/j.1574-695X.2007.00288.x]
13 **Bodger K**, Crabtree JE. Helicobacter pylori and gastric inflammation. *Br Med Bull* 1998; **54**: 139-150 [PMID: 9604438]
14 **O'Connor A**, Gisbert JP, McNamara D, O'Morain C. Treatment of Helicobacter pylori infection 2011. *Helicobacter* 2011; **16** Suppl 1: 53-58 [PMID: 21896086 DOI: 10.1111/j.1523-5378.2011.00881.x]
15 **Megraud F**, Coenen S, Versporten A, Kist M, Lopez-Brea M, Hirschl AM, Andersen LP, Goossens H, Glupczynski Y. Helicobacter pylori resistance to antibiotics in Europe and its relationship to antibiotic consumption. *Gut* 2013; **62**: 34-42 [PMID: 22580412 DOI: 10.1136/gutjnl-2012-302254]
16 **Kawai T**, Akira S. The roles of TLRs, RLRs and NLRs in pathogen recognition. *Int Immunol* 2009; **21**: 317-337 [PMID: 19246554 DOI: 10.1093/intimm/dxp017]
17 **Kawai T**, Akira S. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity* 2011; **34**: 637-650 [PMID: 21616434 DOI: 10.1016/j.immuni.2011.05.006]
18 **Rad R**, Ballhorn W, Voland P, Eisenächer K, Mages J, Rad L, Ferstl R, Lang R, Wagner H, Schmid RM, Bauer S, Prinz C, Kirschning CJ, Krug A. Extracellular and intracellular pattern recognition receptors cooperate in the recognition of Helicobacter pylori. *Gastroenterology* 2009; **136**: 2247-2257 [PMID: 19272387 DOI: 10.1053/j.gastro.2009.02.066]
19 **Smith MF**, Mitchell A, Li G, Ding S, Fitzmaurice AM, Ryan K, Crowe S, Goldberg JB. Toll-like receptor (TLR) 2 and TLR5, but not TLR4, are required for Helicobacter pylori-induced NF-kappa B activation and chemokine expression by epithelial cells. *J Biol Chem* 2003; **278**: 32552-32560 [PMID: 12807870 DOI: 10.1074/jbc.M305536200]
20 **Ding SZ**, Torok AM, Smith MF, Goldberg JB. Toll-like receptor 2-mediated gene expression in epithelial cells during Helicobacter pylori infection. *Helicobacter* 2005; **10**: 193-204 [PMID: 15904477 DOI: 10.1111/j.1523-5378.2005.00311.x]
21 **Sayi A**, Kohler E, Toller IM, Flavell RA, Müller W, Roers A, Müller A. TLR-2-activated B cells suppress Helicobacter-induced preneoplastic gastric immunopathology by inducing T regulatory-1 cells. *J Immunol* 2011; **186**: 878-890 [PMID: 21149607 DOI: 10.4049/jimmunol.1002269]
22 **Tahara T**, Arisawa T, Wang F, Shibata T, Nakamura M, Sakata M, Hirata I, Nakano H. Toll-like receptor 2 (TLR) -196 to 174del polymorphism in gastro-duodenal diseases in Japanese population. *Dig Dis Sci* 2008; **53**: 919-924 [PMID: 17934843 DOI: 10.1007/s10620-007-9950-x]
23 **Tahara T**, Arisawa T, Wang F, Shibata T, Nakamura M, Sakata M, Hirata I, Nakano H. Toll-like receptor 2 -196 to 174del polymorphism influences the susceptibility of Japanese people to gastric cancer. *Cancer Sci* 2007; **98**: 1790-1794 [PMID: 17711514 DOI: 10.1111/j.1349-7006.2007.00590.x]
24 **Mayerle J**, den Hoed CM, Schurmann C, Stolk L, Homuth G, Peters MJ, Capelle LG, Zimmermann K, Rivadeneira F, Gruska S, Völzke H, de Vries AC, Völker U, Teumer A, van Meurs JB, Steinmetz I, Nauck M, Ernst F, Weiss FU, Hofman A, Zenker M, Kroemer HK, Prokisch H, Uitterlinden AG, Lerch MM, Kuipers EJ. Identification of genetic loci associated with Helicobacter pylori serologic status. *JAMA* 2013; **309**: 1912-1920 [PMID: 23652523 DOI: 10.1001/jama.2013.4350]
25 **O'Neill LA**, Golenbock D, Bowie AG. The history of Toll-like receptors - redefining innate immunity. *Nat Rev Immunol* 2013; **13**: 453-460 [PMID: 23681101 DOI: 10.1038/nri3446]
26 **Poltorak A**, He X, Smirnova I, Liu MY, Van Huffel C, Du X, Birdwell D, Alejos E, Silva M, Galanos C, Freudenberg M, Ricciardi-Castagnoli P, Layton B, Beutler B. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* 1998; **282**: 2085-2088 [PMID: 9851930]
27 **Matsuura M**. Structural Modifications of Bacterial Lipopolysaccharide that Facilitate Gram-Negative Bacteria Evasion of Host Innate Immunity. *Front Immunol* 2013; **4**: 109 [PMID: 23745121 DOI: 10.3389/fimmu.2013.00109]
28 **Cullen TW**, Giles DK, Wolf LN, Ecobichon C, Boneca IG, Trent MS. Helicobacter pylori versus the host: remodeling of the bacterial outer membrane is required for survival in the gastric mucosa. *PLoS Pathog* 2011; **7**: e1002454 [PMID: 22216004 DOI: 10.1371/journal.ppat.1002454]
29 **Takeuchi O**, Sato S, Horiuchi T, Hoshino K, Takeda K, Dong Z, Modlin RL, Akira S. Cutting edge: role of Toll-like receptor 1 in mediating immune response to microbial lipoproteins. *J Immunol* 2002; **169**: 10-14 [PMID: 12077222]
30 **Jin MS**, Kim SE, Heo JY, Lee ME, Kim HM, Paik SG, Lee H, Lee JO. Crystal structure of the TLR1-TLR2 heterodimer induced by binding of a tri-acylated lipopeptide. *Cell* 2007; **130**: 1071-1082 [PMID: 17889651 DOI: 10.1016/j.cell.2007.09.008]
31 **Takeuchi O**, Kawai T, Mühlradt PF, Morr M, Radolf JD, Zychlinsky A, Takeda K, Akira S. Discrimination of bacterial lipoproteins by Toll-like receptor 6. *Int Immunol* 2001; **13**: 933-940 [PMID: 11431423]
32 **Kang JY**, Nan X, Jin MS, Youn SJ, Ryu YH, Mah S, Han SH, Lee H, Paik SG, Lee JO. Recognition of lipopeptide patterns by Toll-like receptor 2-Toll-like receptor 6 heterodimer. *Immunity* 2009; **31**: 873-884 [PMID: 19931471 DOI: 10.1016/j.immuni.2009.09.018]
33 **Werts C**, Tapping RI, Mathison JC, Chuang TH, Kravchenko V, Saint Girons I, Haake DA, Godowski PJ, Hayashi F, Ozinsky A, Underhill DM, Kirschning CJ, Wagner H, Aderem A, Tobias PS, Ulevitch RJ. Leptospiral lipopolysaccharide activates cells through a TLR2-dependent mechanism. *Nat Immunol* 2001; **2**: 346-352 [PMID: 11276206 DOI: 10.1038/86354]
34 **Darveau RP**, Pham TT, Lemley K, Reife RA, Bainbridge BW, Coats SR, Howald WN, Way SS, Hajjar AM. Porphyromonas gingivalis lipopolysaccharide contains multiple lipid A species that functionally interact with both toll-like receptors 2 and 4. *Infect Immun* 2004; **72**: 5041-5051 [PMID: 15321997 DOI: 10.1128/IAI.72.9.5041-5051.2004]
35 **Que-Gewirth NL**, Ribeiro AA, Kalb SR, Cotter RJ, Bulach DM, Adler B, Girons IS, Werts C, Raetz CR. A methylated phosphate group and four amide-linked acyl chains in

leptospira interrogans lipid A. The membrane anchor of an unusual lipopolysaccharide that activates TLR2. *J Biol Chem* 2004; **279**: 25420-25429 [PMID: 15044492 DOI: 10.1074/jbc.M400598200]

36 **Hirschfeld M**, Weis JJ, Toshchakov V, Salkowski CA, Cody MJ, Ward DC, Qureshi N, Michalek SM, Vogel SN. Signaling by toll-like receptor 2 and 4 agonists results in differential gene expression in murine macrophages. *Infect Immun* 2001; **69**: 1477-1482 [PMID: 11179315 DOI: 10.1128/IAI.69.3.1477-1482.2001]

37 **Triantafilou M**, Gamper FG, Lepper PM, Mouratis MA, Schumann C, Harokopakis E, Schifferle RE, Hajishengallis G, Triantafilou K. Lipopolysaccharides from atherosclerosis-associated bacteria antagonize TLR4, induce formation of TLR2/1/CD36 complexes in lipid rafts and trigger TLR2-induced inflammatory responses in human vascular endothelial cells. *Cell Microbiol* 2007; **9**: 2030-2039 [PMID: 17419716 DOI: 10.1111/j.1462-5822.2007.00935.x]

38 **Hayashi F**, Smith KD, Ozinsky A, Hawn TR, Yi EC, Goodlett DR, Eng JK, Akira S, Underhill DM, Aderem A. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* 2001; **410**: 1099-1103 [PMID: 11323673 DOI: 10.1038/35074106]

39 **Alexopoulou L**, Holt AC, Medzhitov R, Flavell RA. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. *Nature* 2001; **413**: 732-738 [PMID: 11607032 DOI: 10.1038/35099560]

40 **Hemmi H**, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, Matsumoto M, Hoshino K, Wagner H, Takeda K, Akira S. A Toll-like receptor recognizes bacterial DNA. *Nature* 2000; **408**: 740-745 [PMID: 11130078 DOI: 10.1038/35047123]

41 **Lund J**, Sato A, Akira S, Medzhitov R, Iwasaki A. Toll-like receptor 9-mediated recognition of Herpes simplex virus-2 by plasmacytoid dendritic cells. *J Exp Med* 2003; **198**: 513-520 [PMID: 12900525 DOI: 10.1084/jem.20030162]

42 **Heil F**, Hemmi H, Hochrein H, Ampenberger F, Kirschning C, Akira S, Lipford G, Wagner H, Bauer S. Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. *Science* 2004; **303**: 1526-1529 [PMID: 14976262 DOI: 10.1126/science.1093620]

43 **Diebold SS**, Kaisho T, Hemmi H, Akira S, Reis e Sousa C. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. *Science* 2004; **303**: 1529-1531 [PMID: 14976261 DOI: 10.1126/science.1093616]

44 **Lund JM**, Alexopoulou L, Sato A, Karow M, Adams NC, Gale NW, Iwasaki A, Flavell RA. Recognition of single-stranded RNA viruses by Toll-like receptor 7. *Proc Natl Acad Sci USA* 2004; **101**: 5598-5603 [PMID: 15034168 DOI: 10.1073/pnas.0400937101]

45 **Kawai T**, Akira S. TLR signaling. *Cell Death Differ* 2006; **13**: 816-825 [PMID: 16410796]

46 **Fitzgerald KA**, Palsson-McDermott EM, Bowie AG, Jefferies CA, Mansell AS, Brady G, Brint E, Dunne A, Gray P, Harte MT, McMurray D, Smith DE, Sims JE, Bird TA, O'Neill LA. Mal (MyD88-adapter-like) is required for Toll-like receptor-4 signal transduction. *Nature* 2001; **413**: 78-83 [PMID: 11544529 DOI: 10.1038/35092578]

47 **Horng T**, Barton GM, Medzhitov R. TIRAP: an adapter molecule in the Toll signaling pathway. *Nat Immunol* 2001; **2**: 835-841 [PMID: 11526399 DOI: 10.1038/ni0901-835]

48 **Yamamoto M**, Sato S, Hemmi H, Sanjo H, Uematsu S, Kaisho T, Hoshino K, Takeuchi O, Kobayashi M, Fujita T, Takeda K, Akira S. Essential role for TIRAP in activation of the signalling cascade shared by TLR2 and TLR4. *Nature* 2002; **420**: 324-329 [PMID: 12447441 DOI: 10.1038/nature01182]

49 **Horng T**, Barton GM, Flavell RA, Medzhitov R. The adaptor molecule TIRAP provides signalling specificity for Toll-like receptors. *Nature* 2002; **420**: 329-333 [PMID: 12447442 DOI: 10.1038/nature01180]

50 **Yamamoto M**, Sato S, Mori K, Hoshino K, Takeuchi O, Takeda K, Akira S. Cutting edge: a novel Toll/IL-1 receptor domain-containing adapter that preferentially activates the IFN-beta promoter in the Toll-like receptor signaling. *J Immunol* 2002; **169**: 6668-6672 [PMID: 12471095]

51 **Oshiumi H**, Matsumoto M, Funami K, Akazawa T, Seya T. TICAM-1, an adaptor molecule that participates in Toll-like receptor 3-mediated interferon-beta induction. *Nat Immunol* 2003; **4**: 161-167 [PMID: 12539043 DOI: 10.1038/ni886]

52 **Hoebe K**, Du X, Georgel P, Janssen E, Tabeta K, Kim SO, Goode J, Lin P, Mann N, Mudd S, Crozat K, Sovath S, Han J, Beutler B. Identification of Lps2 as a key transducer of MyD88-independent TIR signalling. *Nature* 2003; **424**: 743-748 [PMID: 12872135 DOI: 10.1038/nature01889]

53 **Yamamoto M**, Sato S, Hemmi H, Hoshino K, Kaisho T, Sanjo H, Takeuchi O, Sugiyama M, Okabe M, Takeda K, Akira S. Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. *Science* 2003; **301**: 640-643 [PMID: 12855817 DOI: 10.1126/science.1087262]

54 **Fitzgerald KA**, Rowe DC, Barnes BJ, Caffrey DR, Visintin A, Latz E, Monks B, Pitha PM, Golenbock DT. LPS-TLR4 signaling to IRF-3/7 and NF-kappaB involves the toll adapters TRAM and TRIF. *J Exp Med* 2003; **198**: 1043-1055 [PMID: 14517278 DOI: 10.1084/jem.20031023]

55 **Yamamoto M**, Sato S, Hemmi H, Uematsu S, Hoshino K, Kaisho T, Takeuchi O, Takeda K, Akira S. TRAM is specifically involved in the Toll-like receptor 4-mediated MyD88-independent signaling pathway. *Nat Immunol* 2003; **4**: 1144-1150 [PMID: 14556004 DOI: 10.1038/ni986]

56 **Oshiumi H**, Sasai M, Shida K, Fujita T, Matsumoto M, Seya T. TIR-containing adapter molecule (TICAM)-2, a bridging adapter recruiting to toll-like receptor 4 TICAM-1 that induces interferon-beta. *J Biol Chem* 2003; **278**: 49751-49762 [PMID: 14519765 DOI: 10.1074/jbc.M305820200]

57 **Maeda S**, Akanuma M, Mitsuno Y, Hirata Y, Ogura K, Yoshida H, Shiratori Y, Omata M. Distinct mechanism of Helicobacter pylori-mediated NF-kappa B activation between gastric cancer cells and monocytic cells. *J Biol Chem* 2001; **276**: 44856-44864 [PMID: 11546774 DOI: 10.1074/jbc.M105381200]

58 **Ishihara S**, Rumi MA, Kadowaki Y, Ortega-Cava CF, Yuki T, Yoshino N, Miyaoka Y, Kazumori H, Ishimura N, Amano Y, Kinoshita Y. Essential role of MD-2 in TLR4-dependent signaling during Helicobacter pylori-associated gastritis. *J Immunol* 2004; **173**: 1406-1416 [PMID: 15240737]

59 **Takenaka R**, Yokota K, Ayada K, Mizuno M, Zhao Y, Fujinami Y, Lin SN, Toyokawa T, Okada H, Shiratori Y, Oguma K. Helicobacter pylori heat-shock protein 60 induces inflammatory responses through the Toll-like receptor-triggered pathway in cultured human gastric epithelial cells. *Microbiology* 2004; **150**: 3913-3922 [PMID: 15583145 DOI: 10.1099/mic.0.27527-0]

60 **Chochi K**, Ichikura T, Kinoshita M, Majima T, Shinomiya N, Tsujimoto H, Kawabata T, Sugasawa H, Ono S, Seki S, Mochizuki H. Helicobacter pylori augments growth of gastric cancers via the lipopolysaccharide-toll-like receptor 4 pathway whereas its lipopolysaccharide attenuates antitumor activities of human mononuclear cells. *Clin Cancer Res* 2008; **14**: 2909-2917 [PMID: 18483357 DOI: 10.1158/1078-0432.CCR-07-4467]

61 **Uno K**, Kato K, Atsumi T, Suzuki T, Yoshitake J, Morita H, Ohara S, Kotake Y, Shimosegawa T, Yoshimura T. Toll-like receptor (TLR) 2 induced through TLR4 signaling initiated by Helicobacter pylori cooperatively amplifies iNOS induction in gastric epithelial cells. *Am J Physiol Gastrointest Liver Physiol* 2007; **293**: G1004-G1012 [PMID: 17855767 DOI: 10.1152/ajpgi.00096.2007]

62 **Bäckhed F**, Rokbi B, Torstensson E, Zhao Y, Nilsson C, Seguin D, Normark S, Buchan AM, Richter-Dahlfors A. Gastric mucosal recognition of Helicobacter pylori is independent of Toll-like receptor 4. *J Infect Dis* 2003; **187**: 829-836

[PMID: 12599057 DOI: 10.1086/367896]
63 **Lepper PM**, Triantafilou M, Schumann C, Schneider EM, Triantafilou K. Lipopolysaccharides from Helicobacter pylori can act as antagonists for Toll-like receptor 4. *Cell Microbiol* 2005; **7**: 519-528 [PMID: 15760452 DOI: 10.1111/j.1462-5822.2005.00482.x]
64 **Torok AM**, Bouton AH, Goldberg JB. Helicobacter pylori induces interleukin-8 secretion by Toll-like receptor 2- and Toll-like receptor 5-dependent and -independent pathways. *Infect Immun* 2005; **73**: 1523-1531 [PMID: 15731050 DOI: 10.1128/IAI.73.3.1523-1531.2005]
65 **Pathak SK**, Basu S, Bhattacharyya A, Pathak S, Banerjee A, Basu J, Kundu M. TLR4-dependent NF-kappaB activation and mitogen- and stress-activated protein kinase 1-triggered phosphorylation events are central to Helicobacter pylori peptidyl prolyl cis-, trans-isomerase (HP0175)-mediated induction of IL-6 release from macrophages. *J Immunol* 2006; **177**: 7950-7958 [PMID: 17114467]
66 **Gobert AP**, Bambou JC, Werts C, Balloy V, Chignard M, Moran AP, Ferrero RL. Helicobacter pylori heat shock protein 60 mediates interleukin-6 production by macrophages via a toll-like receptor (TLR)-2-, TLR-4-, and myeloid differentiation factor 88-independent mechanism. *J Biol Chem* 2004; **279**: 245-250 [PMID: 14573621 DOI: 10.1074/jbc.M307858200]
67 **Obonyo M**, Sabet M, Cole SP, Ebmeyer J, Uematsu S, Akira S, Guiney DG. Deficiencies of myeloid differentiation factor 88, Toll-like receptor 2 (TLR2), or TLR4 produce specific defects in macrophage cytokine secretion induced by Helicobacter pylori. *Infect Immun* 2007; **75**: 2408-2414 [PMID: 17353291 DOI: 10.1128/IAI.01794-06]
68 **Muzio M**, Bosisio D, Polentarutti N, D'amico G, Stoppacciaro A, Mancinelli R, van't Veer C, Penton-Rol G, Ruco LP, Allavena P, Mantovani A. Differential expression and regulation of toll-like receptors (TLR) in human leukocytes: selective expression of TLR3 in dendritic cells. *J Immunol* 2000; **164**: 5998-6004 [PMID: 10820283]
69 **Rad R**, Brenner L, Krug A, Voland P, Mages J, Lang R, Schwendy S, Reindl W, Dossumbekova A, Ballhorn W, Wagner H, Schmid RM, Bauer S, Prinz C. Toll-like receptor-dependent activation of antigen-presenting cells affects adaptive immunity to Helicobacter pylori. *Gastroenterology* 2007; **133**: 150-163.e3 [PMID: 17631139 DOI: 10.1053/j.gastro.2007.04.071]
70 **Kim DJ**, Park JH, Franchi L, Backert S, Núñez G. The Cag pathogenicity island and interaction between TLR2/NOD2 and NLRP3 regulate IL-1β production in Helicobacter pylori infected dendritic cells. *Eur J Immunol* 2013; **43**: 2650-2658 [PMID: 23818043 DOI: 10.1002/eji.201243281]
71 **Rad R**, Brenner L, Bauer S, Schwendy S, Layland L, da Costa CP, Reindl W, Dossumbekova A, Friedrich M, Saur D, Wagner H, Schmid RM, Prinz C. CD25+/Foxp3+ T cells regulate gastric inflammation and Helicobacter pylori colonization in vivo. *Gastroenterology* 2006; **131**: 525-537 [PMID: 16890606 DOI: 10.1053/j.gastro.2006.05.001]
72 **Lundgren A**, Strömberg E, Sjöling A, Lindholm C, Enarsson K, Edebo A, Johnsson E, Suri-Payer E, Larsson P, Rudin A, Svennerholm AM, Lundin BS. Mucosal FOXP3-expressing CD4+ CD25high regulatory T cells in Helicobacter pylori-infected patients. *Infect Immun* 2005; **73**: 523-531 [PMID: 15618192 DOI: 10.1128/IAI.73.1.523-531.2005]
73 **Sun X**, Zhang M, El-Zataari M, Owyang SY, Eaton KA, Liu M, Chang YM, Zou W, Kao JY. TLR2 mediates Helicobacter pylori-induced tolerogenic immune response in mice. *PLoS One* 2013; **8**: e74595 [PMID: 24058595 DOI: 10.1371/journal.pone.0074595]
74 **Peek RM**, Fiske C, Wilson KT. Role of innate immunity in Helicobacter pylori-induced gastric malignancy. *Physiol Rev* 2010; **90**: 831-858 [PMID: 20664074 DOI: 10.1152/physrev.00039.2009]
75 **Tran AX**, Stead CM, Trent MS. Remodeling of Helicobacter pylori lipopolysaccharide. *J Endotoxin Res* 2005; **11**: 161-166 [PMID: 15949144 DOI: 10.1179/096805105X37349]
76 **Moran AP**, Lindner B, Walsh EJ. Structural characterization of the lipid A component of Helicobacter pylori rough- and smooth-form lipopolysaccharides. *J Bacteriol* 1997; **179**: 6453-6463 [PMID: 9335296]
77 **Muotiala A**, Helander IM, Pyhälä L, Kosunen TU, Moran AP. Low biological activity of Helicobacter pylori lipopolysaccharide. *Infect Immun* 1992; **60**: 1714-1716 [PMID: 1548097]
78 **Pérez-Pérez GI**, Shepherd VL, Morrow JD, Blaser MJ. Activation of human THP-1 cells and rat bone marrow-derived macrophages by Helicobacter pylori lipopolysaccharide. *Infect Immun* 1995; **63**: 1183-1187 [PMID: 7890370]
79 **Kawahara T**, Teshima S, Oka A, Sugiyama T, Kishi K, Rokutan K. Type I Helicobacter pylori lipopolysaccharide stimulates toll-like receptor 4 and activates mitogen oxidase 1 in gastric pit cells. *Infect Immun* 2001; **69**: 4382-4389 [PMID: 11401977 DOI: 10.1128/IAI.69.7.4382-4389.2001]
80 **Su B**, Ceponis PJ, Lebel S, Huynh H, Sherman PM. Helicobacter pylori activates Toll-like receptor 4 expression in gastrointestinal epithelial cells. *Infect Immun* 2003; **71**: 3496-3502 [PMID: 12761134]
81 **Cario E**, Brown D, McKee M, Lynch-Devaney K, Gerken G, Podolsky DK. Commensal-associated molecular patterns induce selective toll-like receptor-trafficking from apical membrane to cytoplasmic compartments in polarized intestinal epithelium. *Am J Pathol* 2002; **160**: 165-173 [PMID: 11786410 DOI: 10.1016/S0002-9440(10)64360-X]
82 **Perrais M**, Rousseaux C, Ducourouble MP, Courcol R, Vincent P, Jonckheere N, Van Seuningen I. Helicobacter pylori urease and flagellin alter mucin gene expression in human gastric cancer cells. *Gastric Cancer* 2014; **17**: 235-246 [PMID: 23703470 DOI: 10.1007/s10120-013-0267-5]
83 **Gewirtz AT**, Yu Y, Krishna US, Israel DA, Lyons SL, Peek RM. Helicobacter pylori flagellin evades toll-like receptor 5-mediated innate immunity. *J Infect Dis* 2004; **189**: 1914-1920 [PMID: 15122529 DOI: 10.1086/386289]
84 **Lee SK**, Stack A, Katzowitsch E, Aizawa SI, Suerbaum S, Josenhans C. Helicobacter pylori flagellins have very low intrinsic activity to stimulate human gastric epithelial cells via TLR5. *Microbes Infect* 2003; **5**: 1345-1356 [PMID: 14670447]
85 **Zhao Y**, Yokota K, Ayada K, Yamamoto Y, Okada T, Shen L, Oguma K. Helicobacter pylori heat-shock protein 60 induces interleukin-8 via a Toll-like receptor (TLR)2 and mitogen-activated protein (MAP) kinase pathway in human monocytes. *J Med Microbiol* 2007; **56**: 154-164 [PMID: 17244794 DOI: 10.1099/jmm.0.46882-0]
86 **Basu S**, Pathak SK, Chatterjee G, Pathak S, Basu J, Kundu M. Helicobacter pylori protein HP0175 transactivates epidermal growth factor receptor through TLR4 in gastric epithelial cells. *J Biol Chem* 2008; **283**: 32369-32376 [PMID: 18806258 DOI: 10.1074/jbc.M805053200]
87 **Basak C**, Pathak SK, Bhattacharyya A, Pathak S, Basu J, Kundu M. The secreted peptidyl prolyl cis,trans-isomerase HP0175 of Helicobacter pylori induces apoptosis of gastric epithelial cells in a TLR4- and apoptosis signal-regulating kinase 1-dependent manner. *J Immunol* 2005; **174**: 5672-5680 [PMID: 15843568]
88 **Otani K**, Tanigawa T, Watanabe T, Nadatani Y, Sogawa M, Yamagami H, Shiba M, Watanabe K, Tominaga K, Fujiwara Y, Arakawa T. Toll-like receptor 9 signaling has anti-inflammatory effects on the early phase of Helicobacter pylori-induced gastritis. *Biochem Biophys Res Commun* 2012; **426**: 342-349 [PMID: 22940550 DOI: 10.1016/j.bbrc.2012.08.080]
89 **Guiducci C**, Gong M, Xu Z, Gill M, Chaussabel D, Meeker T, Chan JH, Wright T, Punaro M, Bolland S, Soumelis V, Banchereau J, Coffman RL, Pascual V, Barrat FJ. TLR recognition of self nucleic acids hampers glucocorticoid activity in lupus. *Nature* 2010; **465**: 937-941 [PMID: 20559388 DOI:

10.1038/nature09102]
90 **Arslan F**, Houtgraaf JH, Keogh B, Kazemi K, de Jong R, McCormack WJ, O'Neill LA, McGuirk P, Timmers L, Smeets MB, Akeroyd L, Reilly M, Pasterkamp G, de Kleijn DP. Treatment with OPN-305, a humanized anti-Toll-Like receptor-2 antibody, reduces myocardial ischemia/reperfusion injury in pigs. *Circ Cardiovasc Interv* 2012; **5**: 279-287 [PMID: 22354933 DOI: 10.1161/CIRCINTERVENTIONS.111.967596]
91 **Farrar CA**, Keogh B, McCormack W, O'Shaughnessy A, Parker A, Reilly M, Sacks SH. Inhibition of TLR2 promotes graft function in a murine model of renal transplant ischemia-reperfusion injury. *FASEB J* 2012; **26**: 799-807 [PMID: 22042224 DOI: 10.1096/fj.11-195396]
92 **Bennett-Guerrero E**, Grocott HP, Levy JH, Stierer KA, Hogue CW, Cheung AT, Newman MF, Carter AA, Rossignol DP, Collard CD. A phase II, double-blind, placebo-controlled, ascending-dose study of Eritoran (E5564), a lipid A antagonist, in patients undergoing cardiac surgery with cardiopulmonary bypass. *Anesth Analg* 2007; **104**: 378-383 [PMID: 17242095 DOI: 10.1213/01.ane.0000253501.07183.2a]
93 **Kalil AC**, LaRosa SP, Gogate J, Lynn M, Opal SM. Influence of severity of illness on the effects of eritoran tetrasodium (E5564) and on other therapies for severe sepsis. *Shock* 2011; **36**: 327-331 [PMID: 21701421 DOI: 10.1097/SHK.0b013e318227980e]
94 **Ulevitch RJ**. Therapeutics targeting the innate immune system. *Nat Rev Immunol* 2004; **4**: 512-520 [PMID: 15229470 DOI: 10.1038/nri1396]
95 **Connolly DJ**, O'Neill LA. New developments in Toll-like receptor targeted therapeutics. *Curr Opin Pharmacol* 2012; **12**: 510-518 [PMID: 22748800 DOI: 10.1016/j.coph.2012.06.002]
96 **Persing DH**, Coler RN, Lacy MJ, Johnson DA, Baldridge JR, Hershberg RM, Reed SG. Taking toll: lipid A mimetics as adjuvants and immunomodulators. *Trends Microbiol* 2002; **10**: S32-S37 [PMID: 12377566]
97 **Koch M**, Meyer TF, Moss SF. Inflammation, immunity, vaccines for Helicobacter pylori infection. *Helicobacter* 2013; **18** Suppl 1: 18-23 [PMID: 24011240 DOI: 10.1111/hel.12073]
98 **Altman E**, Chandan V, Harrison BA, Veloso-Pita R, Li J, KuoLee R, Chen W, Vérez-Bencomo V. Design and immunological properties of Helicobacter pylori glycoconjugates based on a truncated lipopolysaccharide lacking Lewis antigen and comprising an α-1,6-glucan chain. *Vaccine* 2012; **30**: 7332-7341 [PMID: 22534169 DOI: 10.1016/j.vaccine.2012.04.035]
99 **Jiang W**, Baker HJ, Smith BF. Mucosal immunization with helicobacter, CpG DNA, and cholera toxin is protective. *Infect Immun* 2003; **71**: 40-46 [PMID: 12496147]
100 **Mori J**, Vranac T, Smrekar B, Cernilec M, Serbec VČ, Horvat S, Ihan A, Benčina M, Jerala R. Chimeric flagellin as the self-adjuvanting antigen for the activation of immune response against Helicobacter pylori. *Vaccine* 2012; **30**: 5856-5863 [PMID: 22819990 DOI: 10.1016/j.vaccine.2012.07.011]
101 **Codolo G**, Fassan M, Munari F, Volpe A, Bassi P, Rugge M, Pagano F, D'Elios MM, de Bernard M. HP-NAP inhibits the growth of bladder cancer in mice by activating a cytotoxic Th1 response. *Cancer Immunol Immunother* 2012; **61**: 31-40 [PMID: 21833592 DOI: 10.1007/s00262-011-1087-2]
102 **Viala J**, Chaput C, Boneca IG, Cardona A, Girardin SE, Moran AP, Athman R, Mémet S, Huerre MR, Coyle AJ, DiStefano PS, Sansonetti PJ, Labigne A, Bertin J, Philpott DJ, Ferrero RL. Nod1 responds to peptidoglycan delivered by the Helicobacter pylori cag pathogenicity island. *Nat Immunol* 2004; **5**: 1166-1174 [PMID: 15489856 DOI: 10.1038/ni1131]
103 **Allison CC**, Kufer TA, Kremmer E, Kaparakis M, Ferrero RL. Helicobacter pylori induces MAPK phosphorylation and AP-1 activation via a NOD1-dependent mechanism. *J Immunol* 2009; **183**: 8099-8109 [PMID: 20007577 DOI: 10.4049/jimmunol.0900664]
104 **Grubman A**, Kaparakis M, Viala J, Allison C, Badea L, Karrar A, Boneca IG, Le Bourhis L, Reeve S, Smith IA, Hartland EL, Philpott DJ, Ferrero RL. The innate immune molecule, NOD1, regulates direct killing of Helicobacter pylori by antimicrobial peptides. *Cell Microbiol* 2010; **12**: 626-639 [PMID: 20039881 DOI: 10.1111/j.1462-5822.2009.01421.x]
105 **Hutton ML**, Kaparakis-Liaskos M, Turner L, Cardona A, Kwok T, Ferrero RL. Helicobacter pylori exploits cholesterol-rich microdomains for induction of NF-kappaB-dependent responses and peptidoglycan delivery in epithelial cells. *Infect Immun* 2010; **78**: 4523-4531 [PMID: 20713621 DOI: 10.1128/IAI.00439-10]
106 **Kaparakis M**, Turnbull L, Carneiro L, Firth S, Coleman HA, Parkington HC, Le Bourhis L, Karrar A, Viala J, Mak J, Hutton ML, Davies JK, Crack PJ, Hertzog PJ, Philpott DJ, Girardin SE, Whitchurch CB, Ferrero RL. Bacterial membrane vesicles deliver peptidoglycan to NOD1 in epithelial cells. *Cell Microbiol* 2010; **12**: 372-385 [PMID: 19888989 DOI: 10.1111/j.1462-5822.2009.01404.x]
107 **Watanabe T**, Asano N, Fichtner-Feigl S, Gorelick PL, Tsuji Y, Matsumoto Y, Chiba T, Fuss IJ, Kitani A, Strober W. NOD1 contributes to mouse host defense against Helicobacter pylori via induction of type I IFN and activation of the ISGF3 signaling pathway. *J Clin Invest* 2010; **120**: 1645-1662 [PMID: 20389019 DOI: 10.1172/JCI39481]
108 **Gringhuis SI**, den Dunnen J, Litjens M, van der Vlist M, Geijtenbeek TB. Carbohydrate-specific signaling through the DC-SIGN signalosome tailors immunity to Mycobacterium tuberculosis, HIV-1 and Helicobacter pylori. *Nat Immunol* 2009; **10**: 1081-1088 [PMID: 19718030 DOI: 10.1038/ni.1778]

P- Reviewer: Matsuo Y, Mavrogiannaki AN **S- Editor**: Wen LL
L- Editor: A **E- Editor:** Lu YJ

World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4291/wjgp.v5.i3.147

World J Gastrointest Pathophysiol 2014 August 15; 5(3): 147-157
ISSN 2150-5330 (online)

TOPIC HIGHLIGHT

WJGP 5th Anniversary Special Issues (3): Pancreatitis

Molecular mechanisms of alcohol associated pancreatitis

Dahn L Clemens, Mark A Wells, Katrina J Schneider, Shailender Singh

Dahn L Clemens, Nebraska-Western Iowa Veterans Administration Medical Center, Omaha, NE 68105, United States
Dahn L Clemens, Mark A Wells, Katrina J Schneider, Shailender Singh, Department of Internal Medicine, University of Nebraska Medical Center, Omaha, NE 68198, United States
Dahn L Clemens, Shailender Singh, Fred and Pamela Buffett Cancer, University of Nebraska Medical Center, Omaha, NE 68198, United States
Author contributions: All the authors solely contributed to this paper.
Correspondence to: Dahn L Clemens, PhD, Department of Internal Medicine, University of Nebraska Medical Center, 4400 Emile St, Omaha, NE 68198, United States. dclemens@unmc.edu
Telephone: +1-402-9953738 Fax: +1-402-4490604
Received: March 21, 2014 Revised: April 26, 2014
Accepted: June 10, 2014
Published online: August 15, 2014

Abstract

Alcohol abuse is commonly associated with the development of both acute and chronic pancreatitis. Despite this close association, the fact that only a small percentage of human beings who abuse alcohol develop pancreatitis indicates that alcohol abuse alone is not sufficient to initiate clinical pancreatitis. This contention is further supported by the fact that administration of ethanol to experimental animals does not cause pancreatitis. Because of these findings, it is widely believed that ethanol sensitizes the pancreas to injury and additional factors trigger the development of overt pancreatitis. How ethanol sensitizes the pancreas to pancreatitis is not entirely known. Numerous studies have demonstrated that ethanol and its metabolites have a number of deleterious effects on acinar cells. Important acinar cells properties that are affected by ethanol include: calcium signaling, secretion of zymogens, autophagy, cellular regeneration, the unfolded protein response, and mitochondrial membrane integrity. In addition to the actions of ethanol on acinar cells, it is apparent that ethanol also affects pancreatic stellate cells. Pancreatic stellate cells have a critical role in normal tissue repair and the pathologic fibrotic response. Given that ethanol and its metabolites affect so many pancreatic functions, and that all of these effects occur simultaneously, it is likely that none of these effects is "THE" effect. Instead, it is most likely that the cumulative effect of ethanol on the pancreas predisposes the organ to pancreatitis. The focus of this article is to highlight some of the important mechanisms by which ethanol alters pancreatic functions and may predispose the pancreas to disease.

Key words: Pancreatitis; Alcoholic pancreatitis; Alcoholic acute pancreatitis; Alcoholic chronic pancreatitis

Core tip: Alcohol abuse is commonly associated with the development of acute and chronic pancreatitis. Despite this close association, the fact that only a small percentage of human beings who abuse alcohol develop pancreatitis indicates that alcohol abuse alone is not sufficient to initiate clinical pancreatitis. It is widely believed that ethanol sensitizes the pancreas to injury and additional factors trigger the development of overt pancreatitis. How ethanol sensitizes the pancreas to pancreatitis in not entirely known. We will review the mechanisms by which ethanol is thought to sensitize human beings to pancreatic injury.

Clemens DL, Wells MA, Schneider KJ, Singh S. Molecular mechanisms of alcohol associated pancreatitis. *World J Gastrointest Pathophysiol* 2014; 5(3): 147-157 Available from: URL: http://www.wjgnet.com/2150-5330/full/v5/i3/147.htm DOI: http://dx.doi.org/10.4291/wjgp.v5.i3.147

INTRODUCTION

The pancreas is a complex organ, containing both exo-

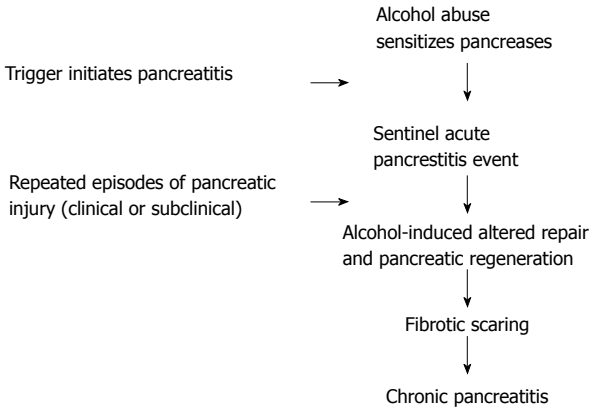

Figure 1 Proposed model for the development of alcoholic chronic pancreatitis. This proposed model incorporated alcohol abuse into the seminal acute pancreatitis event (SAPE) model proposed by Whitcomb. Alcohol metabolism results in biochemical and molecular changes in acinar cells that sensitizes the pancreas to injury. A secondary trigger initiates an initial episode of acute pancreatitis. This is the SAPE. Repeated clinical or subclinical episodes of pancreatitis coupled with ethanol-induced aberrant repair and regeneration of the damaged pancreas leads to fibrotic scarring which eventually results in chronic pancreatitis.

crine and endocrine components. The endocrine component of the pancreas comprises only about 1%-2% of the organ, and is responsible for the production of insulin and glucagon, both of which regulate glucose homeostasis. The exocrine component comprises the vast majority of the pancreas; it is composed of acinar, stellate, and ductal cells. The acinar cells produce digestive enzymes, which facilitate the digestion of carbohydrates, proteins, and lipids. The ductal cells form a network that serves as a conduit for delivery of these enzymes into the duodenum. The pancreatic stellate cells synthesize and degrade extracellular matrix proteins.

Pancreatitis, or inflammation of the pancreas, is a necroinflammatory disease of the pancreas that can manifest as either an acute or chronic disease. Acute pancreatitis is characterized by various degrees of acinar cell damage with concomitant local and systemic inflammation, mediated by inflammatory cytokines and chemokines[1]. Acute pancreatitis is usually a self-limiting condition. Unfortunately, in 10% to 20% of clinical cases, acute pancreatitis progresses to severe acute pancreatitis, a disease with high morbidity and mortality. In the United States alone there are approximately 210000 new clinical cases of acute pancreatitis a year[2]. In 2009, acute pancreatitis was the most common gastrointestinal disease requiring hospitalization. Additionally, it was estimated that acute pancreatitis accounted for more than 2.5 billion dollars in direct and indirect costs[3]. Obviously, pancreatitis is a serious public health concern.

Chronic pancreatitis is a progressive disease characterized by severe pain, persistent pancreatic inflammation, and the development of fibrotic scarring, as well as the loss of endocrine and exocrine function. It has been demonstrated in a long-term prospective study that alcoholic chronic pancreatitis normally progresses from acute pancreatitis. Additionally, this study demonstrated that the progression of acute pancreatitis to chronic pancreatitis is associated with the frequency and severity of the acute attacks[4]. These findings are supported by the observation that individuals who suffer frequent attacks of acute pancreatitis progress to chronic pancreatitis more rapidly[5]. These findings led Whitcomb to propose that a sentinel acute pancreatitis event (SAPE) is required for the development of chronic pancreatitis[6] (Figure 1). Therefore, it appears that although acute and chronic pancreatitis have different clinical manifestations, the mechanisms by which the disease process is initiated is likely similar[7]. Unfortunately, there currently is no treatment, other than palliative care, for either of these diseases.

One of the most common factors associated with both acute and chronic pancreatitis is alcohol abuse[8]. In fact, the association between alcohol abuse and pancreatic disease has been recognized for well over 100 years[9]. It has been known for sometime that the risk of developing pancreatitis increases with increasing alcohol consumption. Recent studies have shown that a threshold of approximately 5 drinks/d (60 g of ethanol) is required for significantly increased risk of developing pancreatitis[10-12]. Although numerous studies have demonstrated direct toxic effects of ethanol and its metabolites on the pancreas, the majority of heavy drinkers (even those consuming more than 5 drinks a day) do not develop pancreatitis[8,12,13]. This fact clearly indicates that alcohol abuse itself is not sufficient to cause pancreatitis, and an additional insult or additional factors are required for the development of clinical pancreatitis. Among the factors suggested to be involved in alcoholic pancreatitis are: smoking, high fat diet, obesity, genetics, and infectious agents[12-16].

Despite the long-standing recognition of the association between alcohol and pancreatitis, the biochemical and molecular processes by which ethanol influences the initiation and progression of these diseases is not well understood. It is thought that the toxic effects of ethanol and/or the by-products of ethanol metabolism sensitize the pancreas; thereby, lowering the threshold to damage from other factors. Ethanol has been shown to affect a number of pathways and functions important in acinar cells. Alteration of these pathways may individually or cumulatively sensitize the pancreas, and lower the threshold of the pancreas to the development of overt pancreatitis. Ethanol has been shown to affect a number of pathways and functions important in acinar cells (Table 1).

Both the rapid course of acute pancreatitis and the relative inaccessibility of pancreatic tissue for examination, prior to the development of fibrotic damage in chronic pancreatitis, have hampered detailed investigations using tissue from human beings. This has contributed to our limited understanding of the mechanisms that lead to the initiation and the progression of alcoholic pancreatitis. Because of this, much of our understand-

Table 1 Mechanisms by which ethanol is thought to sensitize the pancreas to pancreatitis

Alteration of cell death pathways
Altered vesicular trafficking
Impaired autophagy
Impaired tissue repair
ER stress
Mitochondrial dysfunction

ing of pancreatitis in general, and alcoholic pancreatitis in particular, has come from the use of preclinical animal models. Preclinical models used to investigate alcoholic pancreatitis normally utilize mice or rats administered ethanol. Ethanol administration to experimental animals is commonly accomplished through the Tsukamoto-French intragastric method[17], the Lieber-DeCarli pair feeding method[18], or the Cook-Meadows model of providing ethanol in the drinking water[19,20]. Pancreatic cells are either isolated from the animals administered ethanol or pancreatitis is induced. Among the more common methods of inducing pancreatitis in these animals are: bile duct ligation, treatment with supraphysiological concentrations of the cholecystokinin (CCK) analogue caerulein, or treatment with trinitrobenzene sulfonic acid (TNBS)[21]. More recently, methods designed to be more clinically relevant have been reported. These methods include chronic ethanol administration followed by treatment with gram-negative bacterial lipopolysaccharide (LPS)[22,23], or infection with Coxsackievirus CVB3[16,24,25].

Unfortunately, no animal model of chronic pancreatitis recapitulates all of the manifestations of chronic pancreatitis in human beings. It has been demonstrated that alcohol administration to rats and mice results in acinar cell loss and enhanced fibrosis in animals subjected to caerulein-induced pancreatic injury[26,27]. Therefore, these models may be useful in elucidating the mechanisms by which ethanol alters normal pancreatic repair, and predisposes the pancreas to fibrosis.

It is the focus of this article to review and highlight some of the molecular events that may adversely affect the pancreas, and sensitize the pancreas to the initiation or progression of alcoholic pancreatitis.

ETHANOL METABOLISM

Many of the deleterious effects of ethanol are attributed to the by-products produced during its metabolism. Like the hepatocytes of the liver, the pancreatic acinar cells have the ability to metabolize ethanol by both oxidative and nonoxidative pathways. The oxidative metabolism of ethanol is catalyzed by two enzymes: the cytosolic enzyme, alcohol dehydrogenase, and the microsomal enzyme, cytochrome P450 2E1. Ethanol metabolism by both of these enzymes generates acetaldehyde and reactive oxygen species. Although the pancreas expresses both alcohol dehydrogenase and cytochrome P450 2E1, the capacity for ethanol oxidation by the pancreas is significantly less than that of the liver[28,29]. Therefore, the actions of the oxidative metabolites of ethanol oxidation may result from both pancreatic metabolism and systemic metabolism of ethanol.

Nonoxidative metabolism of ethanol is carried out by a number of enzymes, the most important being the fatty acid ethyl ester synthases. Metabolism of ethanol by these enzymes generates fatty acid ethyl esters (FAEEs). The pancreas possesses high fatty acid ester synthase activity. Thus, the capacity for nonoxidative metabolism of ethanol in the pancreas is high[30]. In fact, a study of individuals who were intoxicated at the time of death revealed that the concentration of FAEEs in the pancreas was higher than any other organ analyzed[30]. Thus, because the oxidative metabolism of ethanol in the pancreas is relatively low, the nonoxidative metabolism of ethanol may be more important and the production of FAEEs, and their toxic effects, may be accentuated. Because the by-products of ethanol metabolism have been demonstrated to cause toxicity in other organs, a great deal of work has been performed investigating the actions of the various ethanol metabolites on the pancreas.

CELL DEATH

Cell death during an episode of acute pancreatitis can occur by one of two mechanisms: apoptosis or necrosis. The distinction between the two types of cell death not only has biological implications in the development of acute pancreatitis, but also affects the clinical presentation by influencing the severity of the illness[8]. Clinically, according to the 2012 Atlanta Classification of Acute Pancreatitis, the presence of necrosis and the number of organs affected by the subsequent inflammatory response determines the severity of acute pancreatitis (mild, moderate, severe) and dictates the short-term and long-term management of these patients[31].

While necrosis and apoptosis both lead to cell death, their respective mechanisms of achieving this end are quite different. Apoptosis, or programmed cell death, is a process by which cellular constituents are cleaved by cysteine-dependent, aspartate-directed enzymes, known as caspases. Apoptosis is mediated by caspases 3 and caspases 8. Caspase 8 is the initiator of the caspase cascade and cleaves caspase 3, which mediates many of the cellular changes that lead to apoptotic death. In pancreatitis, these caspases are activated by the release of cytochrome c from mitochondria[32]. The release of cytochrome c is caused by the depolarization of mitochondria. It appears this depolarization is a result of the opening of the mitochondrial permeability transition pore, which is caused by sustained increased calcium levels in the cytosol[33]. Ultimately, there is an organized dismantling of the cell. This leads to cell shrinkage and nuclear chromatin condensation, while preserving the integrity of the plasma membrane. Because the plasma membrane remains intact, there is very little leakage of intracellular material into the extracellular space, and therefore; there is little

activation of inflammatory cytokines.

In contrast to the organized dismantling of the cell in apoptosis, necrosis involves intracellular swelling of organelles and rupture of the plasma membrane. This results in the release of the contents of the cell into the extracellular space, which causes an inflammatory response. It has been shown in a number of preclinical animal models of pancreatitis that the severity of pancreatitis is increased with increasing necrotic cell death[8]. Additionally, perhaps the most important prognostic indicator of the severity of pancreatitis in human beings is the amount of necrosis[31].

In preclinical animal models of pancreatitis, ethanol has been shown to cause a shift in cell death from apoptosis to necrosis. This shift has been shown to occur through several mechanisms. It has been shown that the nonoxidative metabolites of ethanol, FAEEs, activate inositol trisphosphate receptors on the endoplasmic reticulum. Activation of these receptors causes release of calcium into the cytosol. As stated above, the sustained increases in cytosolic calcium results in mitochondria depolarization and loss of ATP production. Without ATP, the cells are unable to complete the apoptotic process and necrosis occurs[8].

Ethanol has also been shown to inhibit the JAK2/STAT1 pathway. Attenuated activity of this pathway leads to decreased activity of both caspase 8 and caspase 3[32]. With lower activity of these caspases, cell death by necrosis is increased while apoptotic cell death is reduced.

Ethanol also increases the pancreatic expression of cathepsin B[32]. Cathepsin B is a cysteine protease that is thought to play a major role in the intrapancreatic conversion of trypsinogen to trypsin. It has been shown that in pancreata of ethanol-fed rats, increased expression of cathepsin B result from increased levels of the transcriptional activators Ets-1 and Sp1[32]. Increases in Sp1 and Ets-1 enhance expression of cathepsin B, which leads to activation of trypsin and a shift from apoptosis to necrosis in pancreatic acinar cells[32]. These findings demonstrate that ethanol can affect the mechanism of cell death in acinar cells, and thereby influence the severity of the disease.

EFFECTS OF ETHANOL ON ZYMOGEN SECRETION

One of the primary roles of the exocrine pancreas is the synthesis and secretion of digestive enzymes. The pancreas is protected from the actions of these potentially dangerous enzymes because they are synthesized as inactive zymogens and packaged into exocytotic vacuoles, known as zymogen granules. Although ethanol has many effects on acinar cells that contribute to the development of pancreatitis, the inappropriate activation of zymogens is likely a critical component of this pathologic process.

Activation of trypsinogen is generally considered a pivotal event in the initiation of pancreatitis[34]. It has been reported by Gorlelich that treatment of isolated acinar cells with intoxicating concentrations of ethanol (25 mmol/L) sensitizes acinar cells to damage by causing the activation of zymogens[35]. The activation of these zymogens required an increase in cytosolic calcium and appeared to involve a low pH compartment (acid granular compartment).

Local cytosolic spikes of calcium in the apical region of acinar cells control the exocytotic secretion of zymogens. These spikes are generated by release of small quantities of calcium from internal stores[36]. In contrast, prolonged, global elevation of calcium results in the formation of empty looking zymogen granules, this is thought to be the site where trypsin is activated. In acinar cells treated with the FAEE palmitoleic acid ethyl ester, calcium was released from both the endoplasmic reticulum (the major calcium storage compartment of the cell) and the acid granular compartment, located near the apical surface. Additionally, it was demonstrated that the calcium release was primarily mediated by type 2 and 3 inositol 1,4,5 trisphosphate receptors[37].

Normally, zymogens are released from acinar cells by fusion of zymogen granules with the apical membrane. This fusion results in their release into the ducts, where they are transported to the duodenum and activated. The components absolutely required for membrane fusion consist of: SNAREs (soluble NSF [N-ethylmaleimide-sensitive fusion proteins] attachment proteins receptors) located on the target membrane, t-SNARES, and v-SNAREs, also known as vesicle-associated membrane proteins (VAMPs), located on the membrane of the vesicle. The t-SNARES syntaxin and synaptosome-associated proteins (SNAPs), form a SNARE complex that binds to its cognate v-SNARE; thus, juxtaposing the two membranes and facilitating the fusion of the membranes.

Interestingly, it has been demonstrated both *in vivo* and *in vitro,* that supramaximal treatment with cholecystokinin (CCK) causes basolateral exocytosis of zymogen granules in acinar cells[38]. Additionally, in both ethanol-fed rats or isolated acinar cells treated with physiologic concentrations of ethanol (20 mmol/L), stimulation with submaximal concentration of CCK or carbachol resulted in the exocytosis being redirected from the apical surface, where zymogens are normally secreted, to the basolateral surface[39]. The authors postulate that the ensuing ectopic activation of the zymogens in the interstitial space results in pancreatitis[39]. More detailed investigations demonstrated that this inappropriate exocytosis was mediated by phosphorylation of mammalian uncoordinated-18c (Munc 18c) by protein kinase C-alpha (PKC-α). Phosphorylation of Munc-18c results in its release from syntaxin-4, which is located on the basolateral surface of acinar cells. Syntaxin-4 is then able to complex with SNAP-23 and VAMP-8, located on the zymogen granules, to form the SNARE complex, which mediates the inappropriate basolateral exocytosis of zymogens[40]. Importantly, basolateral exocytosis has been

observed in tissue samples from a patient suffering from chronic alcoholic pancreatitis[41].

IMPAIRMENT OF AUTOPHAGY

Autophagy is a cellular process in which unnecessary or damaged cellular components or organelles are sequestered in vacuoles and transported to the lysosomes. Upon fusion with the lysosomes, the contents of the autophagic vacuoles, the autophagosomes, are degraded. Not only does this process perform an important role in ridding cells of unneeded components, but during times of low nutrient availability autophagy can provide the cell with needed constituents.

Impaired autophagy has been implicated in the pathogenesis of many diseases, including pancreatitis[15,42-45]. Importantly, it has been shown that ethanol can alter the process of autophagy in a number of organs, including the pancreas[43,46,47].

One of the histological hallmarks of pancreatitis is the accumulation of large vacuoles within acinar cells[48]. In a number of preclinical animal models of pancreatitis, as well as in tissue from a patient with acute pancreatitis, it has been demonstrated that these vacuoles are in fact autophagic vacuoles[44,45]. Further investigation revealed that these vacuoles possessed markers of both autophagosomes and lysosomes, and contained undegraded or partially degraded cellular material[45]. These findings indicate that at least the very late events in the autophagic process, namely the degradation of the components of the autolysosomes, are impaired during pancreatitis[45]. Thus, autophagy is activated during pancreatitis, and it appears that the impairment in the ability to complete this process is responsible for the vacuolization characteristic of this disease.

As mentioned above, trypsin activation is thought to be an early event in the initiation of pancreatitis. How this activation occurs is not well understood. It is generally thought that cathepsin B, is mis-sorted to the zymogens granules, where it co-localizes with trypsinogen. Subsequent cleavage of trypsinogen by cathepsin B results in the production of active trypsin. How trypsinogen and cathepsin B come in contact has always been a mystery. It now appears that the impairment in the completion of the autophagy may have a role in the co-mingling of these two enzymes.

Cathepsin L is an enzyme that degrades trypsinogen and trypsin, and cathepsin B is an enzyme that cleaves trypsinogen forming active trypsin. The two are important lysosomal hydrolases. During pancreatitis, increased levels of these enzymes are found in the zymogen granule fraction. Additionally, in alcoholic pancreatitis, as well as other forms of acute pancreatitis, the processing and activation of cathepsin L and cathepsin B is impaired[45,49]. Furthermore, it appears that the impairment in cathepsin L activity is more severe than the impairment in cathepsin B activity, particularly in the zymogen granule fraction[45]. Importantly, zymogen granules were detected in the autophagosomes/autoloysosomes. The authors propose that it is in these autophagosomes/autoloysosomes that trypsinogen and cathepsin B come in contact[45]. The imbalance between cathepsin B and cathepsin L activity in these vacuoles would favor the activation of trypsin, and the initiation of pancreatitis. Thus, impairment in the completion of the autophagic process and subsequent increase in autolysosomes may contribute not only to the accumulation of vacuoles, but also to the inappropriate intracellular activation of trypsin and the initiation of pancreatitis.

Ethanol has been shown to impair other aspects of autophagy. Using a model of alcoholic pancreatitis in which rats were chronically fed ethanol and then treated with LPS to induce acute pancreatitis, Fortunato *et al*[43] demonstrated that in the pancreata of these animals fusion of autophagosomes with the lysosome was impaired. Additional studies demonstrated that Lamp-2, a lysosomal membrane protein required for the fusion of autophagosomes with lysosomes, was depleted in the pancreata of rats suffering from alcoholic pancreatitis[43,50]. Furthermore, analysis of pancreata from human beings revealed that Lamp-2 was also decreased in the pancreata of patients suffering from chronic alcoholic pancreatitis. These results indicate that the ethanol-mediated reduction in lysosomal proteins, particularly Lamp-2, and subsequent impairment in autophagy may be a contributing factor to alcoholic pancreatitis in human beings. Although not investigated, the authors speculated that disruption in the autophagic pathway may contribute to bioenergenic failure in mitochondria. Lack of mitochondrial ATP would favor necrosis, as opposed to apoptosis. Necrotic cell death would cause inflammation and lead to the initiation of pancreatitis[43].

MITOCHONDRIAL DYSFUNCTION

Pancreatic acinar cells are among the most synthetically active cells in the body[51]. This synthetic activity requires a great deal of energy. Because of this, acinar cells contain an inordinate number of mitochondria. Thus, the actions of toxins, such as ethanol, that affect mitochondria can dramatically affect acinar cells.

Normally, acetylcholine or cholecystokinin bind to G-protein linked receptors that are located on the plasma membrane of acinar cells and stimulate the production of secondary messengers. The secondary messengers bind to inositol trisphosphate or ryanodine receptors located on the endoplasmic reticulum, zymogen granules, and endo-lysosomes. This binding results in the transient release of free calcium. Mitochondria take up this calcium, which results in their activation, the synthesis of ATP, and the secretion of zymogens.

Aberrant calcium signaling has long been considered an important factor in the initiation of pancreatic injury[52]. Pathological calcium signaling in acinar cells results from prolonged global release of calcium from the endoplasmic reticulum, as well as zymogen granules and

endo-lysosomes. In fact, early acinar cell injury (vacuolization, trypsin activation, and basolateral zymogen secretion) does not occur without prolonged, sustained release of calcium[53].

Both nonoxidative and oxidative metabolism of ethanol has been shown to contribute to mitochondrial dysfunction and acinar cell death. FAEEs, the nonoxidative metabolites of ethanol, have been shown to cause pancreatic injury by affecting calcium signaling in acinar cells[54,55]. FAEEs increase the intracellular concentration of calcium to toxic levels. This calcium increase is mediated by activation of the inositol trisphosphate receptors located on the endoplasmic reticulum, and results in global sustained increase in intracellular calcium, which causes mitochondrial membrane permeability. Mitochondrial membrane permeability can lead to cell death by either apoptosis or necrosis[56,57].

Mitochondrial membrane permeability results from opening of the mitochondrial permeability transition pore. The mitochondrial permeability transition pore is thought to have at least three major components, the voltage dependent anion channel (VDAC) located on the outer mitochondrial membrane, adenine nucleotide translocase (ANT) located in the inner mitochondrial membrane and cyclophlin-D located within the mitochondrial matrix[53].

One of the important consequences of the opening of the mitochondrial permeability transition pore and mitochondrial membrane permeability can be loss of the mitochondrial membrane potential ($\Delta\Psi M$). Loss of the $\Delta\Psi M$ results in the decreased ability of TP.

Depleted levels of ATP exacerbate the cells ability to regulate calcium by inhibiting the activity of the important ATP-dependent calcium pumps, the sacroplasmic/endoplasmic reticular calcium ATPase (SERCA) located on the ER, and the plasma membrane calcium ATPase (PMCA) located on the plasma membrane. Thus, mitochondrial membrane permeability can exacerbate the dysregulation of calcium homeostasis and lead to acinar cell necrosis.

The oxidative metabolism of ethanol also has deleterious effects on pancreatic mitochondria. Oxidative metabolism of ethanol by alcohol dehydrogenase requires oxidized nicotinamide adenine dinucleotide (NAD^+) as a cofactor, and results in the production of acetaldehyde and reduced nicotinamide adenine dinucleotide (NADH)[58,59]. Acetaldehyde is then metabolized to acetate, primarily by the mitochondrial enzyme aldehyde dehydrogenase-2. Importantly, this reaction also requires NAD^+ as a cofactor, and also results in the production of NADH[58,59]. Thus, metabolism of acetaldehyde to acetate further depletes the availability of NAD^+.

Using isolated acinar cells treated with ethanol, Shalbueva *et al*[60] demonstrated that ethanol treatment led to a decrease in the NAD^+/NADH ratio. This reduction in NAD^+ resulted in activation of the mitochondrial permeability transition pore, mitochondrial depolarization, ATP depletion, and eventually cellular necrosis[60]. Furthermore, their studies revealed that the ethanol oxidation-mediated polarization of pancreatic mitochondria was attenuated in acinar cells isolated from mice deficient in cyclophilin-D. These results indicate a role for cyclophilin-D in this ethanol metabolism-mediated mitochondrial dysfunction.

Interestingly, it has been shown in mitochondria isolated from the liver that ethanol metabolism sensitizes the mitochondrial permeability transition pore to open, in part, through increased cyclophilin-D activity and increased association of cyclophilin-D with ANT[61]. This increased activity is associated with hyperacetylation of cyclophilin-D. Acetylation of cyclophilin-D is regulated by sirtuin-3, a NAD^+-dependent deacetylase localized in the mitochondrial matrix[62]. The ethanol oxidation-mediated decrease in NAD^+ leads to decreased sirtuin-3 activity and the hyperacetylation of cyclophilin-D. Hyperacetylation of cyclophilin-D results in increased cyclophilin-D activity, increased binding to ANT, and mitochondrial permeability transition pore induction[61]. Thus, it is tempting to speculate that the ethanol oxidation-mediated induction of the mitochondrial permeability transition pore in pancreatic mitochondria is mediated by a similar NAD^+-sirtuin-3-cyclophilin-D axis.

ENDOPLASMIC RETICULUM STRESS AND THE UNFOLDED PROTEIN RESPONSE

Acinar cells are responsible for the production and secretion of large quantities of digestive enzymes. Because of this, in addition to large numbers of mitochondria, acinar cells possess an extensive endoplasmic reticulum network. The endoplasmic reticulum is the major storage site of calcium in the cell, and is the cellular organelle where the proper folding and trafficking of secretory proteins is determined. Endoplasmic reticulum stress resulting from excessive accumulation of proteins, calcium imbalance, oxidative stress, or accumulation of damaged or misfolded protein leads to a response known as the unfolded protein response (UPR)[63].

One hallmark of the UPR is the activation of the IRE1/XBP1 pathway. Inositol-requiring transmembrane kinase/endonuclease 1 (IRE1) splices X-box binding protein 1 (XBP1) messenger RNA, resulting in spliced XBP1. Spliced XBP1 is a transcriptional activator that regulates a number of genes, which encode proteins that act as ER chaperones, are involved in the proper folding of proteins, or are involved in the degradation of damaged or misfolded proteins.

The UPR is activated by pancreatic injury[64]. Additionally, it has been shown that the UPR is activated in acinar cells by long-term ethanol administration to mice[65]. Ethanol mediated UPR was characterized by increased expression of IRE1 and spliced XBP1. Although the UPR was activated, ethanol administration alone did not result in histopathologic changes to the pancreas. In contrast, administration of ethanol to mice with diminished XBP1 expression ($XBP1^{+/-}$ mice) resulted in

acinar vacuolization, cell necrosis, and inflammation[65]. The presence of pathologic changes in the pancreata of XBP1$^{+/-}$ mice led the authors to suggest that the UPR is a protective mechanism in acinar cells during endoplasmic reticulum stress. Exceeding the capacity of the UPR to compensate for endoplasmic reticulum stress results in overt pancreatitis. Thus, if the protective capacity of the UPR is exceeded, this pathway may contribute to the induction and progression of pancreatitis.

THE ROLE OF STELLATE CELLS IN ALCOHOLIC PANCREATITIS

The pancreas, like the liver, has a population of vitamin A storing cells known as stellate cells. Pancreatic stellate cells are periacinar cells located in interacinar and interlobular areas of the pancreas[66,67]. These cells are responsible for the synthesis of extracellular matrix proteins, as well as matrix metalloproteinases (enzymes that degrade extracellular matrix proteins). Thus, it appears that in the healthy organ, pancreatic stellate cells function to maintain the architecture of the pancreas by regulating the deposition and degradation of extracellular matrix components[68]. In response to pancreatic injury, pancreatic stellate cells are activated and transform into myofibroblast-like cells. Activated pancreatic stellate cells synthesize excessive amounts of extracellular matrix proteins. The accumulation of these proteins results in fibrosis. Thus, pancreatic stellate cells are intimately involved in the regulation of both normal and pathologic aspects of the pancreatitis[68,69].

Pancreatic stellate cells of both rat and human origin have the ability to metabolize ethanol through the oxidative pathway[70,71]. Rat pancreatic stellate cells possess alcohol dehydrogenase, the activity of this enzyme is induced when cells are exposed to ethanol concentrations routinely found in the blood of inebriated individuals[70]. Recently, it has also been reported that quiescent pancreatic stellate cells in human beings possess alcohol dehydrogenase activity. Additionally, this activity appeared to be upregulated in pancreatic stellate cells of individuals suffering from chronic pancreatitis and pancreatic cancer[71].

The fact that pancreatic stellate cells possess alcohol dehydrogenase activity may contribute to the development of alcoholic pancreatitis. Pancreatic stellate cells are activated when exposed to concentrations of ethanol detected in the blood of inebriated individuals (10-50 mmol/L)[70,72]. Additionally, pancreatic stellate cells isolated from both rats and human beings are activated by acetaldehyde. Ethanol and acetaldehyde not only activate pancreatic stellate cells, but also elicit responses that may have important biological consequences. Both ethanol and acetaldehyde have been shown to induce the secretion of matrix metalloproteinases in pancreatic stellate cells[73]. Furthermore, treatment of pancreatic stellate cells with ethanol induces the synthesis of interleukin-8 and connective tissue growth factor (CTGF)[72,74]. It has been suggested that these factors act in an autocrine manner to perpetuate the activation of pancreatic stellate cells[13]. This finding may help to explain both the apparent inability of the pancreas to fully recover from injury in the continued presence of ethanol, and the extremely common association between alcohol abuse and chronic pancreatitis.

Although it is well established that pancreatic stellate cells are primarily responsible for the deposition and degradation of components of the extracellular matrix, it appears that acinar cells exposed to ethanol may also contribute to the increase in extracellular matrix deposition. It has been shown that FAEEs can increase the levels of extracellular matrix proteins by inhibiting the acinar cell activity of plasmin and urokinase-type plasminogen activator (uPA) proteins involved in the degradation of the extracellular matrix components[75].

THE ROLE OF THE INFLAMMATORY RESPONSE

Inflammation mediated by cytokines, chemokines, and adhesion molecules is involved in the development of pancreatitis[1,76,77]. Interestingly, it appears that ethanol and its metabolites have a differential effect on the expression of molecules that regulate the inflammatory response. It has been shown that treatment of isolated acini with ethanol or acetaldehyde decreased the activity of two important transcriptional activators involved in the inflammatory response, specifically nuclear factor-κB (NF-κB) and activator protein 1 (AP-1). Conversely, treatment of acini with FAEEs increased the activation of these regulators of the inflammatory response[78].

The activity of NF-κB is also reduced in the pancreata of animals chronically fed ethanol[79]. However, it was demonstrated that induction of pancreatitis in rats chronically administered ethanol resulted in increased NF-κB activity, as well as increases in the mRNA levels of a number of proinflammatory cytokines, including: tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), macrophage inflammatory protein-1 (MIP-1), and monocyte chemotactic protein-1 (MCP-1)[79]. These results led the authors to suggest that the *in vitro* and *in vivo* down-regulation of these factors by ethanol reflected a protective mechanism to prevent the development of alcohol-induced pancreas[78,79].

The role of the inflammatory response in chronic alcoholic pancreatitis has also been investigated[80]. Focusing on the resident mononuclear cells of the pancreas, Deng *et al*[80] demonstrated that chronic ethanol administration reduced the number of these cells present in the pancreas. In agreement with others, they suggested that this reduction likely reflected a general immunologic suppression in the pancreas of ethanol-fed rats, and may explain why animals chronically provided ethanol do not develop chronic pancreatitis in the absence of acute pancreatic damage[80].

Despite this immunologic suppression, when pan-

creatitis was induced by caerulein, the inflammatory response in these animals was enhanced[80]. Furthermore, following repeated caerulein-induced episodes of pancreatitis, it was shown that the expression of both pro-inflammatory cytokines such as TNF-α, MIP-1α, and RANTES (regulated on activation normal T cell expressed and secreted), as well as the anti-inflammatory cytokines tissue growth factor-β (TGF-β) and interleukin-10 (IL-10) was enhanced. The increase in cytokine expression was only observed in rats fed ethanol and subjected to repeated episodes of acute pancreatitis, and was also associated with increased activation of pancreatic stellate cells and fibrosis. These findings led the authors to suggest that ethanol acts not only to sensitize the pancreas to acute pancreatitis, but also aids in the progression of chronic pancreatitis if repeated episodes of acute pancreatitis occur[80].

EFFECTS OF ETHANOL ON PANCREATIC REPAIR

It is generally accepted that fibrosis is an aberrant repair response. It appears that in the presence of ethanol, repair of the damaged pancreas is altered or never fully completed[26,27]. This may help to explain the extremely common association between alcohol abuse and chronic pancreatitis. Because ethanol and acetaldehyde can activate stellate cells, and FAEEs inhibit the degradation of extracellular matrix proteins, it is obvious that ethanol can also influence recovery of the pancreas after damage has occurred[70,72,75].

It has been demonstrated that chronic ethanol administration also delays regeneration of the damaged pancreas[81]. This delay was associated with an ethanol-mediated decrease in the expression of important developmental factors, such as PDX-1 and PTF-1a, as well as impaired activation of the Notch signaling pathway[24]. Normal pancreatic repair requires the dedifferentiation of mature acinar cells followed by their redifferentiation[82]. Thus, ethanol-mediated alterations in the expression of these important developmental factors affect the dedifferentiation/redifferentiation of acinar cells. These alterations may dramatically influence pancreatic repair.

As mentioned above, there is a close association between alcohol abuse and chronic pancreatitis. In fact, in developed countries, alcohol abuse is associated with over 70% of the reported cases[83]. Importantly, individuals suffering from chronic pancreatitis have a 20-fold greater likelihood of developing pancreatic cancer[84], a disease with a dismal prognosis. It is thought that changes that occur in the pancreas during chronic injury are associated with, or predispose the organ to, the initiation of pancreatic neoplasia. Because one of the seminal characteristics of chronic pancreatitis is aberrant tissue repair, resulting in fibrotic scarring, and ethanol consumption alters pancreatic repair, ethanol may have an indirect role in the initiation of pancreatic cancer. Thus, the effects of ethanol on repair of the damaged pancreas may be a contributing factor in pancreatic cancer, as well as alcoholic pancreatitis.

CONCLUSION

Despite the dramatic expansion of our understanding of pancreatitis in general, and how ethanol and its metabolites affect pancreatic cells, we still have not defined the mechanism of alcoholic pancreatitis. Instead, it is evident that ethanol has a plethora of toxic affects on pancreatic cells. Because all of these effects occur simultaneously, it is likely that the cumulative effects of ethanol sensitize the pancreas to damage, and that "alcoholic pancreatitis" is a multifactorial disease. Paradoxically, despite the demonstration that ethanol has numerous toxic effects on the pancreas, data from demographic studies and preclinical animal models has firmly established that ethanol itself does not cause pancreatitis. Because ethanol does not cause pancreatitis, but only sensitizes the pancreas to disease, it appears that the pancreas has developed protective mechanisms that can partially compensate for ethanol-induced cellular damage. Some of these protective mechanisms have been identified. It is likely that additional compensatory mechanisms exist. Further defining the mechanisms of ethanol-induced pancreatic injury may help define these protective mechanisms. It is hoped that this strategy will lead to the development of therapeutic targets that will prevent or reduce the severity of alcoholic pancreatitis.

REFERENCES

1 **Vonlaufen A**, Apte MV, Imhof BA, Frossard JL. The role of inflammatory and parenchymal cells in acute pancreatitis. *J Pathol* 2007; **213**: 239-248 [PMID: 17893879 DOI: 10.1002/path.2231]

2 **Carroll JK**, Herrick B, Gipson T, Lee SP. Acute pancreatitis: diagnosis, prognosis, and treatment. *Am Fam Physician* 2007; **75**: 1513-1520 [PMID: 17555143]

3 **Peery AF**, Dellon ES, Lund J, Crockett SD, McGowan CE, Bulsiewicz WJ, Gangarosa LM, Thiny MT, Stizenberg K, Morgan DR, Ringel Y, Kim HP, Dibonaventura MD, Carroll CF, Allen JK, Cook SF, Sandler RS, Kappelman MD, Shaheen NJ. Burden of gastrointestinal disease in the United States: 2012 update. *Gastroenterology* 2012; **143**: 1179-1187.e1-3 [PMID: 22885331 DOI: 10.1053/j.gastro.2012.08.002]

4 **Ammann RW**, Muellhaupt B. Progression of alcoholic acute to chronic pancreatitis. *Gut* 1994; **35**: 552-556 [PMID: 8174996]

5 **Lankisch PG**, Breuer N, Bruns A, Weber-Dany B, Lowenfels AB, Maisonneuve P. Natural history of acute pancreatitis: a long-term population-based study. *Am J Gastroenterol* 2009; **104**: 2797-2805; quiz 2806 [PMID: 19603011 DOI: 10.1038/ajg.2009.405]

6 **Whitcomb DC**. Hereditary pancreatitis: new insights into acute and chronic pancreatitis. *Gut* 1999; **45**: 317-322 [PMID: 10446089]

7 **Vonlaufen A**, Wilson JS, Apte MV. Molecular mechanisms of pancreatitis: current opinion. *J Gastroenterol Hepatol* 2008; **23**: 1339-1348 [PMID: 18853993 DOI: 10.1111/j.1440-1746.2008.05520.x]

8 **Pandol SJ**, Saluja AK, Imrie CW, Banks PA. Acute pancreatitis: bench to the bedside. *Gastroenterology* 2007; **132**: 1127-1151 [PMID: 17383433 DOI: 10.1053/j.gastro.2007.01.055]

9 **Friederich.** Disease of the Pancreas. Cycloaedia of the Practice of Medicine. Vol 18. New York: William Wood, 1878: 254-312
10 **Yadav D**, Hawes RH, Brand RE, Anderson MA, Money ME, Banks PA, Bishop MD, Baillie J, Sherman S, DiSario J, Burton FR, Gardner TB, Amann ST, Gelrud A, Lawrence C, Elinoff B, Greer JB, O'Connell M, Barmada MM, Slivka A, Whitcomb DC. Alcohol consumption, cigarette smoking, and the risk of recurrent acute and chronic pancreatitis. *Arch Intern Med* 2009; **169**: 1035-1045 [PMID: 19506173 DOI: 10.1001/archinternmed.2009.125]
11 **Kristiansen L**, Grønbaek M, Becker U, Tolstrup JS. Risk of pancreatitis according to alcohol drinking habits: a population-based cohort study. *Am J Epidemiol* 2008; **168**: 932-937 [PMID: 18779386 DOI: 10.1093/aje/kwn222]
12 **Yadav D**, Whitcomb DC. The role of alcohol and smoking in pancreatitis. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 131-145 [PMID: 20125091]
13 **Apte MV**, Pirola RC, Wilson JS. Mechanisms of alcoholic pancreatitis. *J Gastroenterol Hepatol* 2010; **25**: 1816-1826 [PMID: 21091991 DOI: 10.1111/j.1440-1746.2010.06445.x]
14 **Yadav D**, Papachristou GI, Whitcomb DC. Alcohol-associated pancreatitis. *Gastroenterol Clin North Am* 2007; **36**: 219-238, vii [PMID: 17533076]
15 **Gukovsky I**, Li N, Todoric J, Gukovskaya A, Karin M. Inflammation, autophagy, and obesity: common features in the pathogenesis of pancreatitis and pancreatic cancer. *Gastroenterology* 2013; **144**: 1199-1209.e4 [PMID: 23622129 DOI: 10.1053/j.gastro.2013.02.007]
16 **Jerrells TR**, Chapman N, Clemens DL. Animal model of alcoholic pancreatitis: role of viral infections. *Pancreas* 2003; **27**: 301-304 [PMID: 14576491]
17 **Ueno A**, Lazaro R, Wang PY, Higashiyama R, Machida K, Tsukamoto H. Mouse intragastric infusion (iG) model. *Nat Protoc* 2012; **7**: 771-781 [PMID: 22461066 DOI: 10.1038/nprot.2012.014]
18 **Lieber CS**, DeCarli LM. The feeding of alcohol in liquid diets: two decades of applications and 1982 update. *Alcohol Clin Exp Res* 1982; **6**: 523-531 [PMID: 6758624]
19 **Song K**, Coleman RA, Zhu X, Alber C, Ballas ZK, Waldschmidt TJ, Cook RT. Chronic ethanol consumption by mice results in activated splenic T cells. *J Leukoc Biol* 2002; **72**: 1109-1116 [PMID: 12488491]
20 **D'Souza El-Guindy NB**, Kovacs EJ, De Witte P, Spies C, Littleton JM, de Villiers WJ, Lott AJ, Plackett TP, Lanzke N, Meadows GG. Laboratory models available to study alcohol-induced organ damage and immune variations: choosing the appropriate model. *Alcohol Clin Exp Res* 2010; **34**: 1489-1511 [PMID: 20586763 DOI: 10.1111/j.1530-0277.2010.01234.x]
21 **Puig-Diví V**, Molero X, Vaquero E, Salas A, Guarner F, Malagelada J. Ethanol feeding aggravates morphological and biochemical parameters in experimental chronic pancreatitis. *Digestion* 1999; **60**: 166-174 [PMID: 10095159]
22 **Vonlaufen A**, Xu Z, Daniel B, Kumar RK, Pirola R, Wilson J, Apte MV. Bacterial endotoxin: a trigger factor for alcoholic pancreatitis? Evidence from a novel, physiologically relevant animal model. *Gastroenterology* 2007; **133**: 1293-1303 [PMID: 17919500 DOI: 10.1053/j.gastro.2007.06.062]
23 **Vonlaufen A**, Phillips PA, Xu Z, Zhang X, Yang L, Pirola RC, Wilson JS, Apte MV. Withdrawal of alcohol promotes regression while continued alcohol intake promotes persistence of LPS-induced pancreatic injury in alcohol-fed rats. *Gut* 2011; **60**: 238-246 [PMID: 20870739]
24 **Schneider KJ**, Scheer M, Suhr M, Clemens DL. Ethanol administration impairs pancreatic repair after injury. *Pancreas* 2012; **41**: 1272-1279 [PMID: 22617711 DOI: 10.1097/MPA.0b013e31824bde37]
25 **Jerrells TR**, Vidlak D, Strachota JM. Alcoholic pancreatitis: mechanisms of viral infections as cofactors in the development of acute and chronic pancreatitis and fibrosis. *J Leukoc Biol* 2007; **81**: 430-439 [PMID: 17095612]
26 **Gukovsky I**, Lugea A, Shahsahebi M, Cheng JH, Hong PP, Jung YJ, Deng QG, French BA, Lungo W, French SW, Tsukamoto H, Pandol SJ. A rat model reproducing key pathological responses of alcoholic chronic pancreatitis. *Am J Physiol Gastrointest Liver Physiol* 2008; **294**: G68-G79 [PMID: 17884979 DOI: 10.1152/ajpgi.00006.2007]
27 **Perides G**, Tao X, West N, Sharma A, Steer ML. A mouse model of ethanol dependent pancreatic fibrosis. *Gut* 2005; **54**: 1461-1467 [PMID: 15870229 DOI: 10.1136/gut.2004.062919]
28 **Haber PS**, Apte MV, Applegate TL, Norton ID, Korsten MA, Pirola RC, Wilson JS. Metabolism of ethanol by rat pancreatic acinar cells. *J Lab Clin Med* 1998; **132**: 294-302 [PMID: 9794700]
29 **Norton ID**, Apte MV, Haber PS, McCaughan GW, Pirola RC, Wilson JS. Cytochrome P4502E1 is present in rat pancreas and is induced by chronic ethanol administration. *Gut* 1998; **42**: 426-430 [PMID: 9577353]
30 **Laposata EA**, Lange LG. Presence of nonoxidative ethanol metabolism in human organs commonly damaged by ethanol abuse. *Science* 1986; **231**: 497-499 [PMID: 3941913]
31 **Banks PA**, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG, Tsiotos GG, Vege SS. Classification of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus. *Gut* 2013; **62**: 102-111 [PMID: 23100216 DOI: 10.1136/gutjnl-2012-302779]
32 **Wang YL**, Hu R, Lugea A, Gukovsky I, Smoot D, Gukovskaya AS, Pandol SJ. Ethanol feeding alters death signaling in the pancreas. *Pancreas* 2006; **32**: 351-359 [PMID: 16670617 DOI: 10.1097/01.mpa.0000220859.93496.e1]
33 **Odinokova IV**, Sung KF, Mareninova OA, Hermann K, Evtodienko Y, Andreyev A, Gukovsky I, Gukovskaya AS. Mechanisms regulating cytochrome c release in pancreatic mitochondria. *Gut* 2009; **58**: 431-442 [PMID: 18596195 DOI: 10.1136/gut.2007.147207]
34 **Petersen OH**, Gerasimenko OV, Gerasimenko JV. Pathobiology of acute pancreatitis: focus on intracellular calcium and calmodulin. *F1000 Med Rep* 2011; **3**: 15 [PMID: 21876721 DOI: 10.3410/M3-15]
35 **Gorelick FS**. Alcohol and zymogen activation in the pancreatic acinar cell. *Pancreas* 2003; **27**: 305-310 [PMID: 14576492 DOI: 10.1097/00006676-200311000-00006]
36 **Petersen OH**, Tepikin AV. Polarized calcium signaling in exocrine gland cells. *Annu Rev Physiol* 2008; **70**: 273-299 [PMID: 17850212 DOI: 10.1146/annurev.physiol.70.113006.100618]
37 **Gerasimenko JV**, Lur G, Sherwood MW, Ebisui E, Tepikin AV, Mikoshiba K, Gerasimenko OV, Petersen OH. Pancreatic protease activation by alcohol metabolite depends on Ca2+ release via acid store IP3 receptors. *Proc Natl Acad Sci USA* 2009; **106**: 10758-10763 [PMID: 19528657 DOI: 10.1073/pnas.0904818106]
38 **Gaisano HY**, Lutz MP, Leser J, Sheu L, Lynch G, Tang L, Tamori Y, Trimble WS, Salapatek AM. Supramaximal cholecystokinin displaces Munc18c from the pancreatic acinar basal surface, redirecting apical exocytosis to the basal membrane. *J Clin Invest* 2001; **108**: 1597-1611 [PMID: 11733555 DOI: 10.1172/JCI9110]
39 **Cosen-Binker LI**, Lam PP, Binker MG, Reeve J, Pandol S, Gaisano HY. Alcohol/cholecystokinin-evoked pancreatic acinar basolateral exocytosis is mediated by protein kinase C alpha phosphorylation of Munc18c. *J Biol Chem* 2007; **282**: 13047-13058 [PMID: 17324928 DOI: 10.1074/jbc.M611132200]
40 **Cosen-Binker LI**, Binker MG, Wang CC, Hong W, Gaisano HY. VAMP8 is the v-SNARE that mediates basolateral exocytosis in a mouse model of alcoholic pancreatitis. *J Clin Invest* 2008; **118**: 2535-2551 [PMID: 18535671 DOI: 10.1172/JCI34672]

41 **Gaisano HY**, Sheu L, Whitcomb D. Alcoholic chronic pancreatitis involves displacement of Munc18c from the pancreatic acinar basal membrane surface. *Pancreas* 2004; **28**: 395-400 [PMID: 15097857]

42 **Kroemer G**, Mariño G, Levine B. Autophagy and the integrated stress response. *Mol Cell* 2010; **40**: 280-293 [PMID: 20965422 DOI: 10.1016/j.cell.2007.12.018]

43 **Fortunato F**, Bürgers H, Bergmann F, Rieger P, Büchler MW, Kroemer G, Werner J. Impaired autolysosome formation correlates with Lamp-2 depletion: role of apoptosis, autophagy, and necrosis in pancreatitis. *Gastroenterology* 2009; **137**: 350-360, 360.e1-5 [PMID: 19362087 DOI: 10.1053/j.gastro.2009.04.003]

44 **Hashimoto D**, Ohmuraya M, Hirota M, Yamamoto A, Suyama K, Ida S, Okumura Y, Takahashi E, Kido H, Araki K, Baba H, Mizushima N, Yamamura K. Involvement of autophagy in trypsinogen activation within the pancreatic acinar cells. *J Cell Biol* 2008; **181**: 1065-1072 [PMID: 18591426 DOI: 10.1083/jcb.200712156]

45 **Mareninova OA**, Hermann K, French SW, O'Konski MS, Pandol SJ, Webster P, Erickson AH, Katunuma N, Gorelick FS, Gukovsky I, Gukovskaya AS. Impaired autophagic flux mediates acinar cell vacuole formation and trypsinogen activation in rodent models of acute pancreatitis. *J Clin Invest* 2009; **119**: 3340-3355 [PMID: 19805911 DOI: 10.1172/JCI38674]

46 **Ding WX**, Li M, Chen X, Ni HM, Lin CW, Gao W, Lu B, Stolz DB, Clemens DL, Yin XM. Autophagy reduces acute ethanol-induced hepatotoxicity and steatosis in mice. *Gastroenterology* 2010; **139**: 1740-1752 [PMID: 20659474 DOI: 10.1053/j.gastro.2010.07.041]

47 **Thomes PG**, Ehlers RA, Trambly CS, Clemens DL, Fox HS, Tuma DJ, Donohue TM. Multilevel regulation of autophagosome content by ethanol oxidation in HepG2 cells. *Autophagy* 2013; **9**: 63-73 [PMID: 23090141 DOI: 10.4161/auto.22490]

48 **Helin H**, Mero M, Markkula H, Helin M. Pancreatic acinar ultrastructure in human acute pancreatitis. *Virchows Arch A Pathol Anat Histol* 1980; **387**: 259-270 [PMID: 7456314]

49 **Mareninova OA**, Yakubov I, French SW, O'Konski MS, Pandol SJ, Webster P, Erickson AH, Katunuma N, Gorelick FS, Gukovsky I, Gukovskaya AS. Ethanol feeding causes lysosomal dysfunction in exocrine pancreas similar to pancreatitis; but in contrast to pancreatitis, ethanol down-regulates autophagy. *Gastroenterol* 2010; **138**: S-148

50 **Huynh KK**, Eskelinen EL, Scott CC, Malevanets A, Saftig P, Grinstein S. LAMP proteins are required for fusion of lysosomes with phagosomes. *EMBO J* 2007; **26**: 313-324 [PMID: 17245426 DOI: 10.1038/sj.emboj.7601511]

51 **Case RM**. Synthesis, intracellular transport and discharge of exportable proteins in the pancreatic acinar cell and other cells. *Biol Rev Camb Philos Soc* 1978; **53**: 211-354 [PMID: 208670]

52 **Sah RP**, Dawra RK, Saluja AK. New insights into the pathogenesis of pancreatitis. *Curr Opin Gastroenterol* 2013; **29**: 523-530 [PMID: 23892538 DOI: 10.1097/MOG.0b013e328363e399]

53 **Mukherjee R**, Criddle DN, Gukovskaya A, Pandol S, Petersen OH, Sutton R. Mitochondrial injury in pancreatitis. *Cell Calcium* 2008; **44**: 14-23 [PMID: 18207570 DOI: 10.1016/j.ceca.2007.11.013]

54 **Criddle DN**, Murphy J, Fistetto G, Barrow S, Tepikin AV, Neoptolemos JP, Sutton R, Petersen OH. Fatty acid ethyl esters cause pancreatic calcium toxicity via inositol trisphosphate receptors and loss of ATP synthesis. *Gastroenterology* 2006; **130**: 781-793 [PMID: 16530519 DOI: 10.1053/j.gastro.2005.12.031]

55 **Criddle DN**, Raraty MG, Neoptolemos JP, Tepikin AV, Petersen OH, Sutton R. Ethanol toxicity in pancreatic acinar cells: mediation by nonoxidative fatty acid metabolites. *Proc Natl Acad Sci USA* 2004; **101**: 10738-10743 [PMID: 15247419 DOI: 10.1073/pnas.0403431101]

56 **Kroemer G**, Galluzzi L, Brenner C. Mitochondrial membrane permeabilization in cell death. *Physiol Rev* 2007; **87**: 99-163 [PMID: 17237344 DOI: 10.1152/physrev.00013.2006]

57 **Rasola A**, Bernardi P. Mitochondrial permeability transition in Ca(2+)-dependent apoptosis and necrosis. *Cell Calcium* 2011; **50**: 222-233 [PMID: 21601280 DOI: 10.1016/j.ceca.2011.04.007]

58 **Ehrig T**, Bosron WF, Li TK. Alcohol and aldehyde dehydrogenase. *Alcohol Alcohol* 1990; **25**: 105-116 [PMID: 2198030]

59 **Crabb DW**, Bosron WF, Li TK. Ethanol metabolism. *Pharmacol Ther* 1987; **34**: 59-73 [PMID: 3310044]

60 **Shalbueva N**, Mareninova OA, Gerloff A, Yuan J, Waldron RT, Pandol SJ, Gukovskaya AS. Effects of oxidative alcohol metabolism on the mitochondrial permeability transition pore and necrosis in a mouse model of alcoholic pancreatitis. *Gastroenterology* 2013; **144**: 437-446.e6 [PMID: 23103769 DOI: 10.1053/j.gastro.2012.10.037]

61 **Shulga N**, Pastorino JG. Ethanol sensitizes mitochondria to the permeability transition by inhibiting deacetylation of cyclophilin-D mediated by sirtuin-3. *J Cell Sci* 2010; **123**: 4117-4127 [PMID: 21062897 DOI: 10.1242/jcs.073502]

62 **Shulga N**, Wilson-Smith R, Pastorino JG. Sirtuin-3 deacetylation of cyclophilin D induces dissociation of hexokinase II from the mitochondria. *J Cell Sci* 2010; **123**: 894-902 [PMID: 20159966 DOI: 10.1242/jcs.061846]

63 **Kim I**, Xu W, Reed JC. Cell death and endoplasmic reticulum stress: disease relevance and therapeutic opportunities. *Nat Rev Drug Discov* 2008; **7**: 1013-1030 [PMID: 19043451 DOI: 10.1038/nrd2755]

64 **Kubisch CH**, Sans MD, Arumugam T, Ernst SA, Williams JA, Logsdon CD. Early activation of endoplasmic reticulum stress is associated with arginine-induced acute pancreatitis. *Am J Physiol Gastrointest Liver Physiol* 2006; **291**: G238-G245 [PMID: 16574987 DOI: 10.1152/ajpgi.00471.2005]

65 **Lugea A**, Tischler D, Nguyen J, Gong J, Gukovsky I, French SW, Gorelick FS, Pandol SJ. Adaptive unfolded protein response attenuates alcohol-induced pancreatic damage. *Gastroenterology* 2011; **140**: 987-997 [PMID: 21111739 DOI: 10.1053/j.gastro.2010.11.038]

66 **Apte MV**, Haber PS, Applegate TL, Norton ID, McCaughan GW, Korsten MA, Pirola RC, Wilson JS. Periacinar stellate shaped cells in rat pancreas: identification, isolation, and culture. *Gut* 1998; **43**: 128-133 [PMID: 9771417]

67 **Bachem MG**, Schneider E, Gross H, Weidenbach H, Schmid RM, Menke A, Siech M, Beger H, Grünert A, Adler G. Identification, culture, and characterization of pancreatic stellate cells in rats and humans. *Gastroenterology* 1998; **115**: 421-432 [PMID: 9679048]

68 **Apte M**, Pirola R, Wilson J. The fibrosis of chronic pancreatitis: new insights into the role of pancreatic stellate cells. *Antioxid Redox Signal* 2011; **15**: 2711-2722 [PMID: 21728885]

69 **Apte MV**, Pirola RC, Wilson JS. Pancreatic stellate cells: a starring role in normal and diseased pancreas. *Front Physiol* 2012; **3**: 344 [PMID: 22973234 DOI: 10.1089/ars.2011.4079]

70 **Apte MV**, Phillips PA, Fahmy RG, Darby SJ, Rodgers SC, McCaughan GW, Korsten MA, Pirola RC, Naidoo D, Wilson JS. Does alcohol directly stimulate pancreatic fibrogenesis? Studies with rat pancreatic stellate cells. *Gastroenterology* 2000; **118**: 780-794 [PMID: 10734030]

71 **Chiang CP**, Wu CW, Lee SP, Ho JL, Lee SL, Nieh S, Yin SJ. Expression pattern, ethanol-metabolizing activities, and cellular localization of alcohol and aldehyde dehydrogenases in human small intestine. *Alcohol Clin Exp Res* 2012; **36**: 2047-2058 [PMID: 23231010 DOI: 10.1111/j.1530-0277.2009.00927.x]

72 **Masamune A**, Satoh A, Watanabe T, Kikuta K, Satoh M, Suzuki N, Satoh K, Shimosegawa T. Effects of ethanol and its metabolites on human pancreatic stellate cells. *Dig Dis Sci* 2010; **55**: 204-211 [PMID: 19165599 DOI: 10.1007/s10620-008-0695-y]

73 **Phillips PA**, McCarroll JA, Park S, Wu MJ, Pirola R, Korsten

M, Wilson JS, Apte MV. Rat pancreatic stellate cells secrete matrix metalloproteinases: implications for extracellular matrix turnover. *Gut* 2003; **52**: 275-282 [PMID: 12524413]
74 **Lawrencia C**, Charrier A, Huang G, Brigstock DR. Ethanol-mediated expression of connective tissue growth factor (CCN2) in mouse pancreatic stellate cells. *Growth Factors* 2009; **27**: 91-99 [PMID: 19280452 DOI: 10.1080/08977190902786319]
75 **Lugea A**, Gukovsky I, Gukovskaya AS, Pandol SJ. Nonoxidative ethanol metabolites alter extracellular matrix protein content in rat pancreas. *Gastroenterology* 2003; **125**: 1845-1859 [PMID: 14724836]
76 **Bhatia M**, Brady M, Shokuhi S, Christmas S, Neoptolemos JP, Slavin J. Inflammatory mediators in acute pancreatitis. *J Pathol* 2000; **190**: 117-125 [PMID: 10657008 DOI: 10.1002/(SICI)1096-9896(200002)190:2<117::AID-PATH494>3.0.CO;2-K]
77 **Norman J**. The role of cytokines in the pathogenesis of acute pancreatitis. *Am J Surg* 1998; **175**: 76-83 [PMID: 9445247]
78 **Gukovskaya AS**, Mouria M, Gukovsky I, Reyes CN, Kasho VN, Faller LD, Pandol SJ. Ethanol metabolism and transcription factor activation in pancreatic acinar cells in rats. *Gastroenterology* 2002; **122**: 106-118 [PMID: 11781286]
79 **Pandol SJ**, Periskic S, Gukovsky I, Zaninovic V, Jung Y, Zong Y, Solomon TE, Gukovskaya AS, Tsukamoto H. Ethanol diet increases the sensitivity of rats to pancreatitis induced by cholecystokinin octapeptide. *Gastroenterology* 1999; **117**: 706-716 [PMID: 10464148]
80 **Deng X**, Wang L, Elm MS, Gabazadeh D, Diorio GJ, Eagon PK, Whitcomb DC. Chronic alcohol consumption accelerates fibrosis in response to cerulein-induced pancreatitis in rats. *Am J Pathol* 2005; **166**: 93-106 [PMID: 15632003]
81 **Clemens DL**, Jerrells TR. Ethanol consumption potentiates viral pancreatitis and may inhibit pancreas regeneration: preliminary findings. *Alcohol* 2004; **33**: 183-189 [PMID: 15596086]
82 **Jensen JN**, Cameron E, Garay MV, Starkey TW, Gianani R, Jensen J. Recapitulation of elements of embryonic development in adult mouse pancreatic regeneration. *Gastroenterology* 2005; **128**: 728-741 [PMID: 15765408]
83 **Ammann RW**, Heitz PU, Klöppel G. Course of alcoholic chronic pancreatitis: a prospective clinicomorphological long-term study. *Gastroenterology* 1996; **111**: 224-231 [PMID: 8698203]
84 **Krejs GJ**. Pancreatic cancer: epidemiology and risk factors. *Dig Dis* 2010; **28**: 355-358 [PMID: 20814212 DOI: 10.1159/000319414]

P- Reviewer: Pan WS, Pezzilli R, Sakata N **S- Editor**: Song XX
L- Editor: A **E- Editor:** Lu YJ

World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4291/wjgp.v5.i3.158

World J Gastrointest Pathophysiol 2014 August 15; 5(3): 158-168
ISSN 2150-5330 (online)

TOPIC HIGHLIGHT

WJGP 5th Anniversary Special Issues (3): Pancreatitis

Early phase of acute pancreatitis: Assessment and management

Veit Phillip, Jörg M Steiner, Hana Algül

Veit Phillip, Jörg M Steiner, Hana Algül, II. Medizinische Klinik und Poliklinik, Klinikum rechts der Isar der Technischen Universität München, 81675 München, Germany
Jörg M Steiner, Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A and M University, College Station, TX 77843-4474, United States
Author contributions: Phillip V and Algül H collected and analyzed the literature; Phillip V prepared the original draft; Steiner JM and Algül H supplemented the material and revised the manuscript; all authors approved the final version of the paper to be published.
Correspondence to: Hana Algül, MD, MPH, II. Medizinische Klinik und Poliklinik, Klinikum rechts der Isar der Technische Universität München, Ismaninger Straße 22, 81675 München, Germany. hana.alguel@lrz.tum.de
Telephone: +49-89-41405215 Fax: +49-89-41406794
Received: January 29, 2014 Revised: March 25, 2014
Accepted: May 29, 2014
Published online: August 15, 2014

Abstract

Acute pancreatitis (AP) is a potentially life-threatening disease with a wide spectrum of severity. The overall mortality of AP is approximately 5%. According to the revised Atlanta classification system, AP can be classified as mild, moderate, or severe. Severe AP often takes a clinical course with two phases, an early and a late phase, which should both be considered separately. In this review article, we first discuss general aspects of AP, including incidence, pathophysiology, etiology, and grading of severity, then focus on the assessment of patients with suspected AP, including diagnosis and risk stratification, followed by the management of AP during the early phase, with special emphasis on fluid therapy, pain management, nutrition, and antibiotic prophylaxis.

Key words: Acute pancreatitis; Incidence; Pathophysiology; Etiology; Severity; Risk stratification; Fluid therapy; Pain management; Nutrition; Antibiotic prophylaxis

Core tip: Acute pancreatitis is a frequent and potentially life-threatening disease. Therapy is currently mostly symptomatic with fluid resuscitation, pain management, and early oral feeding. Vigorous fluid resuscitation remains a cornerstone of early management of acute pancreatitis. Cross-sectional imaging during the early phase of evaluation has not been associated with improvement in outcome. There is no role for prophylactic antibiotics in the management of the early phase of acute pancreatitis (AP). Enteral nutrition in AP can reduce mortality, systemic infections, and multiorgan dysfunction compared to parenteral nutrition. Immediate endoscopic retrograde cholangiography is indicated only in patients with biliary pancreatitis with common bile duct obstruction and cholangitis.

Phillip V, Steiner JM, Algül H. Early phase of acute pancreatitis: Assessment and management. *World J Gastrointest Pathophysiol* 2014; 5(3): 158-168 Available from: URL: http://www.wjgnet.com/2150-5330/full/v5/i3/158.htm DOI: http://dx.doi.org/10.4291/wjgp.v5.i3.158

INTRODUCTION

Acute pancreatitis (AP) is a potentially life-threatening disease with a wide spectrum of severity. The reported incidence of acute pancreatitis differs depending on geographic location and ranges from 14.7/100000 person years in the Netherlands to 45.1/100000 person years in Japan[1,2]. However, most studies show an incidence between 30 and 45/100000 person years[2-7]. Many studies report an increase in incidence over the last few decades[2,3,8], however, it is a matter of debate whether this

represents a real increase in incidence due to increasing biliary AP in an increasingly obese population or whether this rise in incidence is due to improved diagnostic capabilities, a higher level of suspicion of this disease, or an overestimation of retrospective studies using administrative diagnostic codes[9-11]. In 2009, AP was the most common principal gastrointestinal diagnosis at discharge in the Unite States with estimated inpatient costs of $2.6 billion per year. Furthermore, it was the 14th most common cause of death with a crude rate of 1.0 per 100000 inhabitants[12]. The overall mortality of AP is about 5% and can reach up to 20%-30% in patients with severe AP and infected necrosis[13,14]. While there seems to be an increase in incidence, several studies have reported a decrease in mortality. Again, this could be a real decrease due to an earlier diagnosis and better therapeutic options or it may also be due to an improved sensitivity of diagnostic modalities, leading to an increase in the diagnosis of mild forms of pancreatitis[15].

In this review article, we first discuss general aspects of AP, including pathophysiology, etiology, and grading of severity, then focus on the assessment of patients with suspected AP, including diagnosis and risk stratification, followed by the management of AP during the early phase with special emphasis on fluid therapy, pain management, nutrition, and antibiotic prophylaxis.

PATHOPHYSIOLOGY

The pathophysiology of AP with multi organ failure (MOF) is poorly understood. Researchers have long hypothesized that AP results from premature activation of digestive enzymes within the pancreas, a process referred to as autodigestion. Indeed, inherited mutations in genes encoding for digestive enzymes have been found in patients with a hereditary form of pancreatitis[16]. However, affected patients develop chronic, rather than acute pancreatitis. Therefore, in recent years, a novel concept has evolved, suggesting that systemic complications during AP result from uncontrolled activation of the inflammatory cascade. As indicated above, severe AP is associated with a significant mortality. Thus, early identification of severe forms of AP is crucial for outcome. In an attempt to identify surrogate parameters as predictors for severe AP, several association studies linking cytokines and chemokines with AP severity have been conducted[17]. Among these, serum levels of interleukin (IL)-6 and the IL-6-dependent acute phase protein, C-reactive protein (CRP) were identified as the most reliable predictors for severe AP[18,19]. Recent results from basic research have established that IL-6 or CRP are not only relevant markers to predict the severity of AP, but that the cytokine IL-6 also has a substantial pathophysiological impact on the course of the disease[19]. While excessive stimulation of the inflammatory cascade [hyper-inflammatory state, systemic inflammatory response syndrome (SIRS)] accounts for early systemic complications, paralysis of the inflammatory response, also termed compensatory anti-inflammatory response syndrome (CARS), contributes to local complications and sepsis associated with the late phase of the disease. Although these definitions are largely non-specific, they are undeniably useful in the clinical and research setting. Among the agents contributing to this anti-inflammatory response, IL-10 may be of importance. In fact, the protective role of IL-10 in experimental studies in animal models has been well documented[20]. Thus, the hypo-inflammatory status of CARS might facilitate superinfections that lead to extensive necrosis and/or septic complications. This interplay of these two contrasting phenomena requires an individualized therapeutic approach[20-22].

ETIOLOGY

The identification of the etiology of AP is crucial for the management during the early phase of the disease and also for the prevention of recurrence of AP. Although there is no specific therapy for AP, the causing factor, *e.g.*, choledocholithiasis in biliary AP, must be investigated and eliminated if identified. The most common causes of AP are gallstones and prolonged heavy use of alcohol, which together account for about 60%-80% of all cases. The incidence of biliary etiology differs considerably between different geographic regions. For example, there is a clear predominance for biliary AP over alcoholic AP in Greece (71.4% *vs* 6.0%) whereas the opposite is the case for Finland (6.3% *vs* 79.3%)[23,24]. The regional differences in frequency of biliary and alcoholic etiology are shown in Figure 1[6,7,23-31].

Other causes of AP include ERCP (0.4% to 11%[32,33]), idiosyncratic reactions to drugs (0.1% to 2%)[34], hypertriglyceridemia (1.1%-3.8%)[6,23,35], anatomic alterations[36], genetic predispositions[37], and other rare causes[38,39]. Despite a thorough clinical workup, 10%-25% of all cases remain idiopathic[6,11,23,33].

NATURAL COURSE OF ACUTE PANCREATITIS

The severity of AP can be subclinical, mild without organ dysfunction, or can be severe. Patients with mild disease often improve spontaneously and heal within a few days. However, patients with severe disease may develop life-threatening local and/or systemic complications. According to the revised Atlanta classification system, AP can be classified as mild, moderate, or severe[40]. However, it is important to remember that AP is a rapidly evolving, dynamic condition in which the severity may change rapidly during the course of the disease[40]. Severe AP often takes a clinical course with two phases, an early and a late one, which should both be considered separately[40].

The early phase, which usually lasts for about one week, is characterized by a complex inflammatory reaction. The course of AP starts with a systemic pro-inflammatory phase [systemic inflammatory response

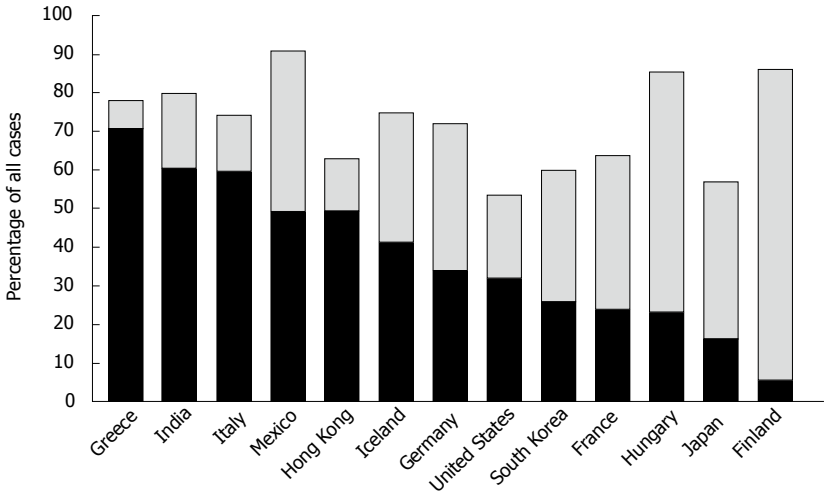

Figure 1 Regional differences in frequency of biliary (black) and alcoholic (gray) etiology of acute pancreatitis.

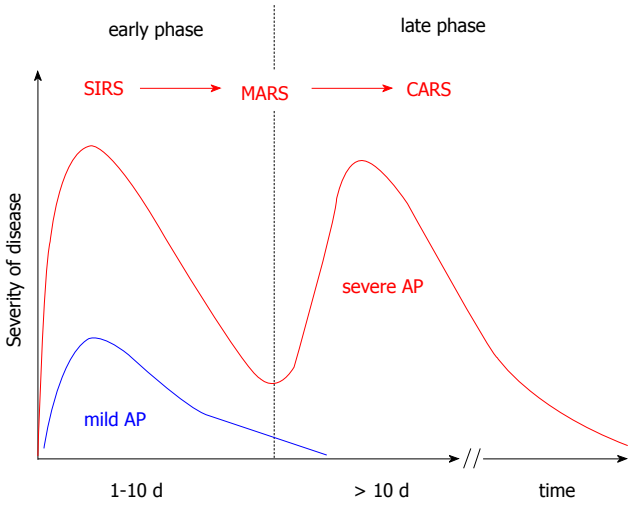

Figure 2 Two-phase course of severe acute pancreatitis. CARS: Compensatory anti-inflammatory response syndrome; MARS: Mixed anti-inflammatory response syndrome; SIRS: Systemic inflammatory response syndrome.

syndrome (SIRS)], followed by a mixed inflammatory response syndrome (mixed antagonist response syndrome, MARS), and finally leads to a phase with a suppressed inflammatory response (compensatory anti-inflammatory response syndrome, CARS)[41-43]. In the phase of CARS, the immune system is downregulated and the chance of an infection of pancreatic and peripancreatic necrotic tissue rises. This is likely the reason why infections usually do not occur earlier than at the end of the first week[44]. During the stage of CARS pathogens can migrate unopposed from the intestinal lumen into necrotic tissue in and around the damaged pancreas. At that point, the clinical course of AP moves towards the second phase, including SIRS, sepsis, local and systemic complications, persistent organ failure, and possibly death. The model of the two-phase course is shown in Figure 2.

Efforts must be made to predict the severity of the disease as early as possible in order to know whether a patient diagnosed with AP can be treated as an outpatient, has to be admitted to a regular ward, to an intermediate care facility, or even to the intensive care unit. While it is generally recognized how important the prediction of severity of the disease is for the management of the individual patient, it is also recognized that such prediction is very difficult. Underestimation of the severity could be harmful for the patient, while overestimation could lead to unnecessary costs and a waste of resources. Therefore, the assessment and prediction of the severity is crucial for the management of the disease. A lot of research has been done over the last few decades trying to identify new tools to accurately predict the severity of pancreatitis, yet no gold standard for such prediction of the course of AP has been identified. An ideal predictor should be fast and easy to obtain, widely available, economical, and associated with a high sensitivity and specificity. Even though there are several clinical scores with a high sensitivity, specificity, positive, and/or negative predictive value, many of them are complicated to asses or can predict severity only after 48 h of admission to the hospital, which effectively means more than 72 h after the onset of disease[45]. This might be too late, as early aggressive fluid resuscitation is a cornerstone of AP therapy.

ASSESSMENT

Diagnosis

The diagnosis of AP can be made if ⩾ 2 of the following three criteria are fulfilled: (1) abdominal pain characteristic of acute pancreatitis; (2) elevation of serum lipase or amylase activity > 3-fold of the upper limit of the reference interval; and (3) characteristic signs of pancreatitis on computed tomography (CT) imaging.

The first step in the diagnosis of AP should be a thorough clinical history. The pain caused by AP is typically dull, located in the epigastrium, may radiate into the back, and is usually severe, leading to hospital admission and often necessitating opioid therapy[45,46]. Furthermore, AP often causes nausea and vomiting. Known cholecystolithiasis and/or colic, alcohol excess within 48 h before the onset of pain, new medications, and the character of the pain should be evaluated. The

Table 1 Prognostic criteria of Ranson

On admission	After 48 h
Age > 55 yr	Hematocrit fall > 10%
White blood cell count > 16000/mL	BUN increase > 1.8 mmol/L
Blood glucose concentration > 11.1 mmol/L	Serum calcium < 2 mmol/L
LDH > 350 IU/L	PaO_2 < 60 mmHg
ASAT > 250 IU/L	Base deficit > 4 mmol/L
	Fluid sequestration > 6 L

ASAT: Aspartate aminotransferase; BUN: Blood urea nitrogen; LDH: Lactate dehydrogenase; PaO_2: Partial pressure of arterial oxygen.

second step of pancreatitis diagnosis is based on clinical chemistry. The measurement of serum lipase activity is generally thought to be more sensitive and specific than that of serum amylase activity and there is no additional value in simultaneous measurement of serum lipase and amylase activities[14,47]. Also, the degree of the elevation of serum pancreatic enzyme activities does not correlate with the severity of the disease, although, some studies would suggest such a correlation between serum enzyme activity and severity[6,45]. Only in patients with characteristic epigastric pain, but serum enzyme activities below 3-fold of the upper limit of the reference interval, a CT scan should be considered to rule out other differential diagnoses or to confirm AP. Apart from that, a CT in the early phase of AP is not recommended by current practice guidelines[14,48,49].

Risk stratification

Risk factors: Obesity favors the development of local and systemic complications in patients with AP[50]. Since assessment for obesity is simple and free it should be assessed in every patient. The same applies for age, as patients 55 years or older are at increased risk for severe disease[14].

Scoring systems: Several single parameters and more or less complex scoring systems for the prediction of the severity of AP have been developed and clinically evaluated and all of them have been shown to be associated with advantages and disadvantages. The HAPScore (harmless acute pancreatitis score) was developed to identify patients with mild AP who can be treated as outpatients. Patients without rebound tenderness and/or guarding, a normal hematocrit, and a normal serum creatinine concentration have a high probability (positive predictive value: 98%-98.7%) to have a harmless course of the disease[51,52].

One of the oldest and probably best known and heavily used scores to predict a severe course of pancreatitis was developed in the early 70ties by John Ranson and colleagues[53]. The Ranson score is based on the presence or absence of simple parameters and is assessed differently at the time of admission (5 parameters; possible scores: 0-5) or 48 h later (6 parameters; possible scores: 0-6; Table 1).

Table 2 Bedside index of severity in acute pancreatitis score and observed mortality by bedside index of severity in acute pancreatitis score score

BUN > 8.9 mmol/L (Age > 60 yr)	
Impaired mental status (Glasgow coma scale < 15)	
SIRS, defined by the presence of two or more	
Temperature	< 36 ℃ or > 38 ℃ (< 96.8 °F or > 100.4 °F)
Heart rate	> 90 per minute
Respiratory rate	> 20 per minute or $PaCO_2$ < 32 mmHg
White blood cell count	< 4000/mL or > 12000/mL or > 10% immature neutrophils
BISAP score	Mortality (%)
0	0.1-0.2
1	0.5-0.7
2	1.9-2.1
3	5.3-8.3
4	12.7-19.3
5	22.5-26.7

BUN: Blood urea nitrogen; $PaCO_2$: Partial pressure of arterial carbon dioxide; SIRS: Systemic inflammatory response syndrome.

Although a score ⩾ 3 has a high sensitivity and specificity regarding a severe course of pancreatitis (83.9% and 78.0%, respectively) and a negative predictive value of 94.5%, the severity can be predicted no earlier than 48 h after admission[25,54]. A modification of the Ranson score by Clemens Imrie and colleagues (Imrie score or Glasgow score) was first reported in 1978 and is still widely used and has a similar accuracy as the Ranson score[25,55].

Currently, the score with the highest sensitivity regarding prediction of a severe course is the Acute Physiology And Chronic Health Evaluation (APACHE) Ⅱ score[14,56]. Originally developed to predict mortality in intensive care patients, a value ⩾ 8 of the APACHE Ⅱ score predicts a severe course of AP with a sensitivity of 65%-83%, specificity of 77%-91%, positive predictive value (PPV) of 23%-69%, and negative predictive value (NPV) of 86%-99%[54,57]. However, the determination of an APACHE Ⅱ score in a clinical patient is complex and time-consuming as it utilizes more than 15 parameters, which limits the clinical value of this score.

A score that was developed and validated more recently in almost 18000 patients, is the BISAP (Bedside Index of Severity in Acute Pancreatitis) score[58]. The main advantage of the BISAP score is its simplicity. One point each is given for blood urea nitrogen (BUN) > 8.9 mmol/L, impaired mental status (Glasgow Coma Scale < 15), presence of SIRS, age > 60 years, and pleural effusion (Table 2). A score ⩾ 3 is predictive for a severe course (observed mortality of > 5%; Table 2) with a sensitivity of 83% and a PPV of 76.9%[58-60]. One disadvantage of the BISAP score is, that this score cannot easily distinguish patients with transient and persistent organ failure and therefore may overestimate severity and preclude differentiation between moderate and severe AP.

In summary, there is currently no ideal predictor of

Table 3 Balthazar score

Grade A	Normal pancreas
Grade B	Focal or diffuse enlargement of the pancreas
Grade C	Pancreatic changes associated with peripancreatic inflammation
Grade D	Single fluid collection
Grade E	Two or more fluid collections and/or presence of gas within the pancreas or within peripancreatic inflammation

Table 4 Computed tomography severity index

Extent of necrosis	Points
Absence of necrosis	0
< 30% necrosis	2
30%-50% necrosis	4
> 50% necrosis	6
Balthazar score	
A	0
B	1
C	2
D	3
E	4

Maximum score 10 points.

severity of AP. All prognostic factors and scores show a good NPV, but suffer from a low PPV. Thus, the main value of severity assessment is to exclude a large number of patients with a low risk of mortality[57].

In addition to the laboratory/clinical scoring systems described above there are scoring systems based on imaging results to assess and predict the severity of AP. A CT scan for diagnostic purposes and severity assessment has been-and probably still is - standard practice in many centers[61]. The Balthazar score, developed in 1985, categorizes patients with AP into 5 groups (A-E) according to pancreatic and peripancreatic changes diagnosed by CT (Table 3)[62]. In 1990, Balthazar *et al*[63] modified this score, including assessment of the extent of pancreatic necrosis and named this score Computed Tomography Severity Index (CTSI) (Table 4). The CTSI is probably the most frequently used imaging score to assess severity in patients with AP and a score ⩾ 4 has a negative predictive value of 94%-97% and a positive predictive value 53%-69% regarding the clinical severity of disease[61,64].

In addition to the Balthazar score and the CTSI, several other scores, *e.g.*, pancreatic size index (PSI), mesenteric edema and peritoneal fluid (MOP) score, extrapancreatic (EP) score, extrapancreatic inflammation on CT (EPIC) score, modified CTSI (MCTSI), and MR severity index (MRSI) have been developed and evaluated[61,65]. However, none of these imaging scores were shown to be superior to clinical scoring systems. Thus, a CT on admission to predict severity of AP cannot be recommended at the current time[61].

In addition to laboratory/clinical and imaging scoring systems, single parameters have been evaluated to assess and predict severity.

A lot of research has been done evaluating hematocrit as an indicator for hemoconcentration. The first prospective cohort study showed a high NPV for a hematocrit ⩾ 44 % (93% on admission and 97% 24 h later) but a poor PPV (26% and 27%, respectively) regarding organ failure in AP[66]. Similar results were obtained by several other studies focusing on the usefulness of hematocrit to predict a severe course of AP, organ failure, pancreatic necrosis, or death[67,68]. Due to its high negative predictive value, its low cost, and the ease of measurement, the hematocrit has value in predicting a non-severe course of AP.

The disruption of water balance can lead to hypoperfusion and a disturbance of pancreatic microcirculation[69], which in turn correlates with the severity of AP[70,71]. Understanding the water balance and the resulting changes in laboratory tests can help to predict severity and outcome of AP. In addition to hematocrit, other parameters, that mirror intravascular volume depletion, can also be helpful.

Serum creatinine has been identified as a predictor for pancreatic necrosis. Also, more recently, an estimated glomerular filtration rate (GFR) < 90 mL/min per 1.73 m^2 on admission has been shown to predict pancreatic necrosis with a sensitivity, specificity, PPV, and NPV of 78.1%, 71%, 64%, and 83%, respectively[72,73]. While only one study has described GFR as a predictor of severity, BUN has been evaluated for many years and has been shown to be a good predictor for severity in AP in several large studies. A rise in BUN > 1.8 mmol/L after 48 h had already been included in the Ranson score 40 some years ago, is one of the 4 parameters used in the BISAP score, and has also been shown to have a high predictive value as a single parameter[74,75].

Besides parameters focusing on water balance and microcirculation, laboratory parameters suggesting the presence of an inflammatory process have been used as a predictor of severity. The most intensively studied parameter is CRP. In one study, a serum CRP concentration of 150 mg/L or greater predicted severe AP at 36 h after admission with a sensitivity, specificity, PPV, and NPV of 86%, 87%, 75%, and 93%, respectively[76]. However, the prediction of severity was only possible more than 24 h after admission, which, on average, is about 50 h after the onset of pain[45]. Also, several other studies showed a high predictive value of CRP during the course of AP in regards to severity, but a very low predictive value on admission[77,78].

Procalcitonin appears to be a valuable tool to discriminate between sterile and infected necrosis within the first days of AP[79,80]. However, data on the ability to predict the course of AP are not consistent. On one hand, a multicenter study from the United Kingdom found a significant difference of procalcitonin concentrations measured within 48 h of the onset of symptoms in patients with mild and severe AP and showed an accuracy of 94% in predicting death[81]. In a study from Slovakia, the PPV for predicting a fatal outcome reached

75% when a cut-off value of 5 ng/mL was used[82]. A third study evaluating procalcitonin showed an accuracy of 76% and a PPV of 75% for predicting a severe course of pancreatitis[83]. On the other hand, two studies reported that procalcitonin is not useful in predicting the severity of AP upon admission[79,84]. However, the time point for determination of procalcitonin concentrations, the assays used, and the cut-off values applied were different for all studies. Finally, measurement of procalcitonin is not widely available and is expensive.

A blood glucose concentration < 6.9 mmol/L on admission has a high negative predictive value (92%) for pancreatic necrosis and also can serve as a predictor for severity[85,86]. Blood glucose is easy, fast, and inexpensive to determine and widely available and therefore should be included in the risk stratification.

In summary, there is no single marker that can adequately predict the severity of AP, but there are several scoring systems that can be used to assess and predict the severity of AP. However, these scoring systems must be applied at the correct time, the correct place, and in the correct patient. Also, it is important to observe patients carefully and reassess severity frequently as the disease course can change rapidly at any given time.

MANAGEMENT

Patients diagnosed with mild AP (according to the HAPScore) and no other risk factors can be treated as outpatients. In contrast, patients with any of the above-mentioned risk factors should be considered for admission to the hospital for close monitoring and timely reassessment of disease severity. In contrast, patients with a Ranson score ⩾ 3, a BISAP score ⩾ 3, an APACHE-II score ⩾ 8, or patients with apparent organ failure should be transferred to an advanced medical care ward or facility.

Therapy

Fluid therapy: Despite a lot of research, there is no pharmacological treatment of AP[87]. Thus, fluid resuscitation, analgesia, supportive care, and management of the local and systemic complications are the key elements of the management of patients with acute pancreatitis. One of the most important components of therapy of AP is early intravenous fluid resuscitation[88]. In fact, the decrease in mortality observed over the last decade might be due to the prevention of pancreatic necrosis by maintenance of microcirculation due to more aggressive fluid resuscitation[89]. Two studies have shown a decrease in mortality by early and aggressive fluid resuscitation[90,91]. However, data on the amount of fluid needed to prevent necrosis or to improve outcome are contradictory and the volume must be adjusted to the patient's age, weight, and pre-existing renal and/or cardiac conditions[92]. The importance of starting fluid resuscitation as early as possible and in fact already in the emergency room was shown by two retrospective studies[90,91]. However, the optimal type of fluid is still a matter of debate. Studies comparing isotonic saline and lactated Ringer's solution and crystalloid *vs* colloid solutions, respectively, showed no differences between both groups regarding clinical outcome as determined by the frequency of pancreatic necrosis, length of hospital stay, or mortality[93,94]. Also, the optimal therapeutic goal of fluid resuscitation is not yet clear. A goal-directed fluid resuscitation algorithm based on changes in BUN measurements, as a mirror of renal function, showed no improvement in outcome in patients with AP[93]. Nonetheless, blood pressure, respiratory function, urine output, and-where appropriate-intraabdominal pressure should be closely monitored. One study showed a less severe course of post-ERCP pancreatitis when patients were treated according to a fluid resuscitation protocol based on vital signs and hematocrit[95]. While questions on the type of fluid, the optimal rate of administration, and the therapeutic goal to reach remain unanswered[96], the time-point appears to be very important - the earlier, the better[90,91].

Causative therapy: Elimination of any potential risk factor is another important approach to AP therapy. In case of suspected alcohol- or drug-induced AP, the intake of the causing agent must be stopped immediately. In case of biliary AP, the indication to perform an endoscopic retrograde cholangiography (ERC) and removal of stones within the bile duct depends on the degree of obstruction of the common bile duct and the presence of cholangitis. Biliary pancreatitis and cholangitis are clear indications for ERC and should be performed as early as possible[49,97,98]. Immediate ERC is indicated in patients with biliary pancreatitis with common bile duct obstruction and cholangitis, arguable in patients with predicted severe pancreatitis but without cholangitis, and not indicated in predicted mild pancreatitis without cholangitis[49].

After biliary pancreatitis, cholecystectomy is recommended within the same hospital stay for mild pancreatitis or after an interval of 6 wk following an episode of severe pancreatitis[49].

Pain management: Given that most patients with AP suffer from severe pain, adequate analgesia is very important. In mild cases, non-opioid drugs might be satisfying, but in many cases, especially severe AP, parenterally administered narcotic agents are warranted and most patients will require the use of opioids to control the pain[99,100]. In contrast to historical reports, there is no evidence or a recommendation for restrictions on the type of pain medications being used[14].

Nutrition: For many years, resting the pancreas by giving the patient nothing per os was an important part of therapy. Nowadays, there is wide agreement that total oral abstinence from food combined with total parenteral nutrition is not beneficial to patients with severe AP,

but may in fact be detrimental. A recent meta-analysis showed a statistically significant association of early enteral nutrition and reductions in systemic infections, pancreatic infections, length of hospital stay, and mortality[101]. Also, in patients with severe AP, enteral nutrition was significantly superior to total parenteral nutrition regarding mortality, infectious complications, and organ failure[102]. Gut barrier function is compromised in patients with acute pancreatitis, likely leading to bacterial translocation and potentially causing infected necrosis or even sepsis[103,104]. Because enteral feeding stabilizes gut barrier function, thereby reducing bacterial translocation, it is important early during the course of AP[14,105].

Therefore, whenever possible, *i.e.*, when dissipating pain allows the patient to eat and infectious parameters do not continue to rise, oral food intake should be initiated as early as possible[49]. If oral food intake is not possible and the patient needs nutritional support, enteral tube feeding is preferred over total parenteral nutrition. However, the composition of an optimal diet has not yet been evaluated.

Antibiotic prophylaxis: There also has been a change regarding prophylactic antibiotic therapy in patients with AP. While in the 90ties, prophylactic antibiotics where thought to improve the outcome in patients with AP, there is no emerging evidence that prophylactic antibiotics reduce infectious complications or mortality[106-108]. Today, there is no clear evidence that supports antibiotic prophylaxis as a routine treatment in patients with severe AP[109-111]. Prophylactic antibiotics may reduce pancreatic infection in special subgroups of patients, but further well-designed and adequately-powered studies are needed to definitively answer the clinical usefulness of antibiotic prophylaxis in these patients[108]. Therefore, antibiotic prophylaxis is currently not recommended by international guidelines for the treatment of acute pancreatitis[14,49].

CONCLUSION

Acute pancreatitis is a frequent and potentially life-threatening disease. Numerous clinical prognostic scoring systems have been developed, and yet tools to discriminate between mild, moderate, and severe AP early during the course of the disease are not well advanced. Therapy is currently mostly symptomatic with fluid resuscitation, pain management, and early oral feeding. However, most of these therapeutic approaches are not well-defined. Vigorous fluid resuscitation remains a cornerstone of early management of acute pancreatitis. Cross-sectional imaging during the early phase of evaluation has not been associated with improvement in outcome. There is no role for prophylactic antibiotics in the management of the early phase of AP. Enteral nutrition in AP can reduce mortality, systemic infections, and multiorgan dysfunction compared to parenteral nutrition. Immediate ERC is indicated only in patients with biliary pancreatitis with common bile duct obstruction and cholangitis. These developments have contributed to an improved outcome for patients with acute pancreatitis, but further studies are still required to tackle the high mortality in this disease.

REFERENCES

1 **Spanier B**, Bruno MJ, Dijkgraaf MG. Incidence and mortality of acute and chronic pancreatitis in the Netherlands: a nationwide record-linked cohort study for the years 1995-2005. *World J Gastroenterol* 2013; **19**: 3018-3026 [PMID: 23716981 DOI: 10.3748/wjg.v19.i20.3018]

2 **Satoh K**, Shimosegawa T, Masamune A, Hirota M, Kikuta K, Kihara Y, Kuriyama S, Tsuji I, Satoh A, Hamada S. Nationwide epidemiological survey of acute pancreatitis in Japan. *Pancreas* 2011; **40**: 503-507 [PMID: 21499203 DOI: 10.1097/MPA.0b013e318214812b]

3 **Roberts SE**, Akbari A, Thorne K, Atkinson M, Evans PA. The incidence of acute pancreatitis: impact of social deprivation, alcohol consumption, seasonal and demographic factors. *Aliment Pharmacol Ther* 2013; **38**: 539-548 [PMID: 23859492 DOI: 10.1111/apt.12408]

4 **Shen HN**, Lu CL, Li CY. Epidemiology of first-attack acute pancreatitis in Taiwan from 2000 through 2009: a nationwide population-based study. *Pancreas* 2012; **41**: 696-702 [PMID: 22699142 DOI: 10.1097/MPA.0b013e31823db941]

5 **Oskarsson V**, Sadr-Azodi O, Orsini N, Andrén-Sandberg Å, Wolk A. High dietary glycemic load increases the risk of non-gallstone-related acute pancreatitis: a prospective cohort study. *Clin Gastroenterol Hepatol* 2014; **12**: 676-682 [PMID: 24100113 DOI: 10.1016/j.cgh.2013.09.058]

6 **Phillip V**, Huber W, Hagemes F, Lorenz S, Matheis U, Preinfalk S, Schuster T, Lippl F, Saugel B, Schmid RM. Incidence of acute pancreatitis does not increase during Oktoberfest, but is higher than previously described in Germany. *Clin Gastroenterol Hepatol* 2011; **9**: 995-1000.e3 [PMID: 21723238 DOI: 10.1016/j.cgh.2011.06.016]

7 **Frey CF**, Zhou H, Harvey DJ, White RH. The incidence and case-fatality rates of acute biliary, alcoholic, and idiopathic pancreatitis in California, 1994-2001. *Pancreas* 2006; **33**: 336-344 [PMID: 17079936 DOI: 10.1097/01.mpa.0000236727.16370.99]

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

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

10 **Yadav D**, Ng B, Saul M, Kennard ED. Relationship of serum pancreatic enzyme testing trends with the diagnosis of acute pancreatitis. *Pancreas* 2011; **40**: 383-389 [PMID: 21283039 DOI: 10.1097/MPA.0b013e3182062970]

11 **Saligram S**, Lo D, Saul M, Yadav D. Analyses of hospital administrative data that use diagnosis codes overestimate the cases of acute pancreatitis. *Clin Gastroenterol Hepatol* 2012; **10**: 805-811.e1 [PMID: 22504004 DOI: 10.1016/j.cgh.2012.03.025]

12 **Peery AF**, Dellon ES, Lund J, Crockett SD, McGowan CE, Bulsiewicz WJ, Gangarosa LM, Thiny MT, Stizenberg K, Morgan DR, Ringel Y, Kim HP, Dibonaventura MD, Carroll CF, Allen JK, Cook SF, Sandler RS, Kappelman MD, Shaheen NJ. Burden of gastrointestinal disease in the United States: 2012 update. *Gastroenterology* 2012; **143**: 1179-1187.e1-e3 [PMID: 22885331 DOI: 10.1053/j.gastro.2012.08.002]

13 **Pavlidis P**, Crichton S, Lemmich Smith J, Morrison D, At-

kinson S, Wyncoll D, Ostermann M. Improved outcome of severe acute pancreatitis in the intensive care unit. *Crit Care Res Pract* 2013; **2013**: 897107 [PMID: 23662207 DOI: 10.1155/2013/897107]
14 **Banks PA**, Freeman ML. Practice guidelines in acute pancreatitis. *Am J Gastroenterol* 2006; **101**: 2379-2400 [PMID: 17032204 DOI: 10.1111/j.1572-0241.2006.00856.x]
15 **Lowenfels AB**, Maisonneuve P, Sullivan T. The changing character of acute pancreatitis: epidemiology, etiology, and prognosis. *Curr Gastroenterol Rep* 2009; **11**: 97-103 [PMID: 19281696]
16 **Mounzer R**, Whitcomb DC. Genetics of acute and chronic pancreatitis. *Curr Opin Gastroenterol* 2013; **29**: 544-551 [PMID: 23872486 DOI: 10.1097/MOG.0b013e3283639383]
17 **Papachristou GI**. Prediction of severe acute pancreatitis: current knowledge and novel insights. *World J Gastroenterol* 2008; **14**: 6273-6275 [PMID: 19009638]
18 **Leser HG**, Gross V, Scheibenbogen C, Heinisch A, Salm R, Lausen M, Rückauer K, Andreesen R, Farthmann EH, Schölmerich J. Elevation of serum interleukin-6 concentration precedes acute-phase response and reflects severity in acute pancreatitis. *Gastroenterology* 1991; **101**: 782-785 [PMID: 1907253]
19 **Zhang H**, Neuhöfer P, Song L, Rabe B, Lesina M, Kurkowski MU, Treiber M, Wartmann T, Regnér S, Thorlacius H, Saur D, Weirich G, Yoshimura A, Halangk W, Mizgerd JP, Schmid RM, Rose-John S, Algül H. IL-6 trans-signaling promotes pancreatitis-associated lung injury and lethality. *J Clin Invest* 2013; **123**: 1019-1031 [PMID: 23426178 DOI: 10.1172/JCI64931]
20 **Van Laethem JL**, Marchant A, Delvaux A, Goldman M, Robberecht P, Velu T, Devière J. Interleukin 10 prevents necrosis in murine experimental acute pancreatitis. *Gastroenterology* 1995; **108**: 1917-1922 [PMID: 7539389]
21 **Lesina M**, Wörmann SM, Neuhöfer P, Song L, Algül H. Interleukin-6 in inflammatory and malignant diseases of the pancreas. *Semin Immunol* 2014; **26**: 80-87 [PMID: 24572992 DOI: 10.1016/j.smim.2014.01.002]
22 **Hoque R**, Malik AF, Gorelick F, Mehal WZ. Sterile inflammatory response in acute pancreatitis. *Pancreas* 2012; **41**: 353-357 [PMID: 22415665 DOI: 10.1097/MPA.0b013e3182321500]
23 **Gullo L**, Migliori M, Oláh A, Farkas G, Levy P, Arvanitakis C, Lankisch P, Beger H. Acute pancreatitis in five European countries: etiology and mortality. *Pancreas* 2002; **24**: 223-227 [PMID: 11893928]
24 **Halonen KI**, Leppaniemi AK, Puolakkainen PA, Lundin JE, Kemppainen EA, Hietaranta AJ, Haapiainen RK. Severe acute pancreatitis: prognostic factors in 270 consecutive patients. *Pancreas* 2000; **21**: 266-271 [PMID: 11039471]
25 **Khanna AK**, Meher S, Prakash S, Tiwary SK, Singh U, Srivastava A, Dixit VK. Comparison of Ranson, Glasgow, MOSS, SIRS, BISAP, APACHE-II, CTSI Scores, IL-6, CRP, and Procalcitonin in Predicting Severity, Organ Failure, Pancreatic Necrosis, and Mortality in Acute Pancreatitis. *HPB Surg* 2013; **2013**: 367581 [PMID: 24204087 DOI: 10.1155/2013/367581]
26 **Sánchez-Lozada R**, Acosta-Rosero AV, Chapa-Azuela O, Hurtado-López LM. [Etiology on determining the severity of acute pancreatitis]. *Gac Med Mex* 2003; **139**: 27-31 [PMID: 12666407]
27 **Sánchez-Lozada R**, Camacho-Hernández MI, Vega-Chavaje RG, Garza-Flores JH, Campos-Castillo C, Gutiérrez-Vega R. [Acute pancreatitis: five year experience at the Hospital General de Mexico]. *Gac Med Mex* 2005; **141**: 123-127 [PMID: 15892460]
28 **Fan ST**, Choi TK, Lai CS, Wong J. Influence of age on the mortality from acute pancreatitis. *Br J Surg* 1988; **75**: 463-466 [PMID: 3390679]
29 **Birgisson H**, Möller PH, Birgisson S, Thoroddsen A, Asgeirsson KS, Sigurjónsson SV, Magnússon J. Acute pancreatitis: a prospective study of its incidence, aetiology, severity, and mortality in Iceland. *Eur J Surg* 2002; **168**: 278-282 [PMID: 12375609 DOI: 10.1002/ejs.46]
30 **Otsuki M**, Matsuno S, Shimosegawa T, Williams JA, Go VL. International symposium: mechanism of pancreatitis--between bedside and laboratory. *Pancreas* 2002; **24**: 391-407 [PMID: 11961493]
31 **Takeyama Y**. Long-term prognosis of acute pancreatitis in Japan. *Clin Gastroenterol Hepatol* 2009; **7**: S15-S17 [PMID: 19896091 DOI: 10.1016/j.cgh.2009.08.022]
32 **Arata S**, Takada T, Hirata K, Yoshida M, Mayumi T, Hirota M, Yokoe M, Hirota M, Kiriyama S, Sekimoto M, Amano H, Wada K, Kimura Y, Gabata T, Takeda K, Kataoka K, Ito T, Tanaka M. Post-ERCP pancreatitis. *J Hepatobiliary Pancreat Sci* 2010; **17**: 70-78 [PMID: 20012323 DOI: 10.1007/s00534-009-0220-5]
33 **Easler J**, Muddana V, Furlan A, Dasyam A, Vipperla K, Slivka A, Whitcomb DC, Papachristou GI, Yadav D. Portosplenomesenteric venous thrombosis in patients with acute pancreatitis is associated with pancreatic necrosis and usually has a benign course. *Clin Gastroenterol Hepatol* 2014; **12**: 854-862 [PMID: 24161350 DOI: 10.1016/j.cgh.2013.09.068]
34 **Nitsche CJ**, Jamieson N, Lerch MM, Mayerle JV. Drug induced pancreatitis. *Best Pract Res Clin Gastroenterol* 2010; **24**: 143-155 [PMID: 20227028 DOI: 10.1016/j.bpg.2010.02.002]
35 **Fortson MR**, Freedman SN, Webster PD. Clinical assessment of hyperlipidemic pancreatitis. *Am J Gastroenterol* 1995; **90**: 2134-2139 [PMID: 8540502]
36 **Wang DB**, Yu J, Fulcher AS, Turner MA. Pancreatitis in patients with pancreas divisum: imaging features at MRI and MRCP. *World J Gastroenterol* 2013; **19**: 4907-4916 [PMID: 23946595 DOI: 10.3748/wjg.v19.i30.4907]
37 **Whitcomb DC**. Genetic risk factors for pancreatic disorders. *Gastroenterology* 2013; **144**: 1292-1302 [PMID: 23622139 DOI: 10.1053/j.gastro.2013.01.069]
38 **DiMagno MJ**, DiMagno EP. New advances in acute pancreatitis. *Curr Opin Gastroenterol* 2007; **23**: 494-501 [PMID: 17762554 DOI: 10.1097/MOG.0b013e3282ba566d]
39 **Löhr JM**, Dinter D, Diehl SJ, Haas SL, Veeser M, Pfützer R, Retter J, Schönberg SO, Düber C, Keim V, Schadendorf D, Witt H. Rapid progression of a splenic aneurysm due to segmental arterial mediolysis: a rare cause of acute pancreatitis. *Pancreatology* 2013; **13**: 553-556 [PMID: 24075524 DOI: 10.1016/j.pan.2013.06.001]
40 **Banks PA**, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG, Tsiotos GG, Vege SS. Classification of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus. *Gut* 2013; **62**: 102-111 [PMID: 23100216 DOI: 10.1136/gutjnl-2012-302779]
41 **Gunjaca I**, Zunic J, Gunjaca M, Kovac Z. Circulating cytokine levels in acute pancreatitis-model of SIRS/CARS can help in the clinical assessment of disease severity. *Inflammation* 2012; **35**: 758-763 [PMID: 21826480 DOI: 10.1007/s10753-011-9371-z]
42 **Isenmann R**, Rau B, Beger HG. Early severe acute pancreatitis: characteristics of a new subgroup. *Pancreas* 2001; **22**: 274-278 [PMID: 11291929]
43 **Cobb JP**, O'Keefe GE. Injury research in the genomic era. *Lancet* 2004; **363**: 2076-2083 [PMID: 15207961 DOI: 10.1016/S0140-6736(04)16460-X]
44 **Besselink MG**, van Santvoort HC, Boermeester MA, Nieuwenhuijs VB, van Goor H, Dejong CH, Schaapherder AF, Gooszen HG. Timing and impact of infections in acute pancreatitis. *Br J Surg* 2009; **96**: 267-273 [PMID: 19125434 DOI: 10.1002/bjs.6447]
45 **Phillip V**, Schuster T, Hagemes F, Lorenz S, Matheis U, Preinfalk S, Lippl F, Saugel B, Schmid RM, Huber W. Time period from onset of pain to hospital admission and patients' awareness in acute pancreatitis. *Pancreas* 2013; **42**: 647-654 [PMID: 23303202 DOI: 10.1097/MPA.0b013e3182714565]

46 **Swaroop VS**, Chari ST, Clain JE. Severe acute pancreatitis. *JAMA* 2004; **291**: 2865-2868 [PMID: 15199038 DOI: 10.1001/jama.291.23.2865]

47 **Keim V**, Teich N, Fiedler F, Hartig W, Thiele G, Mössner J. A comparison of lipase and amylase in the diagnosis of acute pancreatitis in patients with abdominal pain. *Pancreas* 1998; **16**: 45-49 [PMID: 9436862]

48 **American Gastroenterological Association (AGA) Institute on "Management of Acute Pancreatits" Clinical Practice and Economics Committee; AGA Institute Governing Board.** AGA Institute medical position statement on acute pancreatitis. *Gastroenterology* 2007; **132**: 2019-2021 [PMID: 17484893]

49 **Working Group IAP/APA Acute Pancreatitis Guidelines.** IAP/APA evidence-based guidelines for the management of acute pancreatitis. *Pancreatology* 2013; **13**: e1-15 [PMID: 24054878 DOI: 10.1016/j.pan.2013.07.063]

50 **Martínez J**, Sánchez-Payá J, Palazón JM, Suazo-Barahona J, Robles-Díaz G, Pérez-Mateo M. Is obesity a risk factor in acute pancreatitis? A meta-analysis. *Pancreatology* 2004; **4**: 42-48 [PMID: 14988657]

51 **Lankisch PG**, Weber-Dany B, Hebel K, Maisonneuve P, Lowenfels AB. The harmless acute pancreatitis score: a clinical algorithm for rapid initial stratification of nonsevere disease. *Clin Gastroenterol Hepatol* 2009; **7**: 702-705; quiz 607 [PMID: 19245846 DOI: 10.1016/j.cgh.2009.02.020]

52 **Oskarsson V**, Mehrabi M, Orsini N, Hammarqvist F, Segersvärd R, Andrén-Sandberg A, Sadr Azodi O. Validation of the harmless acute pancreatitis score in predicting nonsevere course of acute pancreatitis. *Pancreatology* 2011; **11**: 464-468 [PMID: 21968430 DOI: 10.1159/000331502]

53 **Ranson JH**, Rifkind KM, Roses DF, Fink SD, Eng K, Spencer FC. Prognostic signs and the role of operative management in acute pancreatitis. *Surg Gynecol Obstet* 1974; **139**: 69-81 [PMID: 4834279]

54 **Yeung YP**, Lam BY, Yip AW. APACHE system is better than Ranson system in the prediction of severity of acute pancreatitis. *Hepatobiliary Pancreat Dis Int* 2006; **5**: 294-299 [PMID: 16698595]

55 **Imrie CW**, Benjamin IS, Ferguson JC, McKay AJ, Mackenzie I, O'Neill J, Blumgart LH. A single-centre double-blind trial of Trasylol therapy in primary acute pancreatitis. *Br J Surg* 1978; **65**: 337-341 [PMID: 348250]

56 **Knaus WA**, Zimmerman JE, Wagner DP, Draper EA, Lawrence DE. APACHE-acute physiology and chronic health evaluation: a physiologically based classification system. *Crit Care Med* 1981; **9**: 591-597 [PMID: 7261642]

57 **Gravante G**, Garcea G, Ong SL, Metcalfe MS, Berry DP, Lloyd DM, Dennison AR. Prediction of mortality in acute pancreatitis: a systematic review of the published evidence. *Pancreatology* 2009; **9**: 601-614 [PMID: 19657215 DOI: 10.1159/000212097]

58 **Wu BU**, Johannes RS, Sun X, Tabak Y, Conwell DL, Banks PA. The early prediction of mortality in acute pancreatitis: a large population-based study. *Gut* 2008; **57**: 1698-1703 [PMID: 18519429 DOI: 10.1136/gut.2008.152702]

59 **Cho YS**, Kim HK, Jang EC, Yeom JO, Kim SY, Yu JY, Kim YJ, Do KR, Kim SS, Chae HS. Usefulness of the Bedside Index for severity in acute pancreatitis in the early prediction of severity and mortality in acute pancreatitis. *Pancreas* 2013; **42**: 483-487 [PMID: 23429493 DOI: 10.1097/MPA.0b013e318267c879]

60 **Bone RC**, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, Schein RM, Sibbald WJ. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* 1992; **101**: 1644-1655 [PMID: 1303622]

61 **Bollen TL**, Singh VK, Maurer R, Repas K, van Es HW, Banks PA, Mortele KJ. A comparative evaluation of radiologic and clinical scoring systems in the early prediction of severity in acute pancreatitis. *Am J Gastroenterol* 2012; **107**: 612-619 [PMID: 22186977 DOI: 10.1038/ajg.2011.438]

62 **Balthazar EJ**, Ranson JH, Naidich DP, Megibow AJ, Caccavale R, Cooper MM. Acute pancreatitis: prognostic value of CT. *Radiology* 1985; **156**: 767-772 [PMID: 4023241 DOI: 10.1148/radiology.156.3.4023241]

63 **Balthazar EJ**, Robinson DL, Megibow AJ, Ranson JH. Acute pancreatitis: value of CT in establishing prognosis. *Radiology* 1990; **174**: 331-336 [PMID: 2296641 DOI: 10.1148/radiology.174.2.2296641]

64 **Bollen TL**, Singh VK, Maurer R, Repas K, van Es HW, Banks PA, Mortele KJ. Comparative evaluation of the modified CT severity index and CT severity index in assessing severity of acute pancreatitis. *AJR Am J Roentgenol* 2011; **197**: 386-392 [PMID: 21785084 DOI: 10.2214/AJR.09.4025]

65 **Tang W**, Zhang XM, Xiao B, Zeng NL, Pan HS, Feng ZS, Xu XX. Magnetic resonance imaging versus Acute Physiology And Chronic Healthy Evaluation II score in predicting the severity of acute pancreatitis. *Eur J Radiol* 2011; **80**: 637-642 [PMID: 20843620 DOI: 10.1016/j.ejrad.2010.08.020]

66 **Brown A**, Orav J, Banks PA. Hemoconcentration is an early marker for organ failure and necrotizing pancreatitis. *Pancreas* 2000; **20**: 367-372 [PMID: 10824690]

67 **Lankisch PG**, Mahlke R, Blum T, Bruns A, Bruns D, Maisonneuve P, Lowenfels AB. Hemoconcentration: an early marker of severe and/or necrotizing pancreatitis? A critical appraisal. *Am J Gastroenterol* 2001; **96**: 2081-2085 [PMID: 11467635 DOI: 10.1111/j.1572-0241.2001.03966.x]

68 **Gan SI**, Romagnuolo J. Admission hematocrit: a simple, useful and early predictor of severe pancreatitis. *Dig Dis Sci* 2004; **49**: 1946-1952 [PMID: 15628731]

69 **Knoefel WT**, Kollias N, Warshaw AL, Waldner H, Nishioka NS, Rattner DW. Pancreatic microcirculatory changes in experimental pancreatitis of graded severity in the rat. *Surgery* 1994; **116**: 904-913 [PMID: 7940196]

70 **Bassi D**, Kollias N, Fernandez-del Castillo C, Foitzik T, Warshaw AL, Rattner DW. Impairment of pancreatic microcirculation correlates with the severity of acute experimental pancreatitis. *J Am Coll Surg* 1994; **179**: 257-263 [PMID: 8069418]

71 **Mann O**, Kaifi J, Bloechle C, Schneider CG, Yekebas E, Kluth D, Izbicki JR, Strate T. Therapeutic small-volume resuscitation preserves pancreatic microcirculation in acute experimental pancreatitis of graded severity in rats. *Pancreatology* 2009; **9**: 652-661 [PMID: 19684429 DOI: 10.1159/000212100]

72 **Muddana V**, Whitcomb DC, Khalid A, Slivka A, Papachristou GI. Elevated serum creatinine as a marker of pancreatic necrosis in acute pancreatitis. *Am J Gastroenterol* 2009; **104**: 164-170 [PMID: 19098865 DOI: 10.1038/ajg.2008.66]

73 **Lipinski M**, Rydzewski A, Rydzewska G. Early changes in serum creatinine level and estimated glomerular filtration rate predict pancreatic necrosis and mortality in acute pancreatitis: Creatinine and eGFR in acute pancreatitis. *Pancreatology* 2013; **13**: 207-211 [PMID: 23719589 DOI: 10.1016/j.pan.2013.02.002]

74 **Wu BU**, Johannes RS, Sun X, Conwell DL, Banks PA. Early changes in blood urea nitrogen predict mortality in acute pancreatitis. *Gastroenterology* 2009; **137**: 129-135 [PMID: 19344722 DOI: 10.1053/j.gastro.2009.03.056]

75 **Wu BU**, Bakker OJ, Papachristou GI, Besselink MG, Repas K, van Santvoort HC, Muddana V, Singh VK, Whitcomb DC, Gooszen HG, Banks PA. Blood urea nitrogen in the early assessment of acute pancreatitis: an international validation study. *Arch Intern Med* 2011; **171**: 669-676 [PMID: 21482842 DOI: 10.1001/archinternmed.2011.126]

76 **Pongprasobchai S**, Jianjaroonwong V, Charatcharoenwitthaya P, Komoltri C, Tanwandee T, Leelakusolvong S, Pausawasdi N, Srikureja W, Chainuvati S, Prachayakul V,

Manatsathit S, Kachintorn U. Erythrocyte sedimentation rate and C-reactive protein for the prediction of severity of acute pancreatitis. *Pancreas* 2010; **39**: 1226-1230 [PMID: 20531240 DOI: 10.1097/MPA.0b013e3181deb33e]

77 **Hjalmarsson C**, Stenflo J, Borgström A. Activated protein C-protein C inhibitor complex, activation peptide of carboxypeptidase B and C-reactive protein as predictors of severe acute pancreatitis. *Pancreatology* 2009; **9**: 700-707 [PMID: 19684435 DOI: 10.1159/000215577]

78 **Cardoso FS**, Ricardo LB, Oliveira AM, Canena JM, Horta DV, Papoila AL, Deus JR. C-reactive protein prognostic accuracy in acute pancreatitis: timing of measurement and cutoff points. *Eur J Gastroenterol Hepatol* 2013; **25**: 784-789 [PMID: 23492986 DOI: 10.1097/MEG.0b013e32835fd3f0]

79 **Rau B**, Steinbach G, Gansauge F, Mayer JM, Grünert A, Beger HG. The potential role of procalcitonin and interleukin 8 in the prediction of infected necrosis in acute pancreatitis. *Gut* 1997; **41**: 832-840 [PMID: 9462219]

80 **Rau BM**, Kemppainen EA, Gumbs AA, Büchler MW, Wegscheider K, Bassi C, Puolakkainen PA, Beger HG. Early assessment of pancreatic infections and overall prognosis in severe acute pancreatitis by procalcitonin (PCT): a prospective international multicenter study. *Ann Surg* 2007; **245**: 745-754 [PMID: 17457167]

81 **Ammori BJ**, Becker KL, Kite P, Snider RH, Nylén ES, White JC, Larvin M, McMahon MJ. Calcitonin precursors in the prediction of severity of acute pancreatitis on the day of admission. *Br J Surg* 2003; **90**: 197-204 [PMID: 12555296 DOI: 10.1002/bjs.4036]

82 **Pindak D**, Parrak V, Pechan J, Vavrecka A, Kuzela L, Fuchs D, Irsakova J. The clinical value of the procalcitonin in prediction of severity and outcome in acute pancreatitis. *Hepatogastroenterology* 2003; **50** Suppl 2: ccviii-ccccix [PMID: 15244180]

83 **Kim BG**, Noh MH, Ryu CH, Nam HS, Woo SM, Ryu SH, Jang JS, Lee JH, Choi SR, Park BH. A comparison of the BISAP score and serum procalcitonin for predicting the severity of acute pancreatitis. *Korean J Intern Med* 2013; **28**: 322-329 [PMID: 23682226 DOI: 10.3904/kjim.2013.28.3.322]

84 **Modrau IS**, Floyd AK, Thorlacius-Ussing O. The clinical value of procalcitonin in early assessment of acute pancreatitis. *Am J Gastroenterol* 2005; **100**: 1593-1597 [PMID: 15984987 DOI: 10.1111/j.1572-0241.2005.41456.x]

85 **Lankisch PG**, Blum T, Bruns A, Dröge M, Brinkmann G, Struckmann K, Nauck M, Maisonneuve P, Lowenfels AB. Has blood glucose level measured on admission to hospital in a patient with acute pancreatitis any prognostic value? *Pancreatology* 2001; **1**: 224-229 [PMID: 12120199 DOI: 10.1159/000055815]

86 **Rajaratnam SG**, Martin IG. Admission serum glucose level: an accurate predictor of outcome in gallstone pancreatitis. *Pancreas* 2006; **33**: 27-30 [PMID: 16804409 DOI: 10.1097/01.mpa.0000222315.36490.9b]

87 **Pezzilli R**. Pharmacotherapy for acute pancreatitis. *Expert Opin Pharmacother* 2009; **10**: 2999-3014 [PMID: 19925044 DOI: 10.1517/14656560903382630]

88 **Fisher JM**, Gardner TB. The "golden hours" of management in acute pancreatitis. *Am J Gastroenterol* 2012; **107**: 1146-1150 [PMID: 22858994 DOI: 10.1038/ajg.2012.91]

89 **Wall I**, Badalov N, Baradarian R, Iswara K, Li JJ, Tenner S. Decreased mortality in acute pancreatitis related to early aggressive hydration. *Pancreas* 2011; **40**: 547-550 [PMID: 21499208 DOI: 10.1097/MPA.0b013e318215368d]

90 **Gardner TB**, Vege SS, Chari ST, Petersen BT, Topazian MD, Clain JE, Pearson RK, Levy MJ, Sarr MG. Faster rate of initial fluid resuscitation in severe acute pancreatitis diminishes in-hospital mortality. *Pancreatology* 2009; **9**: 770-776 [PMID: 20110744 DOI: 10.1159/000210022]

91 **Warndorf MG**, Kurtzman JT, Bartel MJ, Cox M, Mackenzie T, Robinson S, Burchard PR, Gordon SR, Gardner TB. Early fluid resuscitation reduces morbidity among patients with acute pancreatitis. *Clin Gastroenterol Hepatol* 2011; **9**: 705-709 [PMID: 21554987 DOI: 10.1016/j.cgh.2011.03.032]

92 **Haydock MD**, Mittal A, Wilms HR, Phillips A, Petrov MS, Windsor JA. Fluid therapy in acute pancreatitis: anybody's guess. *Ann Surg* 2013; **257**: 182-188 [PMID: 23207241 DOI: 10.1097/SLA.0b013e31827773ff]

93 **Wu BU**, Hwang JQ, Gardner TH, Repas K, Delee R, Yu S, Smith B, Banks PA, Conwell DL. Lactated Ringer's solution reduces systemic inflammation compared with saline in patients with acute pancreatitis. *Clin Gastroenterol Hepatol* 2011; **9**: 710-717.e1 [PMID: 21645639 DOI: 10.1016/j.cgh.2011.04.026]

94 **Du XJ**, Hu WM, Xia Q, Huang ZW, Chen GY, Jin XD, Xue P, Lu HM, Ke NW, Zhang ZD, Li QS. Hydroxyethyl starch resuscitation reduces the risk of intra-abdominal hypertension in severe acute pancreatitis. *Pancreas* 2011; **40**: 1220-1225 [PMID: 21775917 DOI: 10.1097/MPA.0b013e3182217f17]

95 **Reddy N**, Wilcox CM, Tamhane A, Eloubeidi MA, Varadarajulu S. Protocol-based medical management of post-ERCP pancreatitis. *J Gastroenterol Hepatol* 2008; **23**: 385-392 [PMID: 18318823 DOI: 10.1111/j.1440-1746.2007.05180.x]

96 **Sarr MG**. Early fluid "resuscitation/therapy" in acute pancreatitis: which fluid? What rate? What parameters to gauge effectiveness? *Ann Surg* 2013; **257**: 189-190 [PMID: 23291660 DOI: 10.1097/SLA.0b013e318280e19e]

97 **Neoptolemos JP**, Carr-Locke DL, London NJ, Bailey IA, James D, Fossard DP. Controlled trial of urgent endoscopic retrograde cholangiopancreatography and endoscopic sphincterotomy versus conservative treatment for acute pancreatitis due to gallstones. *Lancet* 1988; **2**: 979-983 [PMID: 2902491]

98 **Tse F**, Yuan Y. Early routine endoscopic retrograde cholangiopancreatography strategy versus early conservative management strategy in acute gallstone pancreatitis. *Cochrane Database Syst Rev* 2012; **5**: CD009779 [PMID: 22592743 DOI: 10.1002/14651858.CD009779.pub2]

99 **Pezzilli R**, Uomo G, Gabbrielli A, Zerbi A, Frulloni L, De Rai P, Castoldi L, Cavallini G, Di Carlo V. A prospective multicentre survey on the treatment of acute pancreatitis in Italy. *Dig Liver Dis* 2007; **39**: 838-846 [PMID: 17602904 DOI: 10.1016/j.dld.2007.05.014]

100 **Ebbehøj N**, Friis J, Svendsen LB, Bülow S, Madsen P. Indomethacin treatment of acute pancreatitis. A controlled double-blind trial. *Scand J Gastroenterol* 1985; **20**: 798-800 [PMID: 2413519]

101 **Li JY**, Yu T, Chen GC, Yuan YH, Zhong W, Zhao LN, Chen QK. Enteral nutrition within 48 hours of admission improves clinical outcomes of acute pancreatitis by reducing complications: a meta-analysis. *PLoS One* 2013; **8**: e64926 [PMID: 23762266 DOI: 10.1371/journal.pone.0064926]

102 **Yi F**, Ge L, Zhao J, Lei Y, Zhou F, Chen Z, Zhu Y, Xia B. Meta-analysis: total parenteral nutrition versus total enteral nutrition in predicted severe acute pancreatitis. *Intern Med* 2012; **51**: 523-530 [PMID: 22449657]

103 **Ammori BJ**, Becker KL, Kite P, Snider RH, Nylén ES, White JC, Barclay GR, Larvin M, McMahon MJ. Calcitonin precursors: early markers of gut barrier dysfunction in patients with acute pancreatitis. *Pancreas* 2003; **27**: 239-243 [PMID: 14508129]

104 **Rahman SH**, Ammori BJ, Holmfield J, Larvin M, McMahon MJ. Intestinal hypoperfusion contributes to gut barrier failure in severe acute pancreatitis. *J Gastrointest Surg* 2003; **7**: 26-35; discussion 35-6 [PMID: 12559182]

105 **Dervenis C**, Smailis D, Hatzitheoklitos E. Bacterial translocation and its prevention in acute pancreatitis. *J Hepatobiliary Pancreat Surg* 2003; **10**: 415-418 [PMID: 14714160 DOI: 10.1007/s00534-002-0727-5]

106 **Sharma VK**, Howden CW. Prophylactic antibiotic admin-

istration reduces sepsis and mortality in acute necrotizing pancreatitis: a meta-analysis. *Pancreas* 2001; **22**: 28-31 [PMID: 11138967]

107 **Bassi C**, Larvin M, Villatoro E. Antibiotic therapy for prophylaxis against infection of pancreatic necrosis in acute pancreatitis. *Cochrane Database Syst Rev* 2003; **(4)**: CD002941 [PMID: 14583957 DOI: 10.1002/14651858.CD002941]

108 **Villatoro E**, Mulla M, Larvin M. Antibiotic therapy for prophylaxis against infection of pancreatic necrosis in acute pancreatitis. *Cochrane Database Syst Rev* 2010; **(5)**: CD002941 [PMID: 20464721 DOI: 10.1002/14651858.CD002941.pub3]

109 **Wittau M**, Mayer B, Scheele J, Henne-Bruns D, Dellinger EP, Isenmann R. Systematic review and meta-analysis of antibiotic prophylaxis in severe acute pancreatitis. *Scand J Gastroenterol* 2011; **46**: 261-270 [PMID: 21067283 DOI: 10.3109/00365521.2010.531486]

110 **Jiang K**, Huang W, Yang XN, Xia Q. Present and future of prophylactic antibiotics for severe acute pancreatitis. *World J Gastroenterol* 2012; **18**: 279-284 [PMID: 22294832 DOI: 10.3748/wjg.v18.i3.279]

111 **Jafri NS**, Mahid SS, Minor KS, Idstein SR, Hornung CA, Galandiuk S. Meta-analysis: antibiotic prophylaxis to prevent peristomal infection following percutaneous endoscopic gastrostomy. *Aliment Pharmacol Ther* 2007; **25**: 647-656 [PMID: 17311597]

P- Reviewer: Bradley EL, Goral V, Pezzilli R, Sakata N
S- Editor: Ji FF **L- Editor**: A **E- Editor:** Lu YJ

World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4291/wjgp.v5.i3.169

World J Gastrointest Pathophysiol 2014 August 15; 5(3): 169-177
ISSN 2150-5330 (online)

TOPIC HIGHLIGHT

WJGP 5th Anniversary Special Issues (3): Pancreatitis

Potential role of NADPH oxidase in pathogenesis of pancreatitis

Wei-Li Cao, Xiao-Hui Xiang, Kai Chen, Wei Xu, Shi-Hai Xia

Wei-Li Cao, Xiao-Hui Xiang, Kai Chen, Wei Xu, Shi-Hai Xia, Department of Hepatopancreatobiliary and Splenic Medicine, Affiliated Hospital, Logistics University of the Chinese People's Armed Police Forces, Tianjin 300162, China

Author contributions: Cao WL and Xiang XH contributed equally to this work; Xia SH contributed to the conception of this work; Cao WL, Xiang XH, Chen K and Xu W prepared the manuscript; Xia SH revised and approved the manuscript.

Supported by The National Natural Science Foundation of China, No. 81173393; the Natural Science Foundation of Tianjin City, No. 12YFJZJC00800; the Scientific Research Foundation (No. WHM201222, FYM201114) and the Innovation Team Program (No. WHTD201310) from Logistics University of the Chinese People's Armed Police Forces

Correspondence to: Shi-Hai Xia, MD, PhD, Department of Hepatopancreatobiliary and Splenic Medicine, Affiliated Hospital, Logistics University of the Chinese People's Armed Police Forces, 220 Chenglin Road, Hedong District, Tianjin 300162, China. xshhcx@sina.com
Telephone: +86-22-60578765 Fax: +86-22-24370605

Received: December 27, 2013 Revised: June 1, 2014
Accepted: June 14, 2014
Published online: August 15, 2014

Abstract

Studies have demonstrated that reactive oxygen species (ROS) are closely related to inflammatory disorders. Nicotinamide adenine dinucleotide phosphate oxidase (NOX), originally found in phagocytes, is the main source of ROS in nonphagocytic cells. Besides directly producing the detrimental highly reactive ROS to act on biomolecules (lipids, proteins, and nucleic acids), NOX can also activate multiple signal transduction pathways, which regulate cell growth, proliferation, differentiation and apoptosis by producing ROS. Recently, research on pancreatic NOX is no longer limited to inflammatory cells, but extends to the aspect of pancreatic acinar cells and pancreatic stellate cells, which are considered to be potentially associated with pancreatitis. In this review, we summarize the literature on NOX protein structure, activation, function and its role in the pathogenesis of pancreatitis.

Key words: Nicotinamide adenine dinucleotide phosphate oxidase; Reactive oxygen species; Pancreatitis; Pancreatic acinar cells; Pancreatic stellate cells

Core tip: Besides directly producing the detrimental highly reactive reactive oxygen species (ROS) to act on biomolecules, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase can also activate multiple signal transduction pathways, which regulate cell growth, proliferation, differentiation and apoptosis by producing ROS. Recently, research on pancreatic NADPH oxidase is no longer limited to inflammatory cells, but extends to the aspect of pancreatic acinar cells and pancreatic stellate cells, which are considered to be potentially associated with pancreatitis.

Cao WL, Xiang XH, Chen K, Xu W, Xia SH. Potential role of NADPH oxidase in pathogenesis of pancreatitis. *World J Gastrointest Pathophysiol* 2014; 5(3): 169-177 Available from: URL: http://www.wjgnet.com/2150-5330/full/v5/i3/169.htm DOI: http://dx.doi.org/10.4291/wjgp.v5.i3.169

INTRODUCTION

Studies have demonstrated that reactive oxygen species (ROS) are involved in the pathogenesis of pancreatitis[1]. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX), a transmembrane flavoprotein enzyme, uses NADPH as an electron donor to catalyze the univalent reduction of oxygen, resulting in the production of superoxide free radical, which might be a source of oxi-

dants in injured pancreas[1]. NOX is mainly distributed in the phagocytic cell membrane with cytochrome C and *flavin adenine dinucleotide* groups, which can produce ROS, scavenging pathogenic microorganisms such as bacteria[2]. ROS, being generated by NOX, also participate in intracellular signaling processes in the pancreas. Recently, research on NOX is no longer limited to inflammatory cells, but extends to the aspect of pancreatic acinar cells and pancreatic stellate cells (PSCs) in pancreatitis patients[2]. The function of NOX, which is involved in the pathogenesis of inflammation in pancreatic acinar cells and PSCs, has become the hotspot of research. Non-phagocytic NOX derived ROS function as a messenger molecule to participate in the modulation of cell differentiation, proliferation and apoptosis in the pancreas. In this review, we summarize the literature on NOX protein structure, activation, function and its role in the pathogenesis of pancreatitis.

STRUCTURE, LOCATION AND FUNCTION OF NOX IN THE PANCREAS

NOX is a multicomponent enzyme consisting of five different subunits, including the subunits $p22^{phox}$ and $gp91^{phox}$ (also known as NOX2) located in the membrane, together with the cytosolic subunits $p40^{phox}$, $p47^{phox}$ and $p67^{phox}$. The participation of Rac would elicit full oxidase activity[3-5]. Relative to $gp91^{phox}$ (the catalytic subunit of NOX), $p22^{phox}$, $p47^{phox}$, $p40^{phox}$ and $p67^{phox}$ are regulatory subunits. $Gp91^{phox}$ in different types of cells has other six homologues, termed NOX1, NOX3, NOX4, NOX5, DUOX1 and DUOX2, which constitute the NOX family proteins[6-8]. NOX is an enzyme which was initially discovered in phagocytes[4,5]. NOX in neutrophils is composed of constitutive subunits ($p22^{phox}$ and $gp91^{phox}$) positioned in membrane and regulatory subunits ($p47^{phox}$ and $p67^{phox}$, and possibly $p40^{phox}$) stationed in the cytosol[9]. In recent years, NOX has been discovered in several nonphagocytic cells such as fibroblasts[10], vascular smooth muscle cells[11] and hepatic stellate cells[12]. More recently, it has been found that NOX was present in pancreatic β cells[13,14], pancreatic acinar cells[15-18] and PSCs[19,20]. The main intrinsic components of NOX comprising the NOX2 isoform are present in human pancreatic islets[14]. Cytosolic subunits $p47^{phox}$ and $p67^{phox}$ as well as membrane-bound subunits $p22^{phox}$ and NOX1 are constitutively expressed in pancreatic acinar AR42J cells[16,21,22]. The key subunits of NOX including $p22^{phox}$, $p47^{phox}$, NOX activator 1 (a homologue of $p67^{phox}$), NOX1, NOX4, and NOX2 ($gp91^{phox}$) are expressed in PSCs[19,20]. The activation of non-phagocytic NOX is similar to that in neutrophils[23]. Upon activation of NOX, p47 translocates to the membrane and then recruits p67 to interact with the p22 subunit, thus facilitating NADPH-dependent formation of superoxide (O^{2-}), which increases the production of secondary ROS such as hydrogen peroxide (H_2O_2)[21]. Non-phagocytic NOX derived ROS function as a messenger molecule to participate in the modulation of cell differentiation, proliferation and apoptosis[6-8]. NOX protein family can be activated quickly under pathophysiological conditions, leading to high production of ROS, which contributes to oxidative stress and a wide range of diseases.

ACTIVATION AND INHIBITION FACTORS OF NOX IN THE PATHOGENESIS OF PANCREATITIS

Cholecystokinin analogues

Cerulein, an analogue of cholecystokinin (CCK), can stimulate the pancreatic exocrine secretion by binding CCK receptors, causing the autolysis of pancreatic acinar cell[24]. There are two kinds of CCK receptor subtypes, CCK_1 and CCK_2 receptors. CCK_1 receptors regulate pancreatic digestive enzymes, satiety and feeding behavior, while CCK_2 receptors enhance the level of gastric acid, as well as gastrin which has anti-apoptotic effects on pancreatic cells[25]. Experimental pancreatitis induced with high dosages of cerulean, similar to human edematous pancreatitis, is characterized by cytoplasmic vacuolization, formation of edema and acinar cell death as well as elevation in serum levels of digestive enzymes caused by unconventional secretion of digestive enzymes[26]. ROS are involved in the activation of oxidant-sensitive nuclear transcription factor (NF-κB), expression of cytokine, apoptosis and further occurrence of pancreatitis[27]. $P47^{phox}$, $p67^{phox}$, NOX1 and $p22^{phox}$ in pancreatic AR42J cells could produce ROS after cerulein stimulation[21]. Intrapancreatic trypsin is not only activated by high-dose cerulein, but also regulated by neutrophils *via* NADPH oxidase[28]. The mechanism for the activation of NF-κB and expression of cytokines in pancreatic acinar cells stimulated by cerulein may be summarized as the following steps. Cerulein binds to the CCK receptor, a G-protein-coupled receptor, to activate phospholipase C (PLC) and inositol 1,4,5-trisphosphate (IP_3), triggering transient Ca^{2+} release from the endoplasmic reticulum in pancreatic acinar cells. NOX activated by Ca^{2+} produces ROS to activate IκB kinase and then to phosphorylate IκB. Phosphorylated IκB can be ubiquitinated and degraded in a proteasome dependent manner to eliminate the inhibition of NF-κB, a p65/p50 heterodimer in the cytosol. NF-κB then translocates to the nucleus to mediate the expression of cytokines which are involved in the pathogenesis of pancreatitis (Figures 1 and 2)[27].

Renin-angiotensin system

The Renin-angiotensin system (RAS) is generally considered to regulate blood pressure and body fluid homeostasis[29]. The pancreatic RAS activation that is related to the production of ROS might contribute to oxidative stress and tissue injury[30,31]. Angiotensin Ⅱ, an active mediator of RAS, is transformed from angiotensin I by the angiotensin-converting enzyme (ACE)[32]. The effect of angiotensin Ⅱ is regulated by its receptors, including

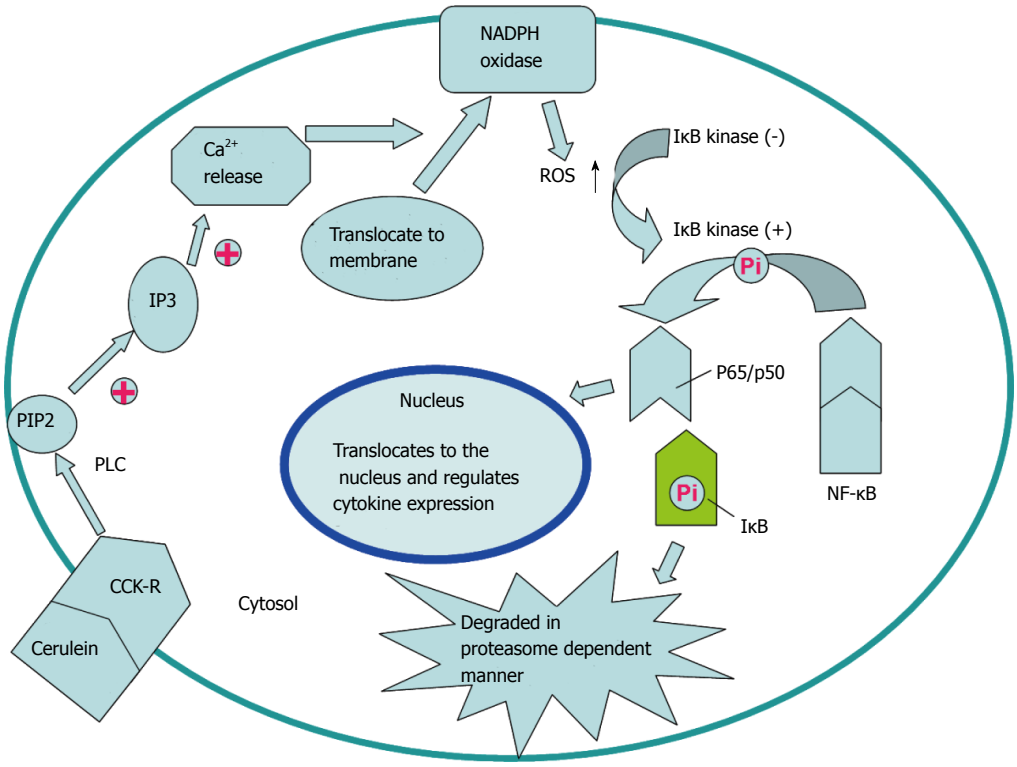

Figure 1 Potential mechanism of nicotinamide adenine dinucleotide phosphate oxidase activation *via* cholecystokinin receptor. Cerulein and cholecystokinin (CCK) receptor binding triggers transient Ca^{2+} release from the endoplasmic reticulum to activate nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which is mediated by PLC and IP3. Reactive oxygen species (ROS) generated by NADPH oxidase activate IκB kinase to phosphorylate IκB in the cytosol. Phosphorylated IκB is ubiquitinated and degraded in a proteasome-dependent manner. NF-κB translocates to the nucleus and regulates expression of cytokines to participate in the pathogenesis of pancreatitis.

angiotensin Ⅱ type 1 receptor (AT_1R) and angiotensin Ⅱ type 2 receptor (AT_2R)[32]. Many reports indicate that interaction of angiotensin Ⅱ with AT_1R promotes superoxide anion production through NOX system[30,31,33,34]. Inhibition of the AT_1R, but not AT_2R, may play a significant role in decreasing the severity of acute pancreatitis. Mechanism of NOX activation by AT_1R and AT_2R might contribute to different effects of AT_1R and AT_2R inhibitors on pancreatic injury induced by cerulein. Activation of pancreatic NOX was associated with oxidative stress which can be indicated by the level of protein oxidation in rats stimulated with cerulein[30,35]. However, further investigations about the potential application of RAS inhibitors including AT_1R in treating acute pancreatitis are needed in the future (Figure 2).

Ethanol and platelet derived growth factor

Alcohol abuse has long been recognized as the most common factor leading to chronic pancreatitis[36]. Activated stellate cells are viewed as vital regulators of chronic alcoholic pancreatitis or fibrosis. Hu *et al*[20] investigated the mechanisms of action of alcohol on PSCs to determine the correlation of NOX system and alcohol with the proliferation of PSCs. The results demonstrated that NOX activity was predominantly located in the cell membrane fraction (95%) compared to the cytosolic fraction (5%) of the stellate cells. platelet derived growth factor (PDGF) could increase NOX activity in a dose- and time-dependent manner. PSC proliferation caused by alcohol is mediated by the activation of PDGF induced NADPH oxidase system. However, ethanol did not show a significant effect on stellate cell DNA synthesis, which provides a new perspective for the mechanism of fibrosis stimulated with alcohol (Figure 2)[20].

Vasoactive intestinal peptide

Previous reports found that vasoactive intestinal peptide (VIP) could decrease the production of cytokines to alleviate experimental acute pancreatitis[37]. VIP could decrease the level of ROS significantly and increase cell viability in acini cells in a dose dependent manner. NOX_1 and NOX_2 markedly increased following treatment with H_2O_2 in pancreatic acini. Besides, H_2O_2 can stimulate the activation of NOX. The production of ROS was affected by VIP *via* NADPH oxidase and the cAMP/PKA pathway because decreased NOX activity by administration of VIP could be abolished by PKA inhibitor H89. Oxidative stress and tissue injury in acini can be decreased by VIP through NOX inhibition (Figure 2)[38].

NOX SIGNAL TRANSDUCTION IN THE PATHOGENESIS OF PANCREATITIS

NOX protein family can be activated quickly under pathophysiological conditions, leading to high produc-

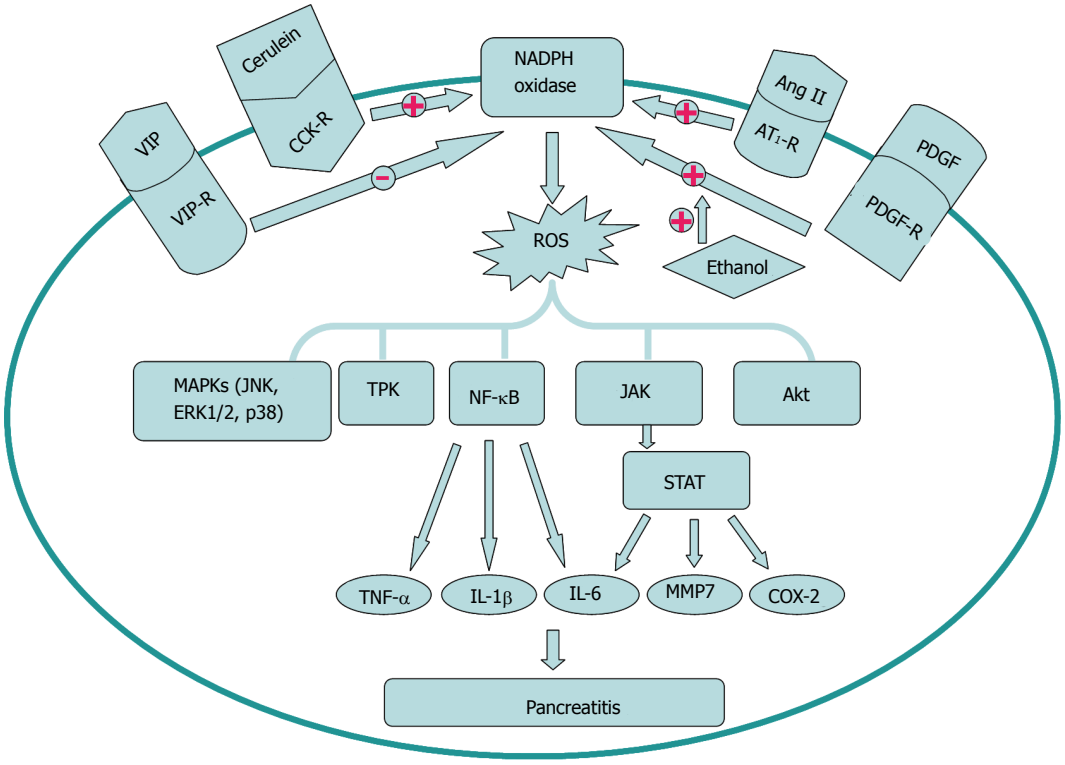

Figure 2 Activation and inhibition factors of nicotinamide adenine dinucleotide phosphate oxidase signal transduction in the pathogenesis of pancreatitis. Cerulein, Ang II and platelet derived growth factor (PDGF) can enhance, while VIP can decrease the activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Ethanol can augment the activation of the cell's NADPH oxidase system stimulated by PDGF. The downstream signal molecules including MAPKs, TPK, NF-κB, JAK/STAT and Akt participate in the pathogenesis of pancreatitis. TNF: Tumor necrosis factor; IL: Interleukin; TPK: Tyrosine protein kinase; MAPK: Mitogen activated protein kinase.

tion of ROS, which contributes to oxidative stress and a wide range of diseases. Furthermore, ROS can act as an intracellular second messenger or chemoattractant to enhance the level of cytokines, resulting in the aggravation of pancreatitis[38]. Studies indicate that pro-inflammation cytokines such as IL-1β, IL-6 and TNF-α mediate the local or systemic manifestations of acute pancreatitis. IL-1β and TNF-α released from activated pancreatic macrophages respond to local tissue damage. Locally, these cytokines may aggravate the severity of acute pancreatitis. Systemically, IL-6 can increase the capillary permeability and accelerate the leukocyte adherence, leading to multiple organ failure (Figure 2)[27].

NF-κB and Janus kinase/signal transducers and activators of transcription

NF-κB, a member of the Rel family of transcription factors, can regulate the activation of cellular stress-related genes or early response genes such as growth factors, cytokines, adhesion molecules, and acute-phase proteins[39,40]. The Janus kinase/signal transducers and activators of transcription (JAK/STAT) signaling pathway was relevant to the immune response mediated by numerous cytokines and non-immune response mediated by hormones and growth factors. The JAK/STAT pathway activated by the family of cytokine receptors regulate a variety of biological processes, such as immune response, cell survival, differentiation, proliferation and oncogenesis[41]. Recently, reports indicated that cerulein could activate the JAK2/STAT3 pathway through NOX in pancreatic acinar cells[27].

NOX may be the source of ROS in pancreatic acinar cells during pancreatitis. ROS can induce expression of cytokines, apoptosis, NF-κB and JAK/STAT pathway activation, thus regulating the inflammation and apoptosis in pancreatic acinar cells. Consequently, NOX, NF-κB and JAK2/STAT3 may be involved in the pathogenesis of acute pancreatitis[27]. Inflammation and apoptosis in pancreatic acinar cells during pancreatitis may be alleviated by inhibition of NOX, NF-κB and JAK/STAT through suppression of inflammatory cytokines, apoptosis and caspase-3 activity. Ju *et al*[23] found that NOX inhibition suppresses STAT3-DNA binding, JAK2/STAT3 activation and TGF-β1 level in AR42J cells stimulated by cerulein. Therefore, ROS may activate NF-κB to induce cytokine production in pancreatic acinar cells through activation of NOX during pancreatitis[21]. NOX, NF-κB and JAK/STAT may be potential targets for treatment of acute pancreatitis.

Mitogen activated protein kinase and tyrosine protein kinase

Recently, studies found that mitogen activated protein kinase (MAPK) and tyrosine protein kinase (TPK) might be involved in NOX signal transduction pathway. ROS induced by the family of NOX can cause protein phosphorylation and cell apoptosis directly or indirectly.

In the direct way, ROS mediate the activation of the MAPK pathway and TPK pathway to promote protein phosphorylation in pancreatic acinar cells. ROS activate the signal transduction pathway which consists of different MAPK family members probably owing to the activation of the upstream ERK1/2 kinase pathway. ROS stimulate TPK signaling pathway through increasing the TPK activity, thereby promoting protein tyrosine phosphorylation and affecting signal transduction to regulate cell proliferation, differentiation, metabolism and apoptosis. Inhibition of NOX or ROS significantly reduced the p38MAPK signaling cascade[42]. Activation of the MAPK signaling pathway including SAPK/JNK, ERK1/2 and p38 by ROS induce cell apoptosis. The activation of the MAPK pathway is mainly dependent on the inhibition of tyrosine phosphatase by ROS[43].

In the indirect way, ROS reduce phosphatase activity, decrease protein dephosphorylation, and thus indirectly increase protein phosphorylation. ROS injure DNA, lipid and protein, thus indirectly inducing apoptosis. In some cases, NOX family can also inhibit cell apoptosis through ROS, which activate the pathway of NF-κB and Akt/ASK1, thereby reducing cell apoptosis[44].

NOX ACTIVATION IN DIFFERENT PANCREATIC CELLS INVOLVED IN THE PATHOGENESIS OF PANCREATITIS

Phagocytes

In support of the involvement of oxygen free radicals in acute pancreatitis, studies have addressed the possibility that the severity of pancreatitis can be reduced by inhibiting the activity of oxygen-derived free radicals[45]. ROS could have different origins, and the role of the NOX system in neutrophils but not pancreatic acinar tissue is originally considered essential. The phagocytic NOX is a multicomponent enzyme complex that is composed of membranous and cytosolic proteins in the resting cell. During activation, approximately 10% of cytosolic proteins including $p47^{phox}$ and $p67^{phox}$ are phosphorylated and translocate to the cell membrane to form active catalytic complexes with $p22^{phox}$ and $gp91^{phox}$, resulting in the generation of ROS[4]. Intrapancreatic trypsin activation and acinar cell trypsin-activation peptide (TAP) labeling induced by high dose cerulein were significantly decreased in neutrophil depleted rats. NOX deficient mice displayed attenuation of the cerulean-induced trypsin activation, while myeloperoxidase (MPO) deficient mice did not. Neutrophils have been considered to be implicated in pathologic activation of digestive enzymes by infiltrating the pancreas in acute pancreatitis, which is mediated by products of NOX[28].

Evidence suggests that inflammatory cell infiltration is an early and vital event in acute pancreatitis, which will lead to local and systemic complications[46]. Many of the pathological failures of acute pancreatitis may be a consequence of the overstimulation of leukocytes[47]. The argument put forward was that once pancreatitis has been initiated, chemoattractants for polymorphonuclear leukocytes, macrophages and platelets are released, possibly *via* the action of oxygen derived free radicals. The chemoattractants induce leukocytes and macrophages to adhere to the endothelium of the postcapillary venule and to migrate into the interstitial spaces. Stimulus-secretion coupling causes synthesis of a range of enzymes including elastase, cathepsins, phospholipase A_2, phospholipase C, platelet-activating factor (PAF) and MPO. When the quantity of material to be digested is excessive, phagocytosis may become so vigorous that the contents of leukocyte and macrophage granules are spilled outside the cell where they increase the severity of inflammation. As a result, large amounts of oxygen-derived free radicals are produced and may exceed the capacity of superoxide dismutase (SOD) and catalase to inactivate them[48].

Pancreatic acinar cells

ROS and apoptosis can be observed in pancreatic acinar cells in cerulein induced pancreatitis[49,50]. NADPH has been considered to be the major source of ROS in pancreatitis[18,21,22]. Oxidative stress induced inflammation and apoptosis have been implicated in pancreatitis[51,52]. Cerulein induced the expression of apoptosis-inducing factor (AIF). AIF is located in the mitochondrial membrane of pancreatic acinar cells. During apoptosis, AIF translocates from mitochondria to the cytoplasm and then enters into the nucleus, resulting in nuclear DNA aggregation and breakage to induce apoptosis of pancreatic acinar cells[53,54]. Antisense oligonucleotides (AS ODN) transfection or Ca^{2+} chelator treatment decreased the expression of AIF induced by cerulein in AR42J cells. These results suggested that intracellular Ca^{2+} increase and NOX activation might be the upstream events of AIF expression, which result in cerulein induced apoptosis of AR42J cells[18,55].

The activation of NOX was inhibited and the production of ROS was decreased when cerulein-stimulated pancreatic acinar cells were treated with Ca^{2+} chelator, which indicates that Ca^{2+} activate NOX and ROS. Transfection with AS ODN for NOX subunits $p22^{phox}$ and $p47^{phox}$ can inhibit the ROS generation, illustrating that NOX mediates the production of ROS. The apoptotic indices including apoptotic genes bax and p53, DNA fragmentation, caspase 3 activity, TUNEL staining and cell viability were inhibited by treatment with Ca^{2+} chelator or AS ODN transfection, indicating that NOX regulates ROS-induced apoptosis in a Ca^{2+} dependent manner in pancreatic acinar cells[22]. Diphenyleneiodonium (DPI), an inhibitor of NOX, reduces the AIF expression and caspase-3 activation, and thus inhibits apoptosis of AR42J cells[16]. During the stimulation with cerulein, the increase of NOX accelerates the formation of ROS in cells and mitochondria, thus further inducing the apoptosis of acinar cells[56,57]. ROS generated by pancreatic acinar cells stimulated with bile acids or cerulein can

induce apoptosis and, at the same time, induce pancreatitis[58-60].

Research indicates that JAK2/STAT3 activation and increases of MAPKs and TGF-β1 induced by administration of cerulein were inhibited by AS ODN transfection in AR42J cells, which shows that NOX can activate JAK2/STAT3, MAPKs and TGF-β1[23]. NOX may regulate the production of cytokines by activating NF-κB in AR42J cells stimulated with cerulein. Rebamipide, an antiulcer agent, can scavenge ROS and decrease the level of superoxide[61,62]. Transfection with AS ODN for NOX subunits or administration of DPI or rebamipide inhibited cerulein induced NF-κB activation and IL-6 expression[21]. Cerulein also could produce large amounts of ROS to activate NF-κB and thus stimulate the expression of cytokines in freshly isolated pancreatic acinar cells without inflammation[63].

Numerous studies have shown that increases of ROS and peroxidation products are accompanied with endogenous antioxidant depletion in the early stage of pancreatitis. Many preclinical antioxidant treatments, including genetic manipulation, significantly reduce pancreatic injury and inflammation[1,64-66]. However, randomized clinical trials of antioxidants have produced conflicting results[67], and treatment of pancreatitis with antioxidants has even been discontinued because of adverse events[68]. Moreover, several studies indicated that NOX was only present in neutrophils but not in pancreatic acinar cells[28,69].

PSCs

PSCs are the major fibrogenic cells in chronic injury of the pancreas, which encircle the acinus[70,71]. PSCs account for approximately 4% of the total pancreatic cells[72]. PSCs are quiescent in normal pancreas and can be identified by the character of vitamin A containing lipid droplets in the cytoplasm. When chronic pancreatitis happens, PSCs are activated and transformed into myofibroblast-like cells. As a result, intracellular lipid droplets disappear and α-smooth muscle actin (α-SMA) and extracellular components such as fibronectin and collagen arise[19,73]. Besides, PSCs may be involved in the pathogenesis of acute pancreatitis[72]. Therefore, suppression of PSC activation is a potential target to treat pancreatic inflammation and fibrosis.

Studies showed that $p22^{phox}$, $p47^{phox}$, NOX1, $gp91^{phox}$ (NOX2), and NOX4 were expressed in rat quiescent and culture-activated PSCs as well as human activated PSCs, while $p67^{phox}$ and NOX3 were not detected. NOX activator 1 was present in human PSCs, while NOX organizer 1 was not detected. NOX can activate PSCs, which can be verified by DPI inhibition experiments. Studies showed that DPI could inhibit the activation of PSCs, that is, to inhibit proliferation, chemokine production, α-SMA and collagen expression. Platelet-derived growth factor BB (PDGF-BB) promoted proliferation of rat PSCs, which was inhibited by DPI in a dose-dependent manner, showing that NOX underlies the PDGF induced PSC proliferation. DPI decreased the chemokine production, which indicates that NOX also regulates the production of chemokines. DPI decreased the levels of α-SMA and collagen, once again, proving that NOX activate PSCs. DPI also inhibited interleukin 1β (IL-1β) and PDGF induced activation of MAPKs in PSCs, and this evidence indicates that NOX mediates the activation of MAPKs induced by IL-1β and PDGF in PSCs[19].

FUTURE RESEARCH ON THE PATHOGENESIS OF PANCREATITIS IN NOX

Accumulated evidence suggested that ROS induced by NOX play a significant role in pancreatitis. The activation of ROS mediates the activation of many cytokines[56,57]. ROS can induce cell apoptosis through direct and indirect pathways[43,44]. ROS induced by bile acids and cerulein can promote apoptosis of pancreatic acinar cells[18,69]. NOX is usually induced by cerulein, inflammatory factors, cytokines and growth factors as well as other stimuli in pancreatic acinar cells and PSCs. NOX can generate ROS, which in turn increase cytokines levels downstream to initiate the next activation cycle. The positive feedback of activation process might be one of the causes of pancreatitis. Although many scholars have made a great deal of research about the pathogenic mechanisms of NOX in the inflammation of pancreatic acinar cells and stellate cells, the relative importance of different pathogenic mechanisms of NOX in the pathogenesis of pancreatitis, the relationship between various pathogenic mechanisms of NOX, the specific pathways involved in each mechanism of NOX in pancreatitis, and the feasibility of NOX targeted therapy applied to pancreatitis are all needed to be studied in the future.

REFERENCES

1 **Leung PS**, Chan YC. Role of oxidative stress in pancreatic inflammation. *Antioxid Redox Signal* 2009; **11**: 135-165 [PMID: 18837654 DOI: 10.1089/ars.2008.2109]

2 **Brown DI**, Griendling KK. Nox proteins in signal transduction. *Free Radic Biol Med* 2009; **47**: 1239-1253 [PMID: 19628035 DOI: 10.1016/j.freeradbiomed.2009.07.023]

3 **Lambeth JD**. NOX enzymes and the biology of reactive oxygen. *Nat Rev Immunol* 2004; **4**: 181-189 [PMID: 15039755 DOI: 10.1038/nri1312]

4 **Babior BM**. NADPH oxidase: an update. *Blood* 1999; **93**: 1464-1476 [PMID: 10029572]

5 **Bedard K**, Krause KH. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol Rev* 2007; **87**: 245-313 [PMID: 17237347 DOI: 10.1152/physrev.00044.2005]

6 **Babior BM**. The respiratory burst oxidase. *Curr Opin Hematol* 1995; **2**: 55-60 [PMID: 9371972 DOI: 10.1097/00062752-199502010-00008]

7 **Lambeth JD**, Cheng G, Arnold RS, Edens WA. Novel homologs of gp91phox. *Trends Biochem Sci* 2000; **25**: 459-461 [PMID: 11050424 DOI: 10.1016/S0968-0004(00)01658-3]

8 **Cheng G**, Cao Z, Xu X, van Meir EG, Lambeth JD. Homologs of gp91phox: cloning and tissue expression of Nox3, Nox4, and Nox5. *Gene* 2001; **269**: 131-140 [PMID: 11376945 DOI: 10.1016/S0378-1119(01)00449-8]

9 **Pongnimitprasert N**, El-Benna J, Foglietti MJ, Gougerot-Pocidalo MA, Bernard M, Braut-Boucher F. Potential role of the "NADPH oxidases" (NOX/DUOX) family in cystic fibrosis. *Ann Biol Clin* (Paris) 2008; **66**: 621-629 [PMID: 19091660 DOI: 10.1684/abc.2008.0285]

10 **Meier B**, Cross AR, Hancock JT, Kaup FJ, Jones OT. Identification of a superoxide-generating NADPH oxidase system in human fibroblasts. *Biochem J* 1991; **275** (Pt 1): 241-245 [PMID: 1850240]

11 **Griendling KK**, Sorescu D, Ushio-Fukai M. NAD(P)H oxidase: role in cardiovascular biology and disease. *Circ Res* 2000; **86**: 494-501 [PMID: 10720409 DOI: 10.1161/01.RES.86.5.494]

12 **Bataller R**, Schwabe RF, Choi YH, Yang L, Paik YH, Lindquist J, Qian T, Schoonhoven R, Hagedorn CH, Lemasters JJ, Brenner DA. NADPH oxidase signal transduces angiotensin II in hepatic stellate cells and is critical in hepatic fibrosis. *J Clin Invest* 2003; **112**: 1383-1394 [PMID: 14597764 DOI: 10.1172/JCI18212]

13 **Mohammed AM**, Syeda K, Hadden T, Kowluru A. Upregulation of phagocyte-like NADPH oxidase by cytokines in pancreatic beta-cells: attenuation of oxidative and nitrosative stress by 2-bromopalmitate. *Biochem Pharmacol* 2013; **85**: 109-114 [PMID: 23092759 DOI: 10.1016/j.bcp.2012.09.024]

14 **Rebelato E**, Mares-Guia TR, Graciano MF, Labriola L, Britto LR, Garay-Malpartida HM, Curi R, Sogayar MC, Carpinelli AR. Expression of NADPH oxidase in human pancreatic islets. *Life Sci* 2012; **91**: 244-249 [PMID: 22820165 DOI: 10.1016/j.lfs.2012.07.004]

15 **Criddle DN**, Gillies S, Baumgartner-Wilson HK, Jaffar M, Chinje EC, Passmore S, Chvanov M, Barrow S, Gerasimenko OV, Tepikin AV, Sutton R, Petersen OH. Menadione-induced reactive oxygen species generation via redox cycling promotes apoptosis of murine pancreatic acinar cells. *J Biol Chem* 2006; **281**: 40485-40492 [PMID: 17088248 DOI: 10.1074/jbc.M607704200]

16 **Yu JH**, Kim KH, Kim DG, Kim H. Diphenyleneiodonium suppresses apoptosis in cerulein-stimulated pancreatic acinar cells. *Int J Biochem Cell Biol* 2007; **39**: 2063-2075 [PMID: 17625947 DOI: 10.1016/j.biocel.2007.05.021]

17 **Kim H**. Inhibitory mechanism of lycopene on cytokine expression in experimental pancreatitis. *Ann N Y Acad Sci* 2011; **1229**: 99-102 [PMID: 21793844 DOI: 10.1111/j.1749-6632.2011.06107.x]

18 **Yu JH**, Kim KH, Kim H. Role of NADPH oxidase and calcium in cerulein-induced apoptosis: involvement of apoptosis-inducing factor. *Ann N Y Acad Sci* 2006; **1090**: 292-297 [PMID: 17384272 DOI: 10.1196/annals.1378.031]

19 **Masamune A**, Watanabe T, Kikuta K, Satoh K, Shimosegawa T. NADPH oxidase plays a crucial role in the activation of pancreatic stellate cells. *Am J Physiol Gastrointest Liver Physiol* 2008; **294**: G99-G108 [PMID: 17962358 DOI: 10.1152/ajpgi.00272.2007]

20 **Hu R**, Wang YL, Edderkaoui M, Lugea A, Apte MV, Pandol SJ. Ethanol augments PDGF-induced NADPH oxidase activity and proliferation in rat pancreatic stellate cells. *Pancreatology* 2007; **7**: 332-340 [PMID: 17627098]

21 **Yu JH**, Lim JW, Kim H, Kim KH. NADPH oxidase mediates interleukin-6 expression in cerulein-stimulated pancreatic acinar cells. *Int J Biochem Cell Biol* 2005; **37**: 1458-1469 [PMID: 15833277 DOI: 10.1016/j.biocel.2005.02.004]

22 **Yu JH**, Lim JW, Kim KH, Morio T, Kim H. NADPH oxidase and apoptosis in cerulein-stimulated pancreatic acinar AR42J cells. *Free Radic Biol Med* 2005; **39**: 590-602 [PMID: 16085178]

23 **Ju KD**, Lim JW, Kim KH, Kim H. Potential role of NADPH oxidase-mediated activation of Jak2/Stat3 and mitogen-activated protein kinases and expression of TGF-β1 in the pathophysiology of acute pancreatitis. *Inflamm Res* 2011; **60**: 791-800 [PMID: 21509626]

24 **Yu JH**, Seo JY, Kim KH, Kim H. Differentially expressed proteins in cerulein-stimulated pancreatic acinar cells: implication for acute pancreatitis. *Int J Biochem Cell Biol* 2008; **40**: 503-516 [PMID: 18024178 DOI: 10.1016/j.biocel.2007.09.001]

25 **Noble F**, Wank SA, Crawley JN, Bradwejn J, Seroogy KB, Hamon M, Roques BP. International Union of Pharmacology. XXI. Structure, distribution, and functions of cholecystokinin receptors. *Pharmacol Rev* 1999; **51**: 745-781 [PMID: 10581329]

26 **Lerch MM**, Adler G. Experimental animal models of acute pancreatitis. *Int J Pancreatol* 1994; **15**: 159-170 [PMID: 7930776]

27 **Kim H**. Cerulein pancreatitis: oxidative stress, inflammation, and apoptosis. *Gut Liver* 2008; **2**: 74-80 [PMID: 20485614 DOI: 10.5009/gnl.2008.2.2.74]

28 **Gukovskaya AS**, Vaquero E, Zaninovic V, Gorelick FS, Lusis AJ, Brennan ML, Holland S, Pandol SJ. Neutrophils and NADPH oxidase mediate intrapancreatic trypsin activation in murine experimental acute pancreatitis. *Gastroenterology* 2002; **122**: 974-984 [PMID: 11910350]

29 **Oparil S**, Haber E. The renin-angiotensin system (first of two parts). *N Engl J Med* 1974; **291**: 389-401 [PMID: 4367917 DOI: 10.1056/NEJM197408222910805]

30 **Sakurai T**, Kudo M, Fukuta N, Nakatani T, Kimura M, Park AM, Munakata H. Involvement of angiotensin II and reactive oxygen species in pancreatic fibrosis. *Pancreatology* 2011; **11** Suppl 2: 7-13 [PMID: 21464581 DOI: 10.1159/000323478]

31 **Chan YC**, Leung PS. Angiotensin II type 1 receptor-dependent nuclear factor-kappaB activation-mediated proinflammatory actions in a rat model of obstructive acute pancreatitis. *J Pharmacol Exp Ther* 2007; **323**: 10-18 [PMID: 17616560 DOI: 10.1124/jpet.107.124891]

32 **Timmermans PB**, Wong PC, Chiu AT, Herblin WF, Benfield P, Carini DJ, Lee RJ, Wexler RR, Saye JA, Smith RD. Angiotensin II receptors and angiotensin II receptor antagonists. *Pharmacol Rev* 1993; **45**: 205-251 [PMID: 8372104]

33 **Dijkhorst-Oei LT**, Stroes ES, Koomans HA, Rabelink TJ. Acute simultaneous stimulation of nitric oxide and oxygen radicals by angiotensin II in humans in vivo. *J Cardiovasc Pharmacol* 1999; **33**: 420-424 [PMID: 10069678 DOI: 10.1097/00005344-199903000-00012]

34 **Landmesser U**, Cai H, Dikalov S, McCann L, Hwang J, Jo H, Holland SM, Harrison DG. Role of p47(phox) in vascular oxidative stress and hypertension caused by angiotensin II. *Hypertension* 2002; **40**: 511-515 [PMID: 12364355 DOI: 10.1161/01.HYP.0000032100.23772.98]

35 **Tsang SW**, Ip SP, Leung PS. Prophylactic and therapeutic treatments with AT 1 and AT 2 receptor antagonists and their effects on changes in the severity of pancreatitis. *Int J Biochem Cell Biol* 2004; **36**: 330-339 [PMID: 14643897]

36 **Lowenfels AB**, Maisonneuve P, Cavallini G, Ammann RW, Lankisch PG, Andersen JR, Dimagno EP, Andrén-Sandberg A, Domellöf L. Pancreatitis and the risk of pancreatic cancer. International Pancreatitis Study Group. *N Engl J Med* 1993; **328**: 1433-1437 [PMID: 8479461 DOI: 10.1056/NEJM199305203282001]

37 **Kojima M**, Ito T, Oono T, Hisano T, Igarashi H, Arita Y, Kawabe K, Coy DH, Jensen RT, Nawata H. VIP attenuation of the severity of experimental pancreatitis is due to VPAC1 receptor-mediated inhibition of cytokine production. *Pancreas* 2005; **30**: 62-70 [PMID: 15632701]

38 **Fujimori N**, Oono T, Igarashi H, Ito T, Nakamura T, Uchida M, Coy DH, Jensen RT, Takayanagi R. Vasoactive intestinal peptide reduces oxidative stress in pancreatic acinar cells through the inhibition of NADPH oxidase. *Peptides* 2011; **32**: 2067-2076 [PMID: 21924308 DOI: 10.1016/j.peptides.2011.08.027]

39 **Wulczyn FG**, Krappmann D, Scheidereit C. The NF-kappa B/Rel and I kappa B gene families: mediators of immune response and inflammation. *J Mol Med* (Berl) 1996; **74**: 749-769

[PMID: 8974017]
40 **Barnes PJ**, Karin M. Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med* 1997; **336**: 1066-1071 [PMID: 9091804 DOI: 10.1056/NEJM199704103361506]
41 **Rawlings JS**, Rosler KM, Harrison DA. The JAK/STAT signaling pathway. *J Cell Sci* 2004; **117**: 1281-1283 [PMID: 15020666 DOI: 10.1242/jcs.00963]
42 **Corbi G**, Conti V, Russomanno G, Longobardi G, Furgi G, Filippelli A, Ferrara N. Adrenergic signaling and oxidative stress: a role for sirtuins? *Front Physiol* 2013; **4**: 324 [PMID: 24265619 DOI: 10.3389/fphys.2013.00324]
43 **Kamata H**, Honda S, Maeda S, Chang L, Hirata H, Karin M. Reactive oxygen species promote TNFalpha-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. *Cell* 2005; **120**: 649-661 [PMID: 15766528 DOI: 10.1016/j.cell.2004.12.041]
44 **Goldstein BJ**, Mahadev K, Wu X. Redox paradox: insulin action is facilitated by insulin-stimulated reactive oxygen species with multiple potential signaling targets. *Diabetes* 2005; **54**: 311-321 [PMID: 15677487 DOI: 10.2337/diabetes.54.2.311]
45 **Otamiri T**, Sjödahl R. Oxygen radicals: their role in selected gastrointestinal disorders. *Dig Dis* 1991; **9**: 133-141 [PMID: 1868620]
46 **Vonlaufen A**, Apte MV, Imhof BA, Frossard JL. The role of inflammatory and parenchymal cells in acute pancreatitis. *J Pathol* 2007; **213**: 239-248 [PMID: 17893879]
47 **Tsuji N**, Watanabe N, Okamoto T, Niitsu Y. Specific interaction of pancreatic elastase and leucocytes to produce oxygen radicals and its implication in pancreatitis. *Gut* 1994; **35**: 1659-1664 [PMID: 7828993]
48 **Dabrowski A**, Konturek SJ, Konturek JW, Gabryelewicz A. Role of oxidative stress in the pathogenesis of caerulein-induced acute pancreatitis. *Eur J Pharmacol* 1999; **377**: 1-11 [PMID: 10448919]
49 **Gukovskaya AS**, Gukovsky I, Zaninovic V, Song M, Sandoval D, Gukovsky S, Pandol SJ. Pancreatic acinar cells produce, release, and respond to tumor necrosis factor-alpha. Role in regulating cell death and pancreatitis. *J Clin Invest* 1997; **100**: 1853-1862 [PMID: 9312187 DOI: 10.1172/JCI119714]
50 **Kimura K**, Shimosegawa T, Sasano H, Abe R, Satoh A, Masamune A, Koizumi M, Nagura H, Toyota T. Endogenous glucocorticoids decrease the acinar cell sensitivity to apoptosis during cerulein pancreatitis in rats. *Gastroenterology* 1998; **114**: 372-381 [PMID: 9453499 DOI: 10.1016/S0016-5085(98)70490-1]
51 **Kaiser AM**, Saluja AK, Sengupta A, Saluja M, Steer ML. Relationship between severity, necrosis, and apoptosis in five models of experimental acute pancreatitis. *Am J Physiol* 1995; **269**: C1295-C1304 [PMID: 7491921]
52 **Sata N**, Klonowski-Stumpe H, Han B, Lüthen R, Häussinger D, Niederau C. Supraphysiologic concentrations of cerulein induce apoptosis in the rat pancreatic acinar cell line AR4-2J. *Pancreas* 1999; **19**: 76-82 [PMID: 10416696 DOI: 10.1097/00006676-199907000-00012]
53 **Wang X**, Yang C, Chai J, Shi Y, Xue D. Mechanisms of AIF-mediated apoptotic DNA degradation in Caenorhabditis elegans. *Science* 2002; **298**: 1587-1592 [PMID: 12446902 DOI: 10.1126/science.1076194]
54 **Cregan SP**, Dawson VL, Slack RS. Role of AIF in caspase-dependent and caspase-independent cell death. *Oncogene* 2004; **23**: 2785-2796 [PMID: 15077142 DOI: 10.1038/sj.onc.1207517]
55 **Yu JH**, Kim H, Kim KH. Calcium-dependent apoptotic gene expression in cerulein-treated AR42J cells. *Ann N Y Acad Sci* 2003; **1010**: 66-69 [PMID: 15033695 DOI: 10.1196/annals.1299.009]
56 **Snook JH**, Li J, Helmke BP, Guilford WH. Peroxynitrite inhibits myofibrillar protein function in an in vitro assay of motility. *Free Radic Biol Med* 2008; **44**: 14-23 [PMID: 18045543 DOI: 10.1016/j.freeradbiomed.2007.09.004]
57 **Crimi E**, Ignarro LJ, Napoli C. Microcirculation and oxidative stress. *Free Radic Res* 2007; **41**: 1364-1375 [PMID: 18075839 DOI: 10.1080/10715760701732830]
58 **Okumura N**, Sakakibara A, Hayakawa T, Noda A. Pancreatic endocrine function in experimental pancreatolithiasis in dogs. *Am J Gastroenterol* 1982; **77**: 392-396 [PMID: 7046428]
59 **Aho HJ**, Nevalainen TJ, Havia VT, Heinonen RJ, Aho AJ. Human acute pancreatitis: a light and electron microscopic study. *Acta Pathol Microbiol Immunol Scand A* 1982; **90**: 367-373 [PMID: 7148455]
60 **Uys CJ**, Bank S, Marks IN. The pathology of chronic pancreatitis in Cape Town. *Digestion* 1973; **9**: 454-468 [PMID: 4784707 DOI: 10.1159/000197474]
61 **Naito Y**, Yoshikawa T, Tanigawa T, Sakurai K, Yamasaki K, Uchida M, Kondo M. Hydroxyl radical scavenging by rebamipide and related compounds: electron paramagnetic resonance study. *Free Radic Biol Med* 1995; **18**: 117-123 [PMID: 7896165 DOI: 10.1016/0891-5849(94)00110-6]
62 **Ogino K**, Hobara T, Ishiyama H, Yamasaki K, Kobayashi H, Izumi Y, Oka S. Antiulcer mechanism of action of rebamipide, a novel antiulcer compound, on diethyldithiocarbamate-induced antral gastric ulcers in rats. *Eur J Pharmacol* 1992; **212**: 9-13 [PMID: 1313372 DOI: 10.1016/0014-2999(92)90065-C]
63 **Yu JH**, Lim JW, Namkung W, Kim H, Kim KH. Suppression of cerulein-induced cytokine expression by antioxidants in pancreatic acinar cells. *Lab Invest* 2002; **82**: 1359-1368 [PMID: 12379770]
64 **Park BK**, Chung JB, Lee JH, Suh JH, Park SW, Song SY, Kim H, Kim KH, Kang JK. Role of oxygen free radicals in patients with acute pancreatitis. *World J Gastroenterol* 2003; **9**: 2266-2269 [PMID: 14562390]
65 **Verlaan M**, Roelofs HM, van-Schaik A, Wanten GJ, Jansen JB, Peters WH, Drenth JP. Assessment of oxidative stress in chronic pancreatitis patients. *World J Gastroenterol* 2006; **12**: 5705-5710 [PMID: 17007026]
66 **Yoo BM**, Oh TY, Kim YB, Yeo M, Lee JS, Surh YJ, Ahn BO, Kim WH, Sohn S, Kim JH, Hahm KB. Novel antioxidant ameliorates the fibrosis and inflammation of cerulein-induced chronic pancreatitis in a mouse model. *Pancreatology* 2005; **5**: 165-176 [PMID: 15849487 DOI: 10.1159/000085268]
67 **Bhardwaj P**, Garg PK, Maulik SK, Saraya A, Tandon RK, Acharya SK. A randomized controlled trial of antioxidant supplementation for pain relief in patients with chronic pancreatitis. *Gastroenterology* 2009; **136**: 149-159.e2 [PMID: 18952082 DOI: 10.1053/j.gastro.2008.09.028]
68 **Siriwardena AK**, Mason JM, Balachandra S, Bagul A, Galloway S, Formela L, Hardman JG, Jamdar S. Randomised, double blind, placebo controlled trial of intravenous antioxidant (n-acetylcysteine, selenium, vitamin C) therapy in severe acute pancreatitis. *Gut* 2007; **56**: 1439-1444 [PMID: 17356040 DOI: 10.1136/gut.2006.115873]
69 **Booth DM**, Murphy JA, Mukherjee R, Awais M, Neoptolemos JP, Gerasimenko OV, Tepikin AV, Petersen OH, Sutton R, Criddle DN. Reactive oxygen species induced by bile acid induce apoptosis and protect against necrosis in pancreatic acinar cells. *Gastroenterology* 2011; **140**: 2116-2125 [PMID: 21354148 DOI: 10.1053/j.gastro.2011.02.054]
70 **Apte MV**, Haber PS, Applegate TL, Norton ID, McCaughan GW, Korsten MA, Pirola RC, Wilson JS. Periacinar stellate shaped cells in rat pancreas: identification, isolation, and culture. *Gut* 1998; **43**: 128-133 [PMID: 9771417 DOI: 10.1136/gut.43.1.128]
71 **Bachem MG**, Schünemann M, Ramadani M, Siech M, Beger H, Buck A, Zhou S, Schmid-Kotsas A, Adler G. Pancreatic carcinoma cells induce fibrosis by stimulating proliferation and matrix synthesis of stellate cells. *Gastroenterology* 2005; **128**: 907-921 [PMID: 15825074 DOI: 10.1053/

j.gastro.2004.12.036]
72 **Omary MB**, Lugea A, Lowe AW, Pandol SJ. The pancreatic stellate cell: a star on the rise in pancreatic diseases. *J Clin Invest* 2007; **117**: 50-59 [PMID: 17200706]
73 **Apte MV**, Phillips PA, Fahmy RG, Darby SJ, Rodgers SC, McCaughan GW, Korsten MA, Pirola RC, Naidoo D, Wilson JS. Does alcohol directly stimulate pancreatic fibrogenesis? Studies with rat pancreatic stellate cells. *Gastroenterology* 2000; **118**: 780-794 [PMID: 10734030 DOI: 10.1016/S0016-5085(00)70148-X]

P- Reviewer: Coelho AMM **S- Editor**: Wen LL
L- Editor: Wang TQ **E- Editor:** Lu YJ

World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4291/wjgp.v5.i3.178

World J Gastrointest Pathophysiol 2014 August 15; 5(3): 178-187
ISSN 2150-5330 (online)

TOPIC HIGHLIGHT

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

Barrett's oesophagus: Evidence from the current meta-analyses

Piers Gatenby, Yuen Soon

Piers Gatenby, Division of Surgery and Interventional Science, University College London, London NW32QG, United Kingdom
Piers Gatenby, Yuen Soon, Regional Oesophagogastric Unit, Royal Surrey County Hospital, Guildford GU2 7XX, United Kingdom
Author contributions: Gatenby P concepted and designed the manuscript; acquisited and analysed data; and drafted the paper; Gatenby P and Soon Y interpreted the data and final approved of the version to be published; Soon Y concepted and revised the article critically.
Supported by Barrett's Oesophagus Campaign; the Wexham Gastrointestinal Trust, the Childwick Trust; the R.L. St J. Harmsworth Memorial Research Fund and the David and Frederick Barclay Foundation
Correspondence to: Piers Gatenby, MA, MD, FRCS, UCL, Division of Surgery and Interventional Science, University College London, Royal Free Campus, Pond Street, London NW32QG, United Kingdom. p.gatenby@ucl.ac.uk
Telephone: +44-020-74726223 Fax: +44-020-74726224
Received: December 31, 2013 Revised: April 5, 2014
Accepted: May 29, 2014
Published online: August 15, 2014

Abstract

Guidelines have been published regarding the management of Barrett's oesophagus (columnar-lined oesophagus). These have examined the role of surveillance in an effort to detect dysplasia and early cancer. The guidelines have provided criteria for enrolment into surveillance and some risk stratification with regard to surveillance interval. The research basis for the decisions reached with regard to cancer risk is weak and this manuscript has examined the available data published from meta-analyses up to 25th April 2013 (much of which has been published since the guidelines and their most recent updates have been written). There were 9 meta-analyses comparing patients with Barrett's oesophagus to control populations. These have demonstrated that Barrett's oesophagus is more common in males than females, in subjects who have ever smoked, in subjects with obesity, in subjects with prolonged symptoms of gastro-oesophageal reflux disease, in subjects who do not have infection with Helicobacter pylori and in subjects with hiatus hernia. These findings should inform public health measures in reducing the risk of Barrett's oesophagus and subsequent surveillance burden and cancer risk. There were 8 meta-analyses comparing different groups of patients with Barrett's oesophagus with regard to cancer risk. These have demonstrated that there was no statistically significant benefit of antireflux surgery over medical therapy, that endoscopic ablative therapy was effective in reducing cancer risk that there was similar cancer risk in patients with Barrett's oesophagus independent of geographic origin, that the adenocarcinoma incidence in males is twice the rate in females, that the cancer risk in long segment disease showed a trend to be higher than in short segment disease, that there was a trend for higher cancer risk in low-grade dysplasia over non-dysplastic Barrett's oesophagus, that there is a lower risk in patients with *Helicobacter pylori* infection and that there is a significant protective effect of aspirin and statins. There were no meta-analyses examining the role of intestinal metaplasia. These results demonstrate that guidance regarding surveillance based on the presence of intestinal metaplasia, segment length and the presence of low-grade dysplasia has a weak basis, and further consideration should be given to gender and helicobacter status, ablation of the metaplastic segment as well as the chemoprotective role of aspirin and statins.

Key words: Barrett esophagus; Esophageal neoplasms; Meta-analysis; Review; Systematic

Core tip: The presence of intestinal metaplasia on biopsy has been regarded as a necessity for enrolment

in a surveillance programme for Barrett's oesophagus and surveillance intervals have been based on segment length and the presence or absence of dysplasia. Evidence from meta-analyses supports male gender and negative *Helicobacter pylori* infection status as important markers of cancer risk and of the role of aspirin, statins and ablation of the Barrett's segment to reduce cancer risk. The evidence from meta-analyses supporting segment length and dysplasia as markers of cancer risk is poor and for intestinal metaplasia has not been shown.

Gatenby P, Soon Y. Barrett's oesophagus: Evidence from the current meta-analyses. *World J Gastrointest Pathophysiol* 2014; 5(3): 178-187 Available from: URL: http://www.wjgnet.com/2150-5330/full/v5/i3/178.htm DOI: http://dx.doi.org/10.4291/wjgp.v5.i3.178

INTRODUCTION

Barrett's columnar-lined oesophagus is a metaplastic change to the squamous mucosa of the oesophagus associated with gastro-oesophageal reflux disease[1]. Guidelines concerning management of patients with Barrett's oesophagus have been published with recommendations on the control of pathological reflux and on periodic surveillance of this pre-malignant condition[2-4]. There has been a rapid increase in the number of meta-analyses published, with over half published in the last 5 years and an increase in the focus of these on pharmacotherapy and reflux control to reduce cancer incidence, associations with smoking and obesity as well as new estimates on cancer incidence. In an attempt to examine the available best evidence since these guidelines were published/updated (in 2013[2], 2011[4] and 2008[3]), this review has conducted a systematic review of the currently published meta-analyses to aid clinicians and patients in optimum decision making for the risk assessment and management of Barrett's oesophagus.

RESEARCH

A search was made of the Pubmed database for the search terms "Barrett's oesophagus" and "meta-analysis". The full search terms are listed in Table 1 with publication dates up to and including 25th April 2013 (including epublication). Papers were included in the analysis if the type of study was a meta-analysis of previously published data concerning Barrett's oesophagus in human subjects and published in English language. Studies were included if they compared subjects with Barrett's oesophagus to control groups or compared different groups of patients with Barrett's oesophagus with respect to cancer risk. Studies were then categorized into the following groups: (1) comparison of patients with Barrett's oesophagus to control groups; and (2) comparison of different groups of patients with Barrett's oesophagus with regard to cancer risk. Where the papers retrieved did not contain meta-analyses, but useful observations were presented, these have been described in this manuscript, but not included in the results tables.

The literature search yielded 50 papers. Of these papers, 10 were excluded after retrieving the abstracts and 6 after retrieving the full papers (2 were letters concerning meta analyses, 1 examined cell culture lines rather than studying human subjects, 4 were in foreign language-3 German and 1 Spanish, 1 was a systematic review without a meta-analysis, 1 was an economic review without a meta-analysis, 2 were reviews only, 3 did not contain a meta-analysis comparing any different groups and 2 were single studies). There were 34 remaining studies and the full manuscript of each was obtained. Eleven studies were excluded as they examined oesophageal cancer compared to control groups without an examination of a comparative risk in Barrett's oesophagus. Three examined diagnostic techniques only and have been excluded. Two examined the risk of adenocarcinoma development within high-grade dysplasia and were excluded. One examined the association of Barrett's oesophagus with colonic tumours (which demonstrated the increased risk of colonic tumours and colorectal cancer in subjects with Barrett's oesophagus[5]).

There were no studies comparing cancer risk in patients with Barrett's oesophagus to control groups. The remaining 17 studies are examined below.

The retrieved studies spanned the last 10 years. As would be anticipated with the growing popularity of meta-analyses, over half of the eligible studies were published since the beginning of 2010. The United States and United Kingdom guidelines were most recently updated in 2013[2], 2011[4] and 2008[3]. With the time required for preparation of these guidelines, this indicates that only a handful of the meta-analyses had been published sufficiently early for their results to be incorporated in the compilation of the American College of Gastroenterology guidelines and a limited number into the American Gastroenterological Association guidelines. In general, the guidelines have not examined differences in cancer risk between individuals beyond segment length, presence of intestinal metaplasia and dysplasia.

Comparison of patients with Barrett's oesophagus to control groups

There were 11 papers comparing patients with Barrett's oesophagus to control groups, usually taken from the general population, but also other endoscopic populations including those with reflux disease but no Barrett's oesophagus. These studies examined gender, smoking habits, obesity, symptom association, presence of *Helicobacter pylori* (*H. pylori*), presence of hiatus hernia and pattern of proton pump inhibitor usage. Of these, 9 were meta-analyses (Table 2).

Gender: The association between male gender and Barrett's oesophagus was demonstrated by Cook *et al*[6]. They

Table 1 Search terms

{"barrett's oesophagus" (All Fields) OR "barrett esophagus" (MeSH Terms) OR ["barrett" (All Fields) AND "esophagus" (All Fields)] OR "barrett esophagus"(All Fields) OR ["barrett's" (All Fields) AND "esophagus" (All Fields)] OR "barrett's esophagus" (All Fields)} OR {"barrett's oesophagus" (All Fields) OR "barrett esophagus" (MeSH Terms) OR ["barrett"(All Fields) AND "esophagus" (All Fields)] OR "barrett esophagus"(All Fields) OR ["barrett's" (All Fields) AND "esophagus" (All Fields)] OR "barrett's esophagus"(All Fields)}	AND	["meta-analysis" (Publication type) OR "meta-analysis as topic" (MeSH Terms) OR "meta-analysis" (All Fields)]

Search strategy: "Barrett's esophagus" or "Barrett's oesophagus".

Table 2 Meta-analyses comparing patients with Barrett's oesophagus to control groups

Subject	Ref.	Comparison	Group	Studies	Results	Outcome
Gender	Cook *et al*[6], 2005	Gender	Barrett's	32	M:F Ratio 1.96:1 (95%CI: 1.77, 2.77)	Higher M:F ratio in Barrett's oesophagus and reflux oesophagitis than in non-erosive reflux disease
			Erosive reflux disease	28	1.57 (95%CI: 1.40, 1.76)	
			Non-erosive reflux disease	14	0.72 (95%CI: 0.62, 0.84)	
Smoking	Andrici *et al*[7], 2013	Ever smoking	Barrett's *vs* GORD	20	OR, 1.18 (95%CI: 0.75, 1.86)	Cigarette smoking associated with increased risk of Barrett's oesophagus
			Barrett's *vs* non-GORD	27	OR, 1.44 (95%CI: 1.20, 1.74)	
Obesity	Cook *et al*[8], 2008	BMI	Barrett's *vs* GORD	9	OR, 0.99/kg per m^2 (95%CI: 0.97, 1.01)	Barrett's oesophagus associated with higher BMI than control but not GORD
			Barrett's *vs* general population	3	OR, 1.02/kg per m^2 (95%CI: 1.01, 1.04)	
	Kamat *et al*[9], 2009	Obesity (BMI ⩾ 30 *vs* BMI < 30)	Barrett's *vs* control (BMI ⩾ 30 *vs* BMI < 30)	9	OR, 1.35 (95%CI: 1.15, 1.59)	Barrett's oesophagus associated with being overweight and obese
		Overweight (BMI ⩾ 25 *vs* BMI < 25)	Barrett's *vs* control	8	OR, 1.49 (95%CI: 1.24, 1.80)	
	Kubo *et al*[10], 2013	Waist circumference	Highest *vs* lowest quartiles	4	Males OR, 2.24 (95%CI: 1.08, 4.65) Females OR, 3.75 (95%CI: 1.47, 9.56)	Barrett's oesophagus associated with higher waist circumference but not BMI
		BMI		4	No significant association	
Symptoms of gastro-oesophageal reflux	Taylor *et al*[11], 2010	Symptoms of GORD	All Barrett's *vs* controls	26	OR, 2.90 (95%CI: 1.86, 4.54)	Symptoms of GORD associated with all Barrett's oesophagus, more strongly with long segment Barrett's oesophagus than with short segment Barrett's oesophagus
			Short segment Barrett's *vs* controls	12	OR, 1.59 (95%CI: 1.07, 2.38)	
			Long segment Barrett's *vs* controls	11	OR, 4.16 (95%CI: 2.43, 7.12)	
Helicobacter pylori	Wang *et al*[12]	Helicobacter pylori infection rate	Barrett's oesophagus *vs* all controls	12	OR, 0.74 (95%CI: 0.40, 1.37)	Similar helicobacter pylori infection rate in Barrett's oesophagus to all controls but lower than in endoscopically normal controls
			Barrett's oesophagus *vs* endoscopically normal	9	OR, 0.50 (95%CI: 0.27, 0.93)	
	Fischbach *et al*[13], 2012	Helicobacter pylori infection rate	Barrett's oesophagus *vs* all controls	49	RR, 0.46 (9%CI: 0.35, 0.60)	Lower helicobacter infection rate in patients with Barrett's oesophagus compared to controls
		Cag A Helicobacter pylori infection rate	Barrett's oesophagus *vs* all controls	7	RR, 0.38 (95%CI: 0.19, 0.78)	
Hiatus hernia	Andrici *et al*[14], 2012	Hiatus hernia presence	Barrett's oesophagus *vs* all controls	31	OR, 3.94 (95%CI: 3.02, 5.13)	Hiatus hernia associated with Barrett's oesophagus and more strongly associated with long-segment Barrett's oesophagus

OR: Odds ratio; BMI: Body mass index; CI: Confidence interval.

examined data from studies on Barrett's oesophagus, erosive reflux disease and non-erosive reflux disease. The overall male: female ratio in Barrett's oesophagus was 1.96 and was similar in erosive reflux disease, but higher than

in non-erosive reflux disease.

Cigarette smoking: The association between cigarette smoking and diagnosis of Barrett's oesophagus was examined by Andrici *et al*[7]. They included a variety of different study designs and control subjects. They demonstrated that having ever smoked was associated with Barrett's oesophagus compared to control subjects who did not have gastro-oesophageal reflux disease or to population-based controls. There was no significant association when compared to controls with gastro-oesophageal reflux disease. There was a dose-related relationship with a higher number of pack-years smoked associated with increased risk of Barrett's oesophagus. The relationships were similar for current, former and ever smokers.

Obesity: Three studies examined the association between obesity and Barrett's oesophagus. Cook *et al*[8] examined studies which compared Barrett's oesophagus to those with reflux disease (those with unknown histology and those with histologically-proven oesophagitis) in 9 studies and to the general population in one study. Their results were similar for all comparison groups with no association noted with obesity and Barrett's oesophagus compared to gastro-oesophageal reflux disease, but in 3 studies comparing Barrett's oesophagus to control subjects there was a small statistically significant association between Barrett's oesophagus and higher body mass index. Kamat *et al*[9] showed that obesity was associated with Barrett's oesophagus and comparing patients who were either overweight or obese showed similar results. More recently, Kubo *et al*[10] showed that from 4 case-control studies that there was no clear association between BMI and Barrett's oesophagus, but that there was an increased risk of Barrett's oesophagus with higher waist circumference.

Symptoms: One study by Taylor *et al*[11] examined the association of Barrett's oesophagus with symptoms of gastro-oesophageal reflux. This analysis included 26 published studies (the majority of which were case-control) and demonstrated that symptoms of gastro-oesophageal reflux were associated with the diagnosis of Barrett's oesophagus, strongly with long segment Barrett's oesophagus and that there was a weaker association with short-segment Barrett's oesophagus.

***Helicobacter pylori*:** Wang *et al*[12] showed that there was no overall difference in *H. pylori* infection between patients with Barrett's oesophagus and control subjects (taken from blood donating populations and subjects with normal findings on endoscopy). When patients with Barrett's oesophagus were compared to those with normal endoscopy only, Barrett's oesophagus was associated with lower rate of *H. pylori* infection. With further data available, Fischbach *et al*[13] found that there was a strong negative association between the presence of *H. pylori* and Barrett's oesophagus. There were a smaller number of studies which examined the effect of virulent Cag A positive *H. pylori* with similar results.

Hiatus hernia: Andrici *et al*[14] examined the relationship between Barrett's oesophagus and hiatus hernia. Barrett's oesophagus was strongly associated with the presence of hiatus hernia compared to all controls, a significant association when compared to the control group of patients with gastro-oesophageal reflux disease and stronger association compared to control subjects without gastro-oesophageal reflux disease. The relationship was stronger for long segment Barrett's oesophagus than for short segment Barrett's oesophagus.

Pattern of proton pump inhibitor usage: There were 2 studies reported in the analysis of Hungin *et al*[15], but this was not undertaken as a meta-analysis. They analysed medication possession rates in patient with Barrett's oesophagus to those with gastro-oesophageal reflux disease and demonstrated higher adherence in those with Barrett's oesophagus. The self-reported adherence was also higher in patients with Barrett's oesophagus than subjects with gastro-oesophageal reflux disease in one of the included studies.

Comparison of different groups of patients with Barrett's oesophagus with regard to cancer risk

There were 12 studies which examined for differences in adenocarcinoma incidence in different groups of patients with Barrett's oesophagus. These studies looked at treatment for control of gastro-oesophageal reflux, endoscopic ablation of the metaplastic segment, demographic factors, segment length, dysplasia, enzyme polymorphisms, infection with *H. pylori* and drugs taken for other conditions. Eight of these studies contained meta-analyses (Table 3).

Treatment of gastro-oesophageal reflux and endoscopic ablation

Corey *et al*[16] examined the question of whether a surgical antireflux procedure was of benefit in reducing cancer risk in patients with Barrett's oesophagus. The cancer incidence was not significantly different between medical and surgical therapy and when the earlier medical cohorts were excluded (those prior to the proton-pump era), the cancer incidence in the medical group remained similar (0.43% per annum) to patients treated with antireflux surgery.

Li *et al*[17] examined randomized controlled trials of medical, surgical and endoscopic therapy for Barrett's oesophagus. There was one study of medical *vs* surgical therapy[18] which showed no significant difference in cancer incidence between patients treated by medical and surgical therapy (5% and 3% respectively), however there was a significantly lower risk of dysplasia development in the surgical arm (2%) compared to the medical arm (20%). There were three studies included of endo-

Table 3 Meta-analyses comparing cancer risk in different groups of patients with Barrett's oesophagus

Subject	Ref.	Comparison	Group	Studies	Results	Outcome
Medical *vs* surgical treatment of reflux	Corey *et al*[16]	Antireflux surgery *vs* medical treatment	Antireflux surgery	34	18 cancers/4678 patient-years (0.38% per annum)	No significant difference in cancer risk between medical and surgical antireflux therapy
			Medical therapy		26 cancers/4906 patient-years (0.53% per annum)	
Endoscopic ablative therapy *vs* surveillance	Wani *et al*[25]	Non-dysplastic Barrett's oesophagus	Surveillance	45	5.98/1000 patient-years	Endoscopic ablative therapy is effective in reducing adenocarcinoma risk in patients with non-dysplastic Barrett's oesophagus, low-grade dysplasia and high-grade dysplasia compared to surveillance alone
			Endoscopic ablative therapy	49	1.63/1000 patient-years	
		Low-grade dysplasia	Surveillance	16	16.98/1000 patient-years	
			Endoscopic ablative therapy	21	1.58/1000 patient-years	
		High-grade dysplasia	Surveillance	4	65.8/1000 patient-years	
			Endoscopic ablative therapy	28	16.76/1000 patient-years	
Demographic factors	Thomas *et al*[26]	Location	United Kingdom	13	7/1000 patient-years	Cancer incidence similar in all geographic areas
			United States	16	7/1000 patient years	
			Europe	10	8/1000 patient-years	
			Australia and New-Zealand	2	5/1000 patient-years	
	Yousef *et al*[27]	Gender	Males	6	10.2/1000 patient-years	Cancer incidence in males is double the rate in females
			Females	5	4.5/1000 patient-years	
Segment length	Thomas *et al*[26]	Segment length	Short segment	6	2.8/1000 patient-years	Trend for lower risk in short segment Barrett's oesophagus (P = 0.25)
			Long segment	6	7.8/1000 patient-years	
	Yousef *et al*[27]	Segment length	Short segment	6	6.1/1000 patient-years	Similar risk in short and long segment disease
			Long segment	26	6.7/1000 patient-years	
Dysplasia	Thomas *et al*[26]	Low-grade dysplasia as a confounding factor	Presence of low-grade dysplasia at index endoscopy	15	P = 0.23	No significant confounding effect on cancer incidence in meta-regression analysis
Helicobacter pylori	Rokkas *et al*[30]	All *Helicobacter pylori*	Cases	10	253/757 (34.3%)	*Helicobacter pylori* associated with lower rate of oesophageal cancer OR, 0.52; (95%CI: 0.37, 0.73)
			Controls	10	1398/2788 (50.1%)	
		Cag A *Helicobacter pylori*	Cases	6	120/462 (26%)	Cag A *Helicobacter pylori* associated with lower rate of oesophageal cancer OR, 0.51; (95%CI: 0.31, 0.82)
			Controls	6	774/1936 (40%)	
Non-steroidal Anti-inflammatory drugs	Wang *et al*[31]	Aspirin and NSAIDs *vs* controls		3	RR 0.64 (95%CI: 0.42, 0.96)	Lower risk of adenocarcinoma in patients taking aspirin or NSAIDs
Statins	Alexandre *et al*[33]	Statins *vs* controls		2	RR, 0.53 (95%CI: 0.36, 0.78)	Protective effect of statins *vs* controls
	Singh *et al*[36]			5	RR, 0.57; (95%CI: 0.44, 0.75)	
Statins and NSAIDs	Singh *et al*[36]	Combined statins and NSAIDs *vs* neither		2	0.28; (95%CI: 0.14, 0.56)	Protective effect of NSAIDs and statins higher than either individually

NSAIDs: Nonsteroidal antiinflammatory drugs; OR: Odds ratio; CI: Confidence interval.

scopic ablative therapy *vs* medical therapy for patients with dysplasia. The studies were heterogenous in their designs and outcome measures. Photodynamic therapy was superior to PPI in reducing the area of Barrett's epithelium[19] and eradication of dysplasia in patients with low-grade dysplasia[20] and high-grade dysplasia[20]. Overholt *et al*[20,21] also showed a lower rate of progression of high-grade dysplasia to cancer in the PDT group. There was one study[22] comparing endoscopic ablation of the metaplastic mucosa (with argon plasma coagulation) after antireflux surgery and showed a trend for superior endoscopic regression of the Barrett's segment after the

ablation, but no difference in cancer incidence[20]. In ablation of the metaplastic mucosa, 3 studies demonstrated that overall argon plasma coagulation was superior to photodynamic therapy with ablation rates of 59.0% and 27.5% respectively [odds ratio (OR), 3.46, 95% confidence interval (CI): 1.67, 7.81]. These studies did not examine long-term cancer incidences. There were 2 studies comparing argon plasma coagulation to multipolar electrocoagulation which demonstrated similar rates of successful ablation of the metaplastic segment (78.6% in patients treated with multipolar electrocoagulation and 64.4% treated with argon plasma coagulation) and again no long-term data on cancer incidence.

Fayter *et al*[23]examined the evidence from 11 randomised controlled trials of photodynamic therapy for Barrett's oesophagus. The trials were heterogeneous in their design, the protocol of therapy used, the patients studied (most studies examined patients with high-grade dysplasia, but some had low-grade dysplasia, non-dysplastic epithelium or a combination of histological findings) and outcome measures. The conclusions drawn from this systematic review were: (1) it was not possible to determine whether there was a significant clinical difference between photodynamic therapy and argon plasma coagulation and which would be the most appropriate treatment; (2) photodynamic therapy was more effective than omeprazole alone in producing long-term ablation of high-grade dysplasia and slowing/preventing progression to cancer; (3) Photodynamic therapy with 5-ALA as the photosensitising agent was more effective than placebo in producing regression of dysplasia and reduction in the area of Barrett's epithelium in patients with low-grade dysplasia; (4) photodynamic therapy with 5-ALA may be more effective than with Photofrin; (5) optimal treatment for patients without dysplasia had yet to be determined; and (6) side effects were similar between 5-ALA and Photofrin with higher levels of photosensitivity with Photofrin.

Rees *et al*[24]examined randomized controlled studies only. They demonstrated that in the 3 studies which examined H_2 receptor antagonists to proton pump inhibitors: cancer risk, eradication of dysplasia or complete regression of the metaplastic segment were not reported. There was a trend towards a reduction in the areas of metaplastic mucosa (but not the length of the Barretts' segment) with PPI. There were no new studies available on antireflux surgery *vs* medical therapy, argon plasma ablation, argon plasma coagulation *vs* multipolar electrocoagulation or argon plasma coagulation *vs* photodynamic therapy since Li *et al*[17].

Wani *et al*[25] compared the rate of development of adenocarcinoma in published series of patients with non-dysplastic Barrett's oesophagus, low-grade dysplasia and high-grade dysplasia comparing cohorts treated with endoscopic ablative therapy to those in surveillance programmes without ablation of the mucosa. They found that there were significantly lower rates of adenocarcinoma incidence in the cohorts treated with ablative therapies compared to the control cohorts. The differences were significant for examinations of non-dysplastic Barrett's oesophagus, low-grade dysplasia and high-grade dysplasia.

Demographic factors: Thomas *et al*[26] showed that age did not influence cancer risk from 41 studies of 9469 patients undergoing surveillance (36635 patient-years follow-up). There was also no significant difference in cancer incidence depending on geographic origin of the included studies. Yousef *et al*[27] showed that the incidence of adenocarcinoma in males was double the rate in females.

Segment length: Thomas *et al*[26] showed that from 6 studies including 960 patients with long-segment Barrett's oesophagus (4130 patient-years of follow-up) and 258 patients (1074 patient-years of follow up) that 32 of the 35 cancers which developed were in long segment Barrett's oesophagus (but this did not reach statistical significance). Yousef *et al*[27] reported a cancer incidence of 0.67% per annum in long segment Barrett's oesophagus and a similar incidence (0.61%) in short segment Barrett's oesophagus in 30 studies.

Dysplasia: Thomas *et al*[26] did not demonstrate an increased cancer risk associated with dysplasia over non-dysplastic Barrett's oesophagus. Desai *et al*[28] examined specifically patients without dysplasia at baseline, but there was no comparison cohort in this study and it has subsequently been excluded from this review.

Intestinal metaplasia: The question of the importance of intestinal metaplasia for cancer risk was not specifically examined by any of the meta-analyses.

Enzyme polymorphisms: Bull *et al*[29] examined enzyme polymorphisms in case-control studies and found an association between Barrett's oesophagus and GSTP1 homozygotes for the Ile105 variant (OR, 1.50, 95%CI: 1.16, 1.95). This genetic variant results in increased IgE and immune-mediated inflammation. There was no other significant association with Barrett's oesophagus and a variety of metabolic gene polymorphisms[29].

***Helicobacter pylori*:** Rokkas *et al*[30] showed similar results in studies of oesophageal cancer to those of Barrett's oesophagus with a negative association between the presence of *H. pylori* and oesophageal cancer. The results were similar in studies of Cag A *H. pylori*.

Other medications

The reduction in cancer risk with aspirin and non-steroidal anti-inflammatory drugs was examined in 3 cohort studies by Wang *et al*[31]. They demonstrated that there was a trend towards lower cancer risk in patients taking aspirin and non-steroidal anti-inflammatory drugs, however 2 case-control studies were excluded for unclear

reasons.

Rees *et al*[24] reported one study[32] comparing celecoxib to placebo and found no difference in cancer risk at 2 years (3/49 and 3/51 patients respectively).

The effects of statins on the risk of oesophageal adenocarcinoma in Barrett's oesophagus were examined by Alexandre *et al*[33], who found two prospective cohort studies. The first was a multicentre study from the Netherlands of 570 patients and demonstrated a hazard ratio of 0.46 (95%CI: 0.21, 0.99) and in patients taking statins and non-steroidal anti-inflammatory drugs the hazards ratio was 0.22 (95%CI: 0.06, 0.85)[34]. Nguyen *et al*[35] examined 812 patients in a case-control cohort in the Veterans Affairs Healthcare System and showed an incidence density ratio of 0.56 (95%CI: 0.36, 0.86) for patients with Barrett's oesophagus taking statins.

Singh *et al*[36] also demonstrated a protective effect of statins in their meta-analysis of 5 studies and a greater protective effect of combining statins with non-steroidal anti-inflammatory drugs with respect to oesophageal cancer risk.

Decision to enrol in surveillance

The American College of Gastroenterology and American Gastroenterological Association have defined Barrett's oesophagus as any length of recognisable columnar mucosa which demonstrates intestinal metaplasia at biopsy[3,4], maintaining the dogma that intestinal metaplasia is necessary for malignant risk on the basis that in many cohort studies intestinal metaplasia has been demonstrated adjacent to adenocarcinoma of the oesophagus. The ACG acknowledge the difficulties associated with sampling error in the detection of intestinal metaplasia and also exclude "ultra-short" segments (< 1 cm) due to poor interobserver reliability of recognition. The BSG broadly agrees with this definition[2] and whilst there is no requirement for the presence of intestinal metaplasia for diagnosis, on the basis of the higher cancer risk in subjects with intestinal metaplasia in the Northern Ireland pathology database cohort[37] and low rate of development of high-grade dysplasia and adenocarcinoma in the Danish pathology database cohort which only included subjects with intestinal metaplasia[38], surveillance is only recommended if intestinal metaplasia is detected during the either the index or the first surveillance endoscopy in patients with short segment (< 3 cm) metaplasia. The rationale for this is that it is felt that the risks of endoscopy probably outweigh the benefits. Both guidelines have excluded very short segments or tongues of metaplasia due to difficulties in clinical assessment rather than on the basis of a proven low risk of complications and there are no good data to support or refute these assertions. The evidence from meta-analyses concerning the role of segment length and intestinal metaplasia is discussed below.

The ACG recommend that the consideration for beginning a surveillance programme should include age, likelihood of survival over the next 5 years, patient's understanding of the process and its limitations for the detection of cancer and the willingness of the patient to adhere to the recommendations.

The ACG supports surveillance of Barrett's oesophagus as in 7 retrospective series the survival in cancers was improved over those detected outside of surveillance programmes. There has not yet been a trial published demonstrating benefits of surveillance in a prospective fashion, however the BOSS study (endoscopic surveillance *vs* endoscopy at time of need) remains underway at present[39].

The ACG, AGA and BSG recommend 4-quadrant biopsies taken every 2 cm throughout the metaplastic segment at index endoscopy and surveillance (if no dysplasia has been previously detected or other macroscopic lesions are present). This biopsy protocol has not yet been tested in a meta-analysis. The difficulties involved in adequately sampling the tissue at risk and variability in histopathological interpretation of the tissue examined should be subject to further studies beyond the initial work done by Levine *et al*[40].

Risk stratification and frequency of surveillance

The ACG recommend that the first two endoscopies are undertaken within a year and if no dysplasia is detected then the surveillance interval is 3 years. If low-grade dysplasia is detected then surveillance interval should be within 6 mo. This recommendation was based upon a poor level of evidence from cohort studies and expert opinion[3].

The BSG note that risk factors for cancer development include the presence of intestinal metaplasia (3 × compared to no intestinal metaplasia), low-grade dysplasia (5.67 × non-dysplastic Barrett's oesophagus), male gender (2 × that of females), smoking (2 × non-smokers). They note that longer segment lengths were associated with a trend to increased risk and no relationship was demonstrated with alcohol consumption and obesity[2].

The ACG and AGA stratify risk based only on the presence of dysplasia after the diagnosis of Barrett's oesophagus and that further work to assess the extent of dysplasia and develop biomarkers is required[3,4]. The BSG note that in future, surveillance intervals will take into account all of the socio-demographic risk factors and characteristics of the Barrett's segment as well as biomarker panels[2] . Until such algorithms are developed, surveillance frequency is based on dysplasia and length only. The ACG also note that a randomised controlled trial to assess the impact of surveillance is required. The BSG also incorporate segment length and allow for consideration of other risk factors (see above)[2]. The BSG have lengthened the recommended surveillance interval for non-dysplastic Barrett's oesophagus (based upon the recent lower cancer incidence estimates) in line with the AGA and allowed some further individualised risk stratification to be incorporated into the frequency of surveillance and in line with the ACG, the interval for low-

grade dysplasia is 6 mo. The AGA recommend surveillance of low-grade dysplasia in 6-12 mo. Inflammatory atypia is difficult to distinguish from true dysplasia[2,41] and the guidelines recommend repeat biopsy after treatment with acid suppression[3] and expert pathological review of biopsies which are dysplastic or have changes indefinite for dysplasia[2-4].

The evidence from the meta-analyses in supporting intestinal metaplasia and low-grade dysplasia as markers of increased risk of malignancy is poor with no significant difference demonstrated in patients with low-grade dysplasia at index endoscopy[26] and no papers on the necessity for the detection of intestinal metaplasia to confer a malignant risk.

Evidence for difference in risk dependent on segment length is also poor with only trends demonstrated[26,27] and it is only on weak evidence that decisions on consideration of surveillance as well as surveillance interval are made on these features.

There is greater evidence for a lower risk of oesophageal cancer development in patients who have *H, pylori* infection[30] and for a higher risk in males over females[27].

What steps to minimise risk of developing Barrett's

The ACG notes that older Caucasian males with chronic reflux symptoms are the group with the highest prevalence of Barrett's oesophagus and there were no direct recommendations from the ACG to reduce the risk of development of Barrett's oesophagus[3]. The BSG state that the known risk factors are male gender, older age and history of reflux symptoms as well as an association with white race, higher waist: hip ration and abdominal circumference. There is a less clear relationship with obesity as measured by body mass index and cigarette smoking. The BSG also note the small degree of familial clustering[2]. The AGA go one step further in recommending consideration of screening for Barrett's oesophagus in patients with multiple risk factors for oesophageal adenocarcinoma (age 50 years or older, male sex, white race, chronic gastro-oesophageal reflux disease, hiatal hernia, elevated body mass index and intra-abdominal distribution of body fat).

The published meta-analyses have demonstrated that the significant risk factors associated with Barrett's oesophagus are male gender[6], smoking[7], obesity[8-10], prolonged symptoms of gastro-oesophageal reflux[11], absence of *H. pylori* infection[12,13] and the presence of hiatus hernia[14]. Age has not been demonstrated to influence cancer risk in the meta-analyses[26].

Minimisation of risk of cancer development in Barrett's

The ACG, AGA and BSG did not recommend fundoplication over medical therapy to reduce cancer development[2-4] and this review supports this strategy[16,17], however there were encouraging data concerning reduction in risk of development of dysplasia with surgical therapy over acid suppression therapy[18].

The question of ablation of the metaplastic mucosa is a complex one requiring further examination, however there are promising results[25] and the SURF trial comparing radiofrequency ablation to surveillance in low-grade dysplasia remains underway[42].

The ACG note that a meta-analysis did demonstrate a lower risk of cancer development in patients taking non-steroidal anti-inflammatory drugs[43] and that the ASPECT study (a randomised study of aspirin and low and high-dose esomeprazole) remains underway[44]. The ACG, AGA and BSG did not recommend chemoprevention with aspirin or non-steroidal anti-inflammatory drugs. The ACG cites two cohort studies demonstrating a lower risk of dysplasia development in patients taking PPI therapy, but no evidence to support a reduction in cancer development[3]. The BSG recommendations are similar to those of the ACG and also do not advocate acid suppression drugs as chemopreventive agents[2], but they are effective in symptom control.

The AGA note that the patients may derive benefit from aspirin if they have cardiovascular risk factors for which aspirin therapy is indicated, but that neither the use of aspirin or non-steroidal anti-inflammatory drugs are recommended solely to prevent oesophageal adenocarcinoma and that the evidence to support the use of PPI therapy to reduce the risk of cancer and dysplasia is indirect and not been proven in a long-term controlled trial[4].The results from the meta-analyses of Alexandre *et al*[33] and Singh *et al*[36] showing the protective effect of aspirin and in particular the effect in conjunction with statins is exciting and may form the basis for effective chemoprevention in the future.

CONCLUSION

The evidence to support the current decisions to enrol patients with Barrett's oesophagus in surveillance programmes and surveillance interval are based on weak evidence on the clinical outcome of features of the metaplastic segment. Further consideration should be given to the role of gender and helicobacter status in examining cancer risk as well as the role of aspirin and statins in chemopreventive strategies and ablation of the metaplastic segment. Public health programmes should also examine measures to reduce the associations of Barrett's oesophagus, notably, smoking and obesity. The relevance of male gender and absence of helicobacter infection should also be considered.

REFERENCES

1 **Gatenby PA**, Caygill CP, Ramus JR, Charlett A, Watson A. Barrett's columnar-lined oesophagus: demographic and lifestyle associations and adenocarcinoma risk. *Dig Dis Sci* 2008; **53**: 1175-1185 [PMID: 17939050]

2 **Fitzgerald RC**, di Pietro M, Ragunath K, Ang Y, Kang JY, Watson P, Trudgill N, Patel P, Kaye PV, Sanders S, O'Donovan M, Bird-Lieberman E, Bhandari P, Jankowski JA, Attwood S, Parsons SL, Loft D, Lagergren J, Moayyedi P, Lyratzopoulos G, de Caestecker J. British Society of Gastro-

enterology guidelines on the diagnosis and management of Barrett's oesophagus. *Gut* 2014; **63**: 7-42 [PMID: 24165758 DOI: 10.1136/gutjnl-2013-305372]
3 **Wang KK**, Sampliner RE. Updated guidelines 2008 for the diagnosis, surveillance and therapy of Barrett's esophagus. *Am J Gastroenterol* 2008; **103**: 788-797 [PMID: 18341497]
4 **Spechler SJ**, Sharma P, Souza RF, Inadomi JM, Shaheen NJ. American Gastroenterological Association medical position statement on the management of Barrett's esophagus. *Gastroenterology* 2011; **140**: 1084-1091 [PMID: 21376940 DOI: 10.1053/j.gastro.2011.01.030]
5 **Andrici J**, Tio M, Cox MR, Eslick GD. Meta-analysis: Barrett's oesophagus and the risk of colonic tumours. *Aliment Pharmacol Ther* 2013; **37**: 401-410 [PMID: 23163592 DOI: 10.1111/apt.12146]
6 **Cook MB**, Wild CP, Forman D. A systematic review and meta-analysis of the sex ratio for Barrett's esophagus, erosive reflux disease, and nonerosive reflux disease. *Am J Epidemiol* 2005; **162**: 1050-1061 [PMID: 16221805 DOI: 10.1093/aje/kwi325]
7 **Andrici J**, Cox MR, Eslick GD. Cigarette smoking and the risk of Barrett's esophagus: a systematic review and meta-analysis. *J Gastroenterol Hepatol* 2013; **28**: 1258-1273 [PMID: 23611750 DOI: 10.1111/jgh.12230]
8 **Cook MB**, Greenwood DC, Hardie LJ, Wild CP, Forman D. A systematic review and meta-analysis of the risk of increasing adiposity on Barrett's esophagus. *Am J Gastroenterol* 2008; **103**: 292-300 [PMID: 17986313 DOI: 10.1111/j.1572-0241.2007.01621.x]
9 **Kamat P**, Wen S, Morris J, Anandasabapathy S. Exploring the association between elevated body mass index and Barrett's esophagus: a systematic review and meta-analysis. *Ann Thorac Surg* 2009; **87**: 655-662 [PMID: 19161814 DOI: 10.1016/j.athoracsur.2008.08.003]
10 **Kubo A**, Cook MB, Shaheen NJ, Vaughan TL, Whiteman DC, Murray L, Corley DA. Sex-specific associations between body mass index, waist circumference and the risk of Barrett's oesophagus: a pooled analysis from the international BEACON consortium. *Gut* 2013; **62**: 1684-1691 [PMID: 23355549 DOI: 10.1136/gutjnl-2012-303753]
11 **Taylor JB**, Rubenstein JH. Meta-analyses of the effect of symptoms of gastroesophageal reflux on the risk of Barrett's esophagus. *Am J Gastroenterol* 2010; **105**: 1729, 1730-1737; quiz 1738 [PMID: 20485283 DOI: 10.1038/ajg.2010.194]
12 **Wang C**, Yuan Y, Hunt RH. Helicobacter pylori infection and Barrett's esophagus: a systematic review and meta-analysis. *Am J Gastroenterol* 2009; **104**: 492-500; quiz 491, 501 [PMID: 19174811 DOI: 10.1038/ajg.2008.37]
13 **Fischbach LA**, Nordenstedt H, Kramer JR, Gandhi S, Dick-Onuoha S, Lewis A, El-Serag HB. The association between Barrett's esophagus and Helicobacter pylori infection: a meta-analysis. *Helicobacter* 2012; **17**: 163-175 [PMID: 22515353 DOI: 10.1111/j.1523-5378.2011.00931.x]
14 **Andrici J**, Tio M, Cox MR, Eslick GD. Hiatal hernia and the risk of Barrett's esophagus. *J Gastroenterol Hepatol* 2013; **28**: 415-431 [PMID: 22694245 DOI: 10.1111/j.1440-1746.2012.07199.x]
15 **Hungin AP**, Hill C, Molloy-Bland M, Raghunath A. Systematic review: Patterns of proton pump inhibitor use and adherence in gastroesophageal reflux disease. *Clin Gastroenterol Hepatol* 2012; **10**: 109-116 [PMID: 21782770 DOI: 10.1016/j.cgh.2011.07.008]
16 **Corey KE**, Schmitz SM, Shaheen NJ. Does a surgical antireflux procedure decrease the incidence of esophageal adenocarcinoma in Barrett's esophagus? A meta-analysis. *Am J Gastroenterol* 2003; **98**: 2390-2394 [PMID: 14638338]
17 **Li YM**, Li L, Yu CH, Liu YS, Xu CF. A systematic review and meta-analysis of the treatment for Barrett's esophagus. *Dig Dis Sci* 2008; **53**: 2837-2846 [PMID: 18427992 DOI: 10.1007/s10620-008-0257-3]
18 **Parrilla P**, Martínez de Haro LF, Ortiz A, Munitiz V, Molina J, Bermejo J, Canteras M. Long-term results of a randomized prospective study comparing medical and surgical treatment of Barrett's esophagus. *Ann Surg* 2003; **237**: 291-298 [PMID: 12616111 DOI: 10.1097/00000658-200303000-00001]
19 **Ackroyd R,** Tam W, Schoeman M, Devitt P, Watson D. Prospective randomized controlled trial of argon plasma coagulation ablation vs. endoscopic surveillance of patients with Barrett's esophagus after antireflux surgery. *Gastrointest Endosc* 2004; **59**: 1-7 [DOI: 10.1016/S0016-5107(03)02528-8]
20 **Overholt BF,** Lightdale CJ, Wang KK, Canto MI, Burdick S, Haggitt RC, Bronner MP, Taylor SL, Grace MGA, Depot M, [On behalf of the International Photodynamic Group for High-Grade Dysplasia in Barrett's Esophagus]. Photodynamic therapy with porfimer sodium for ablation of high-grade dysplasia in Barrett's esophagus: international, partially blinded, randomized phase III trial. *Gastrointestinal Endoscopy* 2005; **62**: 488-498 [DOI: 10.1016/j.gie.2005.06.047]
21 **Overholt BF**, Wang KK, Burdick JS, Lightdale CJ, Kimmey M, Nava HR, Sivak MV, Nishioka N, Barr H, Marcon N, Pedrosa M, Bronner MP, Grace M, Depot M. Five-year efficacy and safety of photodynamic therapy with Photofrin in Barrett's high-grade dysplasia. *Gastrointest Endosc* 2007; **66**: 460-468 [PMID: 17643436 DOI: 10.1016/j.gie.2006.12.037]
22 **Bright T**, Watson DI, Tam W, Game PA, Astill D, Ackroyd R, Wijnhoven BP, Devitt PG, Schoeman MN. Randomized trial of argon plasma coagulation versus endoscopic surveillance for barrett esophagus after antireflux surgery: late results. *Ann Surg* 2007; **246**: 1016-1020 [PMID: 18043104 DOI: 10.1097/SLA.0b013e318133fa85]
23 **Fayter D**, Corbett M, Heirs M, Fox D, Eastwood A. A systematic review of photodynamic therapy in the treatment of pre-cancerous skin conditions, Barrett's oesophagus and cancers of the biliary tract, brain, head and neck, lung, oesophagus and skin. *Health Technol Assess* 2010; **14**: 1-288 [PMID: 20663420 DOI: 10.3310/hta14370]
24 **Rees JR**, Lao-Sirieix P, Wong A, Fitzgerald RC. Treatment for Barrett's oesophagus. *Cochrane Database Syst Rev* 2010; **(1)**: CD004060 [PMID: 20091557 DOI: 10.1002/14651858.CD004060.pub2]
25 **Wani S**, Puli SR, Shaheen NJ, Westhoff B, Slehria S, Bansal A, Rastogi A, Sayana H, Sharma P. Esophageal adenocarcinoma in Barrett's esophagus after endoscopic ablative therapy: a meta-analysis and systematic review. *Am J Gastroenterol* 2009; **104**: 502-513 [PMID: 19174812 DOI: 10.1038/ajg.2008.31]
26 **Thomas T**, Abrams KR, De Caestecker JS, Robinson RJ. Meta analysis: Cancer risk in Barrett's oesophagus. *Aliment Pharmacol Ther* 2007; **26**: 1465-1477 [PMID: 17900269]
27 **Yousef F**, Cardwell C, Cantwell MM, Galway K, Johnston BT, Murray L. The incidence of esophageal cancer and high-grade dysplasia in Barrett's esophagus: a systematic review and meta-analysis. *Am J Epidemiol* 2008; **168**: 237-249 [PMID: 18550563 DOI: 10.1093/aje/kwn121]
28 **Desai TK**, Krishnan K, Samala N, Singh J, Cluley J, Perla S, Howden CW. The incidence of oesophageal adenocarcinoma in non-dysplastic Barrett's oesophagus: a meta-analysis. *Gut* 2012; **61**: 970-976 [PMID: 21997553 DOI: 10.1136/gutjnl-2011-300730]
29 **Bull LM**, White DL, Bray M, Nurgalieva Z, El-Serag HB. Phase I and II enzyme polymorphisms as risk factors for Barrett's esophagus and esophageal adenocarcinoma: a systematic review and meta-analysis. *Dis Esophagus* 2009; **22**: 571-587 [PMID: 19222528 DOI: 10.1111/j.1442-2050.2009.00947.x]
30 **Rokkas T**, Pistiolas D, Sechopoulos P, Robotis I, Margantinis G. Relationship between Helicobacter pylori infection and esophageal neoplasia: a meta-analysis. *Clin Gastroenterol Hepatol* 2007; **5**: 1413-1417, 1413-1417 [PMID: 17997357 DOI: 10.1016/j.cgh.2007.08.010]
31 **Wang F,** Lv ZS, Fu YK. Nonsteroidal anti-inflammatory drugs

and esophageal inflammation - Barrett's esophagus - adenocarcinoma sequence: a meta-analysis. *Dis Esophagus* 2010 Dec 17; Epub ahead of print [PMID: 21166737 DOI: 10.1111/j.1442-2050.2010.01153.x]

32 **Heath EI**, Canto MI, Piantadosi S, Montgomery E, Weinstein WM, Herman JG, Dannenberg AJ, Yang VW, Shar AO, Hawk E, Forastiere AA. Secondary chemoprevention of Barrett's esophagus with celecoxib: results of a randomized trial. *J Natl Cancer Inst* 2007; **99**: 545-557 [PMID: 17405999 DOI: 10.1093/jnci/djk112]

33 **Alexandre L**, Clark AB, Cheong E, Lewis MP, Hart AR. Systematic review: potential preventive effects of statins against oesophageal adenocarcinoma. *Aliment Pharmacol Ther* 2012; **36**: 301-311 [PMID: 22716127 DOI: 10.1111/j.1365-2036.2012.05194.x]

34 **Kastelein F**, Spaander MC, Biermann K, Steyerberg EW, Kuipers EJ, Bruno MJ. Nonsteroidal anti-inflammatory drugs and statins have chemopreventative effects in patients with Barrett's esophagus. *Gastroenterology* 2011; **141**: 2000-2008; quiz 2000-2008 [PMID: 21878200 DOI: 10.1053/j.gastro.2011.08.036]

35 **Nguyen DM**, Richardson P, El-Serag HB. Medications (NSAIDs, statins, proton pump inhibitors) and the risk of esophageal adenocarcinoma in patients with Barrett's esophagus. *Gastroenterology* 2010; **138**: 2260-2266 [PMID: 20188100 DOI: 10.1053/j.gastro.2010.02.045]

36 **Singh S**, Singh AG, Singh PP, Murad MH, Iyer PG. Statins are associated with reduced risk of esophageal cancer, particularly in patients with Barrett's esophagus: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2013; **11**: 620-629 [PMID: 23357487 DOI: 10.1016/j.cgh.2012.12.036]

37 **Bhat S**, Coleman HG, Yousef F, Johnston BT, McManus DT, Gavin AT, Murray LJ. Risk of malignant progression in Barrett's esophagus patients: results from a large population-based study. *J Natl Cancer Inst* 2011; **103**: 1049-1057 [PMID: 21680910 DOI: 10.1093/jnci/djr203]

38 **Hvid-Jensen F**, Pedersen L, Drewes AM, Sørensen HT, Funch-Jensen P. Incidence of adenocarcinoma among patients with Barrett's esophagus. *N Engl J Med* 2011; **365**: 1375-1383 [PMID: 21995385 DOI: 10.1056/NEJMoa1103042]

39 **Jankowski J**, Barr H. Improving surveillance for Barrett's oesophagus: AspECT and BOSS trials provide an evidence base. *BMJ* 2006; **332**: 1512 [PMID: 16793832 DOI: 10.1136/bmj.332.7556.1512]

40 **Levine DS**, Blount PL, Rudolph RE, Reid BJ. Safety of a systematic endoscopic biopsy protocol in patients with Barrett's esophagus. *Am J Gastroenterol* 2000; **95**: 1152-1157 [PMID: 10811320 DOI: 10.1111/j.1572-0241.2000.02002.x]

41 **Gatenby P**, Ramus J, Caygill C, Shepherd N, Winslet M, Watson A. Routinely diagnosed low-grade dysplasia in Barrett's oesophagus: a population-based study of natural history. *Histopathology* 2009; **54**: 814-819 [PMID: 19635100 DOI: 10.1111/j.1365-2559.2009.03316.x]

42 **Alvarez Herrero L**, van Vilsteren FG, Pouw RE, ten Kate FJ, Visser M, Seldenrijk CA, van Berge Henegouwen MI, Fockens P, Weusten BL, Bergman JJ. Endoscopic radiofrequency ablation combined with endoscopic resection for early neoplasia in Barrett's esophagus longer than 10 cm. *Gastrointest Endosc* 2011; **73**: 682-690 [PMID: 21292262 DOI: 10.1016/j.gie.2010.11.016]

43 **Corley DA**, Kerlikowske K, Verma R, Buffler P. Protective association of aspirin/NSAIDs and esophageal cancer: a systematic review and meta-analysis. *Gastroenterology* 2003; **124**: 47-56 [PMID: 12512029]

44 **Jankowski J**, Moayyedi P. Re: Cost-effectiveness of aspirin chemoprevention for Barrett's esophagus. *J Natl Cancer Inst* 2004; **96**: 885-887; author reply 887 [PMID: 15173278]

P- Reviewer: Durand L, Rippe RA, Van Rensburg C
S- Editor: Ji FF **L- Editor**: A **E- Editor:** Lu YJ

World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4291/wjgp.v5.i3.188

World J Gastrointest Pathophysiol 2014 August 15; 5(3): 188-199
ISSN 2150-5330 (online)

TOPIC HIGHLIGHT

WJGP 5th Anniversary Special Issues (5): Cholangiocarcinoma

Review to better understand the macroscopic subtypes and histogenesis of intrahepatic cholangiocarcinoma

Yuichi Sanada, Yujo Kawashita, Satomi Okada, Takashi Azuma, Shigetoshi Matsuo

Yuichi Sanada, Yujo Kawashita, Satomi Okada, Takashi Azuma, Shigetoshi Matsuo, Department of Surgery, Nagasaki Prefecture Shimabara Hospital, Nagasaki 8550861, Japan
Author contributions: Sanada Y contributed most of this review; Kawashita Y, Okada S, Azuma T and Matsuo S contributed equally to the figures.
Correspondence to: Dr. Yuichi Sanada, Department of Surgery, Nagasaki Prefecture Shimabara Hospital, 7895, Shimokawajiri, Shimabara, Nagasaki 8550861, Japan. ysanadasurg@hotmail.com
Telephone: +81-957-631145 Fax: +81-957-634864
Received: January 28, 2014 Revised: May 1, 2014
Accepted: May 28, 2014
Published online: August 15, 2014

Abstract

Intrahepatic cholangiocarcinoma is macroscopically classified into three subtypes, mass-forming-type, periductal infiltrating-type, and intraductal growth-type. Each subtype should be preoperatively differentiated to perform the valid surgical resection. Recent researches have revealed the clinical, radiologic, pathobiological characteristics of each subtype. We reviewed recently published studies covering various aspects of intrahepatic cholangiocarcinoma (ICC), focusing especially on the macroscopic subtypes and stem cell features to better understand the pathophysiology of ICC and to establish the valid therapeutic strategy.

Key words: Intrahepatic cholangiocarcinoma; Combined hepatocellular-cholangiocarcinoma; Hepatic progenitor cells; Macroscopic subtype

Core tip: We reviewed recently published studies covering various aspects of intrahepatic cholangiocarcinoma (ICC), focusing especially on the macroscopic subtypes and stem cell features to better understand the pathophysiology of ICC and to establish the valid therapeutic strategy.

Sanada Y, Kawashita Y, Okada S, Azuma T, Matsuo S. Review to better understand the macroscopic subtypes and histogenesis of intrahepatic cholangiocarcinoma. *World J Gastrointest Pathophysiol* 2014; 5(3): 188-199 Available from: URL: http://www.wjgnet.com/2150-5330/full/v5/i3/188.htm DOI: http://dx.doi.org/10.4291/wjgp.v5.i3.188

INTRODUCTION

The Liver Cancer Study Group of Japan has applied the same TNM staging system used for hepatocellular carcinoma (HCC) to that for intrahepatic cholangiocarcinoma (ICC)[1]. A recent increase in the number of surgically resected cases of ICC has clarified some characteristics inherent in this disease. The most prominent feature of ICC is that of the macroscopic findings reflecting its growth patterns. ICC is grossly classifiable into mass-forming (MF), periductal infiltrating (PI), and intraductal growth (IG) types[2]. The MF type presents as a gray to gray-white, firm and solid mass in the hepatic parenchyma, and of these three subtypes, MF-type ICC is the most common (59%). The PI type shows spreading of the carcinoma along the portal tracts with stricture of the central bile ducts and dilation of the peripheral bile ducts. The IG type presents as a papillary tumor within the dilated bile duct lumen. Some IG-type ICCs are considered to be an intraductal papillary neoplasm of the bile duct. This classification system provides useful information during surgery (Figure 1). For example, the efficacy of hilar resection is not emphasized except in the case of PI-type ICCs. This macroscopic classification cannot be applied to HCC. Therefore, studies focusing on the association of the macroscopic subtypes with biological behavior, clinical features, and radiologic

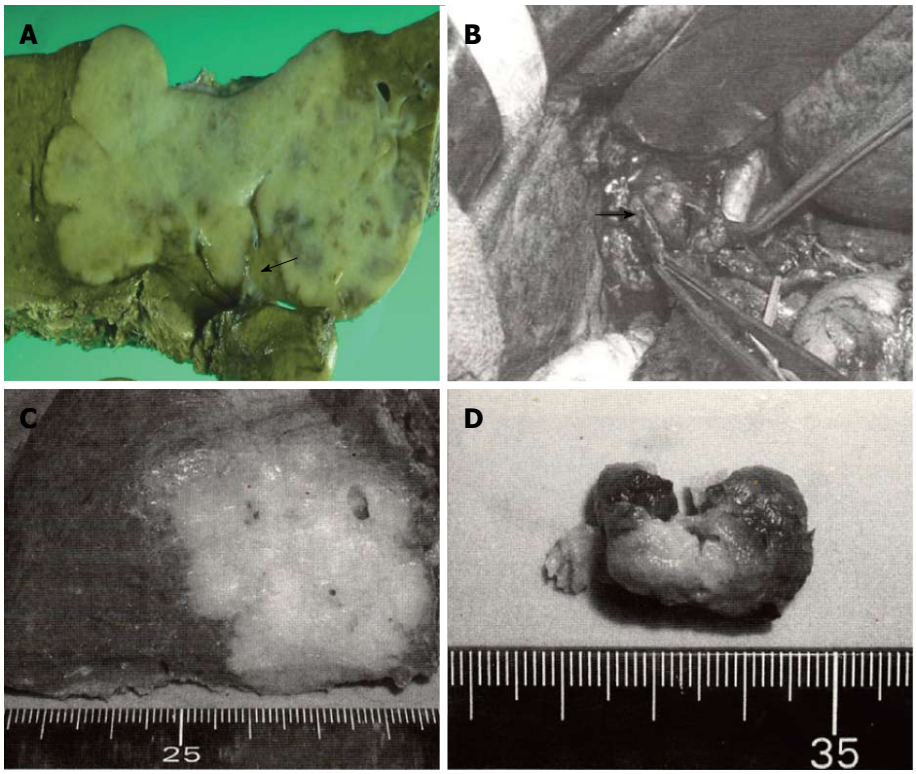

Figure 1 Some intraductal growth-type intrahepatic cholangiocarcinomas are considered to be an intraductal papillary neoplasm of the bile duct, this classification system provides useful information during surgery. A: Gross feature of mass-forming (MF) + periductal infiltrating (PI)-type intrahepatic cholangiocarcinoma (ICC) obtained by left hepatectomy with bile duct resection. The carcinoma spreads along the hilar biliary tree (arrow) in communication with a white firm mass; B-D: Operative findings and resected specimens of MF + intraductal growth (IG)-type ICC. The common hepatic duct is incised, and the soft tumor comprising the intraductal components is easily removed (arrow) without infiltration to the ductal wall; C: Hepatic anterior segmentectomy is performed for MF components; D: IG components are composed of tan-colored soft tissues with necrosis.

findings are needed to establish the therapeutic strategy for ICC. Although the macroscopic features are prominent in ICCs, another aspect of ICCs, in which ICCs cannot be discussed independently of other primary liver cancers, exists. Recent histopathologic and immunohistochemical studies have reported that hepatic progenitor cells (HPC) or stem cells play important roles in liver carcinogenesis including both HCCs and ICCs, supporting the hypothesis that HCCs and ICCs share a common evolutionary origin[3,4]. In 2010, the World Health Organization (WHO) established a new classification system of combined hepatocellular-cholangiocarcinoma (cHCC-CC) based on the presence of stem-cell features[5]. According to this new system, cHCC-CCs are classified into two major subtypes, classic type and subtypes with stem-cell features. Subtypes with stem-cell features are further subclassified into three types: typical type, intermediate-cell type, and cholangiocellular type. In addition, recent reports showed that some cases of HCCs and ICCs are associated with hepatic stem cells. However, little is known about the clinical significance of stem cells in ICCs. This review summarizes recently published studies (from 2011 to 2013) covering various aspects of ICC and cHCC-CC, focusing especially on the macroscopic subtypes and stem-cell features.

CLINICAL STUDIES OF ICC

Recent clinical researches of ICC are summarized in Tables 1 and 2[6-24]. The association between macroscopic subtypes and survival rate and lymph node metastasis has been discussed ever since the macroscopic subtype was established. IG-type ICCs have a favorable outcome because this tumor type shows intraductal growth without invasiveness[2]. Of the three subtypes, MF+PI-type ICCs have the highest incidence of lymph node metastasis (50% to 73%)[15] and are associated with the lowest 5-year survival rate (0% to 19.4%). PI-type and MF-type have relatively favorable outcomes when lymph node metastasis or hilar invasion is absent.

Over the most recent 3 years, 19 studies have been published (Tables 1 and 2). Most of these studies describe the poor prognostic factors of resected cases of ICC. The most significant prognostic factor is lymph node metastasis. However, whether routine lymph node dissection improves postoperative survival is still unclear.

The literature on the macroscopic subtypes is very scant. Uchiyama *et al*[15] and Uno *et al*[17] reported that the PI type showed significantly better survival than the MF and MF+PI types, supporting the results of previous reports. The difference in malignant potential between

Table 1 Clinical study of intrahepatic cholangiocarcinoma

Ref.	*n*	Survival rate (%)	MST (mo)	Prognostic factor
Marubashi *et al*[6]	111	59.7 (3 yr)	-	IM, Hilar inv, LN
Guglielmi *et al*[7]	145	-	19 (LN+), 42 (LN-)	LNR > 0.25, LN
Zhu *et al*[8]	37	-	-	CA19-9, Low prealbmin
Dhanasekaran *et al*[9]	105	-	16	V
Wang *et al*[10]	367	-	-	CEA, CA19-9, Size, V
De Rose *et al*[11]	79 (MF)	-	-	Doubling time < 70 d
Sulpice *et al*[12]	87	-	-	BT, Maj, Size, V, IM
Ribero *et al*[13]	434	39.8 (5 yr)	-	LN, CA19-9, IM
Liu *et al*[14]	132	-	-	Por, CA19-9, Dis(-)
Uchiyama *et al*[15]	334	-	-	Shown in Table 2
Chen *et al*[16]	64	32 (3 yr)	-	LN, PN, Size
Uno *et al*[17]	273	-	-	Shown in Table 2
Morine *et al*[18]	22	-	-	Shown in Table 2
Jiang *et al*[19]	102	-	-	CA19-9, IM
Murakami *et al*[20]	44	47 (5 yr)	-	LN
Clark *et al*[21]	4893	8.4 (5 yr, LN+) 25 (5 yr, LN-)	-	LN
de Jong *et al*[22]	449	31 (5 yr)	27	IM, V, LN
Li *et al*[23]	115	-	-	Cirrhosis
Chen *et al*[24]	320	-	-	-

MST: Median survival time; Prognostic factor: Factor for poor prognosis; IM: Multiple tumors or intrahepatic metastasis; Hilar inv: Hilar invasion; LN: Lymph node metastasis; LNR: Rate of the positive lymph node metastasis; CA19-9: Elevated serum carbohydrate antigen 19-9; CEA: Elevated serum carcinoembryonic antigen; Size: Larger tumor size; V: Vascular invasion; BT: Blood transfusion during operation; Dis: Lymph node dissection; PN: Perineural invasion.

Table 2 Clinical studies of intrahepatic cholangiocarcinoma focused on the macroscopic subtypes

Ref.	*n*	Findings or conclusion
Uchiyama *et al*[15]	334	Lymph node metastasis: MF: 16%; IG: 0%; PI and MF + PI: 60% Survival rate (5 yr): MF: 26%; IG: 79.3%; PI and MF + PI: 19.4%
Uno *et al*[17]	273	Rate of PI-type: 7.9% The PI-type shows significantly better survival than MF- and MF + PI-type
Morine *et al*[18]	22	The PI-type shows a lower incidence of intrahepatic metastasis Routine lymph node dissection do not improve survival in MF-type

MF: Mass-forming type; IG: Intraductal growth type; PI: Periductal infiltrative type.

each subtype emphasizes the importance of the preoperative identification of each subtype.

RADIOLOGIC STUDIES OF ICC

Table 3 summarizes recent radiologic studies of ICC[25-30]. The typical enhancement pattern of ICC on CT and MRI is that of ringed enhancement in the early phase with central delayed enhancement, reflecting the abundant fibrous stroma in ICC. However, Kim *et al*[26] reported that 6 (30%) of 20 ICCs appeared as hypervascular lesions with washout in the delayed phase, resembling HCCs. In addition, Ariizumi *et al*[29] pointed out that MF-type ICCs with hypervascular-type pattern had more favorable prognosis than those with the typical enhancement pattern. The histopathological characteristics of hypervascular-type ICCs have not been clarified. Cholangiocellular carcinoma (CoCC), a subtype of ICC, has been reported to originate from the ductules, or canals of Hering, and appears as a hypervascular mass similar to HCC[31]. These results of recent radiologic studies suggest the possibility that some ICCs share the same origin with that of CoCC, *i.e.*, HPCs. Especially in MF-type ICCs, comparative studies between the enhancement patterns and histopathologic findings are needed for further exploration. However, these descriptions can be applied to only MF-type ICCs. Xu *et al*[28] reported the difference of enhancement patterns on contrast-enhanced ultrasonography between each subtype and demonstrated that most IG-type ICCs appeared as a mass showing homogenous hyperenhancement. This finding provides useful knowledge for preoperative differentiation between IG-type and PI-type ICC.

PATHOBIOLOGICAL STUDIES OF ICCs

During the most recent 3 years, many molecules have been identified as biomarkers for poor prognosis of ICCs (Tables 4-6)[31-71]. Among these, researchers have paid close attention to molecules associated with epithelial-mesenchymal transition (EMT)[32,38,53,55]. The

Table 3 Radiologic studies of intrahepatic cholangiocarcinoma

Ref.	*n*	Method	Findings or conclusion
Nanashima *et al*[25]	42	CT	Factor for poor prognosis: case showing arterial enhancement with lower attenuation
Kim *et al*[26]	20	MRI	6 (30%) of the 20 cases appeared as hypervascular lesions with washout on delayed phase
Kang *et al*[27]	50	MRI	Percentage of relative enhancement on hepatobiliary phase was significantly higher in moderately differentiated tumors than in poorly differentiated tumors and in patients without than in those with lymph node metastasis
Xu *et al*[28]	40	Contrast enhanced ultrasono-graphy	MF-type (n = 32): (1) peripheral rim-like hyperenhancement (n = 19); (2) heterogenous enhancement (n = 10); and (3) homogenous hyperenhancement (n = 3)
Ariizumi *et al*[29]	26	FDG PET	PI-type (n = 4): heterogenous enhancement (n = 4) IG-type (n = 4): (1) homogenous hyperenhancement (n = 3); and (2) heterogenous enhancement (n = 1) FDG PET was able to predict patient outcome after radioembolization treatment

CT: Computed tomography; MRI: Magnetic resonance imaging; FDG PET: ^{18}F-fluorodeoxy glucose positron emission tomography.

Table 4 Pathobiological studies of intrahepatic cholangiocarcinoma

Ref.	*n*	Method	Target	Conclusion
Gu *et al*[32]	85	IHC	E-cadherin	(-)por
			Beta-catenin	(-)por
			Vimentin	(-)por
Yan *et al*[33]	49	IHC	Smad4	(-)por, advanced stage, LN
Kamphues *et al*[34]	65	DNA-Cyto	DNA-index	(+)poor prognosis
Mano *et al*[35]	132	IHC	Roundabout-1	(-)Size, Ki67index, poor prognosis
			Slit-1	(-)PN, LN
Yin *et al*[36]	411	Serum	γ-glutamyl transferase	(+)V, LN, poor prognosis, incomplete encapsulation
Sulpice *et al*[37]	40	mRNA (Stroma)	Osteopontin	(+)poor prognosis
			TGFβ2	(+)poor prognosis
			Laminin	(+)poor prognosis
Zhou *et al*[38]	Cell line	mRNA Western	Notch-1	(+)EMT
Li *et al*[39]	173	IHC	CKAP4	(+)favorable prognosis
Nanashima *et al*[40]	38	IHC	CD44	(+)PI-type, poor prognosis
			Gli1	(+)poor prognosis
Nutthasirikul *et al*[41]	-	mRNA	Δ133p53/TA P53	(+)poor prognosis
	-	IHC	Mutantp53	(+)poor prognosis
Zhang *et al*[42]	33	mRNA Western	Capn4	(+)LN, advanced stage, Poor prognosis
Ding *et al*[43]	20	IHC	Integrinα6	(+)IM, Size, V, poor prognosis
		Cell	Integrinα6	(-)decrease of metastasis
Aishima *et al*[44]	134	IHC	Cox-2	(+)poor prognosis, LN
			iNOS	(-) LN
Chen *et al*[45]	61	IHC	IMP3	(+)Por, advanced stage, V poor prognosis, CA19-9

IHC: Immunohistochemistry; mRNA: Real-time polymerase chain reaction; Western: Western blotting; DNA-cyto: DNA image cytometry; Cell: Functional analyses using cell lines; CKAP4: Cytoskeleton-associated protein4; iNOS: Inducible nitric oxide synthase; IMP3: Insulin-like growth factor II mRNA binding protein.

close association between EMT and the progression of ICC was confirmed not only by immunohistochemistry but also by functional and comprehensive analyses. The fact that EMT induces progression of ICC led us to hypothesize that abundant fibrous stroma in ICCs play an important role in the invasive growth and metastasis of this cancer. In addition, Oishi *et al*[53] reported that activation of miR-200c induced a reduction in EMT and in the expression of neural adhesion molecule (NCAM). Given that NCAM is known to be a hepatic progenitor cell marker, a hypothesis that the hepatic progenitor cell markers and molecules associated with EMT are regulated by common upstream molecules can be proposed. Further functional analyses are needed to confirm this hypothesis.

The literature on the association between macro-

Table 5 Pathobiological studies of intrahepatic cholangiocarcinoma 2

Ref.	*n*	Method	Target	Conclusion
Shi *et al*[46]	138	IHC	DKK-1	(+)poor prognosis elevated sMMP9 and VEGF-C
		Cell	DKK-1	(-)decrease in cell migration and invasiveness (+)LN, Por, advanced stage, V
Yao *et al*[47]	96	IHC	Vimentin and N-cadherin	poor prognosis (+)MF-type
Zhou *et al*[48]	54	IHC	HBx-protein	well differentiated tumor (+)well differentiated tumor, IG-type
Choi *et al*[49]	46	IHC	CK20 MUC6	(+)favorable prognosis (+)Size, LN, V, advanced stage
Jeong *et al*[50]	43	IHC Cell	FABP-5 FABP-5	(-)decrease in cell proliferation and invasion (+)elevated serum CEA and CA
Tsai *et al*[51]	112	IHC	S100P	19-9 value, MUC2 positive poor prognosis (+)perineural invasion
	86	Sequencing	K-ras mutation miR-200c	poor prognosis (+)reduction of EMT
Oishi *et al*[53]	-	Microarray		reduction of NCAM1 expression
Liao *et al*[54]	-	Cell	HCV core protein Angiotensin	(+)enhanced NFAT expression (+)enhanced Angiotensin II receptor expression and fibrogenesis of cancerous stroma, metastasis
Okamoto *et al*[55]	-	Cell	II and SDF1	

DKK1: Dickkopf-related protein1; MMP: Matrix metalloproteinase; FABP-5: Fatty acid-binding protein 5; SDF1: Stromal cell derived factor 1; NCAM1: Neural cell adhesion molecule1; EMT: Epithelial mesenchymal transition; NFAT1: Nuclear factor of activated T-cells.

Table 6 Pathobiological studies of intrahepatic cholangiocarcinoma 3

Source	*n*	Method	Target	Conclusion
Li *et al*[56]	-	Tissues	miR-214	(-)increased expression of Twist(EMT -associated gene)
Gu *et al*[57]	123	IHC	IL-17cells (intratumoral)	(+)poor prognosis
Higashi *et al*[58]	63	IHC	MUC16	(+)poor prognosis
Gu *et al*[59]	83	IHC	E-cadherin Beta-catenin EGFR	(-)poor prognosis (-)V (+)Por
Wang *et al*[60]	77	IHC	P-70S6K 4EBP1	(+)Por (+)poor prognosis
Hirashita *et al*[61]	35	IHC	MMP-7	(+)poor prognosis
Srimunta *et al*[62]	55	IHC	ABCC-1	(+)poor prognosis
Morine *et al*[63]	35	IHC	HDAC	(+)advanced stage, LN poor prognosis
Wakai *et al*[64]	34	IHC	RRM1	(+)gemcitabine resistance
Larbcharoensub *et al*[65]	60	IHC	ABCG2	(-)poor prognosis, LN, Por
Lee *et al*[66]	101	IHC	PTEN P-AKT1 P-MTOR	(+)favorable prognosis (+)favorable prognosis (+)favorable prognosis
Dong *et al*[67]	108	IHC	Beclin1	(-)LN, poor prognosis
Shinozaki *et al*[68]	83	IHC	Claudin-18	(+)LN, PI-type, perineural invasion
Wakai *et al*[69]	34	IHC	NQO1	(-)Por, poor prognosis
Aishima *et al*[70]	110	IHC	S100P S100P(nuc)	(+)PI-type (+)LN, V
Zhou *et al*[71]	89	IHC	MAGE3/4	(+)larger tumor size, poor prognosis

EGFR: Epidermal growth factor receptor; P-70S6K: P70 ribosomal protein S6 kinase; 4EBP1: 4E-binding protein-1; ABCC-1: Adenosine triphosphate binding cassette C1; HDAC: Histone deacetylase; RRM1: Ribonucleotide reductase-M1; ABCG2: Adenosine 5′ triphosphate-binding cassetteG2; PTEN: Phosphatase and tensin homolog on chromosome ten; PAKT: Phosphorylated Akt; PMTOR: Phosphorylated MTOR; NQO1: Quinine oxidoredactase; MAGE: Melanoma antigen.

scopic subtypes and the expression of genes are very scant[48,49,68,70], similar to that in the clinical study literature. Shinozaki *et al*[68] reported that claudin-18 (CLDN18), a tight junction protein specific to the stomach and lung,

Table 7 Clinical studies of combined hepatocellular-cholangiocarcinoma

Source	*n*	Conclusion or findings
Yap *et al*[74]	11	Survival rate: 69.3% (3 yr)
Lee *et al*[75]	65	(1) The clinical characteristics of cHCC-CC are similar to those of HCC (2) Overall survival of cHCC-CC is similar to that of ICC
Yin *et al*[76]	113	(1) Findings similar to HCC: infection with hepatitis virus; presence of cirrhosis; elevated AFP levels (2) Findings similar to ICC: serum CA19-9 elevation; incomplete capsules; lymph node involvement (3) Survival rate: 41.4%(3 yr); 36.4% (5 yr) (4) Factors for poor prognosis: radical liver resection
Ariizumi *et al*[77]	44	(1) Survival rate: 24% (2) Median survival time: 15.4 mo
Yu *et al*[78]	14	(1) Clinical characteristics: hepatitis B virus infection: 13/14; elevated AFP levels: 11/14 (2) Median survival time: 7.9 mo (3) Stem cell markers (IHC): c-Kit 71.4%; CD90: 85.7%; CD133: 92.9%; CK19: 78.6%
Park *et al*[79]	21	Factor for poor prognosis: serum AFP levels
Park *et al*[80]	43	(1) median survival time: 34 mo (2) Survival rate: 18.1% (5 yr) (3) Factors for poor prognosis: Portal vein thrombosis; distant metastasis
Zhan *et al*[81]	27	(1) CK-7: 86.4%; CK19: 90.9% (2) Survival rate: 49.4% (3) Factors for higher recurrence: lymph node metastasis

AFP: Alpha-fetoprotein.

Table 8 Radiologic studies of combined hepatocellular-cholangiocarcinoma

Ref.	*n*	Methods	Conclusion or findings
Ijichi *et al*[82]	3	FDG -PET	(1) SUVmax value of three cHCC-CC cases: 9.9, 12.0, and 13 (2) Median SUVmax value of poorly differentiated HCC: 5.7
de Campos *et al*[83]	11	MRI	(1) 6/11 showed early ring enhancement with progressive enhancement in central portion. (2) 5/11 showed a diffuse heterogenous early enhancement. Characteristics findings of cHCC-CC: irregular shape and strong rim enhancement during early phase; absence of target appearance on hepatobiliary-phase
Hwang *et al*[84]	20	MRI	

cHCC-CC: Combined hepatocellular-cholangiocarcinoma; MRI: Magnetic resonance imaging; FDG-PET: ^{18}F-fluorodeoxy glucose positron emission tomography.

is highly expressed in precancerous lesions of biliary intraepithelial neoplasms and PI components of ICCs. CLDN18 has been reported to be expressed in various gastrointestinal cancer tissues and to be associated with morphogenesis of the histologic subtype and the specific mucin phenotype[72]. In addition, we previously reported the association between the expression of CLDN18 and intestinal-type differentiation in intraductal papillary-mucinous neoplasm of the pancreas[73]. Thus, there is considerable interest in the crucial role of CLDN18 in the development of PI-type morphology in ICCs.

RECENT RESEARCH ON cHCC-CC

There is a large dissociation in the postoperative survival rates of cHCC-CC reported in the recent researches[74-90] (Tables 7-9), probably because the case numbers are limited. In addition, cHCC-CC is associated with many factors that contribute to poor prognosis including lymph node metastasis, higher levels of serum AFP, and portal vein thrombosis, reflecting intermediate features of cHCC-CC between HCC and ICC (Figure 2). The intermingling of findings of cHCC-CCs are also demonstrated by radiologic studies. Based on the new WHO classification system of cHCC-CC, some immunohistochemical research highlighting the expression of HPC markers has been published in the past 3 years in which YAP1 and EpiCAM, are reported to be markers of poor prognosis. These molecules are mainly distributed across the intermediate- and cholangiocellular-type components. Kim *et al*[85] reported that YAP1 is localized in the transitional zone between HCC and ICC components. In addition, Akiba *et al*[87] demonstrated that vimentin is strongly expressed in intermediate-type cHCC-CC. Similar to their role in ICCs, HPC markers may also play a crucial role in the progression of cHCC-CC through EMT. These components may harbor biological instability resembling undifferentiated carcinoma that leads to invasive behavior. However, CoCC, a subtype of ICC, has been known to be a tumor with characteristics resembling those of HCC and to have a relatively favorable prognosis (Figure 3). Given that CoCC is also derived from HPCs[31], a contradictory point exists with regard to the role of HPCs in the progression of ICCs

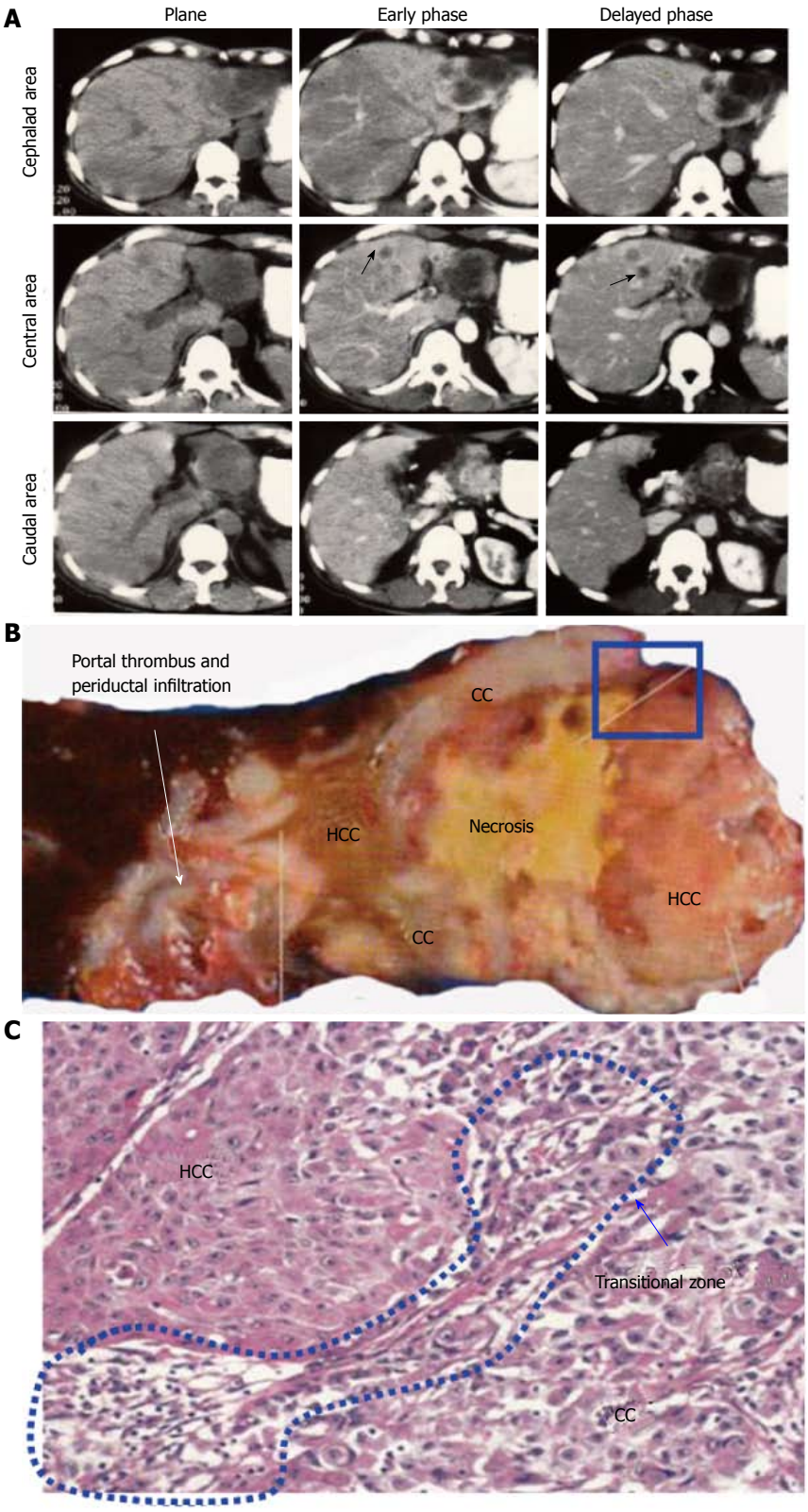

Figure 2 Case presentation of combined hepatocellular-cholangiocarcinoma. A: Preoperative computed tomography shows a large mass composed of two major components that replaces the lateral segment. The mass shows ringed enhancement in the delayed phase in the cephalad area and early enhancement with washout in the delayed phase in the caudal area. Intrahepatic metastases are observed in the S4 segment (arrow); B: Gross features of the resected specimen. Hepatocellular carcinoma (HCC) components are composed of tan-colored soft tissues. Cholangiocarcinoma (CC) components are composed of white firm tissues with central necrosis; C: Small round cells and fibrous stroma are observed at the boundary area between the HCC and CC components (blue flame in panel B).

and cHCC-CCs. We speculate that each HPC marker performs various functions involving progression and metastasis of ICCs and cHCC-CCs to a lesser or greater extent.

CONCLUSION

Recent research in ICC has revealed that each tumor shows different clinical and radiologic characteristics between the macroscopic subtypes. However, there are still many unclear points regarding the molecular mechanisms yielding these subtypes. It is of particular interest to identify the molecular markers inducing invasion, metastasis, and the macroscopic growth patterns of ICC. Many researchers have noted that HPC markers and EMT are involved in the progression of ICCs.

Table 9 Pathobiological studies of combined hepatocellular-cholangiocarcinoma

Ref.	*n*	Method	Target	Conclusion
Kim *et al*[85]	58	IHC	YAP1	(+): transition zone, poor prognosis
			EpiCAM	(-)favorable prognosis
			CK19	(-)favorable prognosis
Ikeda *et al*[86]	36	IHC	DLK1	(+)poor prognosis
Akiba *et al*[87]	54	IHC	CD56	(+): components apart from HCC
			c-Kit	(+): components apart from HCC
			EpiCAM	(+): components apart from HCC
			CD133	(+): intermediate type or cholangiolocellular type
			Vimentin	(+): intermediate type or cholangiolocellular type
Coulouarn *et al*[88]	152	Microarray	-	(1) TGFbeta and beta-catenin are identified as the two major signals in the progression of cHCC-CC/ (2) cHCC-CC shares the characteristics of poorly differentiated HCC. (+)poor prognosis
Cai *et al*[89]	80	IHC	PCNA	Both HCC and CC components of most Of the cHCC-CC express both AFP and CK19
Itoyama *et al*[90]	20	IHC	AFP and CK19	

cHCC-CC: Combined hepatocellular-cholangiocarcinoma; YAP1: Yes-associated protein 1; EpiCAM: Epithelial cell adhesion molecule; DLK1: Delta-like 1 homolog; PCNA: Proliferating cell nuclear antigen index in nontumor ductular reaction.

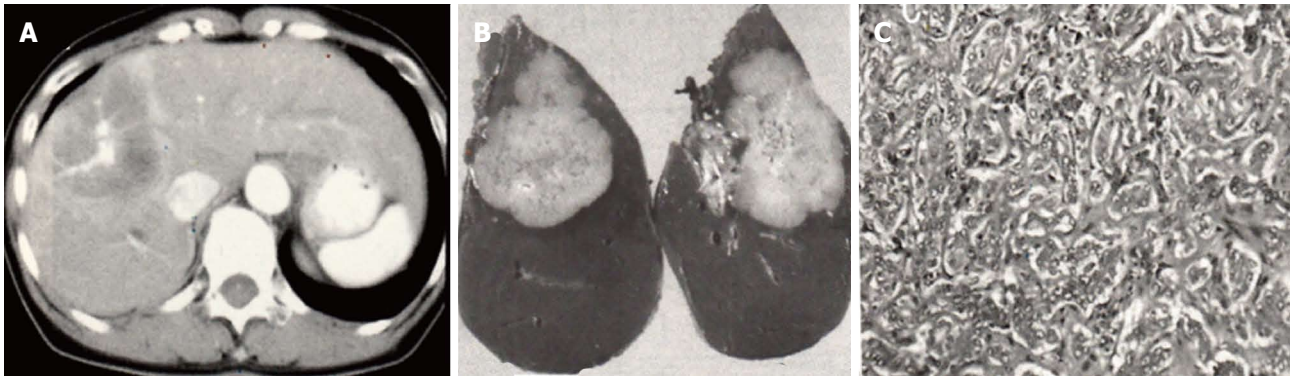

Figure 3 Resected case of cholangiocellular carcinoma. A: Computed tomography (CT) reveals a mass showing ringed enhancement with portal venous penetration; B: CT findings reflect the non-infiltrative growth of the tumor to the portal tract; C: Histopathologically, the size of the carcinoma cells is small, with the cells forming anastomosing patterns with abundant fibrous stroma.

Because most cHCC-CCs show MF-type morphology, we infer that HPC markers are closely associated with the morphogenesis and histogenesis of MF-type ICCs. Therefore, studies of ICC, and especially of its molecular pathology, should be designed in conjunction with those of cHCC-CC.

REFERENCES

1 **Igami T**, Ebata T, Yokoyama Y, Sugawara G, Takahashi Y, Nagino M. Staging of peripheral-type intrahepatic cholangiocarcinoma: appraisal of the new TNM classification and its modifications. *World J Surg* 2011; **35**: 2501-2509 [PMID: 21879422]

2 **Nakanuma Y**, Sato Y, Harada K, Sasaki M, Xu J, Ikeda H. Pathological classification of intrahepatic cholangiocarcinoma based on a new concept. *World J Hepatol* 2010; **2**: 419-427 [PMID: 21191517]

3 **Lo RC**, Ng IO. Hepatic progenitor cells: their role and functional significance in the new classification of primary liver cancers. *Liver Cancer* 2013; **2**: 84-92 [PMID: 24159600]

4 **Zheng YW**, Nie YZ, Taniguchi H. Cellular reprogramming and hepatocellular carcinoma development. *World J Gastroenterol* 2013; **19**: 8850-8860 [PMID: 24379607 DOI: 10.3748/wjg.v19.i47.8850]

5 **Theise ND,** Nakashima O, Park YN. Combined hepatocellular-cholangiocacinoma in WHO classification of tumors of the digestive system; World health organization of tumors. IARC, Lyon, 2010: 225-227

6 **Marubashi S**, Gotoh K, Takahashi H, Ohigashi H, Yano M, Ishikawa O, Sakon M. Prediction of the postoperative prognosis of intrahepatic cholangiocarcinoma (ICC): importance of preoperatively- determined anatomic invasion level and number of tumors. *Dig Dis Sci* 2014; **59**: 201-213 [PMID: 24122559]

7 **Guglielmi A**, Ruzzenente A, Campagnaro T, Valdegamberi A, Bagante F, Bertuzzo F, Conci S, Iacono C. Patterns and prognostic significance of lymph node dissection for surgical treatment of perihilar and intrahepatic cholangiocarcinoma. *J Gastrointest Surg* 2013; **17**: 1917-1928 [PMID: 24048613 DOI: 10.1007/s11605-013-2331-1]

8 **Zhu HF**, Li J, Huang L, Yan YQ. Intrahepatic cholangiocarcinoma: a clinicopathologic study of 37 resected cases. *Hepatogastroenterology* 2013; **60**: 263-267 [PMID: 23574653 DOI:

10.5754/hge12711]
9 **Dhanasekaran R**, Hemming AW, Zendejas I, George T, Nelson DR, Soldevila-Pico C, Firpi RJ, Morelli G, Clark V, Cabrera R. Treatment outcomes and prognostic factors of intrahepatic cholangiocarcinoma. *Oncol Rep* 2013; **29**: 1259-1267 [PMID: 23426976 DOI: 10.3892/or.2013.2290]
10 **Wang Y**, Li J, Xia Y, Gong R, Wang K, Yan Z, Wan X, Liu G, Wu D, Shi L, Lau W, Wu M, Shen F. Prognostic nomogram for intrahepatic cholangiocarcinoma after partial hepatectomy. *J Clin Oncol* 2013; **31**: 1188-1195 [PMID: 23358969 DOI: 10.1200/JCO.2012.41.5984]
11 **De Rose AM**, Cucchetti A, Clemente G, Ardito F, Giovannini I, Ercolani G, Giuliante F, Pinna AD, Nuzzo G. Prognostic significance of tumor doubling time in mass-forming type cholangiocarcinoma. *J Gastrointest Surg* 2013; **17**: 739-747 [PMID: 23292461]
12 **Sulpice L**, Rayar M, Boucher E, Pele F, Pracht M, Meunier B, Boudjema K. Intrahepatic cholangiocarcinoma: impact of genetic hemochromatosis on outcome and overall survival after surgical resection. *J Surg Res* 2013; **180**: 56-61 [PMID: 23183056 DOI: 10.1016/j.jss.2012.10.051]
13 **Ribero D**, Pinna AD, Guglielmi A, Ponti A, Nuzzo G, Giulini SM, Aldrighetti L, Calise F, Gerunda GE, Tomatis M, Amisano M, Berloco P, Torzilli G, Capussotti L. Surgical Approach for Long-term Survival of Patients With Intrahepatic Cholangiocarcinoma: A Multi-institutional Analysis of 434 Patients. *Arch Surg* 2012; **147**: 1107-1113 [PMID: 22910846]
14 **Liu ZH**, Chen Z, Ma LL, Li XH, Wang LX. Factors influencing the prognosis of patients with intrahepatic cholangiocarcinoma. *Acta Gastroenterol Belg* 2012; **75**: 215-218 [PMID: 22870785]
15 **Uchiyama K**, Yamamoto M, Yamaue H, Ariizumi S, Aoki T, Kokudo N, Ebata T, Nagino M, Ohtsuka M, Miyazaki M, Tanaka E, Kondo S, Uenishi T, Kubo S, Yoshida H, Unno M, Imura S, Shimada M, Ueno M, Takada T. Impact of nodal involvement on surgical outcomes of intrahepatic cholangiocarcinoma: a multicenter analysis by the Study Group for Hepatic Surgery of the Japanese Society of Hepato-Biliary-Pancreatic Surgery. *J Hepatobiliary Pancreat Sci* 2011; **18**: 443-452 [PMID: 21132443 DOI: 10.1007/s00534-010-0349-2]
16 **Chen LP**, Li C, Wang C, Wen TF, Yan LN, Li B. Predictive factors of recurrence for patients with intrahepatic cholangiocarcinoma after hepatectomy. *Hepatogastroenterology* 2012; **59**: 1765-1768 [PMID: 22369746 DOI: 10.5754/hge11820]
17 **Uno M**, Shimada K, Yamamoto Y, Nara S, Esaki M, Sakamoto Y, Kosuge T, Ojima H. Periductal infiltrating type of intrahepatic cholangiocarcinoma: a rare macroscopic type without any apparent mass. *Surg Today* 2012; **42**: 1189-1194 [PMID: 22350300 DOI: 10.1007/s00595-012-0145-5]
18 **Morine Y**, Shimada M, Utsunomiya T, Imura S, Ikemoto T, Mori H, Hanaoka J, Kanamoto M, Miyake H. Clinical impact of lymph node dissection in surgery for peripheral-type intrahepatic cholangiocarcinoma. *Surg Today* 2012; **42**: 147-151 [PMID: 22124809 DOI: 10.1007/s00595-011-0057-9]
19 **Jiang BG**, Ge RL, Sun LL, Zong M, Wei GT, Zhang YJ. Clinical parameters predicting survival duration after hepatectomy for intrahepatic cholangiocarcinoma. *Can J Gastroenterol* 2011; **25**: 603-608 [PMID: 22059167]
20 **Murakami Y**, Uemura K, Sudo T, Hashimoto Y, Nakashima A, Sueda T. Intrahepatic cholangiocarcinoma: clinicopathological differences between peripheral type and hilar type. *J Gastrointest Surg* 2012; **16**: 540-548 [PMID: 22012305 DOI: 10.1007/s11605-011-1730-4]
21 **Clark CJ,** Wood-Wentz CM, Reid-Lombardo KM, Kendrick ML, Huebner M, Que FG. Lymphadenectomy in the staging and treatment of intrahepatic cholangiocarcinoma: a population-based study using the National cancer institute SEER database. *HPB* (Oxford) 2011; **13**: 612-620 [DOI: 10.1111/j.1477-2574.2011.00340.x]
22 **de Jong MC**, Nathan H, Sotiropoulos GC, Paul A, Alexandrescu S, Marques H, Pulitano C, Barroso E, Clary BM, Aldrighetti L, Ferrone CR, Zhu AX, Bauer TW, Walters DM, Gamblin TC, Nguyen KT, Turley R, Popescu I, Hubert C, Meyer S, Schulick RD, Choti MA, Gigot JF, Mentha G, Pawlik TM. Intrahepatic cholangiocarcinoma: an international multi-institutional analysis of prognostic factors and lymph node assessment. *J Clin Oncol* 2011; **29**: 3140-3145 [PMID: 21730269 DOI: 10.1200/JCO.2011.35.6519]
23 **Li YY**, Li H, Lv P, Liu G, Li XR, Tian BN, Chen DJ. Prognostic value of cirrhosis for intrahepatic cholangiocarcinoma after surgical treatment. *J Gastrointest Surg* 2011; **15**: 608-613 [PMID: 21246412 DOI: 10.1007/s11605-011-1419-8]
24 **Chen YX**, Zeng ZC, Tang ZY, Fan J, Zhou J, Jiang W, Zeng MS, Tan YS. Prediction of the lymph node status in patients with intrahepatic cholangiocarcinoma: analysis of 320 surgical cases. *Front Oncol* 2011; **1**: 42 [PMID: 22649763 DOI: 10.3389/fonc.2011.00042]
25 **Nanashima A**, Abo T, Murakami G, Matsumoto A, Tou K, Takeshita H, Kunizaki M, Hidaka S, Sakamoto I, Hayashi H, Fukuda T, Kudo T, Nagayasu T. Intrahepatic cholangiocarcinoma: relationship between tumor imaging enhancement by measuring attenuation and clinicopathologic characteristics. *Abdom Imaging* 2013; **38**: 785-792 [PMID: 23232581 DOI: 10.1007/s00261-012-9974-3]
26 **Kim SH**, Lee CH, Kim BH, Kim WB, Yeom SK, Kim KA, Park CM. Typical and atypical imaging findings of intrahepatic cholangiocarcinoma using gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid-enhanced magnetic resonance imaging. *J Comput Assist Tomogr* 2012; **36**: 704-709 [PMID: 23192208 DOI: 10.1097/RCT.0b013e3182706562]
27 **Kang Y**, Lee JM, Kim SH, Han JK, Choi BI. Intrahepatic mass-forming cholangiocarcinoma: enhancement patterns on gadoxetic acid-enhanced MR images. *Radiology* 2012; **264**: 751-760 [PMID: 22798225 DOI: 10.1148/radiol.12112308]
28 **Xu HX**, Chen LD, Liu LN, Zhang YF, Guo LH, Liu C. Contrast-enhanced ultrasound of intrahepatic cholangiocarcinoma: correlation with pathological examination. *Br J Radiol* 2012; **85**: 1029-1037 [PMID: 22374276 DOI: 10.1259/bjr/21653786]
29 **Ariizumi S**, Kotera Y, Takahashi Y, Katagiri S, Chen IP, Ota T, Yamamoto M. Mass-forming intrahepatic cholangiocarcinoma with marked enhancement on arterial-phase computed tomography reflects favorable surgical outcomes. *J Surg Oncol* 2011; **104**: 130-139 [PMID: 21448898]
30 **Haug AR**, Heinemann V, Bruns CJ, Hoffmann R, Jakobs T, Bartenstein P, Hacker M. 18F-FDG PET independently predicts survival in patients with cholangiocellular carcinoma treated with 90Y microspheres. *Eur J Nucl Med Mol Imaging* 2011; **38**: 1037-1045 [PMID: 21308371 DOI: 10.1007/s00259-011-1736-x]
31 **Komuta M**, Spee B, Vander Borght S, De Vos R, Verslype C, Aerts R, Yano H, Suzuki T, Matsuda M, Fujii H, Desmet VJ, Kojiro M, Roskams T. Clinicopathological study on cholangiolocellular carcinoma suggesting hepatic progenitor cell origin. *Hepatology* 2008; **47**: 1544-1556 [PMID: 18393293 DOI: 10.1002/hep.22238]
32 **Gu MJ**, Choi JH. Epithelial-mesenchymal transition phenotypes are associated with patient survival in intrahepatic cholangiocarcinoma. *J Clin Pathol* 2014; **67**: 229-234 [PMID: 24062361 DOI: 10.1136/jclinpath-2013-201806]
33 **Yan XQ**, Zhang W, Zhang BX, Liang HF, Zhang WG, Chen XP. Inactivation of Smad4 is a prognostic factor in intrahepatic cholangiocarcinoma. *Chin Med J* (Engl) 2013; **126**: 3039-3043 [PMID: 23981608]
34 **Kamphues C**, Al-Abadi N, Dürr A, Bova R, Klauschen F, Stenzinger A, Bahra M, Al-Abadi H, Neuhaus P, Seehofer D. The DNA index is a strong predictive marker in intrahepatic cholangiocarcinoma: the results of a five-year prospective study. *Surg Today* 2014; **44**: 1336-1342 [PMID: 23975588]
35 **Mano Y**, Aishima S, Fukuhara T, Tanaka Y, Kubo Y, Mo-

tomura T, Toshima T, Iguchi T, Shirabe K, Maehara Y, Oda Y. Decreased roundabout 1 expression promotes development of intrahepatic cholangiocarcinoma. *Hum Pathol* 2013; **44**: 2419-2426 [PMID: 23953227 DOI: 10.1016/j.humpath.2013.03.022]

36 **Yin X**, Zheng SS, Zhang BH, Zhou Y, Chen XH, Ren ZG, Qiu SJ, Fan J. Elevation of serum γ-glutamyltransferase as a predictor of aggressive tumor behaviors and unfavorable prognosis in patients with intrahepatic cholangiocarcinoma: analysis of a large monocenter study. *Eur J Gastroenterol Hepatol* 2013; **25**: 1408-1414 [PMID: 23839159 DOI: 10.1097/MEG.0b013e328364130f]

37 **Sulpice L**, Rayar M, Desille M, Turlin B, Fautrel A, Boucher E, Llamas-Gutierrez F, Meunier B, Boudjema K, Clément B, Coulouarn C. Molecular profiling of stroma identifies osteopontin as an independent predictor of poor prognosis in intrahepatic cholangiocarcinoma. *Hepatology* 2013; **58**: 1992-2000 [PMID: 23775819 DOI: 10.1002/hep.26577]

38 **Zhou Q**, Wang Y, Peng B, Liang L, Li J. The roles of Notch1 expression in the migration of intrahepatic cholangiocarcinoma. *BMC Cancer* 2013; **13**: 244 [PMID: 23688168 DOI: 10.1186/1471-2407-13-244]

39 **Li MH**, Dong LW, Li SX, Tang GS, Pan YF, Zhang J, Wang H, Zhou HB, Tan YX, Hu HP, Wang HY. Expression of cytoskeleton-associated protein 4 is related to lymphatic metastasis and indicates prognosis of intrahepatic cholangiocarcinoma patients after surgery resection. *Cancer Lett* 2013; **337**: 248-253 [PMID: 23665508 DOI: 10.1016/j.canlet.2013.05.003]

40 **Nanashima A**, Hatachi G, Tsuchiya T, Matsumoto H, Arai J, Abo T, Murakami G, Tominaga T, Takagi K, Nagayasu T. Clinical significances of cancer stem cells markers in patients with intrahepatic cholangiocarcinoma who underwent hepatectomy. *Anticancer Res* 2013; **33**: 2107-2114 [PMID: 23645762]

41 **Nutthasirikul N**, Limpaiboon T, Leelayuwat C, Patrakitkomjorn S, Jearanaikoon P. Ratio disruption of the Δ133p53 and TAp53 isoform equilibrium correlates with poor clinical outcome in intrahepatic cholangiocarcinoma. *Int J Oncol* 2013; **42**: 1181-1188 [PMID: 23404110 DOI: 10.3892/ijo.2013.1818]

42 **Zhang C**, Bai DS, Huang XY, Shi GM, Ke AW, Yang LX, Yang XR, Zhou J, Fan J. Prognostic significance of Capn4 overexpression in intrahepatic cholangiocarcinoma. *PLoS One* 2013; **8**: e54619 [PMID: 23349941 DOI: 10.1371/journal.pone.0054619]

43 **Ding YB**, Deng B, Huang YS, Xiao WM, Wu J, Zhang YQ, Wang YZ, Wu DC, Lu GT, Wu KY. A high level of integrin α6 expression in human intrahepatic cholangiocarcinoma cells is associated with a migratory and invasive phenotype. *Dig Dis Sci* 2013; **58**: 1627-1635 [PMID: 23306848 DOI: 10.1007/s10620-012-2524-6]

44 **Aishima S**, Mano Y, Tanaka Y, Kubo Y, Shirabe K, Maehara Y, Oda Y. Different roles of inducible nitric oxide synthase and cyclooxygenase-2 in carcinogenesis and metastasis of intrahepatic cholangiocarcinoma. *Hum Pathol* 2013; **44**: 1031-1037 [PMID: 23260331 DOI: 10.1016/j.humpath.2012.09.004]

45 **Chen YL**, Jeng YM, Hsu HC, Lai HS, Lee PH, Lai PL, Yuan RH. Expression of insulin-like growth factor II mRNA-binding protein 3 predicts early recurrence and poor prognosis in intrahepatic cholangiocarcinoma. *Int J Surg* 2013; **11**: 85-91 [PMID: 23246869 DOI: 10.1016/j.ijsu.2012.11.021]

46 **Shi RY**, Yang XR, Shen QJ, Yang LX, Xu Y, Qiu SJ, Sun YF, Zhang X, Wang Z, Zhu K, Qin WX, Tang ZY, Fan J, Zhou J. High expression of Dickkopf-related protein 1 is related to lymphatic metastasis and indicates poor prognosis in intrahepatic cholangiocarcinoma patients after surgery. *Cancer* 2013; **119**: 993-1003 [PMID: 23132676 DOI: 10.1002/cncr.27788]

47 **Yao X**, Wang X, Wang Z, Dai L, Zhang G, Yan Q, Zhou W. Clinicopathological and prognostic significance of epithelial mesenchymal transition-related protein expression in intrahepatic cholangiocarcinoma. *Onco Targets Ther* 2012; **5**: 255-261 [PMID: 23091390 DOI: 10.2147/OTT.S36213]

48 **Zhou YM**, Cao L, Li B, Zhang XZ, Yin ZF. Expression of HBx protein in hepatitis B virus-infected intrahepatic cholangiocarcinoma. *Hepatobiliary Pancreat Dis Int* 2012; **11**: 532-535 [PMID: 23060400]

49 **Choi JE**, Noh SJ, Lee JH, Bae JS, Chu HH, Park HS, Jang KY, Chung MJ, Kang MJ, Lee DG, Moon WS. Expression of keratin 20 and its clinicopathological significance in intrahepatic cholangiocarcinoma. *Oncol Lett* 2012; **4**: 534-540 [PMID: 22970052]

50 **Jeong CY**, Hah YS, Cho BI, Lee SM, Joo YT, Jung EJ, Jeong SH, Lee YJ, Choi SK, Ha WS, Park ST, Hong SC. Fatty acid-binding protein 5 promotes cell proliferation and invasion in human intrahepatic cholangiocarcinoma. *Oncol Rep* 2012; **28**: 1283-1292 [PMID: 22825302 DOI: 10.3892/or.2012.1922]

51 **Tsai JH**, Huang WC, Kuo KT, Yuan RH, Chen YL, Jeng YM. S100P immunostaining identifies a subset of peripheral-type intrahepatic cholangiocarcinomas with morphological and molecular features similar to those of perihilar and extrahepatic cholangiocarcinomas. *Histopathology* 2012; **61**: 1106-1116 [PMID: 22882148 DOI: 10.1111/j.1365-2559.2012.04316.x]

52 **Chen TC**, Jan YY, Yeh TS. K-ras mutation is strongly associated with perineural invasion and represents an independent prognostic factor of intrahepatic cholangiocarcinoma after hepatectomy. *Ann Surg Oncol* 2012; **19** Suppl 3: S675-S681 [PMID: 22805857 DOI: 10.1245/s10434-012-2451-y]

53 **Oishi N**, Kumar MR, Roessler S, Ji J, Forgues M, Budhu A, Zhao X, Andersen JB, Ye QH, Jia HL, Qin LX, Yamashita T, Woo HG, Kim YJ, Kaneko S, Tang ZY, Thorgeirsson SS, Wang XW. Transcriptomic profiling reveals hepatic stem-like gene signatures and interplay of miR-200c and epithelial-mesenchymal transition in intrahepatic cholangiocarcinoma. *Hepatology* 2012; **56**: 1792-1803 [PMID: 22707408 DOI: 10.1002/hep.25890]

54 **Liao Q**, Li Z, Chen R, Guo N, Zeng B, Cheng D, Zheng L. [Effect of hepatitis C virus core gene transfection on NFAT1 expression in human intrahepatic cholangiocarcinoma cells]. *Nan Fang Yi Ke Da Xue Xue Bao* 2012; **32**: 789-793 [PMID: 22699055]

55 **Okamoto K**, Tajima H, Nakanuma S, Sakai S, Makino I, Kinoshita J, Hayashi H, Nakamura K, Oyama K, Nakagawara H, Fujita H, Takamura H, Ninomiya I, Kitagawa H, Fushida S, Fujimura T, Harada S, Wakayama T, Iseki S, Ohta T. Angiotensin II enhances epithelial-to-mesenchymal transition through the interaction between activated hepatic stellate cells and the stromal cell-derived factor-1/CXCR4 axis in intrahepatic cholangiocarcinoma. *Int J Oncol* 2012; **41**: 573-582 [PMID: 22664794 DOI: 10.3892/ijo.2012.1499]

56 **Li B**, Han Q, Zhu Y, Yu Y, Wang J, Jiang X. Down-regulation of miR-214 contributes to intrahepatic cholangiocarcinoma metastasis by targeting Twist. *FEBS J* 2012; **279**: 2393-2398 [PMID: 22540680 DOI: 10.1111/j.1742-4658.2012.08618.x]

57 **Gu FM**, Gao Q, Shi GM, Zhang X, Wang J, Jiang JH, Wang XY, Shi YH, Ding ZB, Fan J, Zhou J. Intratumoral IL-17$^+$ cells and neutrophils show strong prognostic significance in intrahepatic cholangiocarcinoma. *Ann Surg Oncol* 2012; **19**: 2506-2514 [PMID: 22411204 DOI: 10.1245/s10434-012-2268-8]

58 **Higashi M**, Yamada N, Yokoyama S, Kitamoto S, Tabata K, Koriyama C, Batra SK, Yonezawa S. Pathobiological implications of MUC16/CA125 expression in intrahepatic cholangiocarcinoma-mass forming type. *Pathobiology* 2012; **79**: 101-106 [PMID: 22286058 DOI: 10.1159/000335164]

59 **Gu MJ**, Choi JH. Clinicopathological significance of E-cadherin, β-catenin and epidermal growth factor receptor expression in intrahepatic cholangiocarcinoma. *Hepato-*

gastroenterology 2012; **59**: 1241-1244 [PMID: 22281980 DOI: 10.5754/hge11881]
60 **Wang Z**, Zheng T, Wu Q, Wang J, Wu C, Wang J. Immunohistochemical analysis of the mTOR pathway in intrahepatic cholangiocarcinoma. *Neoplasma* 2012; **59**: 137-141 [PMID: 22248270]
61 **Hirashita T**, Iwashita Y, Ohta M, Komori Y, Eguchi H, Yada K, Kitano S. Expression of matrix metalloproteinase-7 is an unfavorable prognostic factor in intrahepatic cholangiocarcinoma. *J Gastrointest Surg* 2012; **16**: 842-848 [PMID: 22246855 DOI: 10.1007/s11605-011-1813-2]
62 **Srimunta U**, Sawanyawisuth K, Kraiklang R, Pairojkul C, Puapairoj A, Titipungul T, Hahnvajanawong C, Tassaneeyakul W, Wongkham C, Wongkham S, Vaeteewoottacharn K. High expression of ABCC1 indicates poor prognosis in intrahepatic cholangiocarcinoma. *Asian Pac J Cancer Prev* 2012; **13** Suppl: 125-130 [PMID: 23480753]
63 **Morine Y**, Shimada M, Iwahashi S, Utsunomiya T, Imura S, Ikemoto T, Mori H, Hanaoka J, Miyake H. Role of histone deacetylase expression in intrahepatic cholangiocarcinoma. *Surgery* 2012; **151**: 412-419 [PMID: 21982637 DOI: 10.1016/j.surg.2011.07.038]
64 **Wakai T**, Shirai Y, Sakata J, Takamura M, Matsuda Y, Korita PV, Muneoka K, Sasaki M, Ajioka Y, Hatakeyama K. Ribonucleotide reductase M1 expression in intrahepatic cholangiocarcinoma. *Hepatogastroenterology* 2011; **58**: 1659-1663 [PMID: 21940346 DOI: 10.5754/hge11175]
65 **Larbcharoensub N**, Sornmayura P, Sirachainan E, Wilasrusmee C, Wanmoung H, Janvilisri T. Prognostic value of ABCG2 in moderately and poorly differentiated intrahepatic cholangiocarcinoma. *Histopathology* 2011; **59**: 235-246 [PMID: 21884202 DOI: 10.1111/j.1365-2559.2011.03935.x]
66 **Lee D**, Do IG, Choi K, Sung CO, Jang KT, Choi D, Heo JS, Choi SH, Kim J, Park JY, Cha HJ, Joh JW, Choi KY, Kim DS. The expression of phospho-AKT1 and phospho-MTOR is associated with a favorable prognosis independent of PTEN expression in intrahepatic cholangiocarcinomas. *Mod Pathol* 2012; **25**: 131-139 [PMID: 21874010 DOI: 10.1038/modpathol.2011.133]
67 **Dong LW**, Hou YJ, Tan YX, Tang L, Pan YF, Wang M, Wang HY. Prognostic significance of Beclin 1 in intrahepatic cholangiocellular carcinoma. *Autophagy* 2011; **7**: 1222-1229 [PMID: 21654208 DOI: 10.4161/auto.7.10.16610]
68 **Shinozaki A**, Shibahara J, Noda N, Tanaka M, Aoki T, Kokudo N, Fukayama M. Claudin-18 in biliary neoplasms. Its significance in the classification of intrahepatic cholangiocarcinoma. *Virchows Arch* 2011; **459**: 73-80 [PMID: 21607649 DOI: 10.1007/s00428-011-1092-z]
69 **Wakai T**, Shirai Y, Sakata J, Matsuda Y, Korita PV, Takamura M, Ajioka Y, Hatakeyama K. Prognostic significance of NQO1 expression in intrahepatic cholangiocarcinoma. *Int J Clin Exp Pathol* 2011; **4**: 363-370 [PMID: 21577322]
70 **Aishima S**, Fujita N, Mano Y, Kubo Y, Tanaka Y, Taketomi A, Shirabe K, Maehara Y, Oda Y. Different roles of S100P overexpression in intrahepatic cholangiocarcinoma: carcinogenesis of perihilar type and aggressive behavior of peripheral type. *Am J Surg Pathol* 2011; **35**: 590-598 [PMID: 21412073 DOI: 10.1097/PAS.0b013e31820ffdf1]
71 **Zhou JX**, Li Y, Chen SX, Deng AM. Expression and prognostic significance of cancer-testis antigens (CTA) in intrahepatic cholagiocarcinoma. *J Exp Clin Cancer Res* 2011; **30**: 2 [PMID: 21211023 DOI: 10.1186/1756-9966-30-2]
72 **Sanada Y**, Oue N, Mitani Y, Yoshida K, Nakayama H, Yasui W. Down-regulation of the claudin-18 gene, identified through serial analysis of gene expression data analysis, in gastric cancer with an intestinal phenotype. *J Pathol* 2006; **208**: 633-642 [PMID: 16435283]
73 **Sanada Y**, Hirose Y, Osada S, Tanaka Y, Takahashi T, Yamaguchi K, Yoshida K. Immunohistochemical study of claudin 18 involvement in intestinal differentiation during the progression of intraductal papillary mucinous neoplasm. *Anticancer Res* 2010; **30**: 2995-3003 [PMID: 20683045]
74 **Yap AQ**, Chen CL, Yong CC, Kuo FY, Wang SH, Lin CC, Liu YW, Lin TL, Li WF, Millan CA, Wang CC. Clinicopathological factors impact the survival outcome following the resection of combined hepatocellular carcinoma and cholangiocarcinoma. *Surg Oncol* 2013; **22**: 55-60 [PMID: 23102615 DOI: 10.1016/j.suronc.2012.09.003]
75 **Lee CH**, Hsieh SY, Chang CJ, Lin YJ. Comparison of clinical characteristics of combined hepatocellular-cholangiocarcinoma and other primary liver cancers. *J Gastroenterol Hepatol* 2013; **28**: 122-127 [PMID: 23034166 DOI: 10.1111/j.1440-1746.2012.07289.x]
76 **Yin X**, Zhang BH, Qiu SJ, Ren ZG, Zhou J, Chen XH, Zhou Y, Fan J. Combined hepatocellular carcinoma and cholangiocarcinoma: clinical features, treatment modalities, and prognosis. *Ann Surg Oncol* 2012; **19**: 2869-2876 [PMID: 22451237 DOI: 10.1245/s10434-012-2328-0]
77 **Ariizumi S**, Kotera Y, Katagiri S, Nakano M, Yamamoto M. Combined hepatocellular-cholangiocarcinoma had poor outcomes after hepatectomy regardless of Allen and Lisa class or the predominance of intrahepatic cholangiocarcinoma cells within the tumor. *Ann Surg Oncol* 2012; **19**: 1628-1636 [PMID: 22113592 DOI: 10.1245/s10434-011-2150-0]
78 **Yu XH**, Xu LB, Zeng H, Zhang R, Wang J, Liu C. Clinicopathological analysis of 14 patients with combined hepatocellular carcinoma and cholangiocarcinoma. *Hepatobiliary Pancreat Dis Int* 2011; **10**: 620-625 [PMID: 22146626]
79 **Park HS**, Bae JS, Jang KY, Lee JH, Yu HC, Jung JH, Cho BH, Chung MJ, Moon WS. Clinicopathologic study on combined hepatocellular carcinoma and cholangiocarcinoma: with emphasis on the intermediate cell morphology. *J Korean Med Sci* 2011; **26**: 1023-1030 [PMID: 21860552 DOI: 10.3346/jkms.2011.26.8.1023]
80 **Park H**, Choi KH, Choi SB, Choi JW, Kim do Y, Ahn SH, Kim KS, Choi JS, Han KH, Chon CY, Park JY. Clinicopathological characteristics in combined hepatocellular-cholangiocarcinoma: a single center study in Korea. *Yonsei Med J* 2011; **52**: 753-760 [PMID: 21786439 DOI: 10.3349/ymj.2011.52.5.753]
81 **Zhan Q**, Shen BY, Deng XX, Zhu ZC, Chen H, Peng CH, Li HW. Clinical and pathological analysis of 27 patients with combined hepatocellular-cholangiocarcinoma in an Asian center. *J Hepatobiliary Pancreat Sci* 2012; **19**: 361-369 [PMID: 21744084 DOI: 10.1007/s00534-011-0417-2]
82 **Ijichi H**, Shirabe K, Taketomi A, Yoshizumi T, Ikegami T, Mano Y, Aishima S, Abe K, Honda H, Maehara Y. Clinical usefulness of (18) F-fluorodeoxyglucose positron emission tomography/computed tomography for patients with primary liver cancer with special reference to rare histological types, hepatocellular carcinoma with sarcomatous change and combined hepatocellular and cholangiocarcinoma. *Hepatol Res* 2013; **43**: 481-487 [PMID: 23145869 DOI: 10.1111/j.1872-034X.2012.01107.x]
83 **de Campos RO**, Semelka RC, Azevedo RM, Ramalho M, Heredia V, Armao DM, Woosley JT. Combined hepatocellular carcinoma-cholangiocarcinoma: report of MR appearance in eleven patients. *J Magn Reson Imaging* 2012; **36**: 1139-1147 [PMID: 22782783 DOI: 10.1002/jmri.23754]
84 **Hwang J**, Kim YK, Park MJ, Lee MH, Kim SH, Lee WJ, Rhim HC. Differentiating combined hepatocellular and cholangiocarcinoma from mass-forming intrahepatic cholangiocarcinoma using gadoxetic acid-enhanced MRI. *J Magn Reson Imaging* 2012; **36**: 881-889 [PMID: 22730271 DOI: 10.1002/jmri.23728]
85 **Kim GJ**, Kim H, Park YN. Increased expression of Yes-associated protein 1 in hepatocellular carcinoma with stemness and combined hepatocellular-cholangiocarcinoma. *PLoS One* 2013; **8**: e75449 [PMID: 24086533 DOI: 10.1371/journal.pone.0075449]

86 **Ikeda H**, Harada K, Sato Y, Sasaki M, Yoneda N, Kitamura S, Sudo Y, Ooi A, Nakanuma Y. Clinicopathologic significance of combined hepatocellular-cholangiocarcinoma with stem cell subtype components with reference to the expression of putative stem cell markers. *Am J Clin Pathol* 2013; **140**: 329-340 [PMID: 23955451 DOI: 10.1309/AJCP66AVBANVNTQJ]

87 **Akiba J**, Nakashima O, Hattori S, Tanikawa K, Takenaka M, Nakayama M, Kondo R, Nomura Y, Koura K, Ueda K, Sanada S, Naito Y, Yamaguchi R, Yano H. Clinicopathologic analysis of combined hepatocellular-cholangiocarcinoma according to the latest WHO classification. *Am J Surg Pathol* 2013; **37**: 496-505 [PMID: 23388123 DOI: 10.1097/PAS.0b013e31827332b0]

88 **Coulouarn C**, Cavard C, Rubbia-Brandt L, Audebourg A, Dumont F, Jacques S, Just PA, Clément B, Gilgenkrantz H, Perret C, Terris B. Combined hepatocellular-cholangiocarcinomas exhibit progenitor features and activation of Wnt and TGFβ signaling pathways. *Carcinogenesis* 2012; **33**: 1791-1796 [PMID: 22696594 DOI: 10.1093/carcin/bgs208]

89 **Cai X**, Zhai J, Kaplan DE, Zhang Y, Zhou L, Chen X, Qian G, Zhao Q, Li Y, Gao L, Cong W, Zhu M, Yan Z, Shi L, Wu D, Wei L, Shen F, Wu M. Background progenitor activation is associated with recurrence after hepatectomy of combined hepatocellular-cholangiocarcinoma. *Hepatology* 2012; **56**: 1804-1816 [PMID: 22684921 DOI: 10.1002/hep.25874]

90 **Itoyama M**, Hata M, Yamanegi K, Yamada N, Ohyama H, Hirano H, Terada N, Nakasho K. Expression of both hepatocellular carcinoma and cholangiocarcinoma phenotypes in hepatocellular carcinoma and cholangiocarcinoma components in combined hepatocellular and cholangiocarcinoma. *Med Mol Morphol* 2012; **45**: 7-13 [PMID: 22431178 DOI: 10.1007/s00795-010-0534-z]

P- Reviewer: Basoli A, Lin ZY, Qin JM, Ramia JM, Xu R
S- Editor: Wen LL **L- Editor**: A **E- Editor:** Lu YJ

World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4291/wjgp.v5.i3.200

World J Gastrointest Pathophysiol 2014 August 15; 5(3): 200-204
ISSN 2150-5330 (online)

TOPIC HIGHLIGHT

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

Laparoscopic surgery in the management of Crohn's disease

James Y Lim, Joseph Kim, Scott Q Nguyen

James Y Lim, Joseph Kim, Scott Q Nguyen, Department of Surgery, Icahn School of Medicine at Mount Sinai, New York, NY 10029, United States
Author contributions: Lim JY performed the searches and prepared the initial draft; Kim J and Nguyen SQ edited and supplemented the manuscript.
Correspondence to: Scott Q Nguyen, MD, Department of Surgery, Icahn School of Medicine at Mount Sinai, 1 Gustave Levy Place, New York, NY 10029, United States. scott.nguyen@mountsinai.org
Telephone: +1-212-2418672 Fax: +1-212-2415979
Received: December 29, 2013 Revised: April 9, 2014
Accepted: May 29, 2014
Published online: August 15, 2014

Abstract

Crohn's disease is a chronic inflammatory bowel disease with surgery still frequently necessary in its treatment. Since the 1990's, laparoscopic surgery has become increasingly common for primary resections in patients with Crohn's disease and has now become the standard of care. Studies have shown no difference in recurrence rates when compared to open surgery and benefits include shorter hospital stay, lower rates of wound infection and decreased time to bowel function. This review highlights studies comparing the laparoscopic approach to the open approach in specific situations, including cases of complicated Crohn's disease.

Key words: Crohn's; Laparoscopy; Surgery; Colon; Ileum

Core tip: Laparoscopy is now increasingly used in cases of Crohn's disease. Recurrence rates are similar to that of open surgery and studies have shown benefits of decreased hospital stay as well as earlier bowel function. This review highlights several studies that looked at patients who underwent ileocolic and colon resections as well as more complicated cases of Crohn's.

Lim JY, Kim J, Nguyen SQ. Laparoscopic surgery in the management of Crohn's disease. *World J Gastrointest Pathophysiol* 2014; 5(3): 200-204 Available from: URL: http://www.wjgnet.com/2150-5330/full/v5/i3/200.htm DOI: http://dx.doi.org/10.4291/wjgp.v5.i3.200

INTRODUCTION

Crohn's disease is an autoimmune disorder that causes chronic transmural inflammation of the gastrointestinal tract and makes up one of the two main components of inflammatory bowel disease[1]. The terminal ileum and proximal colon are the most frequently affected and initial diagnosis is made early, between the ages of 20-30[2]. Despite the advances in medical therapies with increasingly new immunomodulator use, the rate of refractory disease requiring surgery has not changed over the years[3]. Surgery is still common and up to 80% of patients with Crohn's disease will require an operation during their lifetime, with 15%-20% requiring an operation within the first year after diagnosis[4-6]. Of those patients that undergo surgery, studies have shown that approximately 40%-50% will likely need additional surgical intervention within 10-15 years[7,8]. The likelihood of a second surgery within one's lifetime is high, with several studies having identified the median age of first surgical resection to be in a patient's third decade[6,9].

Initially, laparoscopic surgery was not attempted for Crohn's disease due to the intraoperative characteristics that made a laparoscopic approach challenging. These findings often included extensive inflammation, enteric fistulae, thickened mesentery, and skip lesions throughout the bowel[10]. This belief has changed over time and laparoscopy has become increasingly accepted in patients with Crohn's disease as the use of laparoscopy in a majority of gastrointestinal procedures has become standard[11]. Crohn's patients are typically young and benefit from a laparoscopic procedure that reduces scar and adhesion formation. In addition, given their high risk of surgical recurrence, Crohn's patients benefit from

surgical approaches that maximize abdominal wall integrity[2,10,12,13].

This article review will evaluate surgical resections as well as common surgical scenarios commonly seen with Crohn's disease and compare the laparoscopic and open approaches. A search was conducted in the PubMed, Cochrane, MEDLINE, and Scopus libraries with the following individual and combined key words: Crohn's disease, laparoscopy, surgery, cost, colon, ileocolic, fistula, recurrent, small bowel, outcome, minimally invasive surgery, inflammatory bowel disease, randomized, metaanalysis. References cited in the articles retrieved were also searched in order to identify other potential sources of information. The results were limited to human studies available in English.

LAPAROSCOPIC ILEOCOLIC RESECTIONS

One of the first randomized trials comparing laparoscopic resections to open resections for refractory ileocolic disease was published in 2001 by Milsom *et al*[14] Sixty patients were randomized to undergo either laparoscopic or open procedures. The authors reported improved morbidity rates and hospital length of stay rates in the laparoscopic group, although the anastomotic leak rate was similar between the two groups. Length of surgery favored the open group. Long term follow up showed no difference between the groups in terms of disease recurrence rates.

A similar long term prospective study undertaken from 1999-2003 in the Netherlands showed similar results of no difference in overall disease recurrence between the laparoscopic and the open groups. Additionally, there were fewer incidences of small bowel obstruction and incisional hernias in the laparoscopic group. Overall, patient quality of life and cosmesis scores favored the laparoscopic group[15].

One of the weaknesses of these randomized prospective studies is that the overall number of patients treated was small. However, metaanalysis studies with a larger number of subjects show that these findings for laparoscopic surgeries are consistent. In a large metaanalysis by Tilney, data from over 15 different studies looking specifically at laparoscopic ileocolic resections was compiled. The analysis included 783 patients, 338 (43.2%) of which had undergone laparoscopic resection. The overall conversion rate to open surgery was 6.8%. As seen in earlier studies, overall surgery duration was longer in the laparoscopic group with a difference of 29.6 min. Perioperative complications and anastomotic leak rates were similar between the two groups. Benefits of laparoscopy were significantly shorter time till bowel function was regained and a shorter hospital stay by 2.7 d[16].

These findings are also supported by other smaller prospective and retrospective studies comparing open *vs* laparoscopic ileocolic resections in patients with Crohn's disease. There were no differences in morbidity and mortality. Furthermore lengths of time till return of bowel function and hospital stay were consistently shorter in the laparoscopic groups[17-20].

More recently, data reviewed from the National Surgical Quality Improvement Program from 2005-2009 compiled perioperative results from over 1900 ileocolic resections for Crohn's disease, 34% of which were performed laparoscopically. On multivariate analysis, the laparoscopic group was associated with an overall decrease in major and minor perioperative complications as well as a significant decrease in overall hospital stay by 1.08 d[21].

Long term studies following open and laparoscopic ileocolic resection patients showed no difference in recurrence rates[22,23]. In one study, the average time to recurrence was 60 mo in the laparoscopic group and 62 mo in the open group. Another study reported the average five year recurrence rates to be 29.1% in laparoscopic patients and 27.7% in open patients. Median times to recurrence were 48 and 56 mo, respectively. These times were not significant with a *P*-value of 0.9104. Of note, the laparoscopic group was found to have lower bowel obstruction rates over that time period[18].

LAPAROSCOPIC COLON RESECTIONS

Much of the literature focuses on laparoscopic surgery at the ileocolic region. Because Crohn's disease can affect any part of the gastrointestinal tract, other anatomical locations can pose different challenges. Acute colitis rates in Crohn's disease patients ranges from 5% to 10%[24]. Given the larger size of the colon and potentially broader thicker diseased mesentery of the colon, laparoscopic surgery for Crohn's colitis was slower to become accepted.

One of the earliest studies comparing minimally invasive surgery in Crohn's colitis to open surgery was in 2007. This study case matched 27 patients based on various patient factors including comorbidities and types of surgery, looking at patients only with disease in the colon. The authors found that although overall surgery was longer in the laparoscopic group, complications and estimated blood loss were the same in both groups. Length of hospital stay was significantly shorter in the laparoscopic group when 30 d readmissions were included[25].

Another study retrospectively looked at 92 patients with Crohn's disease that underwent minimally invasive colon resections. Forty-three cases (47%) were total colectomies, 17 (18%) were subtotal colectomies, 32 (35%) were segmental resections. There were 15 conversions to open resections, but conversions were not associated with longer hospital stay or increased postoperative complications. Five patients required reoperation, three for obstruction and two for anastomotic leak. The only prognostic factor for a complicated hospital course was evidence of perianal disease and 30-d mortality was zero[26].

One of the largest studies looking at laparoscopic colon resections in patients with Crohn's disease prospectively compared 55 laparoscopic resections to 70 open resections. The conversion rate to open resection of 10.9 was similar to that for ileocolic resections. Of note, 34.5% of patients who underwent laparoscopic surgery had had prior abdominal surgery as compared to 65.7% of the open group. This is one of the weaknesses of this study as surgeon preference dictated which procedure was performed. Although there was likely selection bias, the laparoscopic group was associated with similar benefits that were identified in the randomized studies for ileocolic resections. These benefits included less intraoperative blood loss, shorter hospital stays and quicker return of bowel function after surgery in the laparoscopic group[27].

LAPAROSCOPIC SURGERY FOR FISTULIZING DISEASE

Enteric fistulas are challenging complications in Crohn's patients as this finding often implies the presence of a large inflammatory mass, a history of prior surgeries, or use of steroids-all of which can make the surgery technically difficult. Surgery for enteric fistulas requires resection of the involved segment and primary anastomosis in the elective setting. Fistulas involving other organs are treated with bowel resection of the involved segment and primary repair of the other involved organ[10,28]. Some studies have cited intraoperative discovery of an intraabdominal abscess or fistula as an independent risk factor for conversion from a laparoscopic procedure[29]. In addition, a recent consensus conference was unable to recommend a laparoscopic approach for cases of complex Crohn's disease[30].

A laparoscopic approach in these patients with complicated Crohn's can be treacherous but as surgeons have become more skilled with laparoscopy, more studies have shown its feasibility. One retrospective review looked at 72 patients who underwent laparoscopic surgery for enteric fistulas. This study included enterocolic, ileo-ileal, enterocutaneous, ileovesical, colovesical, colocutaneous, and colovaginal fistulas. Prior abdominal surgery was present in 39.7% of the patients. Approximately 30% of the patients had multiple fistulas and 12.3% of those underwent multiple resections. The rate of conversion to open resection was low at 4.1% and overall morbidity was 11%[28].

In a more recent case-matched study 11 patients presenting with 13 fistulas were matched to 22 controls with non-fistulizing disease according to age, sex, nutritional state, steroid use, and type of laparoscopic resection[31]. Although the sample size was small, the authors were unable to show any difference in operative time, conversion rates, or morbidity rates between the two groups.

A larger prospective comparative study compared laparoscopic ileocolonic resections in patients with complex Crohn's disease (abscess and/or fistula) to patients without complex Crohn's disease. There was no significant difference in postoperative complications but overall operative time, conversion rates, and frequency of temporary stoma creation were all significantly increased in the complex Crohn's group[32]. These findings are suggestive of a more challenging operation, although the lack of increased morbidity demonstrates that a laparoscopic approach is still feasible. This is an area that needs to be continued to be studied.

LAPAROSCOPIC SURGERY FOR RECURRENT DISEASE

As indications for laparoscopic surgery in patients with Crohn's disease expands, its use in patients with recurrent disease seems natural. Several studies have compared laparoscopic resection to open resection for recurrent disease with no significant difference in surgical outcomes[33,34]. The reported conversion rates within these studies ranged from 6.7%-42% with the most common reasons for conversion being adhesions, intraoperative discovery of fistula/abscess, or need for associated bowel resection. In general, the conversion rate for recurrent Crohn's disease was similar to numbers seen in surgeries for initial disease. Only in one study was the conversion rate higher in the recurrent disease group and risk factors for conversion were age greater than 40, repeat resection for recurrent disease, and operative findings of an abscess[35].

Laparoscopic surgery is also possible in patients whose primary operation was a midline laparotomy. A recent study compared laparoscopic *vs* open surgery for patients with recurrent Crohn's disease where their primary surgery was a bowel resection through a midline laparotomy. The study was a retrospective case matched study comparing 26 patients who underwent laparoscopic resection to 26 patients that underwent open resection. Both groups had comparable demographics in terms of comorbidities and prior number of abdominal surgeries. Of note, the recovery benefits of shorter hospital stay and earlier return of bowel function that are seen in all other studies were not maintained in the laparoscopic group. However, there was a significant decrease in wound complication rates when compared to the open group[36].

ROLE OF SINGLE INCISION SURGERY IN CROHN'S DISEASE

As the role of laparoscopy has increased in patients with Crohn's disease, other advances such as single incision surgeries have also been studied in these patients. Of the few studies published, there is a significant amount of heterogeneity in terms of the technical aspects of the procedures and long term data is not available. Initial results though, show that the single incision approach is feasible without a large increase in complications and with the benefit of decreased postoperative analgesia.

Other studies have shown that complication profile is similar to laparoscopic surgery with the only advantage appearing to be the decreased number of trocar sites while all other factors were equivalent[8,37-39].

COST EFFECTIVENESS OF LAPAROSCOPIC SURGERY IN CROHN'S DISEASE

The overall cost of care for Crohn's disease continues to increase, with some estimates placing the annual cost in the United States anywhere from $10-15.9 billion and $2.1-16.7 billion in Europe[40-42]. These estimates are expected to increase as newer biologic drugs are increasingly available and used in management[43]. Of these costs, hospitalizations accounted for 53%-66% of the total in the United States with an average of $37459 per hospitalization[41].

Laparoscopy has the potential to decrease these costs per hospitalization as studies have shown that, when compared to open surgeries, laparoscopic surgeries reduce length of hospital stays and concomitant complications. A recent study comparing laparoscopic to open cases found the difference in hospital charges were significantly different, on average $27575 *vs* $38713 respectively[44]. These savings are consistent with those seen in colorectal cancer resections when comparing laparoscopic to open surgeries[45]. These savings can be potentially further reduced with the increasing adoption of single port surgery as well[46].

CONCLUSION

Current literature lacks a large number of randomized trials, but the consistent outcomes seen in the numerous retrospective studies and the small number of randomized studies shows that minimally invasive surgical approaches for Crohn's disease patients are both feasible and safe. It is important to remember that patient selection and surgeon experience are important factors for successful laparoscopic surgery. Complicated Crohn's cases with recurrent disease and enteric fistulas require knowledge of advanced laparoscopic techniques. The primary benefits of laparoscopic surgery over open surgery are quicker return to bowel function, decreased wound infection rates and shorter hospital stays. With no difference in recurrence rates seen, laparoscopy is emerging as the standard approach for patients with Crohn's disease for initial surgery, and even in select cases of patients with recurrent and complicated Crohn's disease.

REFERENCES

1 **Bandzar S**, Gupta S, Platt MO. Crohn's disease: a review of treatment options and current research. *Cell Immunol* 2013; **286**: 45-52 [PMID: 24321565 DOI: 10.1016/j.cellimm.2013.11.003]

2 **Spinelli A**, Sacchi M, Bazzi P, Leone N, Danese S, Montorsi M. Laparoscopic surgery for recurrent Crohn's disease. *Gastroenterol Res Pract* 2012; **2012**: 381017 [PMID: 22253619 DOI: 10.1155/2012/381017]

3 **Rink AD**, Fischer IR, Vestweber B, Vestweber KH. Long-term outcome of laparoscopic ileocecal resection for Crohn's disease before the era of biologics. *Int J Colorectal Dis* 2014; **29**: 127-132 [PMID: 23857597 DOI: 10.1007/s00384-013-1744-3]

4 **Tavernier M**, Lebreton G, Alves A. Laparoscopic surgery for complex Crohn's disease. *J Visc Surg* 2013; **150**: 389-393 [PMID: 24119432 DOI: 10.1016/j.jviscsurg.2013.09.004]

5 **Sica GS**, Biancone L. Surgery for inflammatory bowel disease in the era of laparoscopy. *World J Gastroenterol* 2013; **19**: 2445-2448 [PMID: 23674844 DOI: 10.3748/wjg.v19.i16.2445]

6 **Patel SV**, Patel SV, Ramagopalan SV, Ott MC. Laparoscopic surgery for Crohn's disease: a meta-analysis of perioperative complications and long term outcomes compared with open surgery. *BMC Surg* 2013; **13**: 14 [PMID: 23705825 DOI: 10.1186/1471-2482-13-14]

7 **Hasegawa H**, Watanabe M, Nishibori H, Okabayashi K, Hibi T, Kitajima M. Laparoscopic surgery for recurrent Crohn's disease. *Br J Surg* 2003; **90**: 970-973 [PMID: 12905550 DOI: 10.1002/bjs.4136]

8 **Neumann PA**, Rijcken EJ, Bruewer M. Current status of laparoscopic surgery for patients with Crohn's disease. *Int J Colorectal Dis* 2013; **28**: 599-610 [PMID: 23588872 DOI: 10.1007/s00384-013-1684-y]

9 **Bernell O**, Lapidus A, Hellers G. Risk factors for surgery and recurrence in 907 patients with primary ileocaecal Crohn's disease. *Br J Surg* 2000; **87**: 1697-1701 [PMID: 11122187 DOI: 10.1046/j.1365-2168.2000.01589.x]

10 **Nguyen SQ**, Teitelbaum E, Sabnis AA, Bonaccorso A, Tabrizian P, Salky B. Laparoscopic resection for Crohn's disease: an experience with 335 cases. *Surg Endosc* 2009; **23**: 2380-2384 [PMID: 19263141 DOI: 10.1007/s00464-009-0362-1]

11 **Canin-Endres J**, Salky B, Gattorno F, Edye M. Laparoscopically assisted intestinal resection in 88 patients with Crohn's disease. *Surg Endosc* 1999; **13**: 595-599 [PMID: 10347299]

12 **Marcello PW**. Laparoscopy for inflammatory bowel disease: pushing the envelope. *Clin Colon Rectal Surg* 2006; **19**: 26-32 [PMID: 20011450 DOI: 10.1055/s-2006-939528]

13 **Milsom JW**. Laparoscopic surgery in the treatment of Crohn's disease. *Surg Clin North Am* 2005; **85**: 25-34; vii [PMID: 15619526 DOI: 10.1016/j.suc.2004.10.002]

14 **Milsom JW**, Hammerhofer KA, Böhm B, Marcello P, Elson P, Fazio VW. Prospective, randomized trial comparing laparoscopic vs. conventional surgery for refractory ileocolic Crohn's disease. *Dis Colon Rectum* 2001; **44**: 1-8; discussion 8-9 [PMID: 11805557]

15 **Eshuis EJ**, Slors JF, Stokkers PC, Sprangers MA, Ubbink DT, Cuesta MA, Pierik EG, Bemelman WA. Long-term outcomes following laparoscopically assisted versus open ileocolic resection for Crohn's disease. *Br J Surg* 2010; **97**: 563-568 [PMID: 20175126 DOI: 10.1002/bjs.6918]

16 **Tilney HS**, Constantinides VA, Heriot AG, Nicolaou M, Athanasiou T, Ziprin P, Darzi AW, Tekkis PP. Comparison of laparoscopic and open ileocecal resection for Crohn's disease: a metaanalysis. *Surg Endosc* 2006; **20**: 1036-1044 [PMID: 16715212 DOI: 10.1007/s00464-005-0500-3]

17 **Huilgol RL**, Wright CM, Solomon MJ. Laparoscopic versus open ileocolic resection for Crohn's disease. *J Laparoendosc Adv Surg Tech A* 2004; **14**: 61-65 [PMID: 15107212 DOI: 10.1089/109264204322973808]

18 **Bergamaschi R**, Pessaux P, Arnaud JP. Comparison of conventional and laparoscopic ileocolic resection for Crohn's disease. *Dis Colon Rectum* 2003; **46**: 1129-1133 [PMID: 12907912 DOI: 10.1097/01.DCR.0000074727.07777.FB]

19 **Polle SW**, Wind J, Ubbink DT, Hommes DW, Gouma DJ, Bemelman WA. Short-term outcomes after laparoscopic ileocolic resection for Crohn's disease. A systematic review. *Dig Surg* 2006; **23**: 346-357 [PMID: 17164582 DOI: 10.1159/000097950]

20 **Bemelman WA**, Slors JF, Dunker MS, van Hogezand RA, van Deventer SJ, Ringers J, Griffioen G, Gouma DJ. Laparoscopic-assisted vs. open ileocolic resection for Crohn's disease. A comparative study. *Surg Endosc* 2000; **14**: 721-725 [PMID: 10954817]

21 **Lee Y**, Fleming FJ, Deeb AP, Gunzler D, Messing S, Monson JR. A laparoscopic approach reduces short-term complications and length of stay following ileocolic resection in Crohn's disease: an analysis of outcomes from the NSQIP database. *Colorectal Dis* 2012; **14**: 572-577 [PMID: 21831174 DOI: 10.1111/j.1463-1318.2011.02756.x]

22 **Lowney JK**, Dietz DW, Birnbaum EH, Kodner IJ, Mutch MG, Fleshman JW. Is there any difference in recurrence rates in laparoscopic ileocolic resection for Crohn's disease compared with conventional surgery? A long-term, follow-up study. *Dis Colon Rectum* 2006; **49**: 58-63 [PMID: 16328612 DOI: 10.1007/s10350-005-0214-6]

23 **Stocchi L**, Milsom JW, Fazio VW. Long-term outcomes of laparoscopic versus open ileocolic resection for Crohn's disease: follow-up of a prospective randomized trial. *Surgery* 2008; **144**: 622-627; discussion 622-627 [PMID: 18847647 DOI: 10.1016/j.surg.2008.06.016]

24 **Maggiori L**, Panis Y. Laparoscopy in Crohn's disease. *Best Pract Res Clin Gastroenterol* 2014; **28**: 183-194 [PMID: 24485265 DOI: 10.1016/j.bpg.2013.11.004]

25 **da Luz Moreira A**, Stocchi L, Remzi FH, Geisler D, Hammel J, Fazio VW. Laparoscopic surgery for patients with Crohn's colitis: a case-matched study. *J Gastrointest Surg* 2007; **11**: 1529-1533 [PMID: 17786528 DOI: 10.1007/s11605-007-0284-y]

26 **Holubar SD**, Dozois EJ, Privitera A, Pemberton JH, Cima RR, Larson DW. Minimally invasive colectomy for Crohn's colitis: a single institution experience. *Inflamm Bowel Dis* 2010; **16**: 1940-1946 [PMID: 20848480 DOI: 10.1002/ibd.21265]

27 **Umanskiy K**, Malhotra G, Chase A, Rubin MA, Hurst RD, Fichera A. Laparoscopic colectomy for Crohn's colitis. A large prospective comparative study. *J Gastrointest Surg* 2010; **14**: 658-663 [PMID: 20127200 DOI: 10.1007/s11605-010-1157-3]

28 **Regan JP**, Salky BA. Laparoscopic treatment of enteric fistulas. *Surg Endosc* 2004; **18**: 252-254 [PMID: 14691702 DOI: 10.1007/s00464-003-8904-4]

29 **Alves A**, Panis Y, Bouhnik Y, Marceau C, Rouach Y, Lavergne-Slove A, Vicaut E, Valleur P. Factors that predict conversion in 69 consecutive patients undergoing laparoscopic ileocecal resection for Crohn's disease: a prospective study. *Dis Colon Rectum* 2005; **48**: 2302-2308 [PMID: 16228824 DOI: 10.1007/s10350-005-0190-x]

30 **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]

31 **Beyer-Berjot L**, Mancini J, Bege T, Moutardier V, Brunet C, Grimaud JC, Berdah S. Laparoscopic approach is feasible in Crohn's complex enterovisceral fistulas: a case-match review. *Dis Colon Rectum* 2013; **56**: 191-197 [PMID: 23303147 DOI: 10.1097/DCR.0b013e31826fedeb]

32 **Goyer P**, Alves A, Bretagnol F, Bouhnik Y, Valleur P, Panis Y. Impact of complex Crohn's disease on the outcome of laparoscopic ileocecal resection: a comparative clinical study in 124 patients. *Dis Colon Rectum* 2009; **52**: 205-210 [PMID: 19279413 DOI: 10.1007/DCR.0b013e31819c9c08]

33 **Brouquet A**, Bretagnol F, Soprani A, Valleur P, Bouhnik Y, Panis Y. A laparoscopic approach to iterative ileocolonic resection for the recurrence of Crohn's disease. *Surg Endosc* 2010; **24**: 879-887 [PMID: 19730944 DOI: 10.1007/s00464-009-0682-1]

34 **Chaudhary B**, Glancy D, Dixon AR. Laparoscopic surgery for recurrent ileocolic Crohn's disease is as safe and effective as primary resection. *Colorectal Dis* 2011; **13**: 1413-1416 [PMID: 21087388 DOI: 10.1111/j.1463-1318.2010.02511.x]

35 **Moorthy K**, Shaul T, Foley RJ. Factors that predict conversion in patients undergoing laparoscopic surgery for Crohn's disease. *Am J Surg* 2004; **187**: 47-51 [PMID: 14706585]

36 **Aytac E**, Stocchi L, Remzi FH, Kiran RP. Is laparoscopic surgery for recurrent Crohn's disease beneficial in patients with previous primary resection through midline laparotomy? A case-matched study. *Surg Endosc* 2012; **26**: 3552-3556 [PMID: 22648125 DOI: 10.1007/s00464-012-2361-x]

37 **Gardenbroek TJ**, Verlaan T, Tanis PJ, Ponsioen CY, D'Haens GR, Buskens CJ, Bemelman WA. Single-port versus multiport laparoscopic ileocecal resection for Crohn's disease. *J Crohns Colitis* 2013; **7**: e443-e448 [PMID: 23507422 DOI: 10.1016/j.crohns.2013.02.015]

38 **Rijcken E**, Mennigen R, Argyris I, Senninger N, Bruewer M. Single-incision laparoscopic surgery for ileocolic resection in Crohn's disease. *Dis Colon Rectum* 2012; **55**: 140-146 [PMID: 22228156 DOI: 10.1097/DCR.0b013e31823d0e0d]

39 **Maeda K**, Noda E, Nagahara H, Inoue T, Takii M, Watanabe K, Yamagami H, Sogawa M, Kamata N, Hirakawa K. A comparative study of single-incision versus conventional multiport laparoscopic ileocecal resection for Crohn's disease with strictures. *Asian J Endosc Surg* 2012; **5**: 118-122 [PMID: 22776543 DOI: 10.1111/j.1758-5910.2012.00132.x]

40 **Cohen RD**, Larson LR, Roth JM, Becker RV, Mummert LL. The cost of hospitalization in Crohn's disease. *Am J Gastroenterol* 2000; **95**: 524-530 [PMID: 10685762 DOI: 10.1111/j.1572-0241.2000.01779.x]

41 **Yu AP**, Cabanilla LA, Wu EQ, Mulani PM, Chao J. The costs of Crohn's disease in the United States and other Western countries: a systematic review. *Curr Med Res Opin* 2008; **24**: 319-328 [PMID: 18067689 DOI: 10.1185/030079908X260790]

42 **Feagan BG**, Vreeland MG, Larson LR, Bala MV. Annual cost of care for Crohn's disease: a payor perspective. *Am J Gastroenterol* 2000; **95**: 1955-1960 [PMID: 10950042 DOI: 10.1111/j.1572-0241.2000.02261.x]

43 **Keller DS**, Katz J, Stein SL, Delaney CP. Surgical cost of care in Crohn's disease. *Pol Przegl Chir* 2013; **85**: 511-516 [PMID: 24133109 DOI: 10.2478/pjs-2013-0079]

44 **Lesperance K**, Martin MJ, Lehmann R, Brounts L, Steele SR. National trends and outcomes for the surgical therapy of ileocolonic Crohn's disease: a population-based analysis of laparoscopic vs. open approaches. *J Gastrointest Surg* 2009; **13**: 1251-1259 [PMID: 19301075 DOI: 10.1007/s11605-009-0853-3]

45 **Jensen CC**, Prasad LM, Abcarian H. Cost-effectiveness of laparoscopic vs open resection for colon and rectal cancer. *Dis Colon Rectum* 2012; **55**: 1017-1023 [PMID: 22965399 DOI: 10.1097/DCR.0b013e3182656898]

46 **Moftah M,** Nazour F, Cunningham M, Cahill RA. Single port laparoscopic surgery for patients with complex and recurrent Crohn's disease. *J Crohns Colitis* 2014 Feb 28; Epub ahead of print [PMID: 24589026 DOI: 10.1016/j.crohns.2014.02.003]

P- Reviewer: Agresta F, Amornyotin S, Filik L, Sajid MS
S- Editor: Ji FF **L- Editor**: A **E- Editor:** Lu YJ

World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4291/wjgp.v5.i3.205

World J Gastrointest Pathophysiol 2014 August 15; 5(3): 205-212
ISSN 2150-5330 (online)

TOPIC HIGHLIGHT

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

Pathophysiology of fistula formation in Crohn's disease

Michael Scharl, Gerhard Rogler

Michael Scharl, Gerhard Rogler, Division of Gastroenterology and Hepatology, University Hospital Zürich, 8091 Zürich, Switzerland
Michael Scharl, Gerhard Rogler, Zurich Center for Integrative Human Physiology, University of Zürich, 8057 Zürich, Switzerland
Author contributions: Scharl M and Rogler G wrote and discussed the paper.
Supported by A grant from Fonds zur Förderung des akademischen Nachwuchses (FAN) of the Zürcher Universitätsverein (ZUNIV) to MS; by a research grant from the Swiss Philanthropy Foundation to MS and GR; by a research credit from the University of Zurich to MS; by research grants from the Swiss National Science Foundation (SNF) to MS, No. 314730-146204, GR, No. 310030-120312, and the Swiss IBD Cohort, No. 3347CO-108792; and by the Zurich Center for Integrative Human Physiology (ZIHP) of the University of Zurich
Correspondence to: Dr. Michael Scharl, MD, PhD, Division of Gastroenterology and Hepatology, University Hospital Zürich, Rämistrasse 100, 8091 Zürich, Switzerland. michael.scharl@usz.ch
Telephone: +41-44-2559519 Fax: +41-44-2559497
Received: December 9, 2013 Revised: April 4, 2014
Accepted: May 29, 2014
Published online: August 15, 2014

Abstract

Fistulae represent an important complication in patient suffering from Crohn's disease (CD). Cumulative incidence of fistula formation in CD patients is 17%-50% and about one third of patients suffer from recurring fistulae formation. Medical treatment options often fail and also surgery frequently is not successful. Available data indicate that CD-associated fistulae originate from an epithelial defect that may be caused by ongoing inflammation. Having undergone epithelial to mesenchymal transition (EMT), intestinal epithelial cells (IEC) penetrate into deeper layers of the mucosa and the gut wall causing localized tissue damage formation of a tube like structure and finally a connection to other organs or the body surface. EMT of IEC may be initially aimed to improve wound repair mechanisms since "conventional" wound healing mechanisms, such as migration of fibroblasts, are impaired in CD patients. EMT also enhances activation of matrix remodelling enzymes such as matrix metalloproteinase (MMP)-3 and MMP-9 causing further tissue damage and inflammation. Finally, soluble mediators like TNF and interleukin-13 further induce their own expression in an autocrine manner and enhance expression of molecules associated with cell invasiveness aggravating the process. Additionally, pathogen-associated molecular patterns also seem to play a role for induction of EMT and fistula development. Though current knowledge suggests a number of therapeutic options, new and more effective therapeutic approaches are urgently needed for patients suffering from CD-associated fistulae. A better understanding of the pathophysiology of fistula formation, however, is a prerequisite for the development of more efficacious medical anti-fistula treatments.

Key words: Crohn's disease; Fistula; Tumor necrosis factor; Interleukin-13; Transforming growth factor; Epithelial to mesenchymal transition

Core tip: Fistulae represent an important complication in Crohn's disease (CD) patients. CD-associated fistulae originate from an epithelial defect due to destructive inflammation. Having undergone epithelial-to-mesenchymal transition (EMT), intestinal epithelial cells (IEC) penetrate into deeper layers of the gut wall causing further tissue damage finally forming connections to other organs or the body surface. EMT of IEC results in activation of matrix remodelling enzymes. Soluble mediators like TNF and IL-13 induce their own expression and expression of molecules associated with cell invasiveness. A better understanding of the pathophysiology of fistula formation is a prerequisite for developing more efficacious medical anti-fistula treatments.

Scharl M, Rogler G. Pathophysiology of fistula formation in Crohn's disease. *World J Gastrointest Pathophysiol* 2014; 5(3): 205-212 Available from: URL: http://www.wjgnet.com/2150-5330/full/v5/i3/205.htm DOI: http://dx.doi.org/10.4291/wjgp.v5.i3.205

INTRODUCTION

Crohn's disease (CD) and ulcerative colitis are the two main forms of inflammatory bowel diseases (IBD) and are characterized by chronic intestinal inflammation. An important complication of CD is the formation of fistulae. This frequent clinical problem often causes a severely impaired quality of life in the affected patients. According to population-based studies, the cumulative incidence of fistula formation in CD patients is 17%-50%[1-3]. About 35% of patients suffer from at least one fistula during their disease course[2]. After 10 years of disease, the cumulative incidence for fistulas is reported to be up to 33% and after 20 years it is about 50%[2]. About one third of patients suffer from recurring fistulae[2]. In roughly 10% of the CD patients fistulae are the initial disease presentation and may precede the manifestation of intestinal disease by several years[1]. Therapeutic options are limited: Medical options include antibiotics, immunosuppressives (such as azathioprine or cyclosporine) and anti-TNF antibodies. Their clinical effect is often limited and despite medical treatment more than one third of patients suffers from recurring fistulae[4]. However, also surgical option do not always provide a definitive solution and permanent fistula closure can only be achieved in about 34% of CD patients[5].

MORPHOLOGICAL CHARACTERISATION OF CD FISTULAE

CD fistulae are found perianal in the majority of cases (54%), as well as entero-enteric (24%), recto-vaginal (9%) or in other locations, such as entero-cutaneous or entero-vesical[2]. On a histomorphologic level, CD-associated fistulae reveal a central fissure that penetrates through the lamina propria and muscularis mucosa into deeper layers of the underlying gut wall. In general, fistulae are lined by granulation tissue consisting of histiocytes and a dense network of tender capillaries. The lumen is frequently filled up by nuclear debris, erythrocytes as well as neutrophils and lymphocytes indicating non-specific acute or chronic inflammation. In more than 80% there are signs of moderate to severe acute inflammation. In CD fistulae, the wall of the fistulae is frequently infiltrated by $CD45RO^+$ positive T-cells, followed by a small band of $CD68^+$ positive macrophages and finally a dense infiltrate of $CD20^+$ B-cells. This is in contrast to non-CD fistulae, where often an intense infiltration by $CD68^+$ macrophages, but only a few $CD20^+$ B-lymphocytes and $CD45RO^+$ T-lymphocytes are observed[6].

Independent of the inflammatory infiltrate about one fourth of CD fistulae feature a lining epithelium central in the fistulising inflammation. Depending on the fistula location, this lining epithelium consists of flattened epithelial cells of the small intestine or colon without goblet cells or of a narrow squamous epithelium in cutaneous or perianal fistulae. The cells reveal tight junctions and a basement membrane. In "non-epithelialized" fistulae some areas are lined with myofibroblast-like cells, so-called transitional cells (TC). The region where the mucosal epithelial cells transform into the TC is called transitional zone. The TC are connected by gap junctions to each other and in certain areas a new basement membrane develops between TC and the underlying granulation tissue. The TC and the new basement membrane are connected by fibronexus. However, in adjacent areas there are also disordered myofibroblasts showing no gap junctions and a fragmented basement membrane[6].

ALTERED MIGRATORY POTENTIAL OF COLONIC LAMINA PROPRIA FIBROBLASTS

On a functional level, the migratory potential of colonic lamina propria fibroblasts (CLPF) in the intestine of CD patients is clearly less than in non-IBD or UC patients. Of note, mucosal fibroblasts derived from CD-associated fistulae reveal an even further reduced migratory potential what might contribute to decreased wound healing potential in this disease[7,8]. The decrease in the migratory potential of the CLPF can be induced by pro-inflammatory cytokines, such as TNF or IFN and is a persistent functional change since it is not reversible even after removing the cytokines. Additionally, it is accompanied by a decreased expression and phosphorylation, meaning activation, of the focal adhesion kinase (FAK)[7,9] which is a central regulator of cell migration[10]. Fibronectin is also an essential factor for the induction of migration of CLPF. In CD fistulae, the ED-A and ED-B isoforms of fibronectin are almost absent[11]. This might also critically contribute to the reduced migratory potential of CD fistula CLPF, since the ED-A subunit is usually increased during wound healing and is an important inducer of fibroblast migration[12]. A further stimulator of CLPF migration in the intestine is galectin-3 which is secreted by colonic epithelial cells[13,14]. Though galectin-3 is able to induce the migration of CLPF derived from CD fistulae, its expression is clearly decreased in CD fistulae[14]. These observations might explain the reduced mesenchyme-mediated wound healing potential in patients with CD fistulae. Other mechanisms have to step in to repair the epithelial barrier. Increased IEC migration is a mechanism aiming to replace the malfunctioning fibroblast migration, to improve wound repair and to restore intestinal barrier function. Thus, the migration of epithelial towards the defect might be induced as part of a compensatory mechanism, since the migratory potential of CD fistula CLPF is critically impaired[15].

A KEY ROLE FOR EPITHELIAL TO MESENCHYMAL TRANSITION

In order to migrate, IEC must undergo a conversion into mesenchymal-like cells so-called myofibroblasts. This process is called epithelial-to-mesenchymal transition. During EMT, differentiated IEC characterized by strong intercellular junctions and cell polarity lose their epithelial phenotype and acquire a mesenchymal differentiation featuring reduced cell-cell contacts and a fibroblast-like morphology and function[16,17]. EMT plays an important role during embryogenesis and organ development, but also for tissue remodelling and wound healing[16-18]. However, recent studies also suggested that EMT is critically involved in pathologic conditions, such as cancer growth and fibrosis[16,17]. While TGF is the most potent inducer of EMT *in vivo*[19], there are further markers for the onset of EMT, such as decreased expression of E-cadherin and β-catenin, translocation of membrane-bound-catenin into the cytosol or the nucleus and increased expression of β6-integrin[16,17,20,21].

Available data strongly suggest that EMT might also be critically involved in the formation of the TC layer covering the fistula tracts of CD patients. TGF-1 and TGF-2 expression are both upregulated in fistula lining cells as compared to regular IEC[22]. E-cadherin is involved in the formation of intercellular zonulae adherentes and is found at the lateral cell membrane at the cell-cell contact sites of normal IEC. In the TCs lining the CD fistula tract not only a decreased expression, but also redistribution of membranous E-cadherin is detectable. This change in localization of E-cadherin can especially be observed in the transition zone[22]. In TC-catenin expression is diffuse, much weaker and found in the cytoplasm and nuclei whereas it is located in the lateral cell membrane of normal mucosal IEC[22]. Six integrin expression normally is restricted to epithelial cells during embryonic development and organogenesis. Its re-induction indicates an important role of this molecule during intestinal EMT[20,21]. In contrast to normal IEC, TC localised in the transitional zones feature a strong staining pattern for 6-integrin[22]. IEC as well as mesenchymal-like myofibroblast-like TC expressed both of the epithelial markers, cytokeratin (CK) 8 and CK 20[22]. These staining patterns of the TC strongly suggest an epithelial origin of these myofibroblast-like cells that show characteristic features of EMT.

A further event that is characteristic to EMT, is the nuclear expression of the transcription factors SNAIL1 and SLUG (SNAIL2) which are activated by TGF and are involved in the down-regulation of E-cadherin[16,23,24]. Interestingly, there are different expression pattern for SNAIL1 and SLUG in CD fistulae. While SNAIL1 is heavily expressed in the nuclei of the TC lining the fistula tracts indicating transcriptionally active protein, SLUG is mainly detected in cells around the fistula tract and only to a very limited level in the fistula tract lining TC[25]. CLPF from CD patients with fistulae express higher SLUG mRNA levels than CLPF from patients without fistulae[26]. Expression of fibroblast growth factors 1 and 2 that are also associated with increased SNAIL1 expression, reduced E-cadherin expression and the onset of EMT are detectable in the tissue layers below the fistula tract and in the fistula-associated inflammatory infiltrates[25]. A number of EMT characteristic events are detectable in and around fistula tracts clearly supporting a role for EMT in the pathogenesis of CD-associated fistulae. The detection of epithelial markers in submucosal myofibroblast-like cells further supports this hypothesis and demonstrates the transformation of former IEC into these mesenchymal-like cells.

INVOLVEMENT OF MATRIX REMODELLING MOLECULES

The intercellular matrix is constantly remodelled by a number of enzymes that degrade all components of the extracellular matrix (ECM), namely the matrix metalloproteinases (MMP). Increased MMP activity finally results in immune-mediated tissue injury and is associated with a number of pathologies, such as cancer growth and CD[27-29]. The importance of MMPs for the development of CD is highlighted by the fact that in the murine DSS-induced colitis model, targeted deletion of MMP-9 has a protective effect[30,31] while mice overexpressing MMP-9 in the intestinal epithelium develop more severe colitis when compared to wild type animals[32]. Further, addition of MMP-3 caused extensive tissue injury in an *ex vivo* human fetal model of intestinal inflammation and tissue injury was effectively blocked by inhibiting MMP activity[33]. The physiological inhibitors of MMPs are the tissue inhibitors of MMP (TIMP) which are also secreted by the MMP producing cells[27].

In CD fistulae, strong MMP-3 expression is observed independent of the stage of inflammation. MMP3 mRNA and protein expression is detected in mononuclear cells and fibroblasts[34]. Inactive and active MMP-9 is expressed around CD fistulae and mRNA and protein levels are found in granulocytes and fibroblasts[34,35]. The activated isoform of MMP-13 is present in the supernatant of untreated CD fistula CLPF, but is almost absent in the supernatant of non-fistula CLPF. MMP-13 protein expression also is clearly detectable in mononuclear cells around CD fistulae[26,35]. In contrast, expression of MMP-1 and MMP-7 is only weak around CD fistulae, MMP-10 is not detectable and MMP-2 protein is equally expressed in fistula and control tissue. Activated MMP-2 is only detectable in CD fistulae[34]. Protein levels of TIMP-1, TIMP-2 and TIMP-3 are low around CD fistulae[34]. These observations suggest a critical role for matrix remodelling enzymes in fistula pathogenesis.

MOLECULES ASSOCIATED WITH CELL MIGRATION AND INVASION

The published data on fistula pathogenesis strongly sug-

gest that fistula originate from an epithelial injury and due to defective wound healing mechanisms IEC re-differentiate into mesenchymal-like cells acquiring migratory potential. In line with this assumption, molecules associated with IEC migration, such as 6-integrin, Ets-1 transcription factor and the Wnt-inhibitor Dickkopf-homolog 1 (DKK-1) are detected in CD fistulae.

While regular mucosal epithelial cells do not express 6-integrin, TC located in the transitional zone are strongly positive for β6-integrin staining[22] and fistula CLPF express higher 6-integrin mRNA levels than CLPF from control or CD patients without fistulae[26]. These observations are of particular interest, since 6-integrin expression has been associated with migration, invasion, metastasis and shortened survival in certain carcinomas, such as colorectal cancer, head-and-neck cancer, breast cancer or squamous cell cancer[20,36-39]. Up-regulated β6-integrin is associated with increased levels of EMT[20,38-41], represents a receptor for fibronectin and tenascin what might be important for cell migration, regulates secretion and activation of MMP-9[42,43] and mediates TGF activation and adhesion what has also been implicated in increased survival, progression and metastases of various tumours[44,45]. Additionally, 6-integrin can induce its own expression in an autocrine manner[46]. On a transcriptional level, 6-integrin is regulated by Ets-1 transcription factor[20]. While tissue samples from control and IBD-patients in remission display only low expression levels of Ets-1 protein, a strong staining signal is detected in tissue samples derived from patients with active inflammation and in TC along the fistula tract[47] providing further support for the regulatory effect of Ets-1 on 6-integrin expression.

The Wnt-inhibitor DKK-1 represents an important factor involved in the regulation of cell migration. Loss of DKK-1 has been associated with progression of certain types of carcinomas, such as CRC[48]. The secreted glycoprotein is capable to block IEC migration, is a potent antagonist of the canonical Wnt/β-catenin signalling pathway and has been implicated to act as a mediator of inflammation[49,50]. In the intestinal tissue of non-IBD control patients, Dkk-1 staining is very weak, while tissue samples from CD-patients with a perianal fistulae reveal strong DKK-1 staining in TC lining the fistula tracts and patients with active CD also exhibit considerable DKK-1 expression in inflammatory infiltrates[51].

TGF

TGF is the most important inducer of EMT[19] and expression of TGF-1 and TGF-2 is higher in TC than in normal IEC[22]. TGF induces the mRNA expression of interleukin-13 (IL-13) in the HT-29 IEC spheroid model of EMT and the secretion of IL-13 from fistula CLPF, but not from CLPF from non-IBD control patients or CD patients without fistulae[26]. The effect of TGF on IL-13 expression in IEC is mediated *via* -catenin and DKK-1[51]. In HT-29 IEC, TGF treatment induces DKK-1 levels and this effect is inhibited by knock-down of -catenin. Interestingly, knock-down of either-catenin or DKK-1 prevents the TGF-induced increase in IL-13 expression[51]. These observations fit to the nuclear staining pattern of -catenin in TC. Increased TGF levels in TC induce nuclear accumulation, meaning transcriptional activation, of -catenin what results in enhanced expression of DKK-1 and IL-13. The TGF-induced up-regulation of DKK-1 might serve hereby as a negative feed-back mechanism to control TGF-mediated effects. However, IL-13 decreases the expression of DKK-1 in IEC and fistula CLPF[51] what finally dis-ruptures this regulatory mechanism and might result in uncontrolled secretion of IL-13.

IL-13

IL-13 has been implicated in the pathogenesis of tissue fibrosis, such as in the lung or liver, and, in this context, induces the secretion of TGF[52-54]. It is mainly secreted by Th2-cells and its alpha 1 receptor (IL-13R1) is the signal transducing receptor while the alpha 2 receptor (IL-13R2) acts as a decoy receptor[55,56]. In TC lining the fistula tracts as well as in deformed crypts adjacent to the fistula, IL-13 and IL-13R1 are heavily expressed, while they are almost absent in the intestine of non-IBD patients, UC patients and CD patients without fistula regardless their inflammation status[26]. These observations suggest that IL-13 expression and associated effects might be induced in CLPF and fistula-associated IEC in a positive feedback mechanism. On a functional level, IL-13 induces the expression of SLUG and 6-integrin in HT29 cells grown as monolayers or spheroids. Interestingly the IL-13 induced 6-integrin expression is mediated, at least in part, *via* SLUG transcription factor and SLUG expression is sensitive to anti-IL-13 antibody treatment. However, in contrast to TGF, IL-13 treatment is not sufficient to induce EMT in the HT29-IEC spheroid model[26].

TNF

TNF has been demonstrated to induce EMT in IEC and is able to induce the expression of TGF[47,57,58]. Similar to IL-13, strong staining for TNF and its receptor, TNF-receptor I (TNF-RI) is detected in TC lining the fistula tracts as well as in IEC of adjacent crypts in CD patients. TNF is also expressed in fistula surrounding immune cells[25]. This observation further supports the hypothesis that TNF, similar to IL-13, induces its expression in an autocrine manner. Correlating, TNF induces its own expression in IEC and fistula CLPF *in vitro*[47,57]. In IEC and CLPF, TNF stimulates the expression of 6-integrin and Ets-1 transcription factor and knock-down of Ets-1 results in diminished 6-integrin levels in response to TNF. Of note, while TNF induces TGF and EMT in IEC, it is not sufficient to stimulate IL-13 neither in IEC nor in fistula CLPF[47]. These observations suggest that

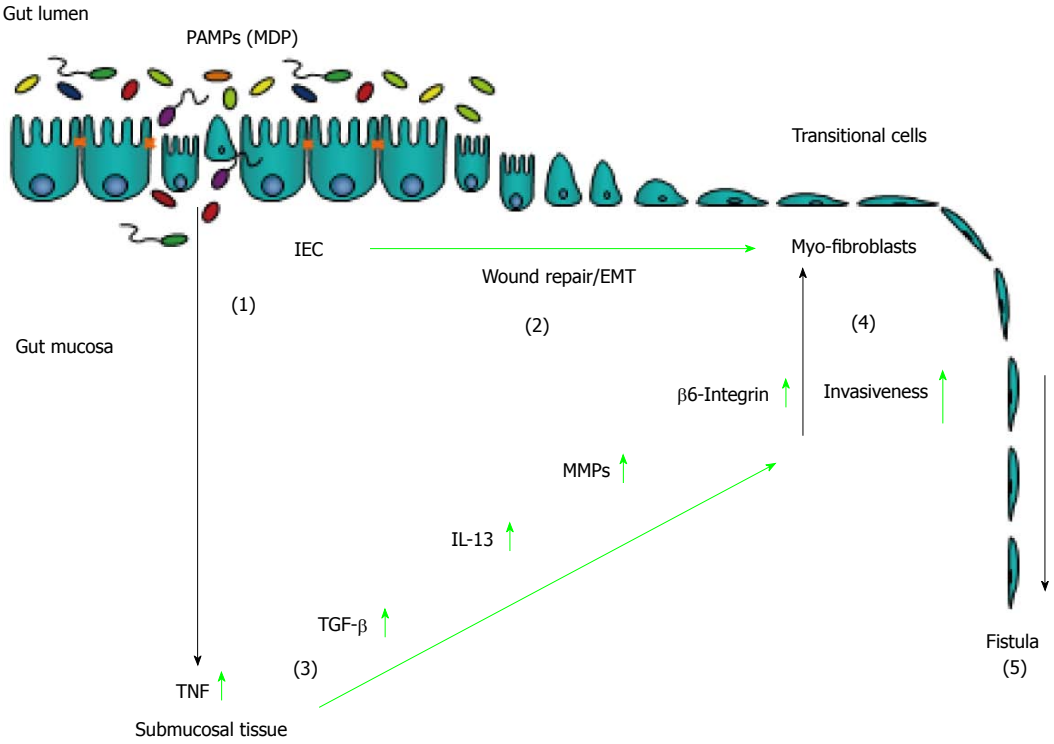

Figure 1 Pathogenesis of Crohn's disease-associated fistulae. An epithelial barrier defect favours the invasion of pathogen-associated pattern (PAMPs) into the gut mucosa (1). On the one hand, for wound healing purposes, intestinal epithelial cells undergo epithelial-to-mesenchymal transition (2). On the other hand, presence of PAMPs induced an inflammatory reaction resulting in increased secretion of TNF (3). TNF is able to induce secretion of TGF as well as to induce EMT and expression of molecules associated with cell invasiveness, such as 6-integrin. TGF-induced IL-13 and elevated activation of matrix remodelling MMPs critically contribute to invasive cell growth (4). Finally, EMT, MMP over-activation and elevated expression of invasive molecules contribute to the development of fistulae (5).

TNF, which is naturally present in acutely and chronically inflamed intestinal tissue, acts *via* two different pathways: TNF induces EMT on the one hand by its own, on the other had *via* TGF, as part of a wound healing mechanism. However, aberrant responses of IEC and/or CLPF to TGF then result in increased expression of IL-13. IL-13, similar to TNF, then stimulates its own expression *via* a positive feedback mechanism what finally causes the expression of molecules being associated with cell migration and invasiveness, such as Ets-1 and 6-integrin. TNF-induced effects can be effectively blocked by administration of an anti-TNF antibody *in vitro*[47], what might also explain, at least in part, the beneficial effect of anti-TNF antibodies for fistula treatment in CD patients.

PATHOGEN-ASSOCIATED MOLECULAR PATTERN

Polymorphisms within the nucleotide oligomerization domain 2 (NOD2) gene are associated with a fistulizing disease course of CD[59]. The bacterial wall component and pathogen-associated molecular pattern (PAMP), muramyl-dipeptide, is the natural ligand for NOD2 and following activation of NOD2, immune cells produce pro-inflammatory cytokines, such as TNF[60]. MDP treatment induces the expression of genes being associated with EMT as well as with cell invasiveness, such as TGF, SNAIL1, IL-13 and 6-Integrin, in IEC. While in non-fistula CLPF, MDP significantly induced mRNA expression of Ets-1, 6-Integrin, TNF, SNAIL1 and TGF, in fistula CLPF MDP treatment only induces mRNA levels of Ets-1 and TGF[47]. Since fistula CLPF express high levels of TNF and IL-13 *via* an autocrine mechanism, it might be that exogenous stimulation of these cells, *i.e.*, by MDP, is not sufficient to further induce TNF or IL-13 levels in these cells. Interestingly, lipopolysaccharide (LPS) does not induce any of the fistula-associated molecules in either IEC or CLPF pointing towards a specific role for the MDP-NOD2 axis in fistula pathogenesis. These observations suggest that distinct PAMPs might play a critical role for fistula pathogenesis by inducing EMT and genes being associated with EMT and cell invasiveness what makes the use of antibiotics in fistula treatment plausible.

CONCLUSION

Taken together, available data demonstrate that CD-associated fistulae originate from an epithelial defect that occurs during chronic inflammation. Having undergone EMT, IEC penetrate into deeper tissue layers causing tissue damage and a connection to other organs or the body surface. EMT of IEC is part of a wound repair mechanism as inflammation causes ongoing tissue damage and conventional wound healing mechanisms, such

as migration of fibroblasts, are impaired. The expression of EMT-associated molecules results in enhanced activation of matrix remodelling enzymes such as MMP-3 and MMP-9 causing further tissue damage and inflammation. Finally, soluble mediators such as TNF and IL-13 promote their own expression in an autocrine manner and enhance expression of molecules being associated with cell invasiveness. Subsequently, fistula formation and "growth" is constantly promoted and further supported by the presence of EMT-inducers, such as TGF, and PAMPs (Figure 1). Though current knowledge suggests a number of therapeutic options, new and more effective therapeutic approaches are urgently needed for patients suffering from CD-associated fistulae.

REFERENCES

1 **Hellers G**, Bergstrand O, Ewerth S, Holmström B. Occurrence and outcome after primary treatment of anal fistulae in Crohn's disease. *Gut* 1980; **21**: 525-527 [PMID: 7429313]
2 **Schwartz DA**, Loftus EV, Tremaine WJ, Panaccione R, Harmsen WS, Zinsmeister AR, Sandborn WJ. The natural history of fistulizing Crohn's disease in Olmsted County, Minnesota. *Gastroenterology* 2002; **122**: 875-880 [PMID: 11910338]
3 **Solomon MJ**. Fistulae and abscesses in symptomatic perianal Crohn's disease. *Int J Colorectal Dis* 1996; **11**: 222-226 [PMID: 8951512]
4 **Nielsen OH**, Rogler G, Hahnloser D, Thomsen OØ. Diagnosis and management of fistulizing Crohn's disease. *Nat Clin Pract Gastroenterol Hepatol* 2009; **6**: 92-106 [PMID: 19153563 DOI: 10.1038/ncpgasthep1340]
5 **Loftus EV**, Schoenfeld P, Sandborn WJ. The epidemiology and natural history of Crohn's disease in population-based patient cohorts from North America: a systematic review. *Aliment Pharmacol Ther* 2002; **16**: 51-60 [PMID: 11856078]
6 **Bataille F**, Klebl F, Rümmele P, Schroeder J, Farkas S, Wild PJ, Fürst A, Hofstädter F, Schölmerich J, Herfarth H, Rogler G. Morphological characterisation of Crohn's disease fistulae. *Gut* 2004; **53**: 1314-1321 [PMID: 15306592 DOI: 10.1136/gut.2003.038208]
7 **Leeb SN**, Vogl D, Gunckel M, Kiessling S, Falk W, Göke M, Schölmerich J, Gelbmann CM, Rogler G. Reduced migration of fibroblasts in inflammatory bowel disease: role of inflammatory mediators and focal adhesion kinase. *Gastroenterology* 2003; **125**: 1341-1354 [PMID: 14598250]
8 **Leeb SN**, Vogl D, Falk W, Schölmerich J, Rogler G, Gelbmann CM. Regulation of migration of human colonic myofibroblasts. *Growth Factors* 2002; **20**: 81-91 [PMID: 12148566]
9 **Meier JK**, Scharl M, Miller SN, Brenmoehl J, Hausmann M, Kellermeier S, Schölmerich J, Rogler G. Specific differences in migratory function of myofibroblasts isolated from Crohn's disease fistulae and strictures. *Inflamm Bowel Dis* 2011; **17**: 202-212 [PMID: 20848526 DOI: 10.1002/ibd.21344]
10 **Schlaepfer DD**, Hauck CR, Sieg DJ. Signaling through focal adhesion kinase. *Prog Biophys Mol Biol* 1999; **71**: 435-478 [PMID: 10354709]
11 **Brenmoehl J**, Lang M, Hausmann M, Leeb SN, Falk W, Schölmerich J, Göke M, Rogler G. Evidence for a differential expression of fibronectin splice forms ED-A and ED-B in Crohn's disease (CD) mucosa. *Int J Colorectal Dis* 2007; **22**: 611-623 [PMID: 17136547 DOI: 10.1007/s00384-006-0188-4]
12 **Ffrench-Constant C**, Van de Water L, Dvorak HF, Hynes RO. Reappearance of an embryonic pattern of fibronectin splicing during wound healing in the adult rat. *J Cell Biol* 1989; **109**: 903-914 [PMID: 2760116]
13 **Lippert E**, Falk W, Bataille F, Kaehne T, Naumann M, Goeke M, Herfarth H, Schoelmerich J, Rogler G. Soluble galectin-3 is a strong, colonic epithelial-cell-derived, lamina propria fibroblast-stimulating factor. *Gut* 2007; **56**: 43-51 [PMID: 16709662 DOI: 10.1136/gut.2005.081646]
14 **Lippert E**, Gunckel M, Brenmoehl J, Bataille F, Falk W, Scholmerich J, Obermeier F, Rogler G. Regulation of galectin-3 function in mucosal fibroblasts: potential role in mucosal inflammation. *Clin Exp Immunol* 2008; **152**: 285-297 [PMID: 18336593 DOI: 10.1111/j.1365-2249.2008.03618.x]
15 **Dignass AU**. Mechanisms and modulation of intestinal epithelial repair. *Inflamm Bowel Dis* 2001; **7**: 68-77 [PMID: 11233665]
16 **Kalluri R**, Neilson EG. Epithelial-mesenchymal transition and its implications for fibrosis. *J Clin Invest* 2003; **112**: 1776-1784 [PMID: 14679171 DOI: 10.1172/JCI20530]
17 **Lee JM**, Dedhar S, Kalluri R, Thompson EW. The epithelial-mesenchymal transition: new insights in signaling, development, and disease. *J Cell Biol* 2006; **172**: 973-981 [PMID: 16567498 DOI: 10.1083/jcb.200601018]
18 **Arias AM**. Epithelial mesenchymal interactions in cancer and development. *Cell* 2001; **105**: 425-431 [PMID: 11371340]
19 **Zavadil J**, Böttinger EP. TGF-beta and epithelial-to-mesenchymal transitions. *Oncogene* 2005; **24**: 5764-5774 [PMID: 16123809 DOI: 10.1038/sj.onc.1208927]
20 **Bates RC**, Bellovin DI, Brown C, Maynard E, Wu B, Kawakatsu H, Sheppard D, Oettgen P, Mercurio AM. Transcriptional activation of integrin beta6 during the epithelial-mesenchymal transition defines a novel prognostic indicator of aggressive colon carcinoma. *J Clin Invest* 2005; **115**: 339-347 [PMID: 15668738 DOI: 10.1172/JCI23183]
21 **Bates RC**. Colorectal cancer progression: integrin alphavbeta6 and the epithelial-mesenchymal transition (EMT). *Cell Cycle* 2005; **4**: 1350-1352 [PMID: 16123591]
22 **Bataille F**, Rohrmeier C, Bates R, Weber A, Rieder F, Brenmoehl J, Strauch U, Farkas S, Fürst A, Hofstädter F, Schölmerich J, Herfarth H, Rogler G. Evidence for a role of epithelial mesenchymal transition during pathogenesis of fistulae in Crohn's disease. *Inflamm Bowel Dis* 2008; **14**: 1514-1527 [PMID: 18626977 DOI: 10.1002/ibd.20590]
23 **Peinado H**, Quintanilla M, Cano A. Transforming growth factor beta-1 induces snail transcription factor in epithelial cell lines: mechanisms for epithelial mesenchymal transitions. *J Biol Chem* 2003; **278**: 21113-21123 [PMID: 12665527 DOI: 10.1074/jbc.M211304200]
24 **Zheng G**, Lyons JG, Tan TK, Wang Y, Hsu TT, Min D, Succar L, Rangan GK, Hu M, Henderson BR, Alexander SI, Harris DC. Disruption of E-cadherin by matrix metalloproteinase directly mediates epithelial-mesenchymal transition downstream of transforming growth factor-beta1 in renal tubular epithelial cells. *Am J Pathol* 2009; **175**: 580-591 [PMID: 19590041 DOI: 10.2353/ajpath.2009.080983]
25 **Scharl M**, Weber A, Fürst A, Farkas S, Jehle E, Pesch T, Kellermeier S, Fried M, Rogler G. Potential role for SNAIL family transcription factors in the etiology of Crohn's disease-associated fistulae. *Inflamm Bowel Dis* 2011; **17**: 1907-1916 [PMID: 21830269 DOI: 10.1002/ibd.21555]
26 **Scharl M**, Frei S, Pesch T, Kellermeier S, Arikkat J, Frei P, Fried M, Weber A, Jehle E, Rühl A, Rogler G. Interleukin-13 and transforming growth factor β synergise in the pathogenesis of human intestinal fistulae. *Gut* 2013; **62**: 63-72 [PMID: 22287592 DOI: 10.1136/gutjnl-2011-300498]
27 **Nelson AR**, Fingleton B, Rothenberg ML, Matrisian LM. Matrix metalloproteinases: biologic activity and clinical implications. *J Clin Oncol* 2000; **18**: 1135-1149 [PMID: 10694567]
28 **Baugh MD**, Evans GS, Hollander AP, Davies DR, Perry MJ, Lobo AJ, Taylor CJ. Expression of matrix metalloproteases in inflammatory bowel disease. *Ann N Y Acad Sci* 1998; **859**: 249-253 [PMID: 9928398]
29 **von Lampe B**, Barthel B, Coupland SE, Riecken EO, Rosewicz S. Differential expression of matrix metalloproteinases

and their tissue inhibitors in colon mucosa of patients with inflammatory bowel disease. *Gut* 2000; **47**: 63-73 [PMID: 10861266]

30 **Castaneda FE**, Walia B, Vijay-Kumar M, Patel NR, Roser S, Kolachala VL, Rojas M, Wang L, Oprea G, Garg P, Gewirtz AT, Roman J, Merlin D, Sitaraman SV. Targeted deletion of metalloproteinase 9 attenuates experimental colitis in mice: central role of epithelial-derived MMP. *Gastroenterology* 2005; **129**: 1991-2008 [PMID: 16344067 DOI: 10.1053/j.gastro.2005.09.017]

31 **Santana A**, Medina C, Paz-Cabrera MC, Díaz-Gonzalez F, Farré E, Salas A, Radomski MW, Quintero E. Attenuation of dextran sodium sulphate induced colitis in matrix metalloproteinase-9 deficient mice. *World J Gastroenterol* 2006; **12**: 6464-6472 [PMID: 17072979]

32 **Liu H**, Patel NR, Walter L, Ingersoll S, Sitaraman SV, Garg P. Constitutive expression of MMP9 in intestinal epithelium worsens murine acute colitis and is associated with increased levels of proinflammatory cytokine Kc. *Am J Physiol Gastrointest Liver Physiol* 2013; **304**: G793-G803 [PMID: 23471340 DOI: 10.1152/ajpgi.00249.2012]

33 **Pender SL**, Tickle SP, Docherty AJ, Howie D, Wathen NC, MacDonald TT. A major role for matrix metalloproteinases in T cell injury in the gut. *J Immunol* 1997; **158**: 1582-1590 [PMID: 9029093]

34 **Kirkegaard T**, Hansen A, Bruun E, Brynskov J. Expression and localisation of matrix metalloproteinases and their natural inhibitors in fistulae of patients with Crohn's disease. *Gut* 2004; **53**: 701-709 [PMID: 15082589]

35 **Frei SM LS,** Jehle EC, Fried M, Rogler G, Scharl M. Expression of Interleukins 22 and 33, Matrix Metalloproteinases 9 and 13, Mast Cell Markers and Hypoxia-Inducible Factor 1α in Crohn's Disease Associated Fistulae Gastroenterology. *DDW* 2013; **144** (5, Supplement 1): S441-S442

36 **Hazelbag S**, Kenter GG, Gorter A, Dreef EJ, Koopman LA, Violette SM, Weinreb PH, Fleuren GJ. Overexpression of the alpha v beta 6 integrin in cervical squamous cell carcinoma is a prognostic factor for decreased survival. *J Pathol* 2007; **212**: 316-324 [PMID: 17503414 DOI: 10.1002/path.2168]

37 **Arihiro K**, Kaneko M, Fujii S, Inai K, Yokosaki Y. Significance of alpha 9 beta 1 and alpha v beta 6 integrin expression in breast carcinoma. *Breast Cancer* 2000; **7**: 19-26 [PMID: 11029766]

38 **Li X**, Yang Y, Hu Y, Dang D, Regezi J, Schmidt BL, Atakilit A, Chen B, Ellis D, Ramos DM. Alphavbeta6-Fyn signaling promotes oral cancer progression. *J Biol Chem* 2003; **278**: 41646-41653 [PMID: 12917446 DOI: 10.1074/jbc.M306274200]

39 **Thomas GJ**, Lewis MP, Hart IR, Marshall JF, Speight PM. AlphaVbeta6 integrin promotes invasion of squamous carcinoma cells through up-regulation of matrix metalloproteinase-9. *Int J Cancer* 2001; **92**: 641-650 [PMID: 11340566 DOI: 10.1002/1097-0215(20010601)92:]

40 **Thomas GJ**, Lewis MP, Whawell SA, Russell A, Sheppard D, Hart IR, Speight PM, Marshall JF. Expression of the alphavbeta6 integrin promotes migration and invasion in squamous carcinoma cells. *J Invest Dermatol* 2001; **117**: 67-73 [PMID: 11442751 DOI: 10.1046/j.0022-202x.2001.01379.x]

41 **Thomas GJ**, Poomsawat S, Lewis MP, Hart IR, Speight PM, Marshall JF. alpha v beta 6 Integrin upregulates matrix metalloproteinase 9 and promotes migration of normal oral keratinocytes. *J Invest Dermatol* 2001; **116**: 898-904 [PMID: 11407978 DOI: 10.1046/j.1523-1747.2001.01352.x]

42 **Agrez M**, Gu X, Turton J, Meldrum C, Niu J, Antalis T, Howard EW. The alpha v beta 6 integrin induces gelatinase B secretion in colon cancer cells. *Int J Cancer* 1999; **81**: 90-97 [PMID: 10077158 DOI: 10.1002/(SICI)1097-0215(19990331)81]

43 **Agrez MV**, Bates RC, Mitchell D, Wilson N, Ferguson N, Anseline P, Sheppard D. Multiplicity of fibronectin-binding alpha V integrin receptors in colorectal cancer. *Br J Cancer* 1996; **73**: 887-892 [PMID: 8611401 DOI: 10.1038/bjc.1996.158]

44 **Weinacker A**, Chen A, Agrez M, Cone RI, Nishimura S, Wayner E, Pytela R, Sheppard D. Role of the integrin alpha v beta 6 in cell attachment to fibronectin. Heterologous expression of intact and secreted forms of the receptor. *J Biol Chem* 1994; **269**: 6940-6948 [PMID: 8120056]

45 **Munger JS**, Huang X, Kawakatsu H, Griffiths MJ, Dalton SL, Wu J, Pittet JF, Kaminski N, Garat C, Matthay MA, Rifkin DB, Sheppard D. The integrin alpha v beta 6 binds and activates latent TGF beta 1: a mechanism for regulating pulmonary inflammation and fibrosis. *Cell* 1999; **96**: 319-328 [PMID: 10025398]

46 **Niu J**, Gu X, Ahmed N, Andrews S, Turton J, Bates R, Agrez M. The alphaVbeta6 integrin regulates its own expression with cell crowding: implications for tumour progression. *Int J Cancer* 2001; **92**: 40-48 [PMID: 11279604 DOI: 10.1002/1097-0215(20010401)92]

47 **Frei SM**, Pesch T, Lang S, Weber A, Jehle E, Vavricka SR, Fried M, Rogler G, Scharl M. A role for tumor necrosis factor and bacterial antigens in the pathogenesis of Crohn's disease-associated fistulae. *Inflamm Bowel Dis* 2013; **19**: 2878-2887 [PMID: 24189042 DOI: 10.1097/01.MIB.0000435760.82705.23]

48 **Aguilera O**, Fraga MF, Ballestar E, Paz MF, Herranz M, Espada J, García JM, Muñoz A, Esteller M, González-Sancho JM. Epigenetic inactivation of the Wnt antagonist DICKKOPF-1 (DKK-1) gene in human colorectal cancer. *Oncogene* 2006; **25**: 4116-4121 [PMID: 16491118 DOI: 10.1038/sj.onc.1209439]

49 **Koch S**, Capaldo CT, Samarin S, Nava P, Neumaier I, Skerra A, Sacks DB, Parkos CA, Nusrat A. Dkk-1 inhibits intestinal epithelial cell migration by attenuating directional polarization of leading edge cells. *Mol Biol Cell* 2009; **20**: 4816-4825 [PMID: 19776352 DOI: 10.1091/mbc.E09-05-0415]

50 **Niehrs C**. Function and biological roles of the Dickkopf family of Wnt modulators. *Oncogene* 2006; **25**: 7469-7481 [PMID: 17143291 DOI: 10.1038/sj.onc.1210054]

51 **Frei SM HC,** Pesch T, Lang S, Weber A, Jehle E, R□hl A, Fried M, Rogler G, Scharl M. The Role for Dickkopf-Homolog-1 in the Pathogenesis of Crohn's Disease-Associated Fistulae. *PLoS One* 2013; In press

52 **Zhu Z**, Homer RJ, Wang Z, Chen Q, Geba GP, Wang J, Zhang Y, Elias JA. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J Clin Invest* 1999; **103**: 779-788 [PMID: 10079098 DOI: 10.1172/JCI5909]

53 **Lee CG**, Homer RJ, Zhu Z, Lanone S, Wang X, Koteliansky V, Shipley JM, Gotwals P, Noble P, Chen Q, Senior RM, Elias JA. Interleukin-13 induces tissue fibrosis by selectively stimulating and activating transforming growth factor beta(1). *J Exp Med* 2001; **194**: 809-821 [PMID: 11560996]

54 **Chiaramonte MG**, Donaldson DD, Cheever AW, Wynn TA. An IL-13 inhibitor blocks the development of hepatic fibrosis during a T-helper type 2-dominated inflammatory response. *J Clin Invest* 1999; **104**: 777-785 [PMID: 10491413 DOI: 10.1172/JCI7325]

55 **Wynn TA**. IL-13 effector functions. *Annu Rev Immunol* 2003; **21**: 425-456 [PMID: 12615888 DOI: 10.1146/annurev.immunol.21.120601.141142]

56 **Donaldson DD**, Whitters MJ, Fitz LJ, Neben TY, Finnerty H, Henderson SL, O'Hara RM, Beier DR, Turner KJ, Wood CR, Collins M. The murine IL-13 receptor alpha 2: molecular cloning, characterization, and comparison with murine IL-13 receptor alpha 1. *J Immunol* 1998; **161**: 2317-2324 [PMID: 9725226]

57 **Bates RC**, Mercurio AM. Tumor necrosis factor-alpha stimulates the epithelial-to-mesenchymal transition of human colonic organoids. *Mol Biol Cell* 2003; **14**: 1790-1800 [PMID: 12802055 DOI: 10.1091/mbc.E02-09-0583]

58 **Sullivan DE**, Ferris M, Pociask D, Brody AR. Tumor necro-

sis factor-alpha induces transforming growth factor-beta1 expression in lung fibroblasts through the extracellular signal-regulated kinase pathway. *Am J Respir Cell Mol Biol* 2005; **32**: 342-349 [PMID: 15653932 DOI: 10.1165/rcmb.2004-0288OC]

59 **Radlmayr M**, Török HP, Martin K, Folwaczny C. The c-insertion mutation of the NOD2 gene is associated with fistulizing and fibrostenotic phenotypes in Crohn's disease. *Gastroenterology* 2002; **122**: 2091-2092 [PMID: 12055616]

60 **Xavier RJ**, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. *Nature* 2007; **448**: 427-434 [PMID: 17653185 DOI: 10.1038/nature06005]

P- Reviewer: Keshteli AH, Kellermayer R, Mullin JM
S- Editor: Ji FF **L- Editor**: A **E- Editor:** Lu YJ

World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4291/wjgp.v5.i3.213

World J Gastrointest Pathophysiol 2014 August 15; 5(3): 213-227
ISSN 2150-5330 (online)

TOPIC HIGHLIGHT

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

Escherichia coli in chronic inflammatory bowel diseases: An update on adherent invasive *Escherichia coli* pathogenicity

Margarita Martinez-Medina, Librado Jesus Garcia-Gil

Margarita Martinez-Medina, Librado Jesus Garcia-Gil, Laboratory of Molecular Microbiology, Biology Department, University of Girona, E-17071 Girona, Spain
Author contributions: Martinez-Medina M wrote the paper; Garcia-Gil LJ revised the paper.
Correspondence to: Margarita Martinez-Medina, PhD, Laboratory of Molecular Microbiology, Biology Department, University of Girona, Campus de Montilivi, E-17071 Girona, Spain. marga.martinez@udg.edu
Telephone: +34-972-418175 Fax: +34-972-418150
Received: January 28, 2014 Revised: April 8, 2014
Accepted: May 29, 2014
Published online: August 15, 2014

Abstract

Escherichia coli (*E. coli*), and particularly the adherent invasive *E. coli* (AIEC) pathotype, has been increasingly implicated in the ethiopathogenesis of Crohn's disease (CD). *E. coli* strains with similar pathogenic features to AIEC have been associated with other intestinal disorders such as ulcerative colitis, colorectal cancer, and coeliac disease, but AIEC prevalence in these diseases remains largely unexplored. Since AIEC was described one decade ago, substantial progress has been made in deciphering its mechanisms of pathogenicity. However, the molecular bases that characterize the phenotypic properties of this pathotype are still not well resolved. A review of studies focused on *E. coli* populations in inflammatory bowel disease (IBD) is presented here and we discuss about the putative role of this species on each IBD subtype. Given the relevance of AIEC in CD pathogenesis, we present the latest research findings concerning AIEC host-microbe interactions and pathogenicity. We also review the existing data regarding the prevalence and abundance of AIEC in CD and its association with other intestinal diseases from humans and animals, in order to discuss the AIEC disease- and host-specificity. Finally, we highlight the fact that dietary components frequently found in industrialized countries may enhance AIEC colonization in the gut, which merits further investigation and the implementation of preventative measures.

Key words: Adherent invasive *Escherichia coli*; Inflammatory bowel disease; Crohn's disease; Pathogenesis; Epidemiology

Core tip: In this review we critically revise the findings on *Escherichia coli* (*E. coli*) populations associated with Crohn's disease and ulcerative colitis. Then we focus on adherent invasive *E. coli* (AIEC), especially in its mechanisms of pathogenicity and epidemiology. We discuss about AIEC disease- and host-specificity and we underline the importance of discovering specific molecular tools to detect AIEC for further epidemiologic studies. Finally we point out to a putative role of diet on AIEC gut colonization.

Martinez-Medina M, Garcia-Gil LJ. *Escherichia coli* in chronic inflammatory bowel diseases: An update on adherent invasive *Escherichia coli* pathogenicity. *World J Gastrointest Pathophysiol* 2014; 5(3): 213-227 Available from: URL: http://www.wjgnet.com/2150-5330/full/v5/i3/213.htm DOI: http://dx.doi.org/10.4291/wjgp.v5.i3.213

ESCHERICHIA COLI IN INFLAMMATORY BOWEL DISEASE

The intestinal microbiota has been implicated in the pathogenesis of Crohn's disease (CD) and ulcerative colitis (UC), the main idiopathic inflammatory bowel diseases (IBDs)[1]. CD patients demonstrate an altered

intestinal microbial community, and the dysbioses present in colonic CD and ileal CD are different[2]. In contrast, a specific dysbiosis in UC is starting to be defined, although differences between studies have hampered attempts to reach a clear consensus to date[2-5]. A number of culture-based and molecular-based studies support the theory that *Escherichia coli* (*E. coli*) is a microbiological factor implicated in CD, but some controversy exists regarding its role in UC[2,6-17]. In this section, we examine data on *E. coli* populations in CD and UC related to abundance, association with disease activity, translocation of the gut mucosa, and pathogenic features of the strains to highlight the evidence that supports or refutes putative implications for this bacterium in each IBD subtype.

Abundance in the intestinal mucosa and correlation with disease activity

Several independent studies based on quantitative Polymerase Chain Reaction (PCR) have indicated that *E. coli* is augmented in CD patients in comparison with controls[2,6,11,13]. However, differences are especially significant for CD patients with ileal disease, and no clear association with colonic or ileocolonic CD has been demonstrated. On average, in our cohort, *E. coli* 16S rRNA gene copies accounted for 14% and 33% of total bacteria 16S rRNA gene copies in healthy subjects and ileal CD patients, respectively ($P < 0.001$)[13]. Of note, a higher abundance of *E. coli* was observed in active CD patients than in patients in remission[6,11,18]. Accordingly, a previous study using Fluorescent In Situ Hybridization (FISH) demonstrated increased *E. coli* numbers in the epithelium and within the lamina propria in active CD patients compared to inactive CD patients[14]. In addition, we determined that higher numbers of this species correlated with a reduced amount of time before relapse[11]. These findings are in agreement with previous data reporting that the higher numbers of *E. coli* isolated from the neoterminal ileum of CD patients are associated with early recurrence of the disease[7], and that high levels of antibodies against the *E. coli* outer membrane protein C (OmpC) correlate with disease progression, longer duration, and greater need for surgery among CD patients[19-21].

There is substantial controversy regarding the abundance of *E. coli* in the colonic mucosa of UC patients (Table 1). Several works have consistently reported no increase with respect to healthy subjects[2,6,7,11-13], arguing against a putative role for *E. coli* in UC, while others have reported increased *E. coli* abundance in UC patients[8,10,14,16,18,22,23]. As in the majority of these studies both CD and UC patients were analyzed, these controversial observations can not be explained by differences in methodology between studies. We postulate that they can be attributable to differences in the disease severity of the patients included in the studies, as increased numbers of *E. coli* have been associated with activity status in UC patients. Using FISH, epithelium-associated *E. coli* were found to be more abundant in active UC compared to inactive UC or controls[14], and quantitative PCR indicated that increased numbers of *E. coli* were present in active UC patients compared to inactive UC patients[22] as well as in inflamed *vs* non-inflamed UC tissue[23].

Altogether, substantial evidence supports an overgrowth of *E. coli* in ileal CD patients, while there is still no convincing data that exists for other IBD subtypes. Further studies aimed at comparing the abundance of *E. coli* in IBD patients categorized by disease subtype and assessing any correlation with activity status of the disease would shed light on the role of this bacterium in each IBD subtype and its putative application as a diagnostic and/or prognostic tool.

E. coli localization in the intestinal mucosa

E. coli has been found in the mucus layer, close to the intestinal epithelial cells and in ulcers of both CD and UC patients[24,25]. Translocation of the intestinal mucosa has been primarily observed in CD[6] and higher amounts of intracellular *E. coli* were detected in inflamed compared to non-inflamed mucosa[6,26]. With FISH and immunohistochemistry, *E. coli* has been detected scattered within the lamina propria, either in the extracellular space or inside macrophages, as well as in the subserosal layer, the perivascular areas of the submucosa, the muscle layer, and in germinal centers of lymph follicles of CD patients[8,14,27]. A recent study using high throughput sequencing indicated a greater proportion of *E. coli* reads in the lymph nodes of ileal CD patients than other CD patients[28]. Interestingly, *E. coli* DNA was also more frequently found in the granulomas of CD patients (80%) than in non-CD control patients (10%) in a study that used Laser Capture Microdissection and PCR[29]. In contrast, *E. coli* has not been frequently found to translocate the mucosa of UC patients[8,24,25], although some controversy exists as some authors have detected *E. coli* in the lamina propria of UC patients[14,27].

The majority of the aforementioned studies are based on techniques that do not distinguish viable bacteria from dead bacteria. Further studies should study the viability of translocated *E. coli*, particularly in lymph nodes and granulomas, as these locations would be more relevant to establish a link between this bacterium and CD pathogenesis. These studies should also focus on UC patients to clarify the existing controversial data. A lack of *E. coli* translocation in UC would suggest that *E. coli* does not play a primary role in UC pathogenesis or that it plays a different role than in CD.

Pathogenic features of the strains

E. coli strains isolated from IBD patients are clonally diverse[6,13,17] and belong to distinct serotypes[6,13,30] and to different sequence types[6,31-33]. Although a close genetic relationship was detected in a study of IBD pediatric patients[34], the hypothesis that there is a particular clone associated with IBD has largely been ruled out.

In turn, *E. coli* strains isolated from IBD patients

Table 1 Controversy about *Escherichia coli* imbalances in ulcerative colitis

Ref.	Method	Samples	Comments
Increased *E. coli* abundance in CD but not UC			
Martin *et al*[12]	Culture	Biopsies	Specially hemagglutinin-positive strains
Martinez-Medina *et al*[13]	qPCR	Biopsies	Specially in ileal CD
Lopez-Siles *et al*[11]	qPCR	Biopsies	Specially in active CD
Darfeuille-Michaud *et al*[7]1	culture	Biopsies	Specially in ileal lesions
Baumgart *et al*[6]1	qPCR	Biopsies	Specially in ileal CD
Willing *et al*[2]1	qPCR	Biopsies	Specially in ileal CD
Increased *E. coli* abundance in CD and UC			
Mylonaki *et al*[14]	FISH	Biopsies	Specially in active UC patients
Kotlowski *et al*[10]	culture	Biopsies	
Rehman *et al*[16]	cloning	Biopsies	
Fujita *et al*[8]	qPCR	Biopsies	
Schwiertz *et al*[18]	qPCR	Feces	Specially in active CD patients
Sha *et al*[22]	qPCR	Feces	Specially in active UC and CD patients
Pilarczyk-Zurek *et al*[23]2	qPCR	Biopsies	Specially in inflamed UC tissue

[1]Increased *E. coli* abundance in CD with respect to controls but UC patients were not included in the study; [2]Increased *E. coli* abundance in UC with respect to controls but CD patients were not included in the study. CD: Crohn's disease; UC: Ulcerative colitis; *E. coli*: *Escherichia coli*.

primarily belong to the B2 and D phylogroups in conjunction with extraintestinal pathogenic *E. coli* (ExPEC). Some works demonstrate major colonization by B2+D phylogroups in IBD patients in comparison with healthy controls[10,31], but in other studies, a similar distribution of phylogroups exist between IBD and healthy subjects[13,29,30,33-36]. Differences between studies could be based on the types of samples analyzed, as it has been reported in healthy individuals that transient *E. coli* (more likely to be found in feces) are principally A and B1, whereas resident *E. coli* (more likely to be found in the mucosa) mainly belong to the B2 and D phylogroups[35]. Therefore, studies based on mucosal samples tend to indicate enrichment of B2 and D strains, even in healthy controls. Another factor that could influence the distribution of phylogroups in IBD is the disease severity of patients analyzed, as an increased proportion of B2 and D isolates has been found in active IBD patients[32], which was significantly associated with the inflammation state of IBD tissues[30]. This denotes a shift in *E. coli* populations to isolates that are better adapted to the inflamed tissue in IBD and/or that are involved in the inflammation itself. Of note, no differences in phylogroup distribution between CD and UC have ever been reported.

E. coli isolated from IBD patients carry different sets of virulence genes that are characteristic of ExPEC strains, whereas intestinal pathogenic *E. coli* are rare or absent[6,10,13,30,32,34,36-39]. These virulence factors are also frequent in *E. coli* from healthy subjects and are considered "colonization factors" necessary for successful establishment in the intestinal mucosa[40]. Virulence gene profiles are inexorably linked with the phylogenetic origins of the strains. Based on the distribution of phylogenetic groups, virulence-associated genes characteristic of ExPEC were more frequently found in IBD patients than in healthy subjects in those studies where B2+D predominated in IBD[10,31], whereas no differences were found in other works[13,36,37,41,42]. A shift in the phylogroup distribution would then lead to an increased proportion of *E. coli* equipped with colonization factors that would facilitate establishment and persistence in IBD patients. However, it is not clear whether this shift occurs specifically in IBD patients or is a general trend taking place in industrialized countries[43]. Although no particular genetic traits distinguish *E. coli* from the intestinal mucosa of CD or UC, some virulence factors have been found to be differentially distributed between these IBDs. For example, a diarrhea-associated hemolytic *E. coli* strain called cell-detaching *E. coli* (CDEC), which commonly harbors hemolysin, cytotoxic necrotizing factor 1, pilus P and S-fimbria genes, was found in 24% of UC *E. coli* and only in 4.7% of CD *E. coli*[44]. The gene *usp* encoding for the uropathogenic-specific protein was also more frequently found in UC *E. coli* than in CD *E. coli*[30]. Recently, *E. coli* carrying the *iroN* gene, which encodes for a receptor for iron-chelating siderophores, was more frequently isolated from inflammatory and unchanged mucosa of active-phase UC patients[23].

On the other hand, approximately one decade ago, Darfeuille-Michaud *et al*[45] discovered a new pathotype of *E. coli* with distinctive phenotypic pathogenic traits that was associated with CD, but not with UC, named adherent invasive *E. coli* (AIEC). Altogether, these observations suggest that specific *E. coli* types could be involved in each IBD. We further discuss this issue in the section dedicated to AIEC prevalence in ulcerative colitis.

ADHERENT INVASIVE *E. COLI*

To date, AIEC is the most likely candidate to cause specific damage to people who are genetically susceptible to the development of CD, and therefore the following sections will focus on discussing the most recent research findings on this pathovar. We review (1) the

latest research regarding AIEC pathogenicity; (2) the prevalence and abundance of the pathotype in several intestinal disorders, discussing its putative contribution to other intestinal diseases in addition to CD; (3) the evidence that supports a lack of host-specificity and thus a risk for zoonosis; and (4) recent research that points to a putative role for environmental factors in the fate of AIEC development in the intestine.

Definition

The AIEC pathotype was defined as *E. coli* strains that (1) are able to adhere to differentiated Caco-2 and/or undifferentiated I-407 intestinal epithelial cells with an adhesion index equal or superior to 1 bacteria per cell; (2) are able to invade I-407 cells with an invasion index equal or superior to 0.1% of the original inoculum; (3) involve host cell actin polymerization and microtubule recruitment in bacterial uptake; (4) do not have known invasive determinants; and (5) are able to survive and replicate within J774-A1 macrophages[45]. Since its definition, invasive determinants characteristic from ExPEC have been detected in some AIEC, but not consistently in all AIEC, and thus are not a particularity of the AIEC pathotype[6,13,36,46-48].

Molecular basis of AIEC pathogenicity

Pathogenicity mechanisms of AIEC have mainly been studied in the reference AIEC strain LF82, and its features have been comprehensively linked to many characteristics of CD pathogenesis.

Adhesion to intestinal epithelial cells is in part mediated by type 1 pili, which interact with the glycoprotein CEACAM6 in a mannose-associated manner[49,50]. CEACAM6 is overexpressed in CD patients with ileal disease, which makes them more susceptible to overcolonization by AIEC. Although type 1 pili is present in almost all *E. coli*, including non-pathogenic strains, we have recently demonstrated that AIEC strains usually present FimH adhesin variants that allow them to more efficiently bind intestinal epithelial cells[31]. Some non-AIEC strains carry these mutations as well, but they do not express type 1 pili. Flagella are also important for adhesion to and invasion of intestinal epithelial cells and elicit the secretion of the pro-inflammatory cytokine IL-8 and chemokine CCL20 in polarized intestinal epithelial cells, which in turn leads to the recruitment of macrophages and dendritic cells to the site of infection[51,52]. The further secretion of INFγ and TNFα by macrophages and lymphocytes leads to CEACAM6 expression, which enhances AIEC colonization. The binding of LF82 type 1 pili to CEACAM6 and flagella to TLR5 in intestinal epithelial cells induces the production of HIF-1a and activation of the classical NF-κB pathway[53]. In turn, these molecules cooperatively control the transcription of IL-8 and pro-angiogenic factors contributing to inflammation and vascularization.

The intermediate filament vimentin, expressed on the host cell surface of mesenchymal cells, has been recently proposed to act as a receptor for AIEC[54]. At the intracellular side, vimentin leucine-rich repeats interact with NOD2 leading to the recruitment of these proteins at the plasma membrane. This is necessary for a proper function of NOD2 in terms of antigen detection, NF-κB activation and autophagy induction. CD patients have specific NOD2 variants (L1007fs and R702W) that are unable to interact with vimentin and, in turn, they localize in the cytosol. That leads to a defective inflammatory response, autophagy induction and handling of CD-associated AIEC. Altogether, NOD2 and vimentin appear to play an important role in AIEC recognition and polymorphisms in these two proteins may have an impact on the ability of AIEC to colonize the host.

A new host-microbe interaction that mediates adhesion of LF82 to intestinal epithelial cells and involves a bacterial and a human chitinase has recently been proposed[55]. Chitinases are enzymes that hydrolyze chitin, a long-chain polymer of an N-acetylglucosamine. The authors demonstrate that specific polymorphisms in two chitin binding domains characteristic of LF82 and other pathogenic *E. coli* are required to interact with an N-glycosylated asparagine of the human chitinase CHI3L1. Interestingly, human chitinases are overexpressed in intestinal epithelial cells and moderately expressed in cells of the lamina propria during inflammation.

Outer membrane vesicles (OMVs) containing the transmembrane protein OmpA play a role in LF82 invasion of intestinal epithelial cells[48]. OmpA binds the endoplasmic reticulum-localized stress response chaperone Gp96 that is overexpressed on the apical surface of ileal epithelial cells in patients with CD. OMVs fuse with host cells, and it is thought that the release of bacterial effectors that are still undefined is involved in the actin polymerization and microtubule recruitment that occurs during invasion. Point mutations in the *ompA* sequence of LF82 and other B2 strains mediate better interactions with Gp96[56]. In turn, Gp96 is overexpressed in the ileum of CD patients, which renders them more susceptible to AIEC infection.

Once inside the host cell, LF82 bacteria can be found in several types of intracellular compartments: individually or in groups within single membrane vacuoles, within damaged vacuoles, or within LC3-positive autophagosomes, which indicates that autophagy restricts a subpopulation of intracellular LF82 bacteria[57]. Nevertheless, it was recently demonstrated that AIEC can abrogate the autophagic process[58]. Intracellular LF82 activates NF-κB, leading to the increased expression of MIR30C and MIR130A in T84 cells and in mouse enterocytes, and the upregulation of these microRNAs reduces levels of ATG5 and ATG16L1, inhibiting autophagy and enhancing the inflammatory response. In turn, defects in autophagy mechanisms related to the ATG16L1 and IRGM genes have been associated with CD patients, and these defects confer an advantage for AIEC to survive inside human cells[57]. Therefore, it is a combination of host deficiency factors and AIEC

pathogenicity that determines the fate of intracellular *E. coli* survival.

In addition to adhesion and invasion capacity, LF82 is also able of translocating *via* the M cells of the Peyer's patches, gaining access to the lamina propria. This interaction is mediated by type 1 pili and long polar fimbriae (Lpf), which can interact independently with GP2, a surface protein specific to M cells. It is of note that the sites of initial inflammation in CD are the Peyer's patches and colonic lymphoid follicles; thus, this mechanism of translocation is consistent with early clinical signs of the disease[59].

Another mechanism that can facilitate bacterial translocation is the ability of LF82 to alter intestinal permeability by inducing the expression of the pore-forming protein claudin-2[60] and by displacing ZO-1 and E-cadherin from apical tight junctions, leading to decreased transepithelial resistance and loss of barrier function[17,61]. Besides, pro-inflammatory cytokines like TNFα can drive alterations in intestinal permeability[62]. As AIEC infection induces the secretion of large amounts of TNFα and IL-8[17]; thus, the loss of barrier function induced by LF82 can in part be mediated by the induction of TNFα secretion.

A novel mechanism of pathogenicity observed in LF82 and two other AIEC strains (O83:H1 and UM146) is the evasion of host immune responses *via* subversion of the IFNγ pathway in intestinal epithelial cells[63]. Phosphorylation of the Signal Transducer and Activator of Transcription STAT-1 is blocked, thus preventing the transcription of IFNγ-dependent genes, which reduces host immune responses and results in an inability to mount an appropriate anti-microbiocidal response. Enterohemorrhagic *E. coli* (EHEC) strain O157:H7, in part through its Shiga toxin, is also able to block tyrosine phosphorylation and activation of STAT1 after IFNγ stimulation, in contrast with enteropathogenic *E. coli* E2348/69 or commensal *E. coli* HB101 which do not present this mechanism of pathogenicity. However, AIEC do not present Shiga toxins. Presumably a small secreted peptide may be responsible for this pathogenic mechanism in AIEC[63].

Once AIEC has gained access to the lamina propria, these bacteria can be engulfed by macrophages. Intramacrophage LF82 do not escape into the cytoplasm but induce the formation of a large vacuole (phagosome) that fuses with lysosomes[64], suggesting that AIEC bacteria have the ability to replicate in an environment with acidic pH, oxidative stress, active proteolytic enzymes, and antimicrobial compounds. Indeed, it was demonstrated *in vitro* that an acidic environment is necessary for replication of AIEC LF82 bacteria[64]. The protease HtrA and the thiol-disulfide oxidoreductase DsbA have been reported to be important for survival and replication within macrophages[65,66]. The authors linked these proteins to the ability of LF82 to resist the stress conditions of the phagolysosomes, as isogenic mutants for these proteins were less efficient in growing in acidic and nutrient-poor medium, and these proteins were overexpressed not only in LF82 during macrophage infection but also in acidic nutrient-poor medium. Interestingly, the overexpression of HtrA is dependent on the LF82 background, as non-pathogenic *E. coli* do not overexpress that protein under similar growth conditions. The RNA-binding protein Hfq, which functions as a global posttranscriptional regulator of gene expression, has also been implicated in survival and replication within macrophages and in stress tolerance but also other aspects of LF82 pathogenicity, such as adhesion and invasion capability[67]. Hfq binds small regulatory RNA molecules, facilitating their interaction with mRNA, but the target genes are still unknown.

Continuous replication of LF82 within macrophages results in the secretion of high levels of TNFα without inducing host cell death[68]. This can explain inflammation and granuloma formation in the gut of CD patients, which has been demonstrated *in vitro*[50,69,70]. A direct role for LF82 in delaying apoptosis of infected macrophages and dendritic cells has recently been reported[71]. LF82 infection was found to alter the function of caspase-3, a protease that plays an essential role in apoptosis, and to increase degradation of this molecule in the proteasome.

Also supporting AIEC capability to replicate within immune cells, strain LF82 was able to replicate within monocytes isolated from CD patients for the first 20 h after infection but then CD monocytes started to clear intracellular bacteria[72]. Interestingly, those patients with polymorphisms in *CARD15* gene (R702W, G908R and 1007fs) showed reduced early inflammatory response towards AIEC infection with decreased levels of IL-1β, IL-6 and IL-10. In contrast, Asp299Gly mutation in TLR4 had no effect on monocyte response to AIEC. Besides, a recent study revealed that CD monocyte-derived dendritic cells stimulated with lipopolysaccharide show an attenuated inflammatory response with decreased levels of IL-6 and IL-1β, as well as an impaired autophagy with reduced LC3 expression[73]. Moreover, these cells had a reduced capacity to support the expansion of allogeneic Th17 cells from CD4+ memory T cells. The authors propose that mucosal Th17 activation in CD patients is a secondary event in response of poor bacterial clearance due to defects in innate immunity. Further studies showing AIEC effects on CD defective dendritic cells regarding, not only cytokine release, but also autophagy function and the level of IL-17A response induction in T cells, are necessary to decipher whether the alterations observed in lipopolysaccharide-stimulated dendritic cells equally occur after AIEC exposure.

AIEC LF82 bacteria are also able to invade and replicate within human neutrophils, but in contrast to its behavior inside macrophages and intestinal epithelial cells, LF82 induces the autophagic death of infected neutrophils, which later undergo an alternative cell death process called NETosis[74]. In neutrophils, LF82 are localized inside autolysosomes, as observed by the colocalization of phagosome and lysosome markers, but there is no

acidification, which suggests that LF82 avoids autolysosome maturation. Infected neutrophils secrete cytokines, in particular IL-8, contributing to mucosal inflammation.

The ability to form biofilms is a pathogenic feature frequently found among AIEC strains. We found that 17 out of 27 AIEC strains and only 9 out of 38 intestinal non-AIEC strains were biofilm producers[75]. Motility and flagellar type are of relevance in biofilm production, as non-motile strains were not able to form biofilms, and all strains with the H1 flagellar antigen were strong biofilm producers. Recently, Chassaing *et al*[76] have demonstrated the ability of the LF82 strain to form biofilms on intestinal epithelial cells using cell culture and animal models.

Genetic factors characteristic of the AIEC pathotype

Despite all the research conducted on AIEC pathogenicity, we still do not know the genetic factors that are characteristic of the AIEC pathotype. The majority of genes related to its pathogenicity are not AIEC-specific, as is the case for *fimH*, *ompA*, *dsbA* or *btrA,* and are present in the majority of *E. coli* strains, including non-pathogenic strains[31,48,65,66]. Point mutations or differential gene expression are involved in the increased fitness and/or virulence of AIEC strains. Unfortunately, these genetic factors have been studied in very few strains or exclusively in the prototype strain LF82. Conversely, virulence genes that are not usually present in non-pathogenic *E. coli*, such as *afaC*, *pks* or *lpf*, have been found frequently, but not consistently, in AIEC strains[13,59,77]. AIEC strains are clonally diverse, belong to different serotypes and carry different sets of virulence genes that are characteristic of ExPEC strains; these features also describe non-AIEC ExPEC-like strains inhabiting the intestinal mucosa[13]. The AIEC pathotype comprises high genotype variability, which complicates the identification of specific genetic factors of the pathotype.

It is of note that despite the genetic similarity between AIEC and ExPEC, the latter generally does not exhibit the AIEC phenotype. We determined that only 4 out of 63 ExPEC strains of different origins were AIEC-like[78], conferring a particular identity on the pathotype. Identification of additional genetic elements or the differential expression of key genes that must be involved in AIEC pathogenicity represents an important milestone that can be achieved through genome- and transcriptome-based studies.

Four AIEC genomes belonging to B2 strains have been sequenced and published to date[46,47,79,80], and comparative genomics have been carried out for the LF82 and NRG857c strains[47]. Although novel virulence factors not previously found in AIEC by PCR genotyping, such as a type-6 secretion system, have been detected in genomic islands of the sequenced strains, genomic studies have corroborated the notion that AIEC resembles ExPEC. Unique sequences for AIEC were found in common between the LF82 and NRG857 strains. However, both strains belong to the same phylogroup and serotype (B2 O83:H1), which indicates they are genetically very close. Given the high variability of AIEC seropathotypes, studying the distribution of these genes in other AIEC strains is essential to confirm whether these elements are common features of the pathotype or are strain-specific. Comparative genomics of phylogenetically distant AIEC strains would presumably reveal a significantly greater number of genetic differences. Although it will complicate the situation, sequencing additional AIEC strains from different phylogenetic origins is crucial to determine the common genetic features involved in the AIEC phenotype.

AIEC localization in the intestinal mucosa

AIEC have generally been isolated from tissue samples, but there is no evidence regarding its exact localization within the intestinal mucosa. Although AIEC are invasive bacteria, they have not been convincingly observed within intestinal epithelial cells or in the lamina propria in resected tissue or mucosal biopsies. The studies conducted by Martin *et al*[12] and Eliott *et al*[36] addressed whether *E. coli* are intraepithelial or mucosa-associated by treating biopsies with gentamicin. This approach has brought indirect evidence of *E. coli* invasion of intestinal epithelial cells in CD but not in UC. However, the complete AIEC phenotype was not studied in the intracellular *E. coli* strains obtained from these studies.

Currently, identifying the exact localization of AIEC strains in the mucosa is nearly impossible to do, as no molecular tools that specifically target the AIEC pathovar are available. Some evidence has been obtained using animal models infected with known reference strains. For example, by staining for the O83 antigen, it has recently been demonstrated that the LF82 and NRG857c strains colonize the ileum, cecum and colon of several mouse models and that they are located at the base of the crypts and within goblet cells[81]. Engineered LF82 with a plasmid containing the GFP protein permitted fluorescence-microscopic examination of the localization of LF82 in the nematode *C. elegans*. In this situation, there was robust gut colonization, but bacteria remained in the lumen and were not attached to intestinal epithelial cells[67]. To visualize the extent of bacterial adhesion and invasion in *in vivo* infection, Low *et al*[55] stained *E. coli* lipopolysaccharides with specific antibodies and compared basal levels of fluorescence in uninfected mice (corresponding to indigenous bacteria) with levels in infected mice. They found higher counts of stained bacteria in the intestinal epithelial cells and lamina propria of infected mice, suggesting AIEC intestinal epithelial cell invasion and translocation.

A pathobiont rather than a true pathogen

Despite the virulence genes that are encoded in the genome of many AIEC strains and the mechanisms of pathogenicity reported for the prototype strain LF82, AIEC are generally considered pathobionts. This assumption is supported by the fact that, although at a

lower frequency than in CD, healthy subjects can carry AIEC in their intestinal mucosa[6,13,17,45,82]. The prevalence varies between studies, ranging from the absence to 15.8% of colonic samples with AIEC and from 6.2% to 18% in ileal samples. Although AIEC bacteria may colonize the intestinal mucosa of non-IBD patients, these bacteria usually do not translocate a healthy mucosal barrier, as bacterial invasion of the mucosa has not frequently been observed in control patients[14] and intracellular *E. coli* was rarely cultivated from the colonic mucosa of healthy subjects (from the absence to 9%)[6,26,83]. AIEC strains are more abundant, and consequently more frequently found, in the ileum than in the colon of healthy subjects. We found that AIEC accounted for 3.58% and 0.95%, respectively, of the ileal and colonic *E. coli* populations[13]. Accordingly, a larger number of AIEC LF82 bacteria were attached to ileal than to colon tissue in *ex vivo* samples from healthy subjects infected with this AIEC strain[84]. Altogether, these data suggest that AIEC can more easily colonize the ileum with respect to other *E. coli*, and at least approximately 1 out of 6 healthy individuals can be considered "asymptomatic carriers".

Genetic or environment-derived host defects at the intestinal barrier may determine the ability of AIEC to colonize and translocate the gut. A number of host deficiencies frequently found in CD patients have been linked with the increased ability of AIEC LF82 to cause infection. For example, these defects include the overexpression of the CEACAM6 and Gp96 receptors in the apical membrane of intestinal epithelial cells, which facilitates AIEC adhesion and invasion[48,49], or defects in autophagy related to NOD2, ATG16L1 and IRGM function and expression, which impair the ability of host cells to resolve infections[57,85]. Additionally, it has been suggested that the altered bile salts metabolism in CD patients could enhance the expression of long polar fimbriae in AIEC, which could permit better translocation *via* M cells[86]. Moreover, decreased levels of the protease meprin, which are characteristic of severe inflammation in IBD patients, have been proposed to determine the fate of AIEC in terms of their ability to colonize the host, as these proteases degrade type 1 pili[87].

PREVALENCE AND ABUNDANCE OF AIEC IN IBD

CD

Intraepithelial *E. coli* with adherent and invasive properties were isolated from the sigmoid colon mucosa in 29% of CD patients[12] and in 90% of CD patients in a cohort composed of ileal, ileocolonic and colonic disease phenotypes[36]. Differences between studies could be explained by the disease activity status of the cohort of patients, who were mainly in the relapse stage in the latter study.

In the last decade, several independent laboratories have reported a higher prevalence of AIEC in CD patients than in healthy subjects[6,13,17,45,82]. Unfortunately, not all of these studies categorized CD patients by their disease subtype or analyzed prevalence based on anatomic location in the gut. The first study was conducted by Darfeuille-Michaud *et al*[45] in 2004 and revealed that 22% of CD patients with ileal involvement harbored AIEC strains in ileal chronic lesions and at a similar frequency in healthy mucosa. However, AIEC bacteria were more likely to be found in the early ileal lesions that occurred in patients after ileostomy (36.4%). AIEC strains were only isolated from the colon of 3.7% of CD patients with a colonic disease phenotype. The authors proposed an association between AIEC and ileal CD and suggested that the pathovar could be involved in the initiation of the inflammatory process. Conversely, Baumgart *et al*[6] reported a prevalence of AIEC strains in the ileum of 38.5% of CD patients with ileal involvement and 37.5% with colonic CD, indicating that AIEC is associated both with ileal and colonic disease phenotypes. Sasaki *et al*[17] demonstrated that 24.3% of CD patients exhibited AIEC strains, but neither the localization of these strains in the gut nor the disease phenotypes of the positive patients were detailed. A similar prevalence was reported by Dogan *et al*[82] in the ileum of CD patients with ileal disease. We detected AIEC strains at a higher frequency in comparison with previous studies, most likely due to the methodological approach used. Whereas other studies analyzed from 1 to 15 *E. coli* colonies per patient, we searched for AIEC strains in a collection of 95 - 150 *E. coli* colonies per patient. This approach not only enabled us to obtain a more accurate prevalence value but also to study the abundance of AIEC strains within the *E. coli* population. We detected AIEC strains in the ileum of 54.5% of CD patients and in the colon of 50% of CD patients[13]. Although data depicted by disease subtype were not reported in the original work, we also found a higher prevalence in CD patients with ileal involvement (66.7% of ileal and 58.3% of colonic samples) than those with colonic disease (50% of ileal and 25% of colonic samples). Colonic CD patients denoted also a high prevalence of AIEC, what supports the observations of Baumgart *et al*[6], but the pathotype was more frequently found in the ileum than in the colon of CD patients, in line with the findings of Darfeuille-Michaud *et al*[45] The abundance of AIEC, defined as the percentage of AIEC within the *E. coli* population, was low and variable, ranging from 1% to 50%. On average, AIEC isolates represented 9.3%, 3.7% and 3.1% of *E. coli* isolates in ileal, ileocolonic and colonic CD patients, respectively. Jensen *et al*[84] supported these data using quantitative PCR targeting indigenous LF82 bacteria. The increased expression of CEACAM6 in the ileum of ileal CD patients may explain the higher prevalence and abundance of AIEC in CD patients with ileal involvement. However, additional host-microbial interactions or environmental factors may be involved in colonization of the colonic mucosa, as no differences

in CEACAM6 expression exist at the level of the colon between CD patients and control subjects[49]. Our work demonstrates that AIEC are more prevalent than expected in all CD disease subtypes, reinforces the hypothesis that the microenvironment of ileal CD specifically favors AIEC expansion, and suggests that the colon is also a niche effectively colonized by AIEC.

UC

More than two decades ago, the adhesion capabilities of *E. coli* from both UC and CD patients were assessed. Mannose-resistant adhesion was characteristic of *E. coli* from both IBDs, which raised the question of whether adhesive *E. coli* could also be involved in UC pathogenesis[88,89]. Recent studies have confirmed that, in UC patients, adherent *E. coli* strains are found as frequently as[90] or even more frequently than[34,91] in CD patients. An undefined adhesion pattern was most prevalent in *E. coli* from both UC and CD patients[42], although aggregative adherence was particularly frequent in UC patients[42,90]. Molecular tools to detect adhesive determinants of IBD *E. coli* did not demonstrate specific adhesion factors in UC *E. coli* in comparison to CD *E. coli*[10,37,41,42], whereas in other studies UC *E. coli* carried some adhesion factors more frequently than CD *E. coli*[30,34,44]. Some of these studies are based on pediatric or newly diagnosed patients, which provides supporting arguments for the early contribution of adherent *E. coli* to IBD rather than being its development a consequence of inflammation. Moreover, the higher frequency of *E. coli* B2 strains with at least one positive adhesion-related gene was correlated with disease activity in UC patients (86% in active *vs.* 13% in non-active patents)[32]. Therefore, there is substantial agreement among studies regarding the adhesion capacity of *E. coli* strains from UC patients.

Intracellular *E. coli* were cultured from 47%[36] and 19%[12] of UC patients in two studies using gentamicin protection assay. However, few works have sought to identify the AIEC pathovar in UC patients, and some controversial results have been obtained. In the first study that searched for AIEC in UC any of UC patients had AIEC bacteria in their colon[45], and similar results were obtained in a later study[92]. In contrast, in studies with larger cohorts, one of them based on pediatric patients, AIEC were detected in 7.2% to 10% of UC patients[17,93]. Other investigators that studied the invasion ability of IBD *E. coli*, but did not study the complete AIEC phenotype, detected a high prevalence of invasive strains in UC patients (37.5%)[44]. Moreover, similar invasion rates in I407 cells were observed for *E. coli* from pediatric UC and CD patients[42], whereas in a previous study the invasion index using differentiated Caco-2 cells was lower in *E. coli* from UC than CD patients[17]. Noteworthy, the intra-macrophage survival capacity of *E. coli* strains was found to be highest in UC patients from a cohort of newly diagnosed IBD patients. Unfortunately, no information about adhesion and invasion abilities was provided[30].

Sasaki *et al*[17] observed that although AIEC from UC were less invasive than CD *E. coli*, they induced the secretion of similar amounts of TNFα and higher amounts of IL-8, suggesting that UC-associated *E. coli* are distinct from those associated with CD. Accordingly, a recent study reported that CD *E. coli* are frequently *lpf*+ *afaC*+, whereas UC *E. coli* do not possess *lpf* gene and frequently harbor the *afaC* and *pks* genes together[77]. Lpf mediate translocation of bacteria via M cells, while the afimbrial adhesin AfaC mediates a diffuse adherence to and invasion of intestinal epithelial cells and also induces vascular endothelial growth factor expression. The polyketide synthase gene complex (*pks*) contains the genes to synthesize the metabolite colibactin, a genotoxin with the ability to cause epithelial DNA damage.

The evidence collected to date suggests that *E. coli* strains with adhesive and other virulence properties could be involved in UC pathogenesis, but further work clarifying the role of these strains in conjunction with host defects in the mucosal barrier is needed. Furthermore, in view of the few studies and conflicting results regarding AIEC prevalence in UC, additional studies characterizing *E. coli* populations from different anatomical sites, and for both affected and unaffected tissue, in active and inactive UC patients would be of relevance to elucidate the possible role of AIEC in UC.

E. COLI POPULATIONS IN OTHER INTESTINAL DISEASES: IS AIEC INVOLVED?

Colorectal cancer

An analysis of fecal bacterial diversity by pyrosequencing demonstrated that the *Escherichia/Shigella* genus was enriched in colorectal cancer (CRC) patients[94]. In contrast, studies conducting quantitative PCR did not find an increase in the *E. coli* population in CRC[8,91]. However, intracellular *E. coli* has frequently been found in CRC patients. Swidsinski and collaborators detected intracellular *E. coli* in 87% of patients with CRC and not in controls using a gentamicin protection assay[95]. Similarly, Martin *et al*[12] isolated intramucosal *E. coli* from 33% of tumors in CRC patients and 9% of control subjects, surpassing the prevalence found among IBD patients, and Bonnet *et al*[96] isolated intramucosal *E. coli* in 86% of colon cancer tumor specimens and 48% of diverticulosis samples. Moreover, high levels of mucosa-associated *E. coli* correlated with poor colorectal carcinoma prognostic factors and a higher proliferative index of epithelial cells, suggesting a role for these bacteria in tumor progression.

E. coli strains isolated from the study by Prorok-Hamon *et al*[77] were hemagglutination-positive, adherent to HT29 and I407 intestinal epithelial cells and frequently able to invade I407 cells, all characteristics that resemble the AIEC pathotype. A recent study conducted by the same research group showed that at least one of the isolates obtained from a patient with CRC shared the com-

plete AIEC phenotype. In addition, *E. coli* isolated from a pediatric cohort with polyposis, who were included as a healthy control group, showed the highest invasion efficiency compared with *E. coli* strains isolated from IBD children[42]. However, as far as we know, there is no data regarding the prevalence of AIEC in patients with CRC.

Several studies have demonstrated that *E. coli* associated with CRC are frequently colibactin-producing[72,93-95]. Not only is the *pks* genomic island encoding for the genotoxin colibactin frequent in CRC, but other cyclomodulins such as CNF, CDT and CIF. Buc *et al*[97] found that cyclomodulin-encoding genes were over-represented among *E. coli* from CRC patients (65.8%), particularly distal colon cancer (76.5%), compared with diverticulosis samples (19.54%). These molecules can be genotoxic and/or modulate cellular differentiation, apoptosis, and proliferation. Prorok-Hamon *et al*[77] observed that CRC *E. coli* frequently harbored the *pks* gene but also the adhesins AfaC and LpfA, partially resembling those *E. coli* isolated from CD and UC. These factors confer the ability to adhere to and invade I407 cells, to upregulate vascular endothelial growth factor expression in intestinal epithelial cells, and presumably, to translocate *via* M cells and cause genotoxicity to host cells. Recently, pathogenic cyclomodulin-positive *E. coli* strains were found to be more prevalent in the mucosa of patients with advanced stages of the disease[96].

Few studies have been focused on *E. coli* populations in CRC patients do date, and the results obtained point to a putative role for a subset of *E. coli* with pathogenic features relevant to CRC pathogenesis. Given that AIEC possessing virulence factors relevant to enterocyte adhesion and invasion, vascular endothelial growth factor expression and carcinogenesis have been detected in CRC patients and the fact that intramucosal *E. coli* with features similar to AIEC have been more frequently found in CRC than in IBD patients, further studies determining the prevalence of AIEC in CRC are needed to corroborate or refute the hypothesis for a putative role for AIEC in CRC.

Coeliac disease

Coeliac disease is a chronic inflammatory disorder exclusively affecting the small intestine, in which genetically predisposed individuals feature a permanent intolerance to dietary gluten. Several studies have provided evidence that coeliac patients exhibit intestinal microbial dysbiosis, similar to what occurs in IBD patients. In a study based on PCR-TGGE of duodenal samples, *E. coli* was found more frequently in coeliac children (92.1%) than in healthy children (20%)[98]. Quantification of *E. coli* by FISH showed also that this species was more abundant in active coeliac patients than in inactive patients and controls[99], but this was not observed in fecal samples[100]. Another study found changes in *Enterobacteriaceae* diversity and increased virulence-gene carriage in *E. coli* isolates from coeliac children[101]. In particular, *E. coli* strains largely belonging to the B2 and D phylogenetic groups and carrying ExPEC-like features, *e.g.*, pilus P and hemolysin A, were found to be more abundant in celiac patients when compared to healthy controls. This dysbiosis of the *E. coli* population is similar to that found in CD patients.

Given the association between *E. coli* and coeliac disease in terms of abundance and the correlation with disease activity, as well as the genetic similarities between isolates from the intestinal mucosa of coeliac patients and CD patients, further studies aimed at identifying the AIEC phenotype amongst coeliac *E. coli* isolates are of interest to better define the disease specificity of the AIEC pathotype.

ADHERENT-INVASIVE *E. COLI* IN ANIMALS WITH INTESTINAL DISEASE

AIEC strains isolated from CD patients genetically resemble avian pathogenic *E. coli* and other animal ExPEC. We studied the AIEC phenotype in a strain collection obtained from animals with extraintestinal infection and intestinal disease to determine the disease and host specificity of the AIEC pathotype. All these strains were classified as ExPEC in terms of their phylogenetic origin and virulence genotype. ExPEC strains of extraintestinal origin rarely shared the AIEC phenotype, whereas ExPEC-like strains of intestinal origin were frequently AIEC-like in cats (82%), dogs (35%) and swine (32%) with intestinal disease[102]. The high prevalence of AIEC in companion and farm animals highlights a putative risk of zoonosis between humans and animals. In a previous study, Simpson *et al*[103] detected AIEC in boxer dogs. Interestingly, these dogs suffered from granulomatous colitis, a disease with pathological features that overlap with CD, which supports the role of AIEC in human CD and analogous diseases in animals.

Altogether, these results suggest that the AIEC pathotype is disease-specific rather than host-specific and raises the question of whether there is a possible route of transmission between animals and humans. Further studies examining the distribution of AIEC strains in different healthy and diseased animals and in the environment would contribute to our understanding of the epidemiology, putative reservoirs, host-specificity and possible routes of transmission of AIEC.

ENVIRONMENTAL FACTORS INVOLVED IN THE SUCCESSFUL COLONIZATION OF AIEC

Recent studies have implicated some emulsifiers and food stabilizers frequently used in developed countries as having a role in AIEC colonization. Maltodextrin, a polysaccharide derived from starch hydrolysis that is used as food additive, has been shown to markedly enhance AIEC biofilm formation and adhesion to intestinal epithelial cells and macrophages[104]. Maltodextrin fa-

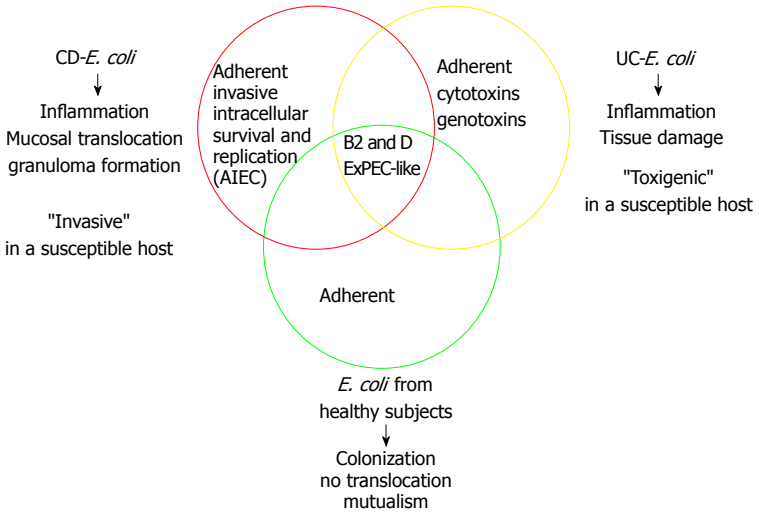

Figure 1 Features of inflammatory bowel disease-associated *Escherichia coli* and impact of this species on Crohn's disease and ulcerative colitis.

vors type 1 pili expression, which is required for biofilm formation and adhesion. Moreover, a higher prevalence of the gene *malX*, which is essential for maltodextrin metabolism, was found in bacteria isolated from ileal CD patients than from healthy controls (71% *vs* 18%, respectively). These observations suggest that a diet rich in maltodextrin would aid maltodextrin-utilizing bacteria, would enhance *E. coli* gut colonization, and thus contribute to dysbiosis. Furthermore, polysorbate-80, an emulsifier commonly used in processed foods, was found to enhance translocation of the AIEC HM605 strain across M cells and intestinal epithelial cells[105]. Using animal models, we also observed that a diet enriched in fat and sugar induced dysbiosis and low grade-inflammation[106]. In this work we also showed that dysbiosis and low-grade inflammation in susceptible individuals lead to increased AIEC colonization, what in turn exacerbated the inflammatory response and epithelial barrier disruption.

The type of dietary fiber intake may influence bile acid metabolism. For example, daily dietary supplementation for four weeks with the purified fiber components pectin and cellulose in humans leads to differential bile acid composition. In cellulose-treated volunteers, cholic acid increased whereas deoxycholic acid decreased, which inversely occurred in pectin-treated individuals[107]. Increased concentrations of cholic acid and chenodeoxycholic acid have been reported in CD patients[108], and lithocholic acid has been reported particularly in ileal CD patients[109]. Interestingly, all of these bile salts induced the expression of the *lpf* operon in AIEC LF82 strain[86]. Therefore, dietary fiber consumption could also influence the tropism of AIEC for CD ileal tissue by altering bile acid composition and thus the expression of *lpf* in AIEC in the gut.

These studies demonstrate that dietary components may impact the success of AIEC in colonizing the host and therefore contribute to disease susceptibility. For that reason, intervention studies are needed to evaluate the effects of diet, probiotics, and/or prebiotics on the intestinal microbial community, including the AIEC population with respect to CD activity status and disease progression.

AIEC, A CAUSE AND A CONSEQUENCE OF INFLAMMATION

Several studies based on animal models have shown that there is a need of microbial dysbiosis and/or intestinal inflammation to succeed with AIEC infection. An effective colonization only occurs in mice that have been treated with antibiotics[50,81,110], dextran sodium sulfate[69] or high-fat/high-sugar diet[106] before infection, having these treatments an effect on gut bacteria composition and mucosal homeostasis. Moreover, Craven *et al*[111], nicely showed that moderate to severe ileitis produced by protozoan infection in mice models induced dysbiosis and proliferation of endogenous mucosally invasive *E. coli*. These works suggest that inflammation and dysbiosis favors AIEC proliferation. Therefore, AIEC overgrowth in the intestine can be seen as a consequence of inflammation.

On the other hand, it has been recently shown that AIEC infection itself induced lasting changes in the intestinal microbiota[112]. This study was conducted on mice lacking flagellin receptor TLR5 (T5KO) which are prone to develope spontaneous colitis. The authors hypothesized that transient colonization of T5KO mice by AIEC results in an altered gut microbiota community with greater proinflammatory potential, which can persist in the host and induce chronic inflammation due to its increased levels of lipopolysaccharide and flagellin. The effects of AIEC infection on host mucosal immunity, barrier integrity and inflammation induction have been demonstrated in multiple animal models[50,60,69,81,106,110] but the work of Chassaing *et al*[112] is the first showing that AIEC infection contribute to intestinal dysbiosis. Overall, these studies suggest that AIEC overgrowth in the intestine can be seen as a cause of inflammation.

Therefore, inflammation can instigate imbalances in *E. coli*, especially the AIEC pathotype and, in turn, these bacteria can be involved in a further dysbiosis and in-

creased intestinal inflammation.

CONCLUSION

Substantial evidence indicates that *E. coli* is involved in CD and growing data suggest that this species is also a contributing factor in UC pathogenesis (Figure 1). Studies focused on defining virulence gene profiles of *E. coli* populations have shown that *E. coli* associated to the mucosa of healthy subjects resemble those of IBD patients. Genes related with adhesion, iron transport, capsule formation and toxins are present in *E. coli* from both healthy subjects and IBD patients. These features are thought to be necessary for an effective colonization of the intestinal tract. However, the intestinal microenvironment in IBD patients, especially those in relapse, would predispose to *E. coli* proliferation. Moreover, *E. coli* from CD patients have probably evolved towards the AIEC pathotype, which has the capacity to adhere to and to invade intestinal epithelial cells, as well as to survive and replicate within a number of cell types. Virulence properties of AIEC described to date can explain several features of CD pathophysiology such as inflammation, mucosal bacterial translocation and granuloma formation. Conversely, *E. coli* strains from UC patients appear to present a "toxigenic" behavior rather than the "invasive" pathogenic mechanism of CD- *E. coli*. Recent research has pointed out that *E. coli* from UC patients frequently carry virulence genes related to cytotoxicity and genotoxicity, which can contribute to mucosal inflammation and tissue damage. This is in accordance with previous works that did not found *E. coli* translocating the epithelial barrier of UC patients, and could be linked with some aspects of UC pathophysiology.

Since the AIEC pathotype was defined one decade ago, substantial research has been conducted focusing on the identification of the mechanisms of pathogenicity and also in the field of epidemiology with regard to CD. However, additional epidemiologic studies are still needed to corroborate the role of AIEC in CD and to clarify the AIEC disease- and host-specificity. An important limitation to epidemiological studies is the absence of specific molecular tools to detect and quantify this pathotype, as the current available techniques to identify the AIEC pathotype are based exclusively on phenotypic screening of cultured bacteria, which is highly time-consuming. The execution of large-scale epidemiologic studies would also provide new insights into its distribution, putative reservoirs and transmission pathways. Moreover, the molecular bases of AIEC pathogenicity are still not fully understood, as only a few model strains have been studied and there is a wide variety of serotypes and phylotypes within the AIEC pathotype. Genomic and transcriptomic studies including wider and more diverse AIEC strain collections could assist in identifying new genetic elements associated with the AIEC phenotype, which may help us to gain a better understanding of the mechanisms of pathogenicity and could result in significant advances in the detection of new therapeutic targets for CD.

REFERENCES

1 **Sartor RB**. Microbial influences in inflammatory bowel diseases. *Gastroenterology* 2008; **134**: 577-594 [PMID: 18242222 DOI: 10.1053/j.gastro.2007.11.059]

2 **Willing B**, Halfvarson J, Dicksved J, Rosenquist M, Järnerot G, Engstrand L, Tysk C, Jansson JK. Twin studies reveal specific imbalances in the mucosa-associated microbiota of patients with ileal Crohn's disease. *Inflamm Bowel Dis* 2009; **15**: 653-660 [PMID: 19023901 DOI: 10.1002/ibd.20783]

3 **Andoh A**, Imaeda H, Aomatsu T, Inatomi O, Bamba S, Sasaki M, Saito Y, Tsujikawa T, Fujiyama Y. Comparison of the fecal microbiota profiles between ulcerative colitis and Crohn's disease using terminal restriction fragment length polymorphism analysis. *J Gastroenterol* 2011; **46**: 479-486 [PMID: 21253779 DOI: 10.1007/s00535-010-0368-4]

4 **Machiels K**, Joossens M, Sabino J, De Preter V, Arijs I, Eeckhaut V, Ballet V, Claes K, Van Immerseel F, Verbeke K, Ferrante M, Verhaegen J, Rutgeerts P, Vermeire S. A decrease of the butyrate-producing species Roseburia hominis and Faecalibacterium prausnitzii defines dysbiosis in patients with ulcerative colitis. *Gut* 2014; **63**: 1275-1283 [PMID: 24021287 DOI: 10.1136/gutjnl-2013-304833]

5 **Martinez-Medina M**, Aldeguer X, Gonzalez-Huix F, Acero D, Garcia-Gil LJ. Abnormal microbiota composition in the ileocolonic mucosa of Crohn's disease patients as revealed by polymerase chain reaction-denaturing gradient gel electrophoresis. *Inflamm Bowel Dis* 2006; **12**: 1136-1145 [PMID: 17119388 DOI: 10.1097/01.mib.0000235828.09305.0c]

6 **Baumgart M**, Dogan B, Rishniw M, Weitzman G, Bosworth B, Yantiss R, Orsi RH, Wiedmann M, McDonough P, Kim SG, Berg D, Schukken Y, Scherl E, Simpson KW. Culture independent analysis of ileal mucosa reveals a selective increase in invasive Escherichia coli of novel phylogeny relative to depletion of Clostridiales in Crohn's disease involving the ileum. *ISME J* 2007; **1**: 403-418 [PMID: 18043660 DOI: 10.1038/ismej.2007.52]

7 **Darfeuille-Michaud A**, Neut C, Barnich N, Lederman E, Di Martino P, Desreumaux P, Gambiez L, Joly B, Cortot A, Colombel JF. Presence of adherent Escherichia coli strains in ileal mucosa of patients with Crohn's disease. *Gastroenterology* 1998; **115**: 1405-1413 [PMID: 9834268 DOI: 10.1016/S0016-5085(98)70019-8]

8 **Fujita H**, Eishi Y, Ishige I, Saitoh K, Takizawa T, Arima T, Koike M. Quantitative analysis of bacterial DNA from Mycobacteria spp., Bacteroides vulgatus, and Escherichia coli in tissue samples from patients with inflammatory bowel diseases. *J Gastroenterol* 2002; **37**: 509-516 [PMID: 12162408 DOI: 10.1007/s005350200079]

9 **Gophna U**, Sommerfeld K, Gophna S, Doolittle WF, Veldhuyzen van Zanten SJ. Differences between tissue-associated intestinal microfloras of patients with Crohn's disease and ulcerative colitis. *J Clin Microbiol* 2006; **44**: 4136-4141 [PMID: 16988016 DOI: 10.1128/JCM.01004-06]

10 **Kotlowski R**, Bernstein CN, Sepehri S, Krause DO. High prevalence of Escherichia coli belonging to the B2+D phylogenetic group in inflammatory bowel disease. *Gut* 2007; **56**: 669-675 [PMID: 17028128 DOI: 10.1136/gut.2006.099796]

11 **Lopez-Siles M,** Martinez-Medina M, Busquets D, Sabat-Mir M, Duncan S, Flint H, Aldeguer X, Garcia-Gil L. Mucosa-associated Faecalibacterium prausnitzii and Escherichia coli co-abundance can distinguish Irritable Bowel Syndrome and Inflammatory Bowel Disease phenotypes. *Int J Med Microbiol* 2014; [DOI: 10.1016/j.ijmm.2014.02.009]

12 **Martin HM**, Campbell BJ, Hart CA, Mpofu C, Nayar M, Singh R, Englyst H, Williams HF, Rhodes JM. Enhanced

Escherichia coli adherence and invasion in Crohn's disease and colon cancer. *Gastroenterology* 2004; **127**: 80-93 [PMID: 15236175 DOI: 10.1053/j.gastro.2004.03.054]

13 **Martinez-Medina M**, Aldeguer X, Lopez-Siles M, González-Huix F, López-Oliu C, Dahbi G, Blanco JE, Blanco J, Garcia-Gil LJ, Darfeuille-Michaud A. Molecular diversity of Escherichia coli in the human gut: new ecological evidence supporting the role of adherent-invasive E. coli (AIEC) in Crohn's disease. *Inflamm Bowel Dis* 2009; **15**: 872-882 [PMID: 19235912 DOI: 10.1002/ibd.20860]

14 **Mylonaki M**, Rayment NB, Rampton DS, Hudspith BN, Brostoff J. Molecular characterization of rectal mucosa-associated bacterial flora in inflammatory bowel disease. *Inflamm Bowel Dis* 2005; **11**: 481-487 [PMID: 15867588 DOI: 10.1097/01.MIB.0000159663.62651.4f]

15 **Neut C**, Bulois P, Desreumaux P, Membré JM, Lederman E, Gambiez L, Cortot A, Quandalle P, van Kruiningen H, Colombel JF. Changes in the bacterial flora of the neoterminal ileum after ileocolonic resection for Crohn's disease. *Am J Gastroenterol* 2002; **97**: 939-946 [PMID: 12003430 DOI: 10.1111/j.1572-0241.2002.05613.x]

16 **Rehman A**, Lepage P, Nolte A, Hellmig S, Schreiber S, Ott SJ. Transcriptional activity of the dominant gut mucosal microbiota in chronic inflammatory bowel disease patients. *J Med Microbiol* 2010; **59**: 1114-1122 [PMID: 20522625 DOI: 10.1099/jmm.0.021170-0]

17 **Sasaki M**, Sitaraman SV, Babbin BA, Gerner-Smidt P, Ribot EM, Garrett N, Alpern JA, Akyildiz A, Theiss AL, Nusrat A, Klapproth JM. Invasive Escherichia coli are a feature of Crohn's disease. *Lab Invest* 2007; **87**: 1042-1054 [PMID: 17660846 DOI: 10.1038/labinvest.3700661]

18 **Schwiertz A**, Jacobi M, Frick JS, Richter M, Rusch K, Köhler H. Microbiota in pediatric inflammatory bowel disease. *J Pediatr* 2010; **157**: 240-244.e1 [PMID: 20400104 DOI: 10.1016/j.jpeds.2010.02.046]

19 **Beaven SW**, Abreu MT. Biomarkers in inflammatory bowel disease. *Curr Opin Gastroenterol* 2004; **20**: 318-327 [PMID: 15703659 DOI: 10.1097/00001574-200407000-00004]

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

21 **Mow WS**, Vasiliauskas EA, Lin YC, Fleshner PR, Papadakis KA, Taylor KD, Landers CJ, Abreu-Martin MT, Rotter JI, Yang H, Targan SR. Association of antibody responses to microbial antigens and complications of small bowel Crohn's disease. *Gastroenterology* 2004; **126**: 414-424 [PMID: 14762777 DOI: 10.1053/j.gastro.2003.11.015]

22 **Sha S**, Xu B, Wang X, Zhang Y, Wang H, Kong X, Zhu H, Wu K. The biodiversity and composition of the dominant fecal microbiota in patients with inflammatory bowel disease. *Diagn Microbiol Infect Dis* 2013; **75**: 245-251 [PMID: 23276768 DOI: 10.1016/j.diagmicrobio.2012.11.022]

23 **Pilarczyk-Zurek M**, Chmielarczyk A, Gosiewski T, Tomusiak A, Adamski P, Zwolinska-Wcislo M, Mach T, Heczko PB, Strus M. Possible role of Escherichia coli in propagation and perpetuation of chronic inflammation in ulcerative colitis. *BMC Gastroenterol* 2013; **13**: 61 [PMID: 23566070 DOI: 10.1186/1471-230X-13-61]

24 **Swidsinski A**, Loening-Baucke V, Lochs H, Hale LP. Spatial organization of bacterial flora in normal and inflamed intestine: a fluorescence in situ hybridization study in mice. *World J Gastroenterol* 2005; **11**: 1131-1140 [PMID: 15754393 DOI: 10.1128/JCM.43.7.3380-3389.2005]

25 **Walmsley RS**, Anthony A, Sim R, Pounder RE, Wakefield AJ. Absence of Escherichia coli, Listeria monocytogenes, and Klebsiella pneumoniae antigens within inflammatory bowel disease tissues. *J Clin Pathol* 1998; **51**: 657-661 [PMID: 9930068 DOI: 10.1136/jcp.51.9.657]

26 **Vasquez N**, Mangin I, Lepage P, Seksik P, Duong JP, Blum S, Schiffrin E, Suau A, Allez M, Vernier G, Tréton X, Doré J, Marteau P, Pochart P. Patchy distribution of mucosal lesions in ileal Crohn's disease is not linked to differences in the dominant mucosa-associated bacteria: a study using fluorescence in situ hybridization and temporal temperature gradient gel electrophoresis. *Inflamm Bowel Dis* 2007; **13**: 684-692 [PMID: 17206669 DOI: 10.1002/ibd.20084]

27 **Kleessen B**, Kroesen AJ, Buhr HJ, Blaut M. Mucosal and invading bacteria in patients with inflammatory bowel disease compared with controls. *Scand J Gastroenterol* 2002; **37**: 1034-1041 [PMID: 12374228 DOI: 10.1080/00365520232037822 0]

28 **O'Brien CL,** Pavli P, Gordon DM, Allison GE. Detection of bacterial DNA in lymph nodes of Crohn's disease patients using high throughput sequencing. *Gut* 2014 Jan 15; Epub ahead of print [PMID: 24429583 DOI: 10.1136/gutjnl-2013-305320]

29 **Ryan P**, Bennett MW, Aarons S, Lee G, Collins JK, O'Sullivan GC, O'Connell J, Shanahan F. PCR detection of Mycobacterium paratuberculosis in Crohn's disease granulomas isolated by laser capture microdissection. *Gut* 2002; **51**: 665-670 [PMID: 12377804 DOI: 10.1136/gut.51.5.665]

30 **Sepehri S**, Khafipour E, Bernstein CN, Coombes BK, Pilar AV, Karmali M, Ziebell K, Krause DO. Characterization of Escherichia coli isolated from gut biopsies of newly diagnosed patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2011; **17**: 1451-1463 [PMID: 21674703 DOI: 10.1002/ibd.21509]

31 **Dreux N**, Denizot J, Martinez-Medina M, Mellmann A, Billig M, Kisiela D, Chattopadhyay S, Sokurenko E, Neut C, Gower-Rousseau C, Colombel JF, Bonnet R, Darfeuille-Michaud A, Barnich N. Point mutations in FimH adhesin of Crohn's disease-associated adherent-invasive Escherichia coli enhance intestinal inflammatory response. *PLoS Pathog* 2013; **9**: e1003141 [PMID: 23358328 DOI: 10.1371/journal.ppat.1003141]

32 **Petersen AM**, Nielsen EM, Litrup E, Brynskov J, Mirsepasi H, Krogfelt KA. A phylogenetic group of Escherichia coli associated with active left-sided inflammatory bowel disease. *BMC Microbiol* 2009; **9**: 171 [PMID: 19695087 DOI: 10.1186/1471-2180-9-171]

33 **Sepehri S**, Kotlowski R, Bernstein CN, Krause DO. Phylogenetic analysis of inflammatory bowel disease associated Escherichia coli and the fimH virulence determinant. *Inflamm Bowel Dis* 2009; **15**: 1737-1745 [PMID: 19462430 DOI: 10.1002/ibd.20966]

34 **Schippa S**, Conte MP, Borrelli O, Iebba V, Aleandri M, Seganti L, Longhi C, Chiarini F, Osborn J, Cucchiara S. Dominant genotypes in mucosa-associated Escherichia coli strains from pediatric patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2009; **15**: 661-672 [PMID: 19067417 DOI: 10.1002/ibd.20818]

35 **Nowrouzian FL**, Adlerberth I, Wold AE. Enhanced persistence in the colonic microbiota of Escherichia coli strains belonging to phylogenetic group B2: role of virulence factors and adherence to colonic cells. *Microbes Infect* 2006; **8**: 834-840 [PMID: 16483819 DOI: 10.1016/j.micinf.2005.10.011]

36 **Elliott TR**, Hudspith BN, Wu G, Cooley M, Parkes G, Quiñones B, Randall L, Mandrell RE, Fagerquist CK, Brostoff J, Rayment NB, Boussioutas A, Petrovska L, Sanderson JD. Quantification and characterization of mucosa-associated and intracellular Escherichia coli in inflammatory bowel disease. *Inflamm Bowel Dis* 2013; **19**: 2326-2338 [PMID: 23989750 DOI: 10.1097/MIB.0b013e3182a38a92]

37 **Gombošová L**, Lazúrová I, Zakuciová M, Curová K, Kmeťová M, Petrášová D, Siegfried L. Genes of intestinal Escherichia coli and their relation to the inflammatory activity in patients with ulcerative colitis and Crohn's disease. *Folia Microbiol* (Praha) 2011; **56**: 367-372 [PMID: 21877213 DOI: 10.1007/s12223-011-0051-z]

38 **Schultsz C**, Moussa M, van Ketel R, Tytgat GN, Dankert J. Frequency of pathogenic and enteroadherent Escherichia coli in patients with inflammatory bowel disease and controls. *J Clin Pathol* 1997; **50**: 573-579 [PMID: 9306938 DOI: 10.1136/jcp.50.7.573]

39 **Vejborg RM**, Hancock V, Petersen AM, Krogfelt KA, Klemm P. Comparative genomics of Escherichia coli isolated from patients with inflammatory bowel disease. *BMC Genomics* 2011; **12**: 316 [PMID: 21676223 DOI: 10.1186/1471-2164-12-316]

40 **Nowrouzian F**, Adlerberth I, Wold AE. P fimbriae, capsule and aerobactin characterize colonic resident Escherichia coli. *Epidemiol Infect* 2001; **126**: 11-18 [PMID: 11293669 DOI: 10.1017/S0950268801005118]

41 **de Souza HL**, de Carvalho VR, Romeiro FG, Sassaki LY, Keller R, Rodrigues J. Mucosa-associated but not luminal Escherichia coli is augmented in Crohn's disease and ulcerative colitis. *Gut Pathog* 2012; **4**: 21 [PMID: 23234341 DOI: 10.1186/1757-4749-4-21]

42 **Sobieszczańska BA**, Duda-Madej AB, Turniak MB, Franiczek R, Kasprzykowska U, Duda AK, Rzeszutko M, Iwańczak B. Invasive properties, adhesion patterns and phylogroup profiles among Escherichia coli strains isolated from children with inflammatory bowel disease. *Adv Clin Exp Med* 2012; **21**: 591-599 [PMID: 23356195]

43 **Tenaillon O**, Skurnik D, Picard B, Denamur E. The population genetics of commensal Escherichia coli. *Nat Rev Microbiol* 2010; **8**: 207-217 [PMID: 20157339 DOI: 10.1038/nrmicro2298]

44 **Curová K**, Kmetová M, Sabol M, Gombosová L, Lazúrová I, Siegfried L. Enterovirulent E. coli in inflammatory and non-inflammatory bowel diseases. *Folia Microbiol* (Praha) 2009; **54**: 81-86 [PMID: 19330549 DOI: 10.1007/s12223-009-0012-y]

45 **Darfeuille-Michaud A**, Boudeau J, Bulois P, Neut C, Glasser AL, Barnich N, Bringer MA, Swidsinski A, Beaugerie L, Colombel JF. High prevalence of adherent-invasive Escherichia coli associated with ileal mucosa in Crohn's disease. *Gastroenterology* 2004; **127**: 412-421 [PMID: 15300573 DOI: 10.1053/j.gastro.2004.04.061]

46 **Miquel S**, Peyretaillade E, Claret L, de Vallée A, Dossat C, Vacherie B, Zineb el H, Segurens B, Barbe V, Sauvanet P, Neut C, Colombel JF, Medigue C, Mojica FJ, Peyret P, Bonnet R, Darfeuille-Michaud A. Complete genome sequence of Crohn's disease-associated adherent-invasive E. coli strain LF82. *PLoS One* 2010; **5**: e12714 [PMID: 20862302 DOI: 10.1371/journal.pone.0012714]

47 **Nash JH**, Villegas A, Kropinski AM, Aguilar-Valenzuela R, Konczy P, Mascarenhas M, Ziebell K, Torres AG, Karmali MA, Coombes BK. Genome sequence of adherent-invasive Escherichia coli and comparative genomic analysis with other E. coli pathotypes. *BMC Genomics* 2010; **11**: 667 [PMID: 21108814 DOI: 10.1186/1471-2164-11-667]

48 **Rolhion N**, Barnich N, Bringer MA, Glasser AL, Ranc J, Hébuterne X, Hofman P, Darfeuille-Michaud A. Abnormally expressed ER stress response chaperone Gp96 in CD favours adherent-invasive Escherichia coli invasion. *Gut* 2010; **59**: 1355-1362 [PMID: 20587550 DOI: 10.1136/gut.2010.207456]

49 **Barnich N**, Carvalho FA, Glasser AL, Darcha C, Jantscheff P, Allez M, Peeters H, Bommelaer G, Desreumaux P, Colombel JF, Darfeuille-Michaud A. CEACAM6 acts as a receptor for adherent-invasive E. coli, supporting ileal mucosa colonization in Crohn disease. *J Clin Invest* 2007; **117**: 1566-1574 [PMID: 17525800 DOI: 10.1172/JCI30504]

50 **Carvalho FA**, Barnich N, Sivignon A, Darcha C, Chan CH, Stanners CP, Darfeuille-Michaud A. Crohn's disease adherent-invasive Escherichia coli colonize and induce strong gut inflammation in transgenic mice expressing human CEACAM. *J Exp Med* 2009; **206**: 2179-2189 [PMID: 19737864 DOI: 10.1084/jem.20090741]

51 **Eaves-Pyles T**, Allen CA, Taormina J, Swidsinski A, Tutt CB, Jezek GE, Islas-Islas M, Torres AG. Escherichia coli isolated from a Crohn's disease patient adheres, invades, and induces inflammatory responses in polarized intestinal epithelial cells. *Int J Med Microbiol* 2008; **298**: 397-409 [PMID: 17900983 DOI: 10.1016/j.ijmm.2007.05.011]

52 **Subramanian S**, Rhodes JM, Hart CA, Tam B, Roberts CL, Smith SL, Corkill JE, Winstanley C, Virji M, Campbell BJ. Characterization of epithelial IL-8 response to inflammatory bowel disease mucosal E. coli and its inhibition by mesalamine. *Inflamm Bowel Dis* 2008; **14**: 162-175 [PMID: 17941093 DOI: 10.1002/ibd.20296]

53 **Mimouna S**, Gonçalvès D, Barnich N, Darfeuille-Michaud A, Hofman P, Vouret-Craviari V. Crohn disease-associated Escherichia coli promote gastrointestinal inflammatory disorders by activation of HIF-dependent responses. *Gut Microbes* 2011; **2**: 335-346 [PMID: 22157238 DOI: 10.4161/gmic.18771]

54 **Stevens C**, Henderson P, Nimmo ER, Soares DC, Dogan B, Simpson KW, Barrett JC, Wilson DC, Satsangi J. The intermediate filament protein, vimentin, is a regulator of NOD2 activity. *Gut* 2013; **62**: 695-707 [PMID: 22684479 DOI: 10.1136/gutjnl-2011-301775]

55 **Low D**, Tran HT, Lee IA, Dreux N, Kamba A, Reinecker HC, Darfeuille-Michaud A, Barnich N, Mizoguchi E. Chitin-binding domains of Escherichia coli ChiA mediate interactions with intestinal epithelial cells in mice with colitis. *Gastroenterology* 2013; **145**: 602-612.e9 [PMID: 23684751 DOI: 10.1053/j.gastro.2013.05.017]

56 **Miquel S**, Claret L, Bonnet R, Dorboz I, Barnich N, Darfeuille-Michaud A. Role of decreased levels of Fis histone-like protein in Crohn's disease-associated adherent invasive Escherichia coli LF82 bacteria interacting with intestinal epithelial cells. *J Bacteriol* 2010; **192**: 1832-1843 [PMID: 20118249 DOI: 10.1128/JB.01679-09]

57 **Lapaquette P**, Glasser AL, Huett A, Xavier RJ, Darfeuille-Michaud A. Crohn's disease-associated adherent-invasive E. coli are selectively favoured by impaired autophagy to replicate intracellularly. *Cell Microbiol* 2010; **12**: 99-113 [PMID: 19747213 DOI: 10.1111/j.1462-5822.2009.01381.x]

58 **Nguyen HT**, Dalmasso G, Müller S, Carrière J, Seibold F, Darfeuille-Michaud A. Crohn's disease-associated adherent invasive Escherichia coli modulate levels of microRNAs in intestinal epithelial cells to reduce autophagy. *Gastroenterology* 2014; **146**: 508-519 [PMID: 24148619 DOI: 10.1053/j.gastro.2013.10.021]

59 **Chassaing B**, Rolhion N, de Vallée A, Salim SY, Prorok-Hamon M, Neut C, Campbell BJ, Söderholm JD, Hugot JP, Colombel JF, Darfeuille-Michaud A. Crohn disease--associated adherent-invasive E. coli bacteria target mouse and human Peyer's patches via long polar fimbriae. *J Clin Invest* 2011; **121**: 966-975 [PMID: 21339647 DOI: 10.1172/JCI44632]

60 **Denizot J**, Sivignon A, Barreau F, Darcha C, Chan HF, Stanners CP, Hofman P, Darfeuille-Michaud A, Barnich N. Adherent-invasive Escherichia coli induce claudin-2 expression and barrier defect in CEABAC10 mice and Crohn's disease patients. *Inflamm Bowel Dis* 2012; **18**: 294-304 [PMID: 21688348 DOI: 10.1002/ibd.21787]

61 **Wine E**, Ossa JC, Gray-Owen SD, Sherman PM. Adherent-invasive Escherichia coli, strain LF82 disrupts apical junctional complexes in polarized epithelia. *BMC Microbiol* 2009; **9**: 180 [PMID: 19709415 DOI: 10.1186/1471-2180-9-180]

62 **Mankertz J**, Amasheh M, Krug SM, Fromm A, Amasheh S, Hillenbrand B, Tavalali S, Fromm M, Schulzke JD. TNFalpha up-regulates claudin-2 expression in epithelial HT-29/B6 cells via phosphatidylinositol-3-kinase signaling. *Cell Tissue Res* 2009; **336**: 67-77 [PMID: 19214581 DOI: 10.1007/s00441-009-0751-8]

63 **Ossa JC**, Ho NK, Wine E, Leung N, Gray-Owen SD, Sherman PM. Adherent-invasive Escherichia coli blocks interferon-γ-induced signal transducer and activator of tran-

scription (STAT)-1 in human intestinal epithelial cells. *Cell Microbiol* 2013; **15**: 446-457 [PMID: 23072252 DOI: 10.1111/cmi.12048]
64 **Bringer MA**, Glasser AL, Tung CH, Méresse S, Darfeuille-Michaud A. The Crohn's disease-associated adherent-invasive Escherichia coli strain LF82 replicates in mature phagolysosomes within J774 macrophages. *Cell Microbiol* 2006; **8**: 471-484 [PMID: 16469058 DOI: 10.1111/j.1462-5822.2005.00639.x]
65 **Bringer MA**, Barnich N, Glasser AL, Bardot O, Darfeuille-Michaud A. HtrA stress protein is involved in intramacrophagic replication of adherent and invasive Escherichia coli strain LF82 isolated from a patient with Crohn's disease. *Infect Immun* 2005; **73**: 712-721 [PMID: 15664909 DOI: 10.1128/IAI.73.2.712-721.2005]
66 **Bringer MA**, Rolhion N, Glasser AL, Darfeuille-Michaud A. The oxidoreductase DsbA plays a key role in the ability of the Crohn's disease-associated adherent-invasive Escherichia coli strain LF82 to resist macrophage killing. *J Bacteriol* 2007; **189**: 4860-4871 [PMID: 17449627 DOI: 10.1128/JB.00233-07]
67 **Simonsen KT**, Nielsen G, Bjerrum JV, Kruse T, Kallipolitis BH, Møller-Jensen J. A role for the RNA chaperone Hfq in controlling adherent-invasive Escherichia coli colonization and virulence. *PLoS One* 2011; **6**: e16387 [PMID: 21298102 DOI: 10.1371/journal.pone.0016387]
68 **Glasser AL**, Boudeau J, Barnich N, Perruchot MH, Colombel JF, Darfeuille-Michaud A. Adherent invasive Escherichia coli strains from patients with Crohn's disease survive and replicate within macrophages without inducing host cell death. *Infect Immun* 2001; **69**: 5529-5537 [PMID: 11500426 DOI: 10.1128/IAI.69.9.5529-5537.2001]
69 **Carvalho FA**, Barnich N, Sauvanet P, Darcha C, Gelot A, Darfeuille-Michaud A. Crohn's disease-associated Escherichia coli LF82 aggravates colitis in injured mouse colon via signaling by flagellin. *Inflamm Bowel Dis* 2008; **14**: 1051-1060 [PMID: 18338780 DOI: 10.1002/ibd.20423]
70 **Meconi S**, Vercellone A, Levillain F, Payré B, Al Saati T, Capilla F, Desreumaux P, Darfeuille-Michaud A, Altare F. Adherent-invasive Escherichia coli isolated from Crohn's disease patients induce granulomas in vitro. *Cell Microbiol* 2007; **9**: 1252-1261 [PMID: 17223928 DOI: 10.1111/j.1462-5822.2006.00868.x]
71 **Dunne KA**, Allam A, McIntosh A, Houston SA, Cerovic V, Goodyear CS, Roe AJ, Beatson SA, Milling SW, Walker D, Wall DM. Increased S-nitrosylation and proteasomal degradation of caspase-3 during infection contribute to the persistence of adherent invasive Escherichia coli (AIEC) in immune cells. *PLoS One* 2013; **8**: e68386 [PMID: 23861899 DOI: 10.1371/journal.pone.0068386]
72 **Peeters H**, Bogaert S, Laukens D, Rottiers P, De Keyser F, Darfeuille-Michaud A, Glasser AL, Elewaut D, De Vos M. CARD15 variants determine a disturbed early response of monocytes to adherent-invasive Escherichia coli strain LF82 in Crohn's disease. *Int J Immunogenet* 2007; **34**: 181-191 [PMID: 17504508 DOI: 10.1111/j.1744-313X.2007.00670.x]
73 **Nieminen JK**, Sipponen T, Färkkilä M, Vaarala O. Monocyte-derived dendritic cells from Crohn's disease patients exhibit decreased ability to activate T helper type 17 responses in memory cells. *Clin Exp Immunol* 2014; **177**: 190-202 [PMID: 24635023 DOI: 10.1111/cei.12326]
74 **Chargui A**, Cesaro A, Mimouna S, Fareh M, Brest P, Naquet P, Darfeuille-Michaud A, Hébuterne X, Mograbi B, Vouret-Craviari V, Hofman P. Subversion of autophagy in adherent invasive Escherichia coli-infected neutrophils induces inflammation and cell death. *PLoS One* 2012; **7**: e51727 [PMID: 23272151 DOI: 10.1371/journal.pone.0051727]
75 **Martinez-Medina M**, Naves P, Blanco J, Aldeguer X, Blanco JE, Blanco M, Ponte C, Soriano F, Darfeuille-Michaud A, Garcia-Gil LJ. Biofilm formation as a novel phenotypic feature of adherent-invasive Escherichia coli (AIEC). *BMC Microbiol* 2009; **9**: 202 [PMID: 19772580 DOI: 10.1186/1471-2180-9-202]
76 **Chassaing B**, Darfeuille-Michaud A. The σE pathway is involved in biofilm formation by Crohn's disease-associated adherent-invasive Escherichia coli. *J Bacteriol* 2013; **195**: 76-84 [PMID: 23104802 DOI: 10.1128/JB.01079-12]
77 **Prorok-Hamon M**, Friswell MK, Alswied A, Roberts CL, Song F, Flanagan PK, Knight P, Codling C, Marchesi JR, Winstanley C, Hall N, Rhodes JM, Campbell BJ. Colonic mucosa-associated diffusely adherent afaC+ Escherichia coli expressing lpfA and pks are increased in inflammatory bowel disease and colon cancer. *Gut* 2014; **63**: 761-770 [PMID: 23846483 DOI: 10.1136/gutjnl-2013-304739]
78 **Martinez-Medina M**, Mora A, Blanco M, López C, Alonso MP, Bonacorsi S, Nicolas-Chanoine MH, Darfeuille-Michaud A, Garcia-Gil J, Blanco J. Similarity and divergence among adherent-invasive Escherichia coli and extraintestinal pathogenic E. coli strains. *J Clin Microbiol* 2009; **47**: 3968-3979 [PMID: 19828750 DOI: 10.1128/JCM.01484-09]
79 **Clarke DJ**, Chaudhuri RR, Martin HM, Campbell BJ, Rhodes JM, Constantinidou C, Pallen MJ, Loman NJ, Cunningham AF, Browning DF, Henderson IR. Complete genome sequence of the Crohn's disease-associated adherent-invasive Escherichia coli strain HM605. *J Bacteriol* 2011; **193**: 4540 [PMID: 21705601 DOI: 10.1128/JB.05374-11]
80 **Krause DO**, Little AC, Dowd SE, Bernstein CN. Complete genome sequence of adherent invasive Escherichia coli UM146 isolated from Ileal Crohn's disease biopsy tissue. *J Bacteriol* 2011; **193**: 583 [PMID: 21075930 DOI: 10.1128/JB.01290-10]
81 **Small CL**, Reid-Yu SA, McPhee JB, Coombes BK. Persistent infection with Crohn's disease-associated adherent-invasive Escherichia coli leads to chronic inflammation and intestinal fibrosis. *Nat Commun* 2013; **4**: 1957 [PMID: 23748852 DOI: 10.1038/ncomms2957]
82 **Dogan B**, Scherl E, Bosworth B, Yantiss R, Altier C, McDonough PL, Jiang ZD, Dupont HL, Garneau P, Harel J, Rishniw M, Simpson KW. Multidrug resistance is common in Escherichia coli associated with ileal Crohn's disease. *Inflamm Bowel Dis* 2013; **19**: 141-150 [PMID: 22508665 DOI: 10.1002/ibd.22971]
83 **Seksik P**, Rigottier-Gois L, Gramet G, Sutren M, Pochart P, Marteau P, Jian R, Doré J. Alterations of the dominant faecal bacterial groups in patients with Crohn's disease of the colon. *Gut* 2003; **52**: 237-242 [PMID: 12524406 DOI: 10.1136/gut.52.2.237]
84 **Jensen SR**, Fink LN, Nielsen OH, Brynskov J, Brix S. Ex vivo intestinal adhesion of Escherichia coli LF82 in Crohn's disease. *Microb Pathog* 2011; **51**: 426-431 [PMID: 21911052 DOI: 10.1016/j.micpath.2011.08.006]
85 **Lapaquette P**, Bringer MA, Darfeuille-Michaud A. Defects in autophagy favour adherent-invasive Escherichia coli persistence within macrophages leading to increased pro-inflammatory response. *Cell Microbiol* 2012; **14**: 791-807 [PMID: 22309232 DOI: 10.1111/j.1462-5822.2012.01768.x]
86 **Chassaing B**, Etienne-Mesmin L, Bonnet R, Darfeuille-Michaud A. Bile salts induce long polar fimbriae expression favouring Crohn's disease-associated adherent-invasive Escherichia coli interaction with Peyer's patches. *Environ Microbiol* 2013; **15**: 355-371 [PMID: 22789019 DOI: 10.1111/j.1462-2920.2012.02824.x]
87 **Vazeille E**, Bringer MA, Gardarin A, Chambon C, Becker-Pauly C, Pender SL, Jakob C, Müller S, Lottaz D, Darfeuille-Michaud A. Role of meprins to protect ileal mucosa of Crohn's disease patients from colonization by adherent-invasive E. coli. *PLoS One* 2011; **6**: e21199 [PMID: 21698174 DOI: 10.1371/journal.pone.0021199]
88 **Burke DA**, Axon AT. Adhesive Escherichia coli in inflammatory bowel disease and infective diarrhoea. *BMJ* 1988; **297**: 102-104 [PMID: 3044496 DOI: 10.1136/bmj.297.6641.102]
89 **Giaffer MH**, Holdsworth CD, Duerden BI. Virulence prop-

erties of Escherichia coli strains isolated from patients with inflammatory bowel disease. *Gut* 1992; **33**: 646-650 [PMID: 1612481 DOI: 10.1136/gut.33.5.646]

90 **Thomazini CM**, Samegima DA, Rodrigues MA, Victoria CR, Rodrigues J. High prevalence of aggregative adherent Escherichia coli strains in the mucosa-associated microbiota of patients with inflammatory bowel diseases. *Int J Med Microbiol* 2011; **301**: 475-479 [PMID: 21616711 DOI: 10.1016/j.ijmm.2011.04.015]

91 **Iebba V**, Conte MP, Lepanto MS, Di Nardo G, Santangelo F, Aloi M, Totino V, Checchi MP, Longhi C, Cucchiara S, Schippa S. Microevolution in fimH gene of mucosa-associated Escherichia coli strains isolated from pediatric patients with inflammatory bowel disease. *Infect Immun* 2012; **80**: 1408-1417 [PMID: 22290143 DOI: 10.1128/IAI.06181-11]

92 **Raso T**, Crivellaro S, Chirillo MG, Pais P, Gaia E, Savoia D. Analysis of Escherichia coli isolated from patients affected by Crohn's disease. *Curr Microbiol* 2011; **63**: 131-137 [PMID: 21626145 DOI: 10.1007/s00284-011-9947-8]

93 **Negroni A**, Costanzo M, Vitali R, Superti F, Bertuccini L, Tinari A, Minelli F, Di Nardo G, Nuti F, Pierdomenico M, Cucchiara S, Stronati L. Characterization of adherent-invasive Escherichia coli isolated from pediatric patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2012; **18**: 913-924 [PMID: 21994005 DOI: 10.1002/ibd.21899]

94 **Wang T**, Cai G, Qiu Y, Fei N, Zhang M, Pang X, Jia W, Cai S, Zhao L. Structural segregation of gut microbiota between colorectal cancer patients and healthy volunteers. *ISME J* 2012; **6**: 320-329 [PMID: 21850056 DOI: 10.1038/ismej.2011.109]

95 **Swidsinski A**, Khilkin M, Kerjaschki D, Schreiber S, Ortner M, Weber J, Lochs H. Association between intraepithelial Escherichia coli and colorectal cancer. *Gastroenterology* 1998; **115**: 281-286 [PMID: 9679033 DOI: 10.1016/S0016-5085(98)70194-5]

96 **Bonnet M**, Buc E, Sauvanet P, Darcha C, Dubois D, Pereira B, Déchelotte P, Bonnet R, Pezet D, Darfeuille-Michaud A. Colonization of the human gut by E. coli and colorectal cancer risk. *Clin Cancer Res* 2014; **20**: 859-867 [PMID: 24334760 DOI: 10.1158/1078-0432.CCR-13-1343]

97 **Buc E**, Dubois D, Sauvanet P, Raisch J, Delmas J, Darfeuille-Michaud A, Pezet D, Bonnet R. High prevalence of mucosa-associated E. coli producing cyclomodulin and genotoxin in colon cancer. *PLoS One* 2013; **8**: e56964 [PMID: 23457644 DOI: 10.1371/journal.pone.0056964]

98 **Schippa S**, Iebba V, Barbato M, Di Nardo G, Totino V, Checchi MP, Longhi C, Maiella G, Cucchiara S, Conte MP. A distinctive 'microbial signature' in celiac pediatric patients. *BMC Microbiol* 2010; **10**: 175 [PMID: 20565734 DOI: 10.1186/1471-2180-10-175]

99 **Nadal I**, Donat E, Ribes-Koninckx C, Calabuig M, Sanz Y. Imbalance in the composition of the duodenal microbiota of children with coeliac disease. *J Med Microbiol* 2007; **56**: 1669-1674 [PMID: 18033837 DOI: 10.1099/jmm.0.47410-0]

100 **De Palma G**, Nadal I, Medina M, Donat E, Ribes-Koninckx C, Calabuig M, Sanz Y. Intestinal dysbiosis and reduced immunoglobulin-coated bacteria associated with coeliac disease in children. *BMC Microbiol* 2010; **10**: 63 [PMID: 20181275 DOI: 10.1186/1471-2180-10-63]

101 **Sánchez E**, Nadal I, Donat E, Ribes-Koninckx C, Calabuig M, Sanz Y. Reduced diversity and increased virulence-gene carriage in intestinal enterobacteria of coeliac children. *BMC Gastroenterol* 2008; **8**: 50 [PMID: 18983674 DOI: 10.1186/1471-230X-8-50]

102 **Martinez-Medina M**, Garcia-Gil J, Barnich N, Wieler LH, Ewers C. Adherent-invasive Escherichia coli phenotype displayed by intestinal pathogenic E. coli strains from cats, dogs, and swine. *Appl Environ Microbiol* 2011; **77**: 5813-5817 [PMID: 21705530 DOI: 10.1128/AEM.02614-10]

103 **Simpson KW**, Dogan B, Rishniw M, Goldstein RE, Klaessig S, McDonough PL, German AJ, Yates RM, Russell DG, Johnson SE, Berg DE, Harel J, Bruant G, McDonough SP, Schukken YH. Adherent and invasive Escherichia coli is associated with granulomatous colitis in boxer dogs. *Infect Immun* 2006; **74**: 4778-4792 [PMID: 16861666 DOI: 10.1128/IAI.00067-06]

104 **Nickerson KP**, McDonald C. Crohn's disease-associated adherent-invasive Escherichia coli adhesion is enhanced by exposure to the ubiquitous dietary polysaccharide maltodextrin. *PLoS One* 2012; **7**: e52132 [PMID: 23251695 DOI: 10.1371/journal.pone.0052132]

105 **Roberts CL**, Keita AV, Duncan SH, O'Kennedy N, Söderholm JD, Rhodes JM, Campbell BJ. Translocation of Crohn's disease Escherichia coli across M-cells: contrasting effects of soluble plant fibres and emulsifiers. *Gut* 2010; **59**: 1331-1339 [PMID: 20813719 DOI: 10.1136/gut.2009.195370]

106 **Martinez-Medina M**, Denizot J, Dreux N, Robin F, Billard E, Bonnet R, Darfeuille-Michaud A, Barnich N. Western diet induces dysbiosis with increased E coli in CEABAC10 mice, alters host barrier function favouring AIEC colonisation. *Gut* 2014; **63**: 116-124 [PMID: 23598352 DOI: 10.1136/gutjnl-2012-304119]

107 **Hillman LC**, Peters SG, Fisher CA, Pomare EW. Effects of the fibre components pectin, cellulose, and lignin on bile salt metabolism and biliary lipid composition in man. *Gut* 1986; **27**: 29-36 [PMID: 3005138 DOI: 10.1136/gut.27.1.29]

108 **Rutgeerts P**, Ghoos Y, Vantrappen G, Fevery J. Biliary lipid composition in patients with nonoperated Crohn's disease. *Dig Dis Sci* 1986; **31**: 27-32 [PMID: 3940821 DOI: 10.1007/BF01347906]

109 **Lapidus A**, Akerlund JE, Einarsson C. Gallbladder bile composition in patients with Crohn 's disease. *World J Gastroenterol* 2006; **12**: 70-74 [PMID: 16440420]

110 **Drouet M**, Vignal C, Singer E, Djouina M, Dubreuil L, Cortot A, Desreumaux P, Neut C. AIEC colonization and pathogenicity: influence of previous antibiotic treatment and pre-existing inflammation. *Inflamm Bowel Dis* 2012; **18**: 1923-1931 [PMID: 22344932 DOI: 10.1002/ibd.22908]

111 **Craven M**, Egan CE, Dowd SE, McDonough SP, Dogan B, Denkers EY, Bowman D, Scherl EJ, Simpson KW. Inflammation drives dysbiosis and bacterial invasion in murine models of ileal Crohn's disease. *PLoS One* 2012; **7**: e41594 [PMID: 22848538 DOI: 10.1371/journal.pone.0041594]

112 **Chassaing B**, Koren O, Carvalho FA, Ley RE, Gewirtz AT. AIEC pathobiont instigates chronic colitis in susceptible hosts by altering microbiota composition. *Gut* 2014; **63**: 1069-1080 [PMID: 23896971 DOI: 10.1136/gutjnl-2013-304909]

P- Reviewer: Gassler N, Silva MA **S- Editor**: Song XX
L- Editor: A **E- Editor:** Lu YJ

World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4291/wjgp.v5.i3.228

World J Gastrointest Pathophysiol 2014 August 15; 5(3): 228-238
ISSN 2150-5330 (online)

TOPIC HIGHLIGHT

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

Similarities and differences between Behçet's disease and Crohn's disease

Veli Yazısız

Veli Yazısız, Division of Rheumatology, Department of Internal Medicine, Medical School, Akdeniz University, Kampus 07058, Antalya, Turkey
Author contributions: Yazısız V contributed to this paper.
Correspondence to: Veli Yazısız, MD, Associate Professor, Division of Rheumatology, Department of Internal Medicine, Medical School, Akdeniz Universitesi, Tıp Fakültesi, İç Hastalıkları AD, Romatoloji BD, Dumlupınar Bulvarı, Kampus 07058, Antalya, Turkey. drvyazisiz@yahoo.com.tr
Telephone: +90-505-3149901 Fax: +90-242-2496040
Received: January 2, 2014 Revised: March 9, 2014
Accepted: May 29, 2014
Published online: August 15, 2014

Abstract

Behçet's disease (BD) is a chronic inflammatory condition with multisystem involvement. Approximately 10%-15% of patients present with gastrointestinal involvement. Involved sites and the endoscopic view usually resemble Crohn's disease (CD). In addition to intestinal involvement, oral mucosa, the eyes, skin, and joints are commonly affected. No pathognomonic laboratory test is available for the diagnosis of either disease. Management approaches are also similar in various aspects. Differentiating BD from CD is highly challenging. In this article, the similarities and differences between BD and CD in terms of epidemiology, etiopathogenesis, clinical and imaging findings, and histopathological and therapeutic approaches are reviewed.

Key words: Behçet's disease; Crohn's disease

Core tip: Behçet's disease and Crohn's disease are chronic inflammatory conditions caused by lesions similar to those seen in the bowels. There are similar and different clinical findings, however both diseases show intestinal inflammation. The differential diagnosis may be difficult when the symptoms of the two disease processes are very similar. This review focuses on the similar and different characteristics of Behçet's disease and Crohn's disease.

Yazısız V. Similarities and differences between Behçet's disease and Crohn's disease. *World J Gastrointest Pathophysiol* 2014; 5(3): 228-238 Available from: URL: http://www.wjgnet.com/2150-5330/full/v5/i3/228.htm DOI: http://dx.doi.org/10.4291/wjgp.v5.i3.228

INTRODUCTION

Behçet's disease (BD), which was first defined by Hulusi Behçet, a Turkish dermatologist, in 1937, is a chronic inflammatory disease with multisystem involvement[1]. It presents with remission and exacerbation of mucocutaneous, ocular, articular, vascular, or gastrointestinal lesions. Crohn's disease (CD), on the other hand, is a chronic relapsing inflammatory disorder of the gastrointestinal tract, presenting with BD-like extra-intestinal manifestations[2]. Both of these chronic immune-mediated inflammatory disorders are likely to affect patients at a younger age accompanied by fluctuating courses. It is possible that a patient with CD meets the criteria for BD. The differential diagnosis in some cases is quite difficult, particularly in the presence of gastrointestinal involvement. Differentiation is usually based on the involvement of different organs. This review aims to investigate the similar and different characteristics of BD and CD.

EPIDEMIOLOGY

The prevalence of BD varies geographically and the

disease is more prevalent in certain groups. It is most common in populations clustered along the ancient Silk Road. Turkey has the highest prevalence (80-370 cases/100000), followed by Asia and the Middle Eastern countries, including Israel, Saudi Arabia and Iran. BD can be seen in all countries worldwide due to immigration[3-6]. The age at onset of the disease is usually between 20.8 and 40 years, as it is more common in young individuals[3]. Patients aged 16 years with initial symptoms, considered as childhood-onset BD, have also been reported. The male-to-female ratio varies regionally. The disease is more common among men in Russia, Saudi Arabia, Iraq, Lebanon, Jordan, Kuwait, Greece, Italy, Turkey, and Iran, while it is more frequent among women in Japan, South Korea, and Israel[3,7].

The incidence of CD may also vary regionally. The incidence of the disease is highest in the United Kingdom, North America, and the northern part of Europe. The prevalence of CD was found to be 133/100000 in the state of Minnesota, United States in 1991. In recent years, several studies showing an increasing incidence ratio have been reported. The incidence is highest in young individuals aged 15 to 29 years. The prevalence of the disease is similar in men and women (the male/female ratio is 2.9-0.76/1)[8-14].

GENETIC FACTORS

There are familial BD cases in the literature, suggesting that genetic factors play a role in the pathogenesis of the disease. The ratio of familial cases is between 0 and 18.2%. The genetic association between the HLA-B51 gene and BD was first reported in 1982 by Ohno[15]. This association has been confirmed in many different ethnic groups. The HLA-B5 gene, particularly the HLA-B5101 allele gene, may be a strong candidate locus responsible for the development of BD and HLA-B51 itself may be the major disease susceptibility gene for BD[16]. It is more likely that the HLA-B51 gene is directly *involved* in the *hyperactivity* of *neutrophils*. Increased neutrophil function has also been reported in HLA-B51-positive BD patients[17,18].

Familial aggregations and a high degree of disease concordance in twins with CD have been recognized for quite some time. The concordance rate has been reported to be 3% in dizygotic twins and up to 35% in monozygotic twins[2]. Recent studies have provided an insight into genetic disorders responsible for susceptibility of the disease. Furthermore, these studies have strengthened the evidence that major cytokines, cytokine receptors and cell types are involved in the underlying pathogenesis of the disease. Nucleotide oligomerization domain 2 (NOD2) is the major susceptibility gene for CD. Genome-wide association studies have demonstrated a number of susceptibility genes where NOD2 is encoded. The nucleotide oligomerization domain 2 gene is located at the CD susceptibility locus on chromosome 16q12[19,20].

PATHOGENESIS

Immunosuppressive agents, which are used in the management of autoimmune disorders, are highly effective in BD, and the role of autoimmunity has been widely discussed in the pathogenesis of the disease[21,22]. However, anti-nuclear antibody (ANA) positivity, anti-Ro, and anti-La antibodies, which are usually found in autoimmune disorders, have not been found in BD. Several studies have demonstrated the presence of anti-endothelial antibodies, anti-lymphocytic antibodies, and heat-shock protein 60 (HSP60) in BD; however, these antibodies have not been strongly associated with the disease[23]. Additionally, major histocompatibility complex (MHC) Class Ⅱ molecules have been associated with autoimmunity. However, BD is strongly associated with HLA-B5, a MHC Class I antigen.

BD is likely to be an autoinflammatory disease, as it presents with mucocutaneous lesions and episodic arthritis without deformity with a very strong acute phase response during these episodes. In BD, neutrophils are implicated in the inflammatory process of natural immune system-mediated disease (caspase pathway, IL-1, IL-18) similar to autoinflammatory diseases[21]. Mediterranean fever (MEFV) gene mutations, which are the main causes of familial Mediterranean fever (FMF), an autoinflammatory disease, are frequently found in BD[24,25]. However, the presence of clinical manifestations including uveitis, vasculitis, and thrombosis, which are not seen in autoinflammatory diseases, and the absence of serositis, a very common pathology in autoinflammatory diseases, does not suggest its autoinflammatory nature. Thus, currently, BD is considered to be neither an autoinflammatory nor an autoimmune disorder[21].

Furthermore, large and small vessel vasculitides may be present in BD. Thrombotic occlusions of the venous branches and aneurysm formations in the arterial vessels may develop. Arterial involvement may lead to bleeding and organ failure, and ultimately death. Immunosuppressive therapies can be effective in the resolution of vasculitis[26]. Vasculitis-related alterations have been observed in biopsy specimens of oral aphthae, genital ulcers, and skin lesions[27]. As vasculitis is considered to be a major component involved in the pathogenesis of BD, it is recommended that the disease should be evaluated under systemic vasculitides[28].

Several microorganisms of the oral microbial flora have been indicated in the pathogenesis of BD[29,30]. Atypical streptococcal colonization is increased in the oral mucosa. A hyperimmune activity against *Streptococci* has been shown in various studies. *Streptococcus sanguinis* causes increased interleukin-6 (IL-6) and interferon gamma (IFN-γ) secretions in the peripheral blood T-cells[31]. *Escherichia coli* and *Staphylococcaceae* species have been reported to increase inflammatory cytokines in BD patients. There are also studies showing regression of BD lesions with antibiotherapy in the literature[32].

Microorganisms that are involved in normal colon

microflora with a mutual relationship with the immune system are also considered to play a role in the underlying pathogenesis of CD. Several products that are produced by these microorganisms such as butyrate and propionate contribute to intestinal inflammation, by affecting immune system cells and cytokines in patients with genetic susceptibility to CD. Reduced mucin production in epithelial cells of the intestinal mucosa is another possible culprit. Genome-wide association studies have revealed a relationship between gene mutations in mucin expression (MUC1, MUC19 and PTGER4) and CD[2,19].

Innate immune system cells are mostly implicated in the immunopathogenesis of CD[2]. Pattern recognition receptors such as Toll-like receptors (TLR) and nucleotide binding domain (NOD) like receptors (NLR) have a critical role in the recognition of the molecular patterns of innate immune system cell pathogens. There is a strong association between NOD2/CARD15 polymorphisms and CD. NOD2/CARD15 encodes an intracellular receptor that is expressed predominantly in monocytes and Paneth cells. These pattern receptors are substantially expressed by dendritic cells lying beneath the intestinal epithelium. Dendritic cells may have reduced regulatory T-cell stimulation, which leads to immune tolerance in CD. These cells are responsible for organization of the relationship between microbial products and immune system cells, and identify immunity or tolerance development. The inflammasome complex of the lamina propria, which is implicated in mononuclear cells, is crucial for the immune response. The stimulation of NLPR3, caspase-1, and pro-interleukin-1 causes a significant increase in pro-inflammatory cytokines including tumor necrosis factor alpha (TNF-alpha) and interleukin (IL)-6. IL-17, IL-23, and IL-27 are crucial players in inflammatory alterations during the disease process[19,33]. Some authorities have adopted CD as an autoinflammatory disease due to its potent inflammation pathways[34]. Unlike patients with BD, the incidence of MEFV gene mutations remains unchanged in patients with CD[35]. The ANA and anti-neutrophil cytoplasmic antibodies (ANCA) positivity is higher in the patient population than healthy individuals. Nearly 40% to 70% of patients also have positive anti-*Saccharomyces cerevisiae* antibodies (ASCA), which are associated with disease severity. The ASCA positivity is higher in patients with CD than BD[36-38].

CLINICAL MANIFESTATIONS

Extra-intestinal manifestations

BD is a multisystem condition usually presenting with oral mucosa, ocular, articular, and vascular involvement. Gastrointestinal, neurological, and cardiac involvement are relatively infrequent. Nearly all patients suffer from recurrent oral ulcers. These ulcers are classified as large, small, or herpetiform, based on their size. They are extremely painful and may involve the buccal mucosa, labial mucosa, tongue, the soft and hard palate, and the pharynx. The incidence of genital ulcers with scar formation is relatively low compared with oral ulcers. These painful ulcers are quite similar to oral ulcers in appearance. They may be found in the scrotum and penis in men, and the vulva, vagina, and cervix in women. Additionally, nearly two-thirds of patients with BD have skin changes including acne-like papules, pustules, pseudofolliculitis, and erythema nodosum-like lesions. Due to superficial thrombophlebitis, shifting, and painful subcutaneous nodules can be palpable[7,39-43].

The pathergy phenomenon is a hyper-reactive response to minor trauma. The test is based on the principle of using a 21-25-gauge needle inserted into the skin. Positive test results show papulopustular lesions in the skin or erythematous reactions of the surrounding tissue within 24-48 h. The positive predictive value of the pathergy test varies regionally as the rate of positive pathergy test differs in different countries, and is highest in countries along the ancient Silk Road (30%-70%). The diagnostic value of diagnostic criteria is reduced when pathergy positivity is excluded. In addition, the pathergy test, which involves intradermal monosodium urate crystals, is more sensitive[7,43].

Ocular involvement in BD includes anterior or posterior uveitis, vitritis, retinal vasculitis, retinal vein thrombosis, corneal ulcers, and retrobulbar neuritis. Ocular disease may be the initial manifestation of the disease. BD-associated uveitis is defined as chronic and recurrent non-granulomatous panuveitis and retinal vasculitis with a bilateral course. The disease usually presents with acute inflammatory episodes that resolve within days or weeks. Recurrent episodes may result in permanent vision loss. Furthermore, as uveitis is rarely accompanied by conjunctivitis, scleritis, episcleritis, or sicca syndrome, other conditions should be suspected in patients with ocular involvement[40-44].

Musculoskeletal disorders are also common in patients with BD. Palindromic asymmetric arthritic exacerbations involving the knee, wrist, and ankle may develop. Chronic erosive arthritis is relatively rare. The incidence of sacroiliitis has been reported to increase in patients with BD. Due to peripheral arthritis characteristics and sacroiliac joint involvement, BD is evaluated in the spectrum of seronegative spondyloarthropathy. An overlap of relapsing polychondritis and BD, known as mouth and genital ulcers with inflamed cartilage (MAGIC) syndrome, may also develop in patients with cartilaginous inflammation[42-45].

BD-related vasculopathy differs from other vasculitides, due to its pattern of arterial and venous involvement. Venous thrombus may develop. It may present with superficial thrombophlebitis or involve deep veins, as well as the inferior/superior vena cava, the right atrium, or intracranial large sinuses. The major hepatobiliary disease is Budd-Chiari syndrome, which is one of the leading causes of mortality[46]. Unlike other thrombotic events, embolism is not anticipated. Primary thrombosis,

which is often accompanied by right atrial thrombi, may occur in the pulmonary artery and its thin branches. In addition, arterial aneurysms are common. Pulmonary artery aneurysms may lead to massive bleeding and a fatal outcome[40-42].

Moreover, central nervous system (CNS)-related symptoms may develop secondary to vascular events, such as sinus thrombus and intracranial aneurysms. Primary parenchymal involvement including meningitis and encephalitis, mostly in the pons and mesencephalon, is also seen in patients with BD. It is also known as Neuro-BD, accounting for 10% of patients. In addition, longitudinal extensive transverse myelitis (LETM), characterized by spinal cord lesions, may occur. Histopathological examination of neurological lesions typically shows an inflammatory cellular infiltration of the surrounding vessels[40,41].

CD is a complex disorder, which primarily involves the small intestine and the colon. However, various extra-intestinal manifestations of the disease including oral and genital ulcers, erythema nodosum, uveitis, and arthritis may also be observed[2]. Skin changes may be seen in 5%-10% of patients. Erythema nodosum (5.6%-13.5%), pyoderma gangrenosum (0.75%-0.15%), and acute neutrophilic dermatoses, also termed Sweet' s disease, are among the main skin lesions. Other skin conditions include oral aphthous lesions, perianal lesions, large ulcers, fissures, fistulas, and aseptic abscesses[47,48]. Pathergy positivity is extremely low in patients with CD, compared to those with BD[49].

The most common ocular conditions are uveitis, episcleritis, conjunctivitis, and blepharitis. Non-granulomatous anterior uveitis may develop and recurrent episodes may result in permanent vision loss. Ocular complications are not associated with disease severity. Additionally, retinal vasculitis, which is extremely rare, has been reported in the literature as case studies[47-51].

The clinical association between spondyloarthropathy and CD has been well-established. Nearly 10%-15% of CD patients are complicated by spondyloarthropathy. Both peripheral and axial arthropathies may be seen in CD. Peripheral arthropathies often present as asymmetric pauciarticular involvement. It is usually acute and self-limited, and the severity of the disease is reduced in parallel with decreased disease activity without sequelae. Persistent erosive monoarthritis has been described. Axial involvement resembles ankylosing spondylitis. Bilateral sacroiliitis, as well as spondylitis of the lumbar vertebrae and syndesmophytes may be seen. Chronic low back pain is the main symptom. It is frequent in asymptomatic sacroiliitis. Half of patients with CD have sacroiliac joint abnormalities, as evidenced by X-ray images[52,53].

There are several studies showing a 1.5- to 3.5-fold increase in the risk of venous thromboembolism in CD. Some authors have suggested that it can be attributed to increased hospitalization and surgical interventions. On the other hand, the risk of arterial aneurysm and thromboembolism remain unchanged. However, mesenteric ischemia may occur[54,55]. The incidence of Takayasu's arteritis has been reported to increase in patients with CD[56].

Primary sclerosing cholangitis is common in patients with CD, which has been reported in up to 10% of cases[48]. Although neurological signs of CD are not evident, neuroradiological imaging studies have demonstrated alterations in brain morphology[57].

Intestinal manifestations

Gastrointestinal manifestations are quite common in patients with BD. The most frequently observed signs include abdominal pain, diarrhea, nausea, anorexia, and abdominal distension. Despite the diffuse nature of the symptoms, ulcerations known as intestinal BD are relatively few. Gastrointestinal involvement varies regionally and according to the diagnostic method used. The incidence ranges from 15% to 50% based on symptoms alone and from 0.7% to 30% based on imaging or endoscopic findings[58]. Gastrointestinal involvement is higher in patients with childhood-onset BD[59]. BD-associated gastrointestinal involvement may affect all areas from the mouth to the anus. The terminal ileum and cecum are the main sites of ulcers, while few ulcers are seen in the esophagus and gastric duodenum. The most common site of involvement is in the segmental colon. Less than 15% of patients have diffuse intestinal involvement. The differentiation of intestinal BD from inflammatory bowel disease is sometimes quite challenging. The disease can be misdiagnosed as CD or ulcerative colitis during endoscopic examination. Fistulas, hemorrhage, and perforations mimicking CD may also be present. The shape of ulcers varies endoscopically from irregular to round and oval with a punched-out appearance, they are large (> 1 cm) and typically located in the deep layers. Longitudinal ulcers are rarely seen. The presence of less than six round and focal ulcers strongly indicate intestinal BD. Colonic ulcers include volcano-type and aphthous type lesions. Rectal and anal lesions are extremely rare[36,60-62].

Abdominal pain, diarrhea with or without bleeding, fatigue, weight loss, and fever are common manifestations of CD. Odynophagia, dysphagia, and dyspeptic symptoms are also seen in the case of esophageal and gastroduodenal involvement. Diarrhea is a common presentation, but often fluctuates over a long period of time. Fibrotic strictures may lead to repeated episodes of small bowel, or less commonly colonic, obstruction. Transmural bowel inflammation is associated with the development of sinus tracts, which may give rise to a fistula or abscess formation. Perianal disease, such as anal fissures, perirectal abscesses, and anorectal fistulas, occur in more than one-third of patients with CD[63]. CD may affect all areas from lips to the anus. Lesions were located in the terminal ileum in 40%-83%, colon in 32%, perianal region in 10%-15%, and the upper gastrointestinal tract in 4%[2,58,63]. Endoscopic findings of proximal CD include mucosal edema, focal and diffuse erythema, nodular lesions, erosion, and ulcers[64]. A diagnosis of CD should be considered in any patient who presents

with isolated terminal ileum involvement and ileoscopy should be performed in all patients. The earliest lesions in CD consist of tiny punched-out ulcers. Deeper ulcers can occur throughout the entire wall of the colon. Cobblestoning–Serpiginous and linear ulcers are seen along the longitudinal axis of the entire colon. CD lesions are discontinuous and can be adjacent to normal tissue. Rectal involvement is suggestive of ulcerative colitis rather than CD. In addition, perianal lesions are frequently seen in CD with fistula formation[36,65].

PATHOLOGY

In BD, neutrophilic infiltration, lymphocyte aggregation of the surrounding vessels, and vascular proliferation have been observed in biopsy specimens of oral apthae and genital ulcers. Neutrophil-predominating infiltration, abscess formation and vasculitis-related changes may be present in skin lesions. Aggregation of lymphocytes, neutrophils, and eosinophils as well as edema and leukocytoclasia occur in the pathergy test site within the first 12 h. In the presence of large vessel involvement such as aortic involvement, medial elastic fiber ruptures or loss may be seen, while proliferation of the vaso vasorum and lymphocytic infiltration of the surrounding tissue may develop. Lymphocytic and necrotizing vasculitides are other conditions involving pulmonary arteries, veins, and septal capillaries. In addition, transmural necrosis and aneurysms of great vessels and pulmonary arteries may arise. Despite the non-specific nature, perivascular lymphocyte/plasma cell infiltration and myelin loss of parenchymal CNS lesions may develop[26,27,66-68].

Furthermore, inflamed intestinal BD may lead to mesenteric vasculitis with ischemia or necrosis of the intestines. Ulcer specimens often show non-specific patterns, including fibrinopurulent exudates and necrotic debris in active ulcers and transmural fibrosis in chronic ulcers. Inflammation from the lumen to the serosa is present in the perforated site with mural necrosis. Vasculitic changes secondary to the inflamed surrounding tissue and thrombus formation in the small vessels including both arteries and veins are other critical manifestations. Lymphoid follicles may be seen due to mucosal erosion in some cases. The differential diagnosis of these lesions, which are histopathologically suggestive of CD is highly challenging[26,68].

Histopathological characteristics of CD are discontinuous cryptic architectural abnormalities, mucin preservation at active sites, discontinuous inflammation, focal cryptitis, and epithelioid granulomas. Granulomas in histological sections are key features of CD, but are not necessary for diagnosis. In the submucosa, fibromuscular obliteration, nerve fiber hyperplasia and transmural lymphoid aggregates are found. Transmucosal increases in lamina propria cellularity and neutrophils are an indicator of disease activity[69].

Both BD and CD may present with transmural enteritis and colitis. Longitudinal ulcers, cobblestone appearance, and anorectal fistula are usual findings in Crohn's colitis. The presence of granulomas in biopsy specimens indicates CD, while vasculitides are suggestive of BD[36].

DIAGNOSTIC CRITERIA

Although there is no specific diagnostic test for BD, diagnostic criteria sets described at different time points are available. The International Study Group (ISG) criteria[70], which were defined in 1990, are the most commonly used criteria for the diagnosis of BD (Table 1). These criteria are based on the most frequent clinical signs of BD. In addition, some cases of CD meet these criteria[71].

Several diagnostic and classification criteria for CD have been proposed[8,72-75] (Table 1). The location and appearance of lesions are important for the diagnosis of CD. According to the Vienna[74] and Montreal[75] classifications, the diagnosis of CD is established by three variables: (1) age at diagnosis; (2) disease location; and (3) behavior of the disease. The Lennard-Jones criteria are based on endoscopic, surgical/histopathological, radiological and clinical findings[73]. The Copenhagen criteria include histopathological confirmation of CD[8]. A diagnostic criteria set for CD based on alterations in gastrointestinal morphology was published in 2011[72]. However, no validated and widely adopted criteria set is currently available for the diagnosis of CD in clinical practice. The diagnosis usually relies on the patient history, physical examination, laboratory results, imaging studies, and endoscopic findings in combination with histopathological examination. Patients with BD, particularly with intestinal involvement, may be misdiagnosed and mismanaged as CD by clinicians with insufficient experience and knowledge on BD.

MANAGEMENT

As BD is a multisystem condition, effective management of the disease requires a multidisciplinary approach. Although the disease should be primarily managed by a rheumatologist, consultation is provided by a dermatologist, neurologist, gastroenterologist and cardiovascular surgeon, if necessary. The disease is inflammatory; therefore, immunosuppressive and immunomodulatory agents are first-line therapies. Due to the limited number of randomized-controlled clinical trials, management usually depends on the clinical experience of the treating physician. In 2008, the European League Against Rheumatism (EULAR) published a recommendation guideline for the management of BD[76].

The management of patients with BD is based on the presence of organ involvement and disease severity. Colchicine is a widely used treatment for BD. Corticosteroids and azathioprine can be prescribed if colchicine monotherapy is inadequate. Colchicine is used for the management of mucocutaneous and musculoskeletal findings. Corticosteroids and azathioprine can be com-

Table 1 Diagnostic criteria for Behçet's disease and Crohn's disease

	International Study Group Diagnostic Criteria for Behçet's disease[70]	Proposed diagnostic criteria for Crohn's disease		
		Japan Criteria[72]	Lennard-Jones Criteria[73]	Copenhagen Criteria[8]
Major findings	Recurrent oral ulcerations	A: Longitudinal ulcer B: Cobblestone-like appearance C: Noncaseating epithelioid cell granuloma	Typical diarrhea history for at least 2 mo; 1 Radiological features of CD: segmental distribution, deep ulcerations or cobblestone pattern, thickened bowel wall, coarse mucosal relief, stenotic segments and fistulae; 2 Macroscopic diagnosis by endoscopy: patchy penetrating lesions, fissuring and strictures 3 Fistulas and/or abscesses with typical intestinal disease	1 History of abdominal pain, weight loss and/or diarrhea for more than 3 mo 2 Characteristic endoscopic findings of ulceration (aphtous lesions, snail track ulceration) or cobble stoning or radiological features of stricture or cobble stoning 3 Histopathology consistent with Crohn's disease (epitheloid granuloma of Langerhans type or transmural discontinuous focal or patchy inflammation) 4 Fistula and/or abscess in relation to affected bowel segments
Minor findings	Recurrent genital ulcerations Eye lesions Skin lesions Positive pathergy test	(1) Irregular-shaped and/or quasi-circular ulcers or aphthous ulcerations found extensively in the gastrointestinal tract (2) Characteristic perianal lesions (3) Characteristic gastric and/or duodenal lesions		
Definite	Major finding plus two minor findings	1 Major finding A or B 2 Major finding C, with minor finding (1) or (2) 3 All minor findings (1), (2), and (3)	Positive findings or one positive plus the finding of granuloma	At least two of the criteria present

bined in patients who are unresponsive to colchicine treatment and who have ocular, vascular, neurological, or intestinal involvement. Cyclosporine A and interferon-alpha are immunosuppressive agents used in the management of refractory uveitis and retinal vasculitis. A small number or patients with inadequate response may require mycophenolate mofetil and infliximab. Currently, these agents are used experimentally in the management of vascular involvement. In addition, cyclophosphamide is an effective immunosuppressive agent with increased side effects in patients with arterial, venous and neurological involvement who are refractory to other agents. Other agents that are preferred in unresponsive arthritis with a chronicity tendency include methotrexate and sulfasalazine. The latter is the most widely preferred agent in patients with intestinal BD, after corticosteroids and azathioprine. On the other hand, there are no randomized-controlled clinical trials in BD patients. Observational studies and case series have revealed that steroids, mesalazine, azathioprine, and sulfasalazine are likely to be used in the management of inflammatory bowel diseases. Recently, experience related to the use of anti-TNF agents have increased and some patients respond well to treatment. The efficacy of drugs in the treatment of CD and BD are compared in Table 2. In addition to immunosuppressive agents, antiaggregants, and anti-coagulants can be initiated in patients with venous and neurological involvement. However, no consensus on the use of antiaggregants and anti-coagulants has been reached yet, due to the low embolization tendency of BD-associated thrombosis and high bleeding risk secondary to arterial aneurysms. In clinical practice, these agents are prescribed in patients with low bleeding risk[7,41,76,77].

Corticosteroids have been used in the management of CD for over five decades. Corticosteroids are the most effective therapeutic agents in relieving disease exacerbations. They exert remarkable effects in suppressing pro-inflammatory cytokines and active lymphocytes and inhibiting inflammatory processes of the intestinal lamina propria. Although corticosteroids are more effective in higher concentrations, treatment-related side effects are likely to increase. Prednisolone treatment is usually initiated at 40-60 mg/d and reduced on a gradual basis. Nearly 48%-58% of the patients achieve complete remission, while 26%-32% achieve partial remission following 30 d of treatment. Approximately 16%-20% of patients are unresponsive. Six-mercaptopurine and its pro-drug azathioprine are the most commonly used agents in patients unresponsive to corticosteroids and maintenance therapy. Methotrexate is an alternative agent in patients who are intolerant or unresponsive to these agents. On the other hand, controversial data are available on the efficacy of 5-aminosalicylic acid (5-ASA) preparations. In several meta-analyses, mesalazine 4 g/d significantly reduced disease activity in patients with mild to moderate activity. All these agents are frequently prescribed due to their low side-effect potential[78,79]. Anti-TNF agents including infliximab, adalimumab, and certolizumab pegol can be used in refractory patients with relapsing disease. Meta-analyses have demonstrated that anti-TNF agents are effective as both induction

Table 2 Treatment options for Behçet's and Crohn's disease

	BD		CD	
	Extraintestinal BD	Intestinal BD	Extraintestinal CD	Intestinal CD
Colchicine	S, M, A	-	-	-
Corticosteroids	All manifestations	+	All manifestations	+
Azathioprine	S, M, O, V, N	+	S	+
6-mercaptopurine	-	??	-	+
Cyclosporine A	O	-	-	-
Interferon-alpha	O, N	-	-	-
Mycophenolate Mofetil	O	-	-	-
Cyclophosphamide	O, V, N	-	-	-
Methotrexate	A, N	-	A, S	-
Sulfasalazine	A	+	A	+
Mesalazine	-	+	-	+
Anti-TNF agents	A, O, N	+	A, S, O	+

A: Arthritis; S: Skin; M: Mucosal; O: Ocular; V: Vascular; N: Neurogical Involvement; (+): Effective; (-): Non-Effective; BD: Behçet's Disease; CD: Crohn's disease.

Table 3 Distribution of similarities and differences in the differential diagnosis of Behçet's disease and Crohn's disease[2,3,6,8,9,14,58,60,62,68,81]

	Behçet's Disease	Crohn's Disease
Gender (M/F)	4.9-0.57	2.9-0.76
Symptoms onset age (yr)	20.8-40	15-29
Average age at diagnosis (yr)	24.7-35.7	29.5-31
Oral aphtous ulcers (%)	Approximately 100	< 10
Uveitis (%)	57-69	< 10
Skin lesions (%)	61-87	< 10
Arthritis (%)	30-57	2-24.7
Gastrointestinal involvement (%)		
Ileocecal area	50-94	40-83
Colon	10-15	32-50
Upper GI	1-3	4
Perianal	1-2	10-15
Intestinal complications (%)		
Perforation	12.7	8.7
Fistula	7.6	24.7
Stricture	7.2	38.3
Abscess	3.3	19.6
Endoscopic Morphology	Round-oval shape, Focal, solitary Volcano-shaped Deep ulcers	Longitudinal ulcers with a cobblestone appearance (segmental and diffuse distribution)
Mucosal Biopsy	Vasculitis Neutrophilic infiltration Fibrinopurulent exudates Necrotic debris	Granuloma Focal cryptitis Nerve fiberhyperplasia Lymphoid aggregates

and maintenance therapy in CD patients with fistulizing disease[80]. Surgery is indicated in patients with perianal involvement, fistulas, fissures, and intra-abdominal abscesses.

Medical and surgical management approaches for CD and intestinal BD are similar. Recently, a retrospective case series with long-term outcomes for both diseases was reported[81]. Ten year-follow-up data after diagnosis showed no significant difference in the need for surgery between the study groups with CD and intestinal BD. However, CD patients required a higher dose of corticosteroids and immunosuppressive agents. The doses of biological agents were also higher in CD patients compared to patients with intestinal BD (14.2% *vs* 1.4%). Based on these results, long-term prognosis appears to be similar in patients with CD and intestinal BD.

CONCLUSION

CD primarily involves the gastrointestinal system and can present with various extra-intestinal signs and symptoms. However, BD is a condition or syndrome that presents with multisystem involvement. The gastrointestinal tract is also one of the main sites of involvement in these patients. Both diseases have a true overlap, affecting the gastrointestinal tract. Furthermore, both

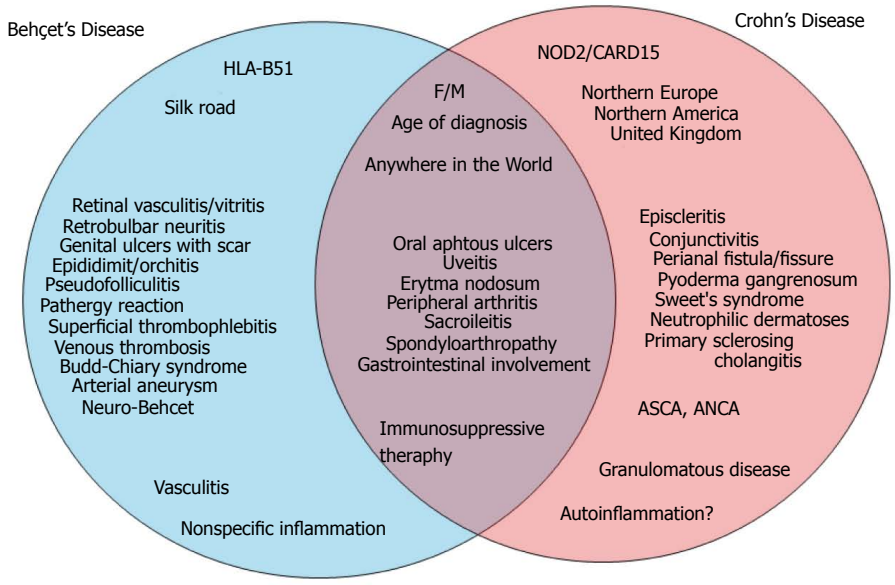

Figure 1 Similar and different characteristics of Behçet's disease and Crohn's disease. F: Female; M: Male; ASCA: Anti-Saccharomyces cerevisiae antibodies; ANCA: Anti-neutrophil cytoplasmic antibodies.

conditions share similar characteristics with respect to age of onset, gender, and inflammation biomarkers such as erythrocyte sedimentation rate and C-reactive protein (increased levels). Despite these similarities, the immunopathogenesis, genetic factors, and regional distribution are quite different. Although both diseases involve similar systems, they have distinct histopathological characteristics. For instance, uveitis is more common in BD, and CD patients are more likely to suffer from episcleritis or conjunctivitis. Figure 1 shows the similarities and differences in BD and CD. Table 3 summarizes the incidence of similarities, the distribution of gastrointestinal involvement, and endoscopic and histopathological differences.

As mentioned above, BD is more common in Asian and Mediterranean populations, while CD is more frequently seen in north European and American individuals. However, given the fact that we live in a globalizing world, the number of patients in whom the differential diagnosis of both conditions is of the utmost importance has increased. Therefore, rheumatologists and gastroenterologists who are mainly involved in the diagnosis and management of BD and CD should be well aware of the typical characteristics of both diseases.

REFERENCES

1 **Behçet H.** Uber rezidivierende, aphthose, durch ein Virus verursachte Geschwure am Mund, am Auge und an den Genitalien. *Dermatol Wochenschr* 1937; **36**: 1152-1157

2 **Baumgart DC**, Sandborn WJ. Crohn's disease. *Lancet* 2012; **380**: 1590-1605 [PMID: 22914295 DOI: 10.1016/S0140-6736(12)60026-9]

3 **Davatchi F**, Shahram F, Chams-Davatchi C, Shams H, Nadji A, Akhlaghi M, Faezi T, Ghodsi Z, Faridar A, Ashofteh F, Sadeghi Abdollahi B. Behcet's disease: from East to West. *Clin Rheumatol* 2010; **29**: 823-833 [PMID: 20354748 DOI: 10.1007/s10067-010-1430-6]

4 **Mahr A**, Belarbi L, Wechsler B, Jeanneret D, Dhote R, Fain O, Lhote F, Ramanoelina J, Coste J, Guillevin L. Population-based prevalence study of Behçet's disease: differences by ethnic origin and low variation by age at immigration. *Arthritis Rheum* 2008; **58**: 3951-3959 [PMID: 19035493 DOI: 10.1002/art.24149]

5 **Yurdakul S**, Günaydin I, Tüzün Y, Tankurt N, Pazarli H, Ozyazgan Y, Yazici H. The prevalence of Behçet's syndrome in a rural area in northern Turkey. *J Rheumatol* 1988; **15**: 820-822 [PMID: 3172095]

6 **Yurdakul S**, Yazıcı Y. Epidemiology of Behçet's Syndrome and Regional Differences in Disease Expression. 1st ed. In: Yazici Y, Yazici H, editors. Behçet's Syndrome (NY): Springer, 2010: 35-53

7 **Ambrose NL**, Haskard DO. Differential diagnosis and management of Behçet syndrome. *Nat Rev Rheumatol* 2013; **9**: 79-89 [PMID: 23007742]

8 **Vind I**, Riis L, Jess T, Knudsen E, Pedersen N, Elkjaer M, Bak Andersen I, Wewer V, Nørregaard P, Moesgaard F, Bendtsen F, Munkholm P. Increasing incidences of inflammatory bowel disease and decreasing surgery rates in Copenhagen City and County, 2003-2005: a population-based study from the Danish Crohn colitis database. *Am J Gastroenterol* 2006; **101**: 1274-1282 [PMID: 16771949]

9 **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]

10 **Yapp TR**, Stenson R, Thomas GA, Lawrie BW, Williams GT, Hawthorne AB. Crohn's disease incidence in Cardiff from 1930: an update for 1991-1995. *Eur J Gastroenterol Hepatol* 2000; **12**: 907-911 [PMID: 10958218]

11 **Hovde Ø**, Moum BA. Epidemiology and clinical course of Crohn's disease: results from observational studies. *World J Gastroenterol* 2012; **18**: 1723-1731 [PMID: 22553396 DOI:

10.3748/wjg.v18.i15.1723]
12 **Fonager K**, Sørensen HT, Olsen J. Change in incidence of Crohn's disease and ulcerative colitis in Denmark. A study based on the National Registry of Patients, 1981-1992. *Int J Epidemiol* 1997; **26**: 1003-1008 [PMID: 9363521]
13 **Loftus EV**, Silverstein MD, Sandborn WJ, Tremaine WJ, Harmsen WS, Zinsmeister AR. Crohn's disease in Olmsted County, Minnesota, 1940-1993: incidence, prevalence, and survival. *Gastroenterology* 1998; **114**: 1161-1168 [PMID: 9609752]
14 **Bernstein CN**, Wajda A, Svenson LW, MacKenzie A, Koehoorn M, Jackson M, Fedorak R, Israel D, Blanchard JF. The epidemiology of inflammatory bowel disease in Canada: a population-based study. *Am J Gastroenterol* 2006; **101**: 1559-1568 [PMID: 16863561]
15 **Ohno S**, Ohguchi M, Hirose S, Matsuda H, Wakisaka A, Aizawa M. Close association of HLA-Bw51 with Behçet's disease. *Arch Ophthalmol* 1982; **100**: 1455-1458 [PMID: 6956266]
16 **Durrani K**, Papaliodis GN. The genetics of Adamantiades-Behcet's disease. *Semin Ophthalmol* 2008; **23**: 73-79 [PMID: 18214795 DOI: 10.1080/08820530701745264]
17 **Takeno M**, Kariyone A, Yamashita N, Takiguchi M, Mizushima Y, Kaneoka H, Sakane T. Excessive function of peripheral blood neutrophils from patients with Behçet's disease and from HLA-B51 transgenic mice. *Arthritis Rheum* 1995; **38**: 426-433 [PMID: 7880197]
18 **Kaneoka H**, Furukawa H, Takeno M, Mizushima Y, Sakane T. HLA-B51 involved in the hyperfunction of peripheral blood neutrophils from patients Behcet's disease. In: Wechsler B, Godeau P, editors. Behcet's Disease Amsterdam: Excerpta Medica, 1993: 29-32
19 **Knights D**, Lassen KG, Xavier RJ. Advances in inflammatory bowel disease pathogenesis: linking host genetics and the microbiome. *Gut* 2013; **62**: 1505-1510 [PMID: 24037875 DOI: 10.1136/gutjnl-2012-303954]
20 **Parkes M**. The genetics universe of Crohn's disease and ulcerative colitis. *Dig Dis* 2012; **30** Suppl 1: 78-81 [PMID: 23075873 DOI: 10.1159/000341130]
21 **Direskeneli H**. Autoimmunity vs autoinflammation in Behcet's disease: do we oversimplify a complex disorder? *Rheumatology* (Oxford) 2006; **45**: 1461-1465 [PMID: 16998234]
22 **Yazici H**. The place of Behçet's syndrome among the autoimmune diseases. *Int Rev Immunol* 1997; **14**: 1-10 [PMID: 9203022]
23 **Kibaroglu A**, Eksioglu-Demiralp E, Akoglu T, Direskeneli H. T and NK cell subset changes with microbial extracts and human HSP60-derived peptides in Behçet's disease. *Clin Exp Rheumatol* 2004; **22**: S59-S63 [PMID: 15515788]
24 **Atagunduz P**, Ergun T, Direskeneli H. MEFV mutations are increased in Behçet's disease (BD) and are associated with vascular involvement. *Clin Exp Rheumatol* 2003; **21**: S35-S37 [PMID: 14727457]
25 **Tasliyurt T**, Yigit S, Rustemoglu A, Gul U, Ates O. Common MEFV gene mutations in Turkish patients with Behcet's disease. *Gene* 2013; **530**: 100-103 [PMID: 23973724 DOI: 10.1016/j.gene.2013.08.026]
26 **Hamuryudan V**, Melikoğlu M. Vascular Disease in Behçet's Syndrome. 1st ed. In: Yazici Y, Yazici H, editors. Behçet's Syndrome (NY): Springer, 2010: 115-135
27 **Chun SI**, Su WP, Lee S. Histopathologic study of cutaneous lesions in Behçet's syndrome. *J Dermatol* 1990; **17**: 333-341 [PMID: 2384635]
28 **Jennette JC**, Falk RJ, Bacon PA, Basu N, Cid MC, Ferrario F, Flores-Suarez LF, Gross WL, Guillevin L, Hagen EC, Hoffman GS, Jayne DR, Kallenberg CG, Lamprecht P, Langford CA, Luqmani RA, Mahr AD, Matteson EL, Merkel PA, Ozen S, Pusey CD, Rasmussen N, Rees AJ, Scott DG, Specks U, Stone JH, Takahashi K, Watts RA. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. *Arthritis Rheum* 2013; **65**: 1-11 [PMID: 23045170 DOI: 10.1002/art.37715]
29 **Mumcu G**, Ergun T, Inanc N, Fresko I, Atalay T, Hayran O, Direskeneli H. Oral health is impaired in Behçet's disease and is associated with disease severity. *Rheumatology* (Oxford) 2004; **43**: 1028-1033 [PMID: 15161982]
30 **Direskeneli H**. Behçet's disease: infectious aetiology, new autoantigens, and HLA-B51. *Ann Rheum Dis* 2001; **60**: 996-1002 [PMID: 11602462]
31 **Hirohata S**, Oka H, Mizushima Y. Streptococcal-related antigens stimulate production of IL6 and interferon-gamma by T cells from patients with Behcet's disease. *Cell Immunol* 1992; **140**: 410-419 [PMID: 1544169]
32 **Calgüneri M**, Kiraz S, Ertenli I, Benekli M, Karaarslan Y, Celik I. The effect of prophylactic penicillin treatment on the course of arthritis episodes in patients with Behçet's disease. A randomized clinical trial. *Arthritis Rheum* 1996; **39**: 2062-2065 [PMID: 8961912]
33 **Franke A**, McGovern DP, Barrett JC, Wang K, Radford-Smith GL, Ahmad T, Lees CW, Balschun T, Lee J, Roberts R, Anderson CA, Bis JC, Bumpstead S, Ellinghaus D, Festen EM, Georges M, Green T, Haritunians T, Jostins L, Latiano A, Mathew CG, Montgomery GW, Prescott NJ, Raychaudhuri S, Rotter JI, Schumm P, Sharma Y, Simms LA, Taylor KD, Whiteman D, Wijmenga C, Baldassano RN, Barclay M, Bayless TM, Brand S, Büning C, Cohen A, Colombel JF, Cottone M, Stronati L, Denson T, De Vos M, D'Inca R, Dubinsky M, Edwards C, Florin T, Franchimont D, Gearry R, Glas J, Van Gossum A, Guthery SL, Halfvarson J, Verspaget HW, Hugot JP, Karban A, Laukens D, Lawrance I, Lemann M, Levine A, Libioulle C, Louis E, Mowat C, Newman W, Panés J, Phillips A, Proctor DD, Regueiro M, Russell R, Rutgeerts P, Sanderson J, Sans M, Seibold F, Steinhart AH, Stokkers PC, Torkvist L, Kullak-Ublick G, Wilson D, Walters T, Targan SR, Brant SR, Rioux JD, D'Amato M, Weersma RK, Kugathasan S, Griffiths AM, Mansfield JC, Vermeire S, Duerr RH, Silverberg MS, Satsangi J, Schreiber S, Cho JH, Annese V, Hakonarson H, Daly MJ, Parkes M. Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. *Nat Genet* 2010; **42**: 1118-1125 [PMID: 21102463 DOI: 10.1038/ng.717]
34 **Masters SL**, Simon A, Aksentijevich I, Kastner DL. Horror autoinflammaticus: the molecular pathophysiology of autoinflammatory disease (*). *Annu Rev Immunol* 2009; **27**: 621-668 [PMID: 19302049 DOI: 10.1146/annurev]
35 **Fidder H**, Chowers Y, Ackerman Z, Pollak RD, Crusius JB, Livneh A, Bar-Meir S, Avidan B, Shinhar Y. The familial Mediterranean fever (MEVF) gene as a modifier of Crohn's disease. *Am J Gastroenterol* 2005; **100**: 338-343 [PMID: 15667491]
36 **Grigg EL**, Kane S, Katz S. Mimicry and deception in inflammatory bowel disease and intestinal behçet disease. *Gastroenterol Hepatol* (N Y) 2012; **8**: 103-112 [PMID: 22485077]
37 **Filik L**, Biyikoglu I. Differentiation of Behcet's disease from inflammatory bowel diseases: anti-Saccharomyces cerevisiae antibody and anti-neutrophilic cytoplasmic antibody. *World J Gastroenterol* 2008; **14**: 7271 [PMID: 19084948]
38 **Choi CH**, Kim TI, Kim BC, Shin SJ, Lee SK, Kim WH, Kim HS. Anti-Saccharomyces cerevisiae antibody in intestinal Behçet's disease patients: relation to clinical course. *Dis Colon Rectum* 2006; **49**: 1849-1859 [PMID: 17080284]
39 **Shang Y**, Han S, Li J, Ren Q, Song F, Chen H. The clinical feature of Behçet's disease in Northeastern China. *Yonsei Med J* 2009; **50**: 630-636 [PMID: 19881965 DOI: 10.3349/ymj.2009.50.5.630]
40 **Tursen U**, Gurler A, Boyvat A. Evaluation of clinical findings according to sex in 2313 Turkish patients with Behçet's disease. *Int J Dermatol* 2003; **42**: 346-351 [PMID: 12755969]
41 **Yilmaz S**, Karadag O, Yazisiz V, Altun B, Gezer M, Karaman M, Cinar M, Erdem H, Pay S, Dinc A. Systemic involve-

ments and preferred treatments in a large cohort of Behçet's disease. *Rheumatol Int* 2013; **33**: 3025-3030 [PMID: 23881265]
42 **Alpsoy E**, Donmez L, Onder M, Gunasti S, Usta A, Karincaoglu Y, Kandi B, Buyukkara S, Keseroglu O, Uzun S, Tursen U, Seyhan M, Akman A. Clinical features and natural course of Behçet's disease in 661 cases: a multicentre study. *Br J Dermatol* 2007; **157**: 901-906 [PMID: 17711526]
43 **Mat MC,** Bang D, Melikoğlu M. The Mucocutaneous Manifestations and Pathergy Reaction in Behcet's Disease. 1st ed. In: Yazici Y, Yazici H, editors. Behçet's Syndrome (NY): Springer, 2010: 53-73
44 **Tugal-Tutkun I**, Gupta V, Cunningham ET. Differential diagnosis of behçet uveitis. *Ocul Immunol Inflamm* 2013; **21**: 337-350 [PMID: 23730816]
45 **Benamour S**, Zeroual B, Alaoui FZ. Joint manifestations in Behçet's disease. A review of 340 cases. *Rev Rhum Engl Ed* 1998; **65**: 299-307 [PMID: 9636948]
46 **Ben Ghorbel I**, Ennaifer R, Lamloum M, Khanfir M, Miled M, Houman MH. Budd-Chiari syndrome associated with Behçet's disease. *Gastroenterol Clin Biol* 2008; **32**: 316-320 [PMID: 18400436 DOI: 10.1016/j.gcb.2007.12.022]
47 **Larsen S**, Bendtzen K, Nielsen OH. Extraintestinal manifestations of inflammatory bowel disease: epidemiology, diagnosis, and management. *Ann Med* 2010; **42**: 97-114 [PMID: 20166813 DOI: 10.3109/07853890903559724]
48 **Ardizzone S**, Puttini PS, Cassinotti A, Porro GB. Extraintestinal manifestations of inflammatory bowel disease. *Dig Liver Dis* 2008; **40** Suppl 2: S253-S259 [PMID: 18598997 DOI: 10.1016/S1590-8658(08)60534-4]
49 **Hatemi I**, Hatemi G, Celik AF, Melikoglu M, Arzuhal N, Mat C, Ozyazgan Y, Yazici H. Frequency of pathergy phenomenon and other features of Behçet's syndrome among patients with inflammatory bowel disease. *Clin Exp Rheumatol* 2008; **26**: S91-S95 [PMID: 19026122]
50 **Lanna CC**, Ferrari Mde L, Rocha SL, Nascimento E, de Carvalho MA, da Cunha AS. A cross-sectional study of 130 Brazilian patients with Crohn's disease and ulcerative colitis: analysis of articular and ophthalmologic manifestations. *Clin Rheumatol* 2008; **27**: 503-509 [PMID: 18097711]
51 **Yilmaz S**, Aydemir E, Maden A, Unsal B. The prevalence of ocular involvement in patients with inflammatory bowel disease. *Int J Colorectal Dis* 2007; **22**: 1027-1030 [PMID: 17262200]
52 **Colombo E**, Latiano A, Palmieri O, Bossa F, Andriulli A, Annese V. Enteropathic spondyloarthropathy: a common genetic background with inflammatory bowel disease? *World J Gastroenterol* 2009; **15**: 2456-2462 [PMID: 19468994]
53 **Gravallese EM**, Kantrowitz FG. Arthritic manifestations of inflammatory bowel disease. *Am J Gastroenterol* 1988; **83**: 703-709 [PMID: 3289378]
54 **Miehsler W**, Reinisch W, Valic E, Osterode W, Tillinger W, Feichtenschlager T, Grisar J, Machold K, Scholz S, Vogelsang H, Novacek G. Is inflammatory bowel disease an independent and disease specific risk factor for thromboembolism? *Gut* 2004; **53**: 542-548 [PMID: 15016749]
55 **Fumery M**, Xiaocang C, Dauchet L, Gower-Rousseau C, Peyrin-Biroulet L, Colombel JF. Thromboembolic events and cardiovascular mortality in inflammatory bowel diseases: A meta-analysis of observational studies. *J Crohns Colitis* 2014; **8**: 469-479 [PMID: 24183231 DOI: 10.1016/j.crohns.2013.09.021]
56 **Reny JL**, Paul JF, Lefèbvre C, Champion K, Emmerich J, Blétry O, Piette JC, Fiessinger JN. Association of Takayasu's arteritis and Crohn's disease. Results of a study on 44 Takayasu patients and review of the literature. *Ann Med Interne* (Paris) 2003; **154**: 85-90 [PMID: 12746644]
57 **Agostini A**, Benuzzi F, Filippini N, Bertani A, Scarcelli A, Farinelli V, Marchetta C, Calabrese C, Rizzello F, Gionchetti P, Ercolani M, Campieri M, Nichelli P. New insights into the brain involvement in patients with Crohn's disease: a voxel-based morphometry study. *Neurogastroenterol Motil* 2013; **25**: 147-e82 [PMID: 22998431 DOI: 10.1111/nmo.12017]
58 **Cheon JH,** Çelik AF, Kim WH. Behçet's Disease: Gastrointestinal Involvement. 1st ed. In: Yazici Y, Yazici H, editors. Behçet's Syndrome (NY): Springer, 2010: 165-189
59 **Hung CH**, Lee JH, Chen ST, Yang YH, Lin YT, Wang LC, Yu HH, Chiang BL. Young children with Behçet disease have more intestinal involvement. *J Pediatr Gastroenterol Nutr* 2013; **57**: 225-229 [PMID: 23880628 DOI: 10.1097/MPG.0b013e3182936ec4]
60 **Wu QJ**, Zhang FC, Zhang X. Adamantiades-Behcet's disease-complicated gastroenteropathy. *World J Gastroenterol* 2012; **18**: 609-615 [PMID: 22363131 DOI: 10.3748/wjg.v18.i7.609]
61 **Lee HJ**, Kim YN, Jang HW, Jeon HH, Jung ES, Park SJ, Hong SP, Kim TI, Kim WH, Nam CM, Cheon JH. Correlations between endoscopic and clinical disease activity indices in intestinal Behcet's disease. *World J Gastroenterol* 2012; **18**: 5771-5778 [PMID: 23155319 DOI: 10.3748/wjg.v18.i40.5771]
62 **Lee SK**, Kim BK, Kim TI, Kim WH. Differential diagnosis of intestinal Behçet's disease and Crohn's disease by colonoscopic findings. *Endoscopy* 2009; **41**: 9-16 [PMID: 19160153 DOI: 10.1055/s-0028-1103481]
63 **Mekhjian HS**, Switz DM, Melnyk CS, Rankin GB, Brooks RK. Clinical features and natural history of Crohn's disease. *Gastroenterology* 1979; **77**: 898-906 [PMID: 381094]
64 **Annunziata ML**, Caviglia R, Papparella LG, Cicala M. Upper gastrointestinal involvement of Crohn's disease: a prospective study on the role of upper endoscopy in the diagnostic work-up. *Dig Dis Sci* 2012; **57**: 1618-1623 [PMID: 22350786]
65 **Pera A**, Bellando P, Caldera D, Ponti V, Astegiano M, Barletti C, David E, Arrigoni A, Rocca G, Verme G. Colonoscopy in inflammatory bowel disease. Diagnostic accuracy and proposal of an endoscopic score. *Gastroenterology* 1987; **92**: 181-185 [PMID: 3781186]
66 **Matsumoto T**, Uekusa T, Fukuda Y. Vasculo-Behçet's disease: a pathologic study of eight cases. *Hum Pathol* 1991; **22**: 45-51 [PMID: 1985077]
67 **Hamuryudan V**, Oz B, Tüzün H, Yazici H. The menacing pulmonary artery aneurysms of Behçet's syndrome. *Clin Exp Rheumatol* 2004; **22**: S1-S3 [PMID: 15515774]
68 **Demirkesen C**, Oz B, Göksal S. Behçet's Disease: Pathology. 1st ed. In: Yazici Y, Yazici H, editors. Behçet's Syndrome (NY): Springer, 2010: 215-243
69 **Geboes K**. What histologic features best differentiate Crohn's disease from ulcerative colitis? *Inflamm Bowel Dis* 2008; **14 Suppl 2**: S168-S169 [PMID: 18816725]
70 Criteria for diagnosis of Behçet's disease. International Study Group for Behçet's Disease. *Lancet* 1990; **335**: 1078-1080 [PMID: 1970380]
71 **Tunç R**, Uluhan A, Melikoğlu M, Ozyazgan Y, Ozdoğan H, Yazici H. A reassessment of the International Study Group criteria for the diagnosis (classification) of Behçet's syndrome. *Clin Exp Rheumatol* 2001; **19**: S45-S47 [PMID: 11760398]
72 **Matsui T**, Hirai F, Hisabe T. Proposed diagnostic criteria for Crohn's disease. Annual reports of the research group of intractable inflammatory bowel disease subsidized by the Ministry of Health, Labour, and Welfare of Japan, Tokyo, Japan, 2011: 52-54
73 **Lennard-Jones JE**. Classification of inflammatory bowel disease. *Scand J Gastroenterol Suppl* 1989; **170**: 2-6; discussion 16-19 [PMID: 2617184]
74 **Gasche C**, Scholmerich J, Brynskov J, D'Haens G, Hanauer SB, Irvine EJ, Jewell DP, Rachmilewitz D, Sachar DB, Sandborn WJ, Sutherland LR. A simple classification of Crohn's disease: report of the Working Party for the World Congresses of Gastroenterology, Vienna 1998. *Inflamm Bowel Dis* 2000; **6**: 8-15 [PMID: 10701144]

75 **Silverberg MS**, Satsangi J, Ahmad T, Arnott ID, Bernstein CN, Brant SR, Caprilli R, Colombel JF, Gasche C, Geboes K, Jewell DP, Karban A, Loftus EV, Peña AS, Riddell RH, Sachar DB, Schreiber S, Steinhart AH, Targan SR, Vermeire S, Warren BF. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. *Can J Gastroenterol* 2005; **19** Suppl A: 5A-36A [PMID: 16151544]

76 **Hatemi G**, Silman A, Bang D, Bodaghi B, Chamberlain AM, Gul A, Houman MH, Kötter I, Olivieri I, Salvarani C, Sfikakis PP, Siva A, Stanford MR, Stübiger N, Yurdakul S, Yazici H. EULAR recommendations for the management of Behçet disease. *Ann Rheum Dis* 2008; **67**: 1656-1662 [PMID: 18245110 DOI: 10.1136/ard.2007.080432]

77 **Arida A**, Fragiadaki K, Giavri E, Sfikakis PP. Anti-TNF agents for Behçet's disease: analysis of published data on 369 patients. *Semin Arthritis Rheum* 2011; **41**: 61-70 [PMID: 21168186 DOI: 10.1016/j.semarthrit.2010.09.002]

78 **Girardin M**, Manz M, Manser C, Biedermann L, Wanner R, Frei P, Safroneeva E, Mottet C, Rogler G, Schoepfer AM. First-line therapies in inflammatory bowel disease. *Digestion* 2012; **86** Suppl 1: 6-10 [PMID: 23051720 DOI: 10.1159/000341951]

79 **Ueno F**, Matsui T, Matsumoto T, Matsuoka K, Watanabe M, Hibi T. Evidence-based clinical practice guidelines for Crohn's disease, integrated with formal consensus of experts in Japan. *J Gastroenterol* 2013; **48**: 31-72 [PMID: 23090001 DOI: 10.1007/s00535-012-0673-1]

80 **Kawalec P**, Mikrut A, Wiśniewska N, Pilc A. Tumor necrosis factor-α antibodies (infliximab, adalimumab and certolizumab) in Crohn's disease: systematic review and meta-analysis. *Arch Med Sci* 2013; **9**: 765-779 [PMID: 24273556]

81 **Jung YS**, Cheon JH, Park SJ, Hong SP, Kim TI, Kim WH. Long-term clinical outcomes of Crohn's disease and intestinal Behcet's disease. *Inflamm Bowel Dis* 2013; **19**: 99-105 [PMID: 22508364 DOI: 10.1002/ibd.22991]

P- Reviewer: Ahluwalia NK, Castro FJ, Garip Y, Lakatos PL
S- Editor: Ji FF **L- Editor**: Webster JR **E- Editor:** Lu YJ

World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4291/wjgp.v5.i3.239

World J Gastrointest Pathophysiol 2014 August 15; 5(3): 239-251
ISSN 2150-5330 (online)

TOPIC HIGHLIGHT

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

Multidisciplinary and evidence-based management of fistulizing perianal Crohn's disease

Ricardo Sordo-Mejia, Wolfgang B Gaertner

Ricardo Sordo-Mejia, Wolfgang B Gaertner, Division of Colon and Rectal Surgery, Department of Surgery, ABC Medical Center, 01120 Mexico City, Mexico
Author contributions: Sordo-Mejia R and Gaertner WB contributed to literature search and manuscript preparation.
Correspondence to: Wolfgang B Gaertner, MSc, MD, Division of Colon and Rectal Surgery, Department of Surgery, ABC Medical Center, Sur 136. No. 116-1A, Colonia Las Americas, 01120 Mexico City, Mexico. wolfganggaertnermd@gmail.com
Telephone: +52-55-10406569
Received: November 28, 2013 Revised: May 7, 2014
Accepted: May 28, 2014
Published online: August 15, 2014

Abstract

Perianal symptoms are common in patients with Crohn's disease and cause considerable morbidity. The etiology of these symptoms include skin tags, ulcers, fissures, abscesses, fistulas or stenoses. Fistula is the most common perianal manifestation. Multiple treatment options exist although very few are evidence-based. The phases of treatment include: drainage of infection, assessment of Crohn's disease status and fistula tracts, medical therapy, and selective operative management. The impact of biological therapy on perianal Crohn's disease is uncertain given that outcomes are conflicting. Operative treatment to eradicate the fistula tract can be attempted once infection has resolved and Crohn's disease activity is controlled. The operative approach should be tailored according to the anatomy of the fistula tract. Definitive treatment is challenging with medical and operative treatment rarely leading to true healing with frequent complications and recurrence. Treatment success must be weighed against the risk of complications, specially anal sphincter injury. A full understanding of the etiology and all potential therapeutic options is critical for success. Multidisciplinary management of fistulizing perianal Crohn's disease is crucial to improve outcomes.

Key words: Perianal Crohn's disease; Fistula; Abscess; Management; Review

Core tip: This manuscript is a comprehensive review that focuses on the multidisciplinary management of fistulizing perianal Crohn's disease. The treatment options discussed in this review are based on a current literature review as well as our experience with the disease. Diagnostic and treatment algorithms are also provided.

Sordo-Mejia R, Gaertner WB. Multidisciplinary and evidence-based management of fistulizing perianal Crohn's disease. *World J Gastrointest Pathophysiol* 2014; 5(3): 239-251 Available from: URL: http://www.wjgnet.com/2150-5330/full/v5/i3/239.htm DOI: http://dx.doi.org/10.4291/wjgp.v5.i3.239

INTRODUCTION

Although Gabriel[1] first described patients with granulomatous perianal disease 17 years before the formal description of the disease by Burrill Crohn's[2] in 1932, Bissell[3] was the first to describe the associated perianal manifestations of Crohn's disease (CD). Furthermore, Morson *et al*[4] documented the appearance of perianal non-caseating granulomas and fistulas many years before the onset of intestinal CD.

The reported prevalence of anorectal involvement in patients with CD has varied but the most current population-based series have found involvement in 14 to 38 percent of patients[5-7], with isolated perianal disease seen in only five percent[8]. The prevalence of perianal mani-

festations increases as the disease progresses distally, with up to 92 percent of patients with CD involving the colon and rectum developing fistulas[9]. In most cases, bowel involvement precedes perianal disease[9], but up to 40 percent of patients can experience perianal symptoms before intestinal manifestations[10]. There does not seem to be a predilection for age but a younger age of onset increases the odds of developing perianal disease over time[11-12].

The most common presentation of perianal CD is abscess and fistula. However, patients with CD are frequently affected by other perianal pathologies including hemorrhoids, fissures, skin tags, ulcers, and strictures. Perianal CD has been associated with a disabling natural history[13], with common extraintestinal manifestations[14] and greater steroid resistance[15]. Perianal disease is often recurrent, with 35 to 59 percent of patients relapsing within two years[16]. More than 80 percent of patients require operative treatment, and up to 20 percent may require proctectomy[5,7]. Patients with perianal CD have also shown an increased risk for anal malignancies[17,18], with active and long duration of disease being identified risk factors[19-21].

The treatment of perianal CD continues to be a challenge, especially with the plethora of literature addressing both medical and operative treatment strategies. The purpose of this review is to summarize the efficacy of currently described methods for the management of fistulizing perianal CD and its complications.

ABSCESS

Abscesses usually occur with active perianal CD with an incidence of up to 62 percent during the course of the disease[22]. Ischiorectal abscesses account for 40 percent of all perianal abscesses[23]. Fistula tract location can influence abscess development and transsphincteric fistulas pose the greatest risk[23].

Abscesses are uncommon with superficial fistula tracts. Makowiec *et al*[24] evaluated 61 patients with perianal CD and found that 73 percent of all abscesses were related to an ischiorectal fistula and 50 percent with a transsphincteric fistula. Recurrences occurred in 53 percent with a median time to recurrence of 14 mo. No patients with superficial fistula tracts had a second abscess, whereas about two thirds of patients with transsphincteric and ischiorectal fistulas recurred after 36 mo.

A detailed anorectal exam should be performed before any type of treatment is initiated. This frequently requires evaluation under anesthesia (EUA) with evaluation of the rectum to rule-out active disease. Perianal infection can occur in any anatomic plane (superficial, intersphincteric, ischiorectal, or supralevator), and requires immediate drainage and treatment of systemic symptoms with broad-spectrum antibiotics[6,25]. Many authors recommend drain placement or partial sphincter division to facilitate drainage, but these have not been associated with better outcomes[26,27]. In the setting of persistent perianal sepsis, imaging modalities such as magnetic resonance imaging (MRI) and computed tomography (CT) are used to guide the drainage of deep or complex abscesses[28,29].

When a fistula is encountered, a non-cutting seton should be placed to facilitate drainage and prevent recurrent infection, with improvement seen in 79 to 100 percent of patients[30-35]. Long-term drainage with non-cutting setons without definitive therapy has been reported to result in fistula recurrence in 20 to 80 percent of cases[33,36,37]. The combination of non-cutting setons and anti-tumor necrosis factor (TNF) therapy has been associated with fistula healing rates of up to 67 percent and will be discussed below[38,39]. Fecal diversion to increase fistula healing and control perianal sepsis continues to be controversial with no level A data supporting its role but in the setting of persistent perianal sepsis, a temporary diverting stoma can be effective. Patients should be aware that these stomas are rarely reversed[40].

Cryptoglandular abscess/fistulas can and do occur in patients with CD and should be recognized as so because treatment differs. These abscess/fistulas tend to be superficial and are not associated with active anorectal CD; therefore, anti-TNF therapy is not indicated. Abscess drainage should follow the same principles as mentioned above. Placement of a non-cutting seton is encouraged and any attempt of local surgical treatment should take into consideration the patients underlying continence and CD status. Supplemental imaging studies, such as endoanal ultrasound (EAUS), are very helpful even when cryptoglandular etiology is suspected.

FISTULA

A population-based study[7] with up to 20 years of follow-up showed that one out every two patients with CD develop perianal fistulas. The etiology of perianal fistula formation in CD is not completely clear but genetic, microbiological, and immunological factors play a role. Most authors believe that fistulas originate either from the penetration of a rectal ulcer or from cryptitis spreading to the intersphincteric space. Intersphincteric and transsphincteric fistulas are the most common fistula tracts of cryptoglandular origin that occur in patients with CD. Suprasphincteric fistulas result from cryptoglandular disease or rectal ulceration, and extra sphincteric fistulas are frequently seen in patients with severe proctitis or iatrogenic injury.

At St. Marks Hospital, Tozer[41] studied biopsy samples from Crohn's and idiopathic anal fistulas. Although immunological analysis showed no significant differences in interleukin (IL)-2, IL-4, IL-6, IL-10, TNF, and interferon levels, CD patients had significantly higher interleukin 17 levels and significantly lower CD65 levels. The authors showed data suggesting aberrant expression of homing molecules on dendritic cells in Crohn's anal fistulas suggesting a non-directed immune response, which may contribute to the pathophysiology.

Pelvic MRI is the preferred imaging study to assess fistulizing perianal disease. It has an accuracy of 90 percent for evaluating fistula tracts and of 97 percent for characterizing complex abscesses[42,43]. Furthermore, operative management may be altered in ten to 20 percent of patients by the addition of MRI to EUA, and this increases to up to 40 percent in patients with CD[44,45].

Once the anorectal disease is delineated, evaluation for proximal CD with endoscopy should also be considered. Although some studies have found an association between proximal fistulizing disease and perianal fistulas[46], other investigators have not observed this finding[47,48]. In patients with fistulizing perianal CD it is our practice to combine a pelvic MRI with EUA and rigid proctoscopy to evaluate for rectal inflammation.

Medical treatment

Once perianal infection is controlled, the fistula tract is characterized, and CD status is assessed; combined definitive medical and surgical therapy should be initiated (Figure 1). When active proctitis is encountered, this must be aggressively treated. Medical therapy includes antibiotics (metronidozole and ciprofloxacin), immunosuppressives (6-mercaptopurine, azathioprine, cyclosporine, and tacrolimus), and immunomodulators (infliximab, adalimumab, and certolizumab pegol). Although steroids are frequently used to manage concomitant luminal disease, there is no demonstrable role for corticosteroids in perianal CD. Medical treatment of perianal CD demands significant cooperation between gastroenterologists and surgeons as patient management is challenging and requires frequent feedback between medical professionals to optimize therapeutic strategies.

Antibiotic therapy

Antibiotics are commonly initiated when perianal infection is diagnosed and are frequently continued until immunosuppressive therapy is initiated[49], with 70 to 95 percent of patients having a positive response within six weeks[50,51]. It is our practice to continue antibiotic therapy for two weeks with perianal infection, and for three to four weeks with active proctitis. Metronidazole is the most common antibiotic prescribed for perianal CD and has been associated with fistula healing rates ranging from zero to 56 percent[50,52,53]. Seventy-five percent of patients relapse after suspending treatment and side effects which include nausea and peripheral neuropathy commonly limit its long-term use.

Ciprofloxacin has been studied in small, uncontrolled series of patients with perianal CD[54,55]. Improvement has been shown in approximately half of patients without detailed data on fistula healing. Ciprofloxacin was compared to metronidazole and placebo in a small randomized study including 25 patients[53]. After receiving treatment for ten weeks, clinical remission and response were 30 percent and 40 percent with ciprofloxacin, 12.5 percent and 12.5 percent with placebo, and 0 percent and 14 percent with metronidazole; none of these differences being significant.

Immunomodulators

The definitive medical treatment of perianal CD includes immunomodulation. A meta-analysis of five randomized controlled trials evaluated the efficacy of 6-mercaptopurine and azathioprine[56]. Fistula healing occurred in 54 percent of patients *vs* 21 percent of controls (OR 3.09; 95%CI, 2.45 to 3.91). Intravenous cyclosporine has also shown to have a good response in up to 83 percent of patients[57,58], but the effect is short-lasting when it is discontinued or transitioned to oral formulations[59]. Tacrolimus has also been effective in the treatment of perianal CD, as shown in one randomized controlled trial. Clinical improvement was seen in 43 percent of patients *vs* 8 percent receiving placebo ($P = 0.004$)[60].

Anti-TNF therapy

Anti-TNF therapy, which includes monoclonal antibodies that are given intravenously [Infliximab (chimeric – murine/human)] or subcutaneously [Adalimumab and Certolizumab pegol (human)], has shown good results in the multidisciplinary management of perianal CD. Most patients who receive anti-TNF therapy receive concomitant immunomodulators. This combination has been poorly studied, specifically in perianal CD, but may be associated with less perianal complications and increased fistula healing[61]. What must be taken into consideration is that most studies evaluating anti-TNF therapy in the setting of perianal CD are of small numbers that involve heterogeneous patient groups with short follow-up. These studies also use varying definitions of fistula healing, disease improvement and "response".

Infliximab alone: Present *et al*[62] reported that three infusions of infliximab resulted in closure of perianal fistulas in 46 percent of patients over 3 mo follow-up. A large study from Hungary including 148 patients with CD reported a perianal fistula closure rate of 49 percent at a mean of 3 mo follow-up[63]. A multicenter Italian study evaluating the impact of infliximab alone in 188 patients with perianal CD reported an initial response in 76 percent of patients with a 44 percent fistula closure rate[64]. Ng *et al*[65] prospectively evaluated the response to infliximab therapy with MRI in 34 patients with perianal Crohn's fistulas. At six months, 58 percent of patients showed fistula closure, with 37 percent showing good clinical response.

Infliximab plus surgery: Regueiro *et al*[66] demonstrated an improved clinical response and less fistula recurrence when patients had EUA and seton placement before starting infliximab compared to patients who received infliximab alone. Topstad *et al*[38] also achieved improved outcomes with combined seton drainage, infliximab infusion, and immunosuppressives in 29 patients. At a mean follow-up of nine months, 67 percent of patients showed a complete response. Hyder *et al*[67] evaluated

long-term healing rates with this approach in 22 patients. At a median follow-up of 21 mo, the authors only observed an 18% fistula closure rate. Van der Hagen *et al*[68] developed a multistep multidisciplinary approach that involved EUA with seton placement, fecal diversion when fistulas and abscesses recurred, infliximab therapy in case of persistent proctitis, and definitive fistula surgery. At a mean follow-up of 23 mo, fistula healing occurred in 90 percent of patients who received infliximab (9/10) compared to 71 percent in those who did not (5/7).

At the University of Minnesota, Gaertner *et al*[39] evaluated the outcomes of 226 patients who underwent operative treatment for perianal Crohn's fistulas, with 79 of these patients also receiving preoperative infliximab. Fistula healing rates were similar regardless of infliximab therapy (59% *vs* 60%). However, patients who underwent surgery plus infliximab healed faster than those who did not receive infliximab (6.5 mo *vs* 12.1 mo; $P < 0.0001$), and seton placement plus infliximab infusion resulted in significantly improved fistula healing rates compared to seton placement alone ($P = 0.001$). Regardless of infliximab therapy, lay-open fistulotomy was the operation with the best healing rates. Active proctitis did not significantly impact healing after fistula surgery.

Adalimumab alone: Adalimumab has shown similar efficacy to infliximab in randomized controlled trials. In the CHARM (Crohn's trial of the fully Human Antibody Adalimumab for Remission Maintenance) study, 113 patients with perianal Crohn's fistulas received induction adalimumab; with subsequent maintenance adalimumab or placebo[69]. Evaluation at 26 wk showed complete fistula closure in 30 percent of patients treated with adalimumab, with improved outcomes at 56 wk compared to placebo (33% *vs* 13%). The durability of these results have been confirmed at two years follow-up[70]. In the CLASSIC-1 (Clinical Assessment of Adalimumab Safety and Efficacy Studied as an Induction Therapy in Crohn's disease) trial, adalimumab was compared to placebo with the aim to evaluate short-term outcomes[71]. Thirty-two of 299 patients had perianal fistulas and no significant differences were observed in fistula healing.

Adalimumab has also been used in patients who have failed to respond to other anti-TNF agents, specially infliximab. In the GAIN (Gauging Adalimumab efficacy in Infliximab Nonresponders) trial, CD patients who were intolerant or who had lost response to infliximab received adalimumab or placebo[72]. Forty-five of 325 patients had perianal fistulas and no significant differences in fistula healing were found between placebo and adalimumab. Based on these results, most physicians consider that a second biological agent has minimal efficacy in patients who have already failed anti-TNF therapy.

Adalimumab plus surgery: As the experience with anti-TNF therapy expands, many authors have reported on a combined approach with adalimumab and local anorectal procedures. Tozer *et al*[73] reviewed the outcomes of 41 consecutive patients with fistulizing perianal CD treated with infliximab ($n = 32$) or adalimumab ($n = 9$), and followed radiologically with MRI. Fifty-eight percent of all patients (66% infliximab and 43% adalimumab) demonstrated remission or response at three years. Fistula healing, as demonstrated by MRI, lagged behind clinical healing by a median of 12 mo. All patients who achieved radiological healing maintained fistula closure while on anti-TNF therapy but only 43 percent maintained fistula closure after cessation of anti-TNF agents. El-Gazzaz *et al*[74] reviewed the Cleveland Clinic experience with combined anti-TNF therapy and anorectal surgery in 218 patients. Mean follow-up was 3.2 years. Two hundred and eighteen patients underwent operative treatment, 101 with anti-TNF therapy (74 infliximab and 27 adalimumab). Patient groups were comparable in demographic data and CD history but operative treatment was significantly heterogeneous. Patients who received combined anti-TNF therapy and surgery had significant overall improvement compared to patients who underwent surgery alone (36% *vs* 71%, $P = 0.001$).

Local anti-TNF therapy: Local injections of anti-TNF agents have also been attempted in fistulizing perianal CD, specifically in patients with contraindications to systemic treatment and resistance to infliximab. Poggioli *et al*[75] performed three to 12 local injections of infliximab (15-20 mg) adjacent to both internal and external openings and fistulous tract in 15 patients. Fistula closure occurred in ten patients at a mean follow-up of 18 mo. Asteria *et al*[76] achieved clinical response in six of eleven patients treated with local infliximab. Four of the eleven remained healed at a median of ten months of follow-up.

Tonelli *et al*[77] reviewed the outcomes of 12 patients with fistulizing perianal CD who underwent local injection of Adalimumab. Each patient received a median of seven (range, 4-16) injections. At a mean follow-up of 17.5 mo, 75 percent of patients (9 of 12) no longer had fistula drainage, and three patients (25%) showed clinical improvement. No adverse side effects were noted.

Certolizumab pegol: Certolizumab pegol is a humanized monoclonal antibody directed against TNF alpha. The antibody is fused with polyethylene glycol, which does not cross the placenta, so it should be safe in pregnancy. In 2008, the Food and Drug Administration approved Certolizumab pegol for the treatment of CD. Schreiber *et al*[78] evaluated its impact in patients with fistulizing CD. Patients with fistulizing CD from a randomized controlled study (PRECiSE 2, $n = 108$) comparing certolizumab pegol *vs* placebo were further randomized (if a good initial response was noted) to certolizumab pegol ($n = 28$) or placebo ($n = 30$) every four weeks until 24 wk. The majority of patients (55/58) had perianal fistulas. At week 26, fistula closure occurred in 36 percent of patients in the certolizumab pegol group compared to 17 percent of patients receiving placebo ($P = 0.038$).

Operative treatment

If the attempt to heal a fistula has significant impact on

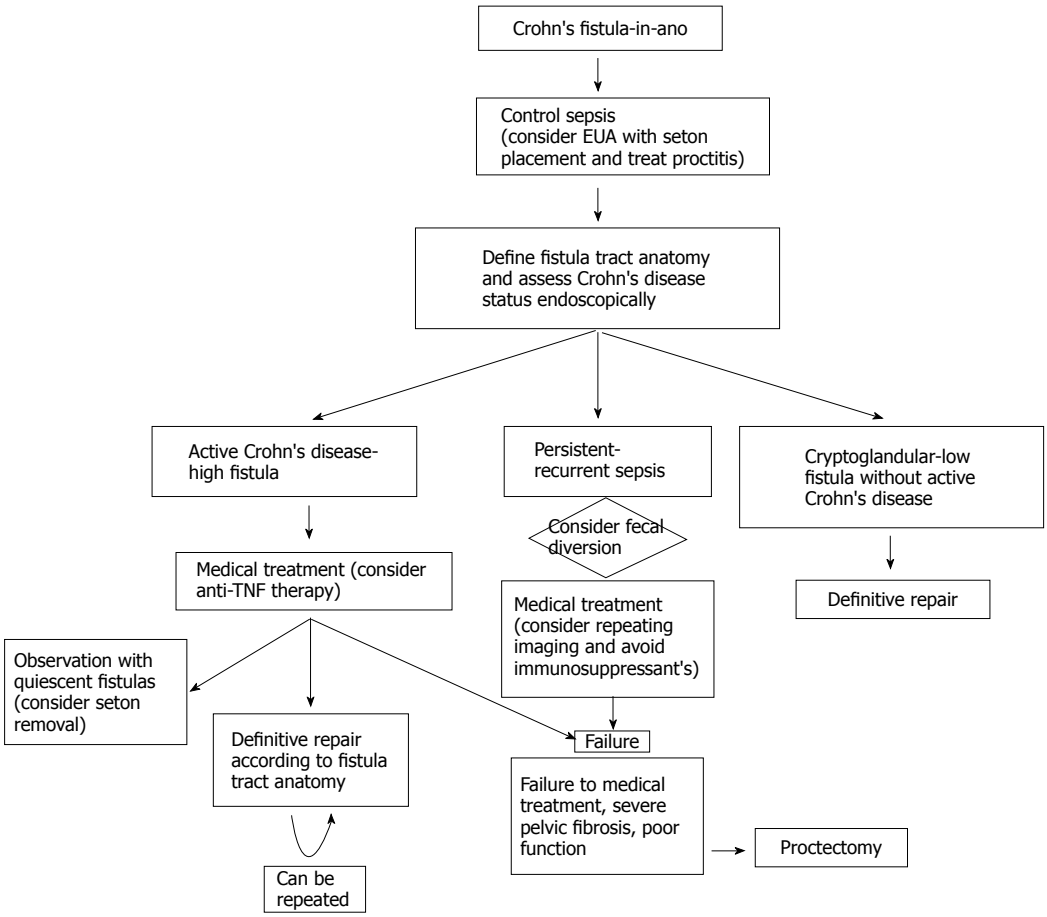

Figure 1 Diagnostic and treatment algorithm for fistulizing perianal Crohn's disease.

a patient's quality of life, operative treatment should be undertaken. Currently, the majority of operations for fistulizing perianal CD are performed in conjunction with medical therapy (immunomodulators or anti-TNF agents), and because this approach has been covered above, this section will focus on operative indications and efficacy of the most popular surgical techniques.

Most low, simple fistulas can be treated by fistulotomy. Healing rates from 80 to 100 percent have been reported with this technique[27,31,79,80]. Despite careful patient selection, an occasional fistulotomy wound may result in a chronic ulcer. In this situation, medical treatment is preferred as further operations have been associated with recurrent infection, fistula, and sphincter damage.

If partial sphincter division would compromise fecal continence, one can choose between minimally invasive techniques and anorectal repairs. Minimally invasive techniques include fibrin glue injection and collagen plug insertion. These techniques have no significant effect on fecal continence, are well tolerated by the patient, can be repeated, and are associated with fistula healing rates between 38 and 71 percent[81-84]. Fistula recurrence is common and occurs in approximately 50 to 70 percent of patients[81-84]. Video-assisted anal fistula treatment (VAAFT) and local injection of adipose-derived stem cells are recently described minimally invasive techniques that have been employed in patients with fistulizing perianal CD[85,86]. VAAFT involves performing fistuloscopy to identify the etiologic crypt and rule-out secondary tracts and then excise the internal opening. After this, the fistula tract is fulgurated. Stem cell therapy is a novel and promising approach for the treatment of chronic inflammatory conditions, and its use in fistulizing perianal CD has increased in Europe. Fistula healing rates between 30 and 82 percent have been reported with these techniques but the long-term safety and outcomes have not been adequately studied in the Crohn's population. Overall, studies assessing the efficacy of minimally invasive techniques for Crohn's perianal fistulas tend to be of small patient numbers, non-comparative and heterogeneous patient groups, retrospective nature, and with short duration of follow-up.

The most commonly employed anorectal operation for transsphincteric Crohn's fistulas is a rectal advancement flap. This procedure has been associated with incontinence rates between five and nine percent but has not been associated with an increased risk for proctectomy[87]. Contraindications include significant proctitis, a cavitating ulcer or anal stenosis. Crohn's fistula healing rates reported in the literature average 64 percent[87]. A recently described technique, ligation of intersphinc-

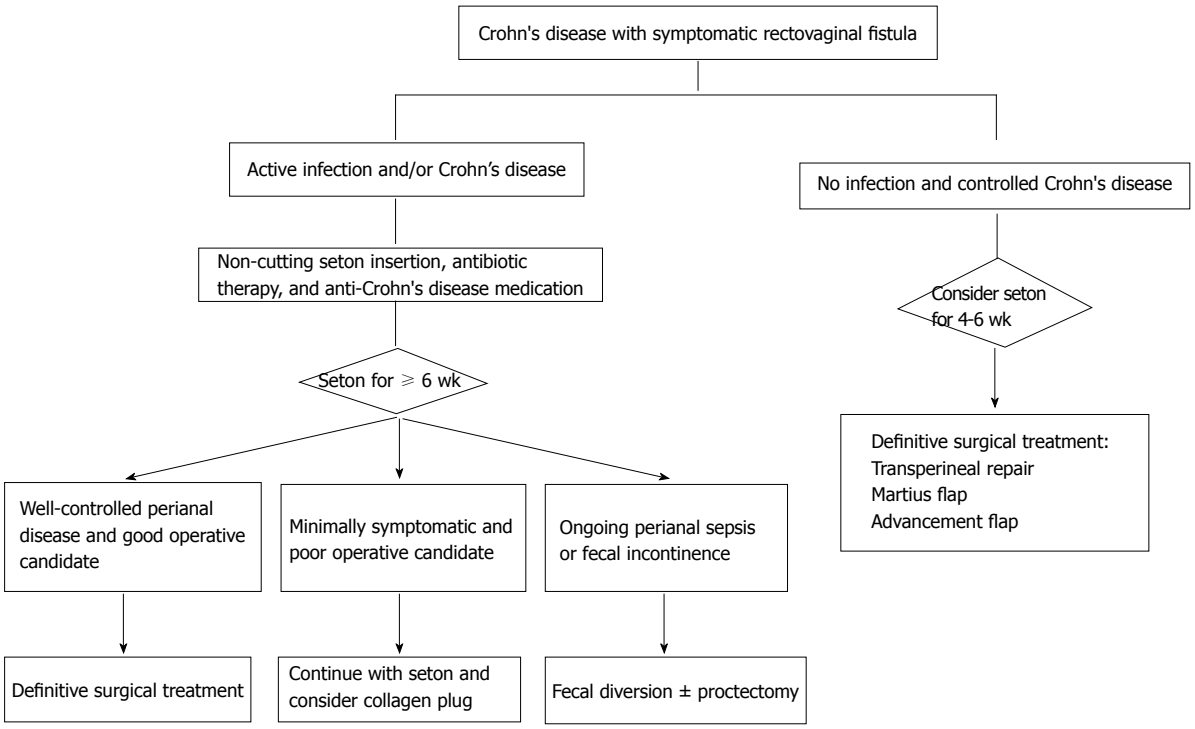

Figure 2 Treatment algorithm for patients with Crohn's disease and symptomatic recto-vaginal fistulae.

teric fistula tract (LIFT) which involves the identification and ligation/transection of the fistula tract in the intersphincteric plane, is being increasingly employed in patients with transsphincteric Crohn's fistulas[88]. This technique also has minimal to no repercussion on fecal continence but does involve a perianal wound. Although encouraging results have been reported in complex fistulas of cryptoglandular origin, experience in CD patients is limited[89,90].

In the setting of a large anal canal ulcer or severe stricture, an endorectal advancement flap can be performed in selective patients[91]. After the ulcer or stricture is excised, a full-thickness circumferential sleeve is mobilized and a formal rectoanal anastomosis is performed in combination with a diverting loop ileostomy.

SPECIFIC SITUATIONS

Rectovaginal fistulas

After obstetric trauma, CD is the second most common cause of rectovaginal fistula (RVF)[92], occurring in five to 23 percent of CD patients[93-95]. The majority of RVF's in the setting of CD are low and transsphincteric, and arise from rectal ulceration or infection of anterior anal glands[94,96].

The management of RVF in CD is challenging. Treatment depends on the degree of symptoms, CD activity, and the anatomy of the fistulous tract (Figure 2). Minimally symptomatic patients may not require any treatment[7,94,97,98]. However, carefully selected symptomatic patients should be treated with a step-wise multidisciplinary approach. Drainage of local infection, seton placement and medical therapy are the initial steps before any attempts at fistula closure[92,94].

Patient selection is very important. Women with extensive anorectal CD are not good candidates for definitive fistula operations without first eradicating local infection and controlling the activity of underlying CD. Contrast to what has been reported in non-CD patients, a previous failed repair does not dictate a worse outcome with a subsequent operation. Healing rates reported after secondary operations are similar to those seen after a first attempt repair (29%-54%)[99-101]. Fecal diversion to protect a repair is also controversial. Penninckx *et al*[99] evaluated the impact of fecal diversion and parenteral nutrition in 32 consecutive patients undergoing RVF repair and did not find any significant role for either of these interventions. However, when O'Leary *et al*[102] selectively used fecal diversion in a step-wise approach that included initial seton placement and delayed repair, fistula healing occurred in 80 percent of patients. A diverting stoma does not ensure fistula healing and should only be performed in complex and recurrent cases.

Most of current treatment algorithms include combined medical and operative treatment. Present *et al*[103] found that 6-mercaptopurine was more effective than placebo, when combined with surgery (31% *vs* 6%). Most RVF's recurred after discontinuation of 6-mercaptopurine. Similar results were observed with cyclosporine in two studies that included a total of six patients with RVF[104,105].

El-Gazzaz *et al*[106] evaluated long-term outcomes in 65 women with Crohn's RVF´s who underwent a variety of different procedures. At a median follow-up of

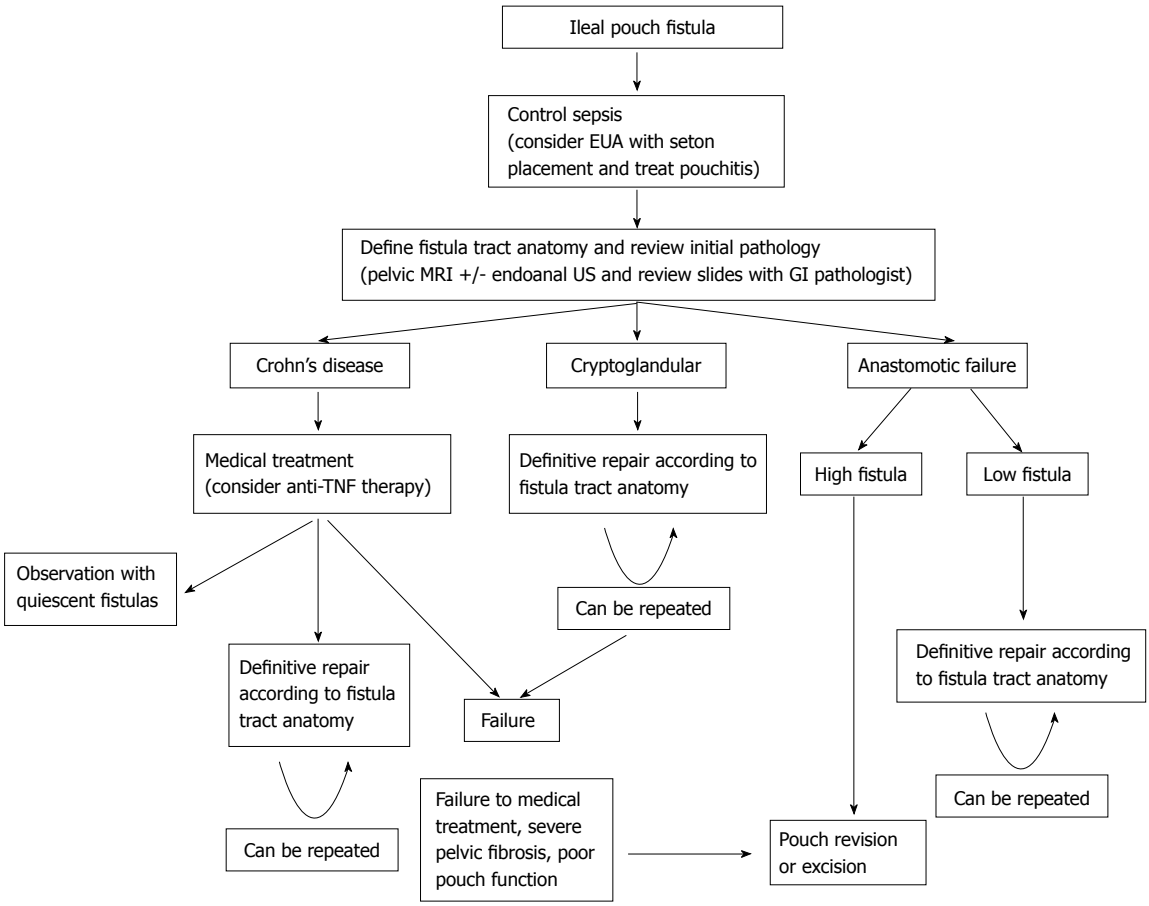

Figure 3 Diagnostic and treatment algorithm for patients with ileal pouch fistulas.

47 mo, 46 percent healed. Multivariate analysis showed that immunomodulators were associated with successful healing ($P = 0.009$); and smoking and steroids were associated with failure ($P = 0.04$).

The efficacy of infliximab in RVF and CD has been controversial[38,62,66-68,107-109]. In the ACCENT Ⅱ study[109], the initial response rate to infliximab was 64 percent. Rectovaginal fistula closure was maintained for longer with maintenance infliximab compared to placebo (46 wk *vs* 33 wk). Gaertner *et al*[110] reviewed the outcomes of 51 patients with Crohn's RVF's who underwent combined medical and operative treatment, 26 received preoperative infliximab. At a mean follow up of 38.6 mo, 27 fistulas (53%) healed. Transperineal repair was the operation with the highest healing rate regardless of infliximab therapy. Preoperative fecal diversion, active proctitis and infliximab therapy did not significantly impact fistula healing.

The definition of fistula healing tends to raise controversy when reviewing the RVF literature and seems to be influenced by the type of treatment, method of evaluation, and follow-up period. Rasul *et al*[111] assessed RVF healing by endoanal ultrasound in patients who clinically healed with infliximab therapy. Only five of 35 women demonstrated improvement but none showed fistula closure on ultrasound. Bell *et al*[112] found good correlation between clinical assessment and MRI in seven of ten patients treated with infliximab. Only two of these patients had RVF.

Ileal pouch fistulas

Patients who develop CD after restorative proctocolectomy with ileoanal anastomosis are at particularly high risk of developing pouch-anal fistulas. Although preoperative colorectal pathology, operative technique, and postoperative pelvic sepsis have also been identified as risk factors, CD is considered the most common[113-115]. Several operative techniques have been described to control pelvic and perianal sepsis and ultimately eliminate the fistula tract[116-120], but because of the low incidence of these fistulas, the optimal management continues to be controversial (Figure 3). Gaertner *et al*[121] reviewed the outcomes of 25 patients who presented with symptomatic ileal pouch fistulas over a 22-year period. Fistulas were classified as pouch-anal (48%), pouch-vaginal (28%), complex (16%), and pouch-perineal (8%). The most common etiology was CD. Overall fistula closure with a variety of local anorectal and abdominal procedures was 64 percent at a median follow-up of 29 mo. Postoperative pelvic sepsis, fecal diversion, and anti-TNF therapy did not significantly impact fistula healing. Three patients (12%) required pouch excision with end ileostomy.

Fistula-associated cancer

In 1934, Rosser[122] first described carcinoma associated

with a chronic perianal fistula. Fistula-associated adenocarcinoma is a rare but increasingly reported malignancy[18,21,123-131] that is commonly found in CD patients with chronic anal fistulas[18,21]. This malignancy is frequently associated with chronic, complex fistulas and can be particularly difficult to diagnose. High clinical suspicion is crucial to avoid any delay in diagnosis and treatment. Chronic infection and inflammation (*i.e.*, CD and radiation) are the most frequently associated risk factors but even when the diagnosis is suspected clinically, confirmation requires EUA with biopsy. Misdiagnosis commonly occurs in elderly patients and patients with long-standing anorectal disease. Once the diagnosis of cancer has been established, EAUS and MRI are recommended for staging[132].

Mucinous adenocarcinoma is the most common malignancy reported in long-standing perianal fistulas. It is typically a slow growing, locally aggressive neoplasm that mainly spreads *via* the inguinal lymphatic's[133]. Outcomes are good when malignancy is diagnosed early[131,133-136]. Oncologic resection remains the standard treatment option. Abdominoperineal resection is the most frequently employed operation[131,137,138]. The role of neoadjuvant chemoradiotherapy in the treatment of this neoplasm has not been well studied, probably because of its rarity, but results are promising[21,131]. Neoadjuvant therapy may play a significant role to improve outcomes but remains investigational.

Gaertner *et al*[131] identified 14 patients with fistula-associated anal adenocarcinoma. The most common presentation was persistent perianal fistula (n = 9). Ten patients (71%) had CD. Abdominoperineal resection was performed in eleven patients, seven following neoadjuvant chemoradiotherapy. At a mean follow-up of 64 mo, ten patients were alive without evidence of disease and four patients died with metastatic disease. All seven patients who received neoadjuvant chemoradiotherapy had a complete pathologic response. In a systematic review by Iesalnieks *et al*[21], a total of 23 publications including 65 patients with fistula-associated adenocarcinoma and CD were reviewed. Abdominoperineal resection was performed in 56 patients with an overall 3-year survival rate of 54 percent.

We recommend that tissue from refractory, recurrent and chronic anal fistula tracts, regardless of their etiology, should be submitted for pathologic evaluation. All patients with long-standing perianal CD should undergo cancer surveillance. Although the impact of neoadjuvant chemoradiotherapy remains controversial, oncologic resection continues to be the standard treatment option for fistula-associated adenocarcinoma.

Proctectomy

Proctectomy is appropriate in patients in whom repeated medical and operative strategies fail. Historically, it is required in ten to 20 percent of patients with perianal CD[6], and is commonly associated with perineal wound breakdown, chronic open wounds and sinus formation in up to half of patients[139,140]. In our experience, intersphincteric proctectomy (when feasible) and the use of rectus abdominal and gracilis flaps can help with avoiding these complications.

A low Hartmann's procedure is an alternative approach that may result in a healed perineum in up to 60 percent of patients with perianal CD[141]. Despite this approach, Guillem *et al*[142] reported a 54 percent completion proctectomy rate in 28 patients who underwent rectal exclusion, plus an additional nine patients had persistent active disease at the rectal stump.

CONCLUSION

The appropriate treatment of fistulizing perianal CD must be individualized to each patient. The primary goals are to ameliorate symptoms and prevent complications. Overall, a less aggressive approach is preferred as many patients will require repetitive operations that can often result in outcomes that are worse than the disease itself.

Based on the current literature, multidisciplinary treatment includes: eradication of infection, assessment of CD status and fistula tract(s), medical therapy, and selective operative management. The first phase of treatment is to drain the perianal infection. This typically involves an EUA, seton drainage and a short course of antibiotics. The second phase consists of endoscopically evaluating the extent of CD and delimiting the anatomy of the fistula tract with EUA and either EAUS or MRI, or both. During this phase, medical therapy with immunomodulators and anti-TNF agents is typically initiated but if the fistula is thought to be of cryptoglandular etiology, CD medications are rarely required.

The third phase should ideally involve healing of the perianal pathology. Many patients who have minimal symptoms elect to continue with a non-cutting seton or removal and expect healing in some cases. On many occasions a non-cutting seton may actually act as a cutting seton, specially in low superficial fistula tracts. The extensive range of operations highlights the complexity of operative treatment. These include a variety of minimally invasive techniques and anorectal operations. Sphincter injury and fecal incontinence should be the main concern with any anorectal operation. The operative approach depends on the anatomy of the fistula tract, CD status, and the patients' functional status. Attempts to heal a fistula in the setting of active infection and proctitis are likely to fail. If the patient's symptoms persist or increase despite adequate medical and surgical treatment, a diverting stoma or proctectomy should be considered.

REFERENCES

1 **Gabriel WB**. Results of an Experimental and Histological Investigation into Seventy-five Cases of Rectal Fistulæ. *Proc R Soc Med* 1921; **14**: 156-161 [PMID: 20906483]
2 **Crohn BB,** Ginzberg L, Oppenheimer GD. Regional ileitis: a pathologic and clinical entity. *JAMA* 1932; **99**: 1323 [DOI:

10.1001/jama.1932.02740680019005]
3 **Bissell AD**. Localized Chronic Ulcerative Ileitis. *Ann Surg* 1934; **99**: 957-966 [PMID: 17867210 DOI: 10.1097/00000658-193406000-00011]
4 **Morson BC**, Lockhart-Mummery HE. Anal lesions in Crohn's disease. *Lancet* 1959; **2**: 1122-1123 [PMID: 14424442 DOI: 10.1016/S0140-6736(59)90104-7]
5 **Hellers G**, Bergstrand O, Ewerth S, Holmström B. Occurrence and outcome after primary treatment of anal fistulae in Crohn's disease. *Gut* 1980; **21**: 525-527 [PMID: 7429313 DOI: 10.1136/gut.21.6.525]
6 **Sandborn WJ**, Fazio VW, Feagan BG, Hanauer SB. AGA technical review on perianal Crohn's disease. *Gastroenterology* 2003; **125**: 1508-1530 [PMID: 14598268 DOI: 10.1016/j.gastro.2003.08.025]
7 **Schwartz DA**, Loftus EV, Tremaine WJ, Panaccione R, Harmsen WS, Zinsmeister AR, Sandborn WJ. The natural history of fistulizing Crohn's disease in Olmsted County, Minnesota. *Gastroenterology* 2002; **122**: 875-880 [PMID: 11910338 DOI: 10.1053/gast.2002.32362]
8 **Lockhart-Mummery HE**. Symposium. Crohn's disease: anal lesions. *Dis Colon Rectum* 1975; **18**: 200-202 [PMID: 1140047 DOI: 10.1007/BF02587272]
9 **Williams DR**, Coller JA, Corman ML, Nugent FW, Veidenheimer MC. Anal complications in Crohn's disease. *Dis Colon Rectum* 1981; **24**: 22-24 [PMID: 7472097 DOI: 10.1007/BF02603444]
10 **Gray BK**, Lockhartmummery HE, Morson BC. Crohn's disease of the anal region. *Gut* 1965; **6**: 515-524 [PMID: 5857889 DOI: 10.1136/gut.6.6.515]
11 **Cosnes J**, Cattan S, Blain A, Beaugerie L, Carbonnel F, Parc R, Gendre JP. Long-term evolution of disease behavior of Crohn's disease. *Inflamm Bowel Dis* 2002; **8**: 244-250 [PMID: 12131607 DOI: 10.1097/00054725-200207000-00002]
12 **Roberts PL**, Schoetz DJ, Pricolo R, Veidenheimer MC. Clinical course of Crohn's disease in older patients. A retrospective study. *Dis Colon Rectum* 1990; **33**: 458-462 [PMID: 2350997 DOI: 10.1007/BF02052138]
13 **Beaugerie L**, Seksik P, Nion-Larmurier I, Gendre JP, Cosnes J. Predictors of Crohn's disease. *Gastroenterology* 2006; **130**: 650-656 [PMID: 16530505 DOI: 10.1053/j.gastro.2005.12.019]
14 **Rankin GB**, Watts HD, Melnyk CS, Kelley ML. National Cooperative Crohn's Disease Study: extraintestinal manifestations and perianal complications. *Gastroenterology* 1979; **77**: 914-920 [PMID: 467943]
15 **Gelbmann CM**, Rogler G, Gross V, Gierend M, Bregenzer N, Andus T, Schölmerich J. Prior bowel resections, perianal disease, and a high initial Crohn's disease activity index are associated with corticosteroid resistance in active Crohn's disease. *Am J Gastroenterol* 2002; **97**: 1438-1445 [PMID: 12094862 DOI: 10.1111/j.1572-0241.2002.05685.x]
16 **Makowiec F**, Jehle EC, Starlinger M. Clinical course of perianal fistulas in Crohn's disease. *Gut* 1995; **37**: 696-701 [PMID: 8549948 DOI: 10.1136/gut.37.5.696]
17 **Sjödahl RI**, Myrelid P, Söderholm JD. Anal and rectal cancer in Crohn's disease. *Colorectal Dis* 2003; **5**: 490-495 [PMID: 12925087 DOI: 10.1046/j.1463-1318.2003.00510.x]
18 **Ky A,** Sohn N, Weinstein MA, Korelitz BI. Carcinoma arising in anorectal fistulas of Crohn's disease. *Dis Colon Rectum* 1998; **41**: 992-996 [DOI: 10.1007/BF02237388]
19 **Connell WR**, Sheffield JP, Kamm MA, Ritchie JK, Hawley PR, Lennard-Jones JE. Lower gastrointestinal malignancy in Crohn's disease. *Gut* 1994; **35**: 347-352 [PMID: 8150345 DOI: 10.1136/gut.35.3.347]
20 **Weedon DD**, Shorter RG, Ilstrup DM, Huizenga KA, Taylor WF. Crohn's disease and cancer. *N Engl J Med* 1973; **289**: 1099-1103 [PMID: 4754948 DOI: 10.1056/NEJM197311222892101]
21 **Iesalnieks I**, Gaertner WB, Glass H, Strauch U, Hipp M, Agha A, Schlitt HJ. Fistula-associated anal adenocarcinoma in Crohn's disease. *Inflamm Bowel Dis* 2010; **16**: 1643-1648 [PMID: 20186945 DOI: 10.1002/ibd.21228]
22 **Wolff BG**, Culp CE, Beart RW, Ilstrup DM, Ready RL. Anorectal Crohn's disease. A long-term perspective. *Dis Colon Rectum* 1985; **28**: 709-711 [PMID: 4053875 DOI: 10.1007/BF02560279]
23 **Solomon MJ**. Fistulae and abscesses in symptomatic perianal Crohn's disease. *Int J Colorectal Dis* 1996; **11**: 222-226 [PMID: 8951512 DOI: 10.1007/s003840050051]
24 **Makowiec F**, Jehle EC, Becker HD, Starlinger M. Perianal abscess in Crohn's disease. *Dis Colon Rectum* 1997; **40**: 443-450 [PMID: 9106694 DOI: 10.1007/BF02258390]
25 **Singh B**, McC Mortensen NJ, Jewell DP, George B. Perianal Crohn's disease. *Br J Surg* 2004; **91**: 801-814 [PMID: 15227686 DOI: 10.1002/bjs.4613]
26 **Pritchard TJ**, Schoetz DJ, Roberts PL, Murray JJ, Coller JA, Veidenheimer MC. Perirectal abscess in Crohn's disease. Drainage and outcome. *Dis Colon Rectum* 1990; **33**: 933-937 [PMID: 2226080 DOI: 10.1007/BF02139102]
27 **Sohn N**, Korelitz BI, Weinstein MA. Anorectal Crohn's disease: definitive surgery for fistulas and recurrent abscesses. *Am J Surg* 1980; **139**: 394-397 [PMID: 7362011 DOI: 10.1016/0002-9610(80)90301-3]
28 **Giovannini M**, Bories E, Moutardier V, Pesenti C, Guillemin A, Lelong B, Delpéro JR. Drainage of deep pelvic abscesses using therapeutic echo endoscopy. *Endoscopy* 2003; **35**: 511-514 [PMID: 12783350 DOI: 10.1055/s-2003-39673]
29 **Schratter-Sehn AU**, Lochs H, Vogelsang H, Schurawitzki H, Herold C, Schratter M. Endoscopic ultrasonography versus computed tomography in the differential diagnosis of perianorectal complications in Crohn's disease. *Endoscopy* 1993; **25**: 582-586 [PMID: 8119208 DOI: 10.1055/s-2007-1010409]
30 **Williams JG**, Rothenberger DA, Nemer FD, Goldberg SM. Fistula-in-ano in Crohn's disease. Results of aggressive surgical treatment. *Dis Colon Rectum* 1991; **34**: 378-384 [PMID: 2022142 DOI: 10.1007/BF02053687]
31 **Halme L**, Sainio AP. Factors related to frequency, type, and outcome of anal fistulas in Crohn's disease. *Dis Colon Rectum* 1995; **38**: 55-59 [PMID: 7813346 DOI: 10.1007/BF02053858]
32 **Sangwan YP**, Schoetz DJ, Murray JJ, Roberts PL, Coller JA. Perianal Crohn's disease. Results of local surgical treatment. *Dis Colon Rectum* 1996; **39**: 529-535 [PMID: 8620803 DOI: 10.1007/BF02058706]
33 **Faucheron JL**, Saint-Marc O, Guibert L, Parc R. Long-term seton drainage for high anal fistulas in Crohn's disease--a sphincter-saving operation? *Dis Colon Rectum* 1996; **39**: 208-211 [PMID: 8620789 DOI: 10.1007/BF02068077]
34 **Pearl RK**, Andrews JR, Orsay CP, Weisman RI, Prasad ML, Nelson RL, Cintron JR, Abcarian H. Role of the seton in the management of anorectal fistulas. *Dis Colon Rectum* 1993; **36**: 573-577; discussion 573-577 [PMID: 8500375 DOI: 10.1007/BF02049864]
35 **Thornton M**, Solomon MJ. Long-term indwelling seton for complex anal fistulas in Crohn's disease. *Dis Colon Rectum* 2005; **48**: 459-463 [PMID: 15719188 DOI: 10.1007/s10350-004-0830-6]
36 **Buchanan GN**, Owen HA, Torkington J, Lunniss PJ, Nicholls RJ, Cohen CR. Long-term outcome following loose-seton technique for external sphincter preservation in complex anal fistula. *Br J Surg* 2004; **91**: 476-480 [PMID: 15048751 DOI: 10.1002/bjs.4466]
37 **Eitan A**, Koliada M, Bickel A. The use of the loose seton technique as a definitive treatment for recurrent and persistent high trans-sphincteric anal fistulas: a long-term outcome. *J Gastrointest Surg* 2009; **13**: 1116-1119 [PMID: 19238493 DOI: 10.1007/s11605-009-0826-6]
38 **Topstad DR**, Panaccione R, Heine JA, Johnson DR, MacLean AR, Buie WD. Combined seton placement, infliximab infusion, and maintenance immunosuppressives improve healing rate in fistulizing anorectal Crohn's disease: a single center experience. *Dis Colon Rectum* 2003; **46**: 577-583 [PMID:

12792431 DOI: 10.1007/s10350-004-6611-4]
39 **Gaertner WB**, Decanini A, Mellgren A, Lowry AC, Goldberg SM, Madoff RD, Spencer MP. Does infliximab infusion impact results of operative treatment for Crohn's perianal fistulas? *Dis Colon Rectum* 2007; **50**: 1754-1760 [PMID: 17899271 DOI: 10.1007/s10350-007-9077-3]
40 **Edwards CM**, George BD, Jewell DP, Warren BF, Mortensen NJ, Kettlewell MG. Role of a defunctioning stoma in the management of large bowel Crohn's disease. *Br J Surg* 2000; **87**: 1063-1066 [PMID: 10931051 DOI: 10.1046/j.1365-2168.2000.01467.x]
41 **Tozer P.** Dendritic cell homing and immune cell function in Crohn's anal fistulae. *Gut* 2011; **60**: A220-1 [DOI: 10.1136/gut.2011.239301.465]
42 **Buchanan GN**, Halligan S, Bartram CI, Williams AB, Tarroni D, Cohen CR. Clinical examination, endosonography, and MR imaging in preoperative assessment of fistula in ano: comparison with outcome-based reference standard. *Radiology* 2004; **233**: 674-681 [PMID: 15498901 DOI: 10.1148/radiol.2333031724]
43 **Schaefer O**, Lohrmann C, Langer M. Assessment of anal fistulas with high-resolution subtraction MR-fistulography: comparison with surgical findings. *J Magn Reson Imaging* 2004; **19**: 91-98 [PMID: 14696225 DOI: 10.1002/jmri.10436]
44 **Beets-Tan RG**, Beets GL, van der Hoop AG, Kessels AG, Vliegen RF, Baeten CG, van Engelshoven JM. Preoperative MR imaging of anal fistulas: Does it really help the surgeon? *Radiology* 2001; **218**: 75-84 [PMID: 11152782 DOI: 10.1148/radiology.218.1.r01dc0575]
45 **Buchanan GN**, Halligan S, Williams AB, Cohen CR, Tarroni D, Phillips RK, Bartram CI. Magnetic resonance imaging for primary fistula in ano. *Br J Surg* 2003; **90**: 877-881 [PMID: 12854117 DOI: 10.1002/bjs.4125]
46 **Sachar DB**, Bodian CA, Goldstein ES, Present DH, Bayless TM, Picco M, van Hogezand RA, Annese V, Schneider J, Korelitz BI, Cosnes J. Is perianal Crohn's disease associated with intestinal fistulization? *Am J Gastroenterol* 2005; **100**: 1547-1549 [PMID: 15984979 DOI: 10.1111/j.1572-0241.2005.40980.x]
47 **Marks CG**, Ritchie JK, Lockhart-Mummery HE. Anal fistulas in Crohn's disease. *Br J Surg* 1981; **68**: 525-527 [PMID: 7272665 DOI: 10.1002/bjs.1800680802]
48 **Orkin BA**, Telander RL. The effect of intra-abdominal resection or fecal diversion on perianal disease in pediatric Crohn's disease. *J Pediatr Surg* 1985; **20**: 343-347 [PMID: 4045658 DOI: 10.1016/S0022-3468(85)80216-5]
49 **Dejaco C**, Harrer M, Waldhoer T, Miehsler W, Vogelsang H, Reinisch W. Antibiotics and azathioprine for the treatment of perianal fistulas in Crohn's disease. *Aliment Pharmacol Ther* 2003; **18**: 1113-1120 [PMID: 14653831 DOI: 10.1046/j.1365-2036.2003.01793.x]
50 **Bernstein LH**, Frank MS, Brandt LJ, Boley SJ. Healing of perineal Crohn's disease with metronidazole. *Gastroenterology* 1980; **79**: 599 [PMID: 7429123]
51 **Turunen UM**, Färkkilä MA, Hakala K, Seppälä K, Sivonen A, Ogren M, Vuoristo M, Valtonen VV, Miettinen TA. Long-term treatment of ulcerative colitis with ciprofloxacin: a prospective, double-blind, placebo-controlled study. *Gastroenterology* 1998; **115**: 1072-1078 [PMID: 9797360 DOI: 10.1016/S0016-5085(98)70076-9]
52 **Brandt LJ**, Bernstein LH, Boley SJ, Frank MS. Metronidazole therapy for perineal Crohn's disease: a follow-up study. *Gastroenterology* 1982; **83**: 383-387 [PMID: 7084615]
53 **Thia KT**, Mahadevan U, Feagan BG, Wong C, Cockeram A, Bitton A, Bernstein CN, Sandborn WJ. Ciprofloxacin or metronidazole for the treatment of perianal fistulas in patients with Crohn's disease: a randomized, double-blind, placebo-controlled pilot study. *Inflamm Bowel Dis* 2009; **15**: 17-24 [PMID: 18668682 DOI: 10.1002/ibd.20608]
54 **Turunen U,** Farkkila M, Seppala K. What is New and What is Established in Inflammatory Bowel Diseases: Proceedings of the Athens International Meeting on Inflammatory Bowel Diseases Athens, 19–23 April, 1989. Longterm treatment of peri-anal or fistulous Crohn's disease with ciprofloxacin. *Scand J Gastroenterol* 1989; **24**: 144
55 **Solomon M,** McLeod R, O'Connor B, Steinhart A, Greenberg G, Cohen Z. Combination ciprofloxacin and metronidazole in severe perineal Crohn's disease. *Can J Gastroenterol* 1993; **7**: 571-573
56 **Pearson DC**, May GR, Fick GH, Sutherland LR. Azathioprine and 6-mercaptopurine in Crohn disease. A meta-analysis. *Ann Intern Med* 1995; **123**: 132-142 [PMID: 7778826 DOI: 10.7326/0003-4819-123-2-199507150-00009]
57 **Hinterleitner TA**, Petritsch W, Aichbichler B, Fickert P, Ranner G, Krejs GJ. Combination of cyclosporine, azathioprine and prednisolone for perianal fistulas in Crohn's disease. *Z Gastroenterol* 1997; **35**: 603-608 [PMID: 9297775]
58 **Egan LJ**, Sandborn WJ, Tremaine WJ. Clinical outcome following treatment of refractory inflammatory and fistulizing Crohn's disease with intravenous cyclosporine. *Am J Gastroenterol* 1998; **93**: 442-448 [PMID: 9517654 DOI: 10.1111/j.1572-0241.1998.00442.x]
59 **Gurudu SR**, Griffel LH, Gialanella RJ, Das KM. Cyclosporine therapy in inflammatory bowel disease: short-term and long-term results. *J Clin Gastroenterol* 1999; **29**: 151-154 [PMID: 10478875 DOI: 10.1097/00004836-199909000-00009]
60 **Sandborn WJ**, Present DH, Isaacs KL, Wolf DC, Greenberg E, Hanauer SB, Feagan BG, Mayer L, Johnson T, Galanko J, Martin C, Sandler RS. Tacrolimus for the treatment of fistulas in patients with Crohn's disease: a randomized, placebo-controlled trial. *Gastroenterology* 2003; **125**: 380-388 [PMID: 12891539 DOI: 10.1016/S0016-5085(03)00877-1]
61 **Sokol H**, Seksik P, Carrat F, Nion-Larmurier I, Vienne A, Beaugerie L, Cosnes J. Usefulness of co-treatment with immunomodulators in patients with inflammatory bowel disease treated with scheduled infliximab maintenance therapy. *Gut* 2010; **59**: 1363-1368 [PMID: 20587545 DOI: 10.1136/gut.2010.212712]
62 **Present DH**, Rutgeerts P, Targan S, Hanauer SB, Mayer L, van Hogezand RA, Podolsky DK, Sands BE, Braakman T, DeWoody KL, Schaible TF, van Deventer SJ. Infliximab for the treatment of fistulas in patients with Crohn's disease. *N Engl J Med* 1999; **340**: 1398-1405 [PMID: 10228190 DOI: 10.1056/NEJM199905063401804]
63 **Miheller P**, Lakatos PL, Horváth G, Molnár T, Szamosi T, Czeglédi Z, Salamon A, Czimmer J, Rumi G, Palatka K, Papp M, Jakab Z, Szabó A, Gelley A, Lakatos L, Barta Z, Balázs C, Rácz I, Zeher M, Döbrönte Z, Altorjay I, Hunyady B, Simon L, Papp J, Banai J, Nagy F, Lonovics J, Ujszászy L, Muzes G, Herszényi L, Tulassay Z. Efficacy and safety of infliximab induction therapy in Crohn's Disease in Central Europe--a Hungarian nationwide observational study. *BMC Gastroenterol* 2009; **9**: 66 [PMID: 19740450 DOI: 10.1186/1471-230X-9-66]
64 **Orlando A**, Colombo E, Kohn A, Biancone L, Rizzello F, Viscido A, Sostegni R, Benazzato L, Castiglione F, Papi C, Meucci G, Riegler G, Mocciaro F, Cassinotti A, Cosintino R, Geremia A, Morselli C, Angelucci E, Lavagna A, Rispo A, Bossa F, Scimeca D, Cottone M. Infliximab in the treatment of Crohn's disease: predictors of response in an Italian multicentric open study. *Dig Liver Dis* 2005; **37**: 577-583 [PMID: 15886081 DOI: 10.1016/j.dld.2005.01.019]
65 **Ng SC**, Plamondon S, Gupta A, Burling D, Swatton A, Vaizey CJ, Kamm MA. Prospective evaluation of anti-tumor necrosis factor therapy guided by magnetic resonance imaging for Crohn's perineal fistulas. *Am J Gastroenterol* 2009; **104**: 2973-2986 [PMID: 19755971 DOI: 10.1038/ajg.2009.509]
66 **Regueiro M**, Mardini H. Treatment of perianal fistulizing Crohn's disease with infliximab alone or as an adjunct to exam under anesthesia with seton placement. *Inflamm Bowel*

Dis 2003; **9**: 98-103 [PMID: 12769443 DOI: 10.1097/00054725-200303000-00003]
67 **Hyder SA**, Travis SP, Jewell DP, McC Mortensen NJ, George BD. Fistulating anal Crohn's disease: results of combined surgical and infliximab treatment. *Dis Colon Rectum* 2006; **49**: 1837-1841 [PMID: 17041753 DOI: 10.1007/s10350-006-0656-5]
68 **van der Hagen SJ**, Baeten CG, Soeters PB, Russel MG, Beets-Tan RG, van Gemert WG. Anti-TNF-alpha (infliximab) used as induction treatment in case of active proctitis in a multistep strategy followed by definitive surgery of complex anal fistulas in Crohn's disease: a preliminary report. *Dis Colon Rectum* 2005; **48**: 758-767 [PMID: 15750797 DOI: 10.1007/s10350-004-0828-0]
69 **Colombel JF**, Sandborn WJ, Rutgeerts P, Enns R, Hanauer SB, Panaccione R, Schreiber S, Byczkowski D, Li J, Kent JD, Pollack PF. Adalimumab for maintenance of clinical response and remission in patients with Crohn's disease: the CHARM trial. *Gastroenterology* 2007; **132**: 52-65 [PMID: 17241859 DOI: 10.1053/j.gastro.2006.11.041]
70 **Colombel JF**, Schwartz DA, Sandborn WJ, Kamm MA, D'Haens G, Rutgeerts P, Enns R, Panaccione R, Schreiber S, Li J, Kent JD, Lomax KG, Pollack PF. Adalimumab for the treatment of fistulas in patients with Crohn's disease. *Gut* 2009; **58**: 940-948 [PMID: 19201775 DOI: 10.1136/gut.2008.159251]
71 **Hanauer SB**, Sandborn WJ, Rutgeerts P, Fedorak RN, Lukas M, MacIntosh D, Panaccione R, Wolf D, Pollack P. Human anti-tumor necrosis factor monoclonal antibody (adalimumab) in Crohn's disease: the CLASSIC-I trial. *Gastroenterology* 2006; **130**: 323-333; quiz 591 [PMID: 16472588 DOI: 10.1053/j.gastro.2005.11.030]
72 **Sandborn WJ**, Rutgeerts P, Enns R, Hanauer SB, Colombel JF, Panaccione R, D'Haens G, Li J, Rosenfeld MR, Kent JD, Pollack PF. Adalimumab induction therapy for Crohn disease previously treated with infliximab: a randomized trial. *Ann Intern Med* 2007; **146**: 829-838 [PMID: 17470824 DOI: 10.7326/0003-4819-146-12-200706190-00159]
73 **Tozer P**, Ng SC, Siddiqui MR, Plamondon S, Burling D, Gupta A, Swatton A, Tripoli S, Vaizey CJ, Kamm MA, Phillips R, Hart A. Long-term MRI-guided combined anti-TNF-α and thiopurine therapy for Crohn's perianal fistulas. *Inflamm Bowel Dis* 2012; **18**: 1825-1834 [PMID: 22223472 DOI: 10.1002/ibd.21940]
74 **El-Gazzaz G**, Hull T, Church JM. Biological immunomodulators improve the healing rate in surgically treated perianal Crohn's fistulas. *Colorectal Dis* 2012; **14**: 1217-1223 [PMID: 22251452 DOI: 10.1111/j.1463-1318.2012.02944.x]
75 **Poggioli G**, Laureti S, Pierangeli F, Rizzello F, Ugolini F, Gionchetti P, Campieri M. Local injection of Infliximab for the treatment of perianal Crohn's disease. *Dis Colon Rectum* 2005; **48**: 768-774 [PMID: 15768185 DOI: 10.1007/s10350-004-0832-4]
76 **Asteria CR**, Ficari F, Bagnoli S, Milla M, Tonelli F. Treatment of perianal fistulas in Crohn's disease by local injection of antibody to TNF-alpha accounts for a favourable clinical response in selected cases: a pilot study. *Scand J Gastroenterol* 2006; **41**: 1064-1072 [PMID: 16938720 DOI: 10.1080/00365520600609941]
77 **Tonelli F**, Giudici F, Asteria CR. Effectiveness and safety of local adalimumab injection in patients with fistulizing perianal Crohn's disease: a pilot study. *Dis Colon Rectum* 2012; **55**: 870-875 [PMID: 22810472 DOI: 10.1097/DCR.0b013e31825af532]
78 **Schreiber S**, Lawrance IC, Thomsen OØ, Hanauer SB, Bloomfield R, Sandborn WJ. Randomised clinical trial: certolizumab pegol for fistulas in Crohn's disease - subgroup results from a placebo-controlled study. *Aliment Pharmacol Ther* 2011; **33**: 185-193 [PMID: 21083671 DOI: 10.1111/j.1365-2036.2010.04509.x]
79 **Fry RD**, Shemesh EI, Kodner IJ, Timmcke A. Techniques and results in the management of anal and perianal Crohn's disease. *Surg Gynecol Obstet* 1989; **168**: 42-48 [PMID: 2909131]
80 **Levien DH**, Surrell J, Mazier WP. Surgical treatment of anorectal fistula in patients with Crohn's disease. *Surg Gynecol Obstet* 1989; **169**: 133-136 [PMID: 2756461]
81 **Lindsey I**, Smilgin-Humphreys MM, Cunningham C, Mortensen NJ, George BD. A randomized, controlled trial of fibrin glue vs. conventional treatment for anal fistula. *Dis Colon Rectum* 2002; **45**: 1608-1615 [PMID: 12473883 DOI: 10.1007/s10350-004-7247-0]
82 **Grimaud JC**, Munoz-Bongrand N, Siproudhis L, Abramowitz L, Sénéjoux A, Vitton V, Gambiez L, Flourié B, Hébuterne X, Louis E, Coffin B, De Parades V, Savoye G, Soulé JC, Bouhnik Y, Colombel JF, Contou JF, François Y, Mary JY, Lémann M. Fibrin glue is effective healing perianal fistulas in patients with Crohn's disease. *Gastroenterology* 2010; **138**: 2275-2281, 2281.e1 [PMID: 20178792 DOI: 10.1053/j.gastro.2010.02.013]
83 **Vitton V**, Gasmi M, Barthet M, Desjeux A, Orsoni P, Grimaud JC. Long-term healing of Crohn's anal fistulas with fibrin glue injection. *Aliment Pharmacol Ther* 2005; **21**: 1453-1457 [PMID: 15948812 DOI: 10.1111/j.1365-2036.2005.02456.x]
84 **O'Riordan JM**, Datta I, Johnston C, Baxter NN. A systematic review of the anal fistula plug for patients with Crohn's and non-Crohn's related fistula-in-ano. *Dis Colon Rectum* 2012; **55**: 351-358 [PMID: 22469804 DOI: 10.1097/DCR.0b013e318239d1e4]
85 **de la Portilla F**, Alba F, García-Olmo D, Herrerías JM, González FX, Galindo A. Expanded allogeneic adipose-derived stem cells (eASCs) for the treatment of complex perianal fistula in Crohn's disease: results from a multicenter phase I/IIa clinical trial. *Int J Colorectal Dis* 2013; **28**: 313-323 [PMID: 23053677 DOI: 10.1007/s00384-012-1581-9]
86 **Schwandner O**. Video-assisted anal fistula treatment (VAAFT) combined with advancement flap repair in Crohn's disease. *Tech Coloproctol* 2013; **17**: 221-225 [PMID: 23179892 DOI: 10.1007/s10151-012-0921-7]
87 **Soltani A**, Kaiser AM. Endorectal advancement flap for cryptoglandular or Crohn's fistula-in-ano. *Dis Colon Rectum* 2010; **53**: 486-495 [PMID: 20305451 DOI: 10.1007/DCR.0b013e3181ce8b01]
88 **Vergara-Fernandez O**, Espino-Urbina LA. Ligation of intersphincteric fistula tract: what is the evidence in a review? *World J Gastroenterol* 2013; **19**: 6805-6813 [PMID: 24187455 DOI: 10.3748/wjg.v19.i40.6805]
89 **Abcarian AM**, Estrada JJ, Park J, Corning C, Chaudhry V, Cintron J, Prasad L, Abcarian H. Ligation of intersphincteric fistula tract: early results of a pilot study. *Dis Colon Rectum* 2012; **55**: 778-782 [PMID: 22706130 DOI: 10.1097/DCR.0b013e318255ae8a]
90 **Ellis CN**. Outcomes with the use of bioprosthetic grafts to reinforce the ligation of the intersphincteric fistula tract (BioLIFT procedure) for the management of complex anal fistulas. *Dis Colon Rectum* 2010; **53**: 1361-1364 [PMID: 20847616 DOI: 10.1007/DCR.0b013e3181ec4470]
91 **Marchesa P**, Hull TL, Fazio VW. Advancement sleeve flaps for treatment of severe perianal Crohn's disease. *Br J Surg* 1998; **85**: 1695-1698 [PMID: 9876077 DOI: 10.1046/j.1365-2168.1998.00959.x]
92 **Saclarides TJ**. Rectovaginal fistula. *Surg Clin North Am* 2002; **82**: 1261-1272 [PMID: 12516853 DOI: 10.1016/S0039-6109(02)00055-5]
93 **Platell C**, Mackay J, Collopy B, Fink R, Ryan P, Woods R. Anal pathology in patients with Crohn's disease. *Aust N Z J Surg* 1996; **66**: 5-9 [PMID: 8629983 DOI: 10.1111/j.1445-2197.1996.tb00690.x]
94 **Radcliffe AG**, Ritchie JK, Hawley PR, Lennard-Jones JE, Northover JM. Anovaginal and rectovaginal fistulas in Crohn's disease. *Dis Colon Rectum* 1988; **31**: 94-99 [PMID: 3338350 DOI: 10.1007/BF02562636]
95 **Galandiuk S**, Kimberling J, Al-Mishlab TG, Stromberg AJ.

Perianal Crohn disease: predictors of need for permanent diversion. *Ann Surg* 2005; **241**: 796-801; discussion 801-802 [PMID: 15849515 DOI: 10.1097/01.sla.0000161030.25860.c1]
96 **McClane SJ**, Rombeau JL. Anorectal Crohn's disease. *Surg Clin North Am* 2001; **81**: 169-183, ix [PMID: 11218163 DOI: 10.1016/S0039-6109(05)70279-6]
97 **Tuxen PA**, Castro AF. Rectovaginal fistula in Crohn's disease. *Dis Colon Rectum* 1979; **22**: 58-62 [PMID: 421652 DOI: 10.1007/BF02586761]
98 **Morrison JG**, Gathright JB, Ray JE, Ferrari BT, Hicks TC, Timmcke AE. Results of operation for rectovaginal fistula in Crohn's disease. *Dis Colon Rectum* 1989; **32**: 497-499 [PMID: 2791787 DOI: 10.1007/BF02554505]
99 **Penninckx F**, Moneghini D, D'Hoore A, Wyndaele J, Coremans G, Rutgeerts P. Success and failure after repair of rectovaginal fistula in Crohn's disease: analysis of prognostic factors. *Colorectal Dis* 2001; **3**: 406-411 [PMID: 12790939 DOI: 10.1046/j.1463-1318.2001.00274.x]
100 **Lowry AC**, Thorson AG, Rothenberger DA, Goldberg SM. Repair of simple rectovaginal fistulas. Influence of previous repairs. *Dis Colon Rectum* 1988; **31**: 676-678 [PMID: 3168676 DOI: 10.1007/BF02552581]
101 **MacRae HM**, McLeod RS, Cohen Z, Stern H, Reznick R. Treatment of rectovaginal fistulas that has failed previous repair attempts. *Dis Colon Rectum* 1995; **38**: 921-925 [PMID: 7656738 DOI: 10.1007/BF02049726]
102 **O'Leary DP**, Milroy CE, Durdey P. Definitive repair of anovaginal fistula in Crohn's disease. *Ann R Coll Surg Engl* 1998; **80**: 250-252 [PMID: 9771223]
103 **Present DH**, Korelitz BI, Wisch N, Glass JL, Sachar DB, Pasternack BS. Treatment of Crohn's disease with 6-mercaptopurine. A long-term, randomized, double-blind study. *N Engl J Med* 1980; **302**: 981-987 [PMID: 6102739 DOI: 10.1056/NEJM198005013021801]
104 **Present DH**, Lichtiger S. Efficacy of cyclosporine in treatment of fistula of Crohn's disease. *Dig Dis Sci* 1994; **39**: 374-380 [PMID: 8313821 DOI: 10.1007/BF02090211]
105 **Hanauer SB**, Smith MB. Rapid closure of Crohn's disease fistulas with continuous intravenous cyclosporin A. *Am J Gastroenterol* 1993; **88**: 646-649 [PMID: 8480725]
106 **El-Gazzaz G**, Hull T, Mignanelli E, Hammel J, Gurland B, Zutshi M. Analysis of function and predictors of failure in women undergoing repair of Crohn's related rectovaginal fistula. *J Gastrointest Surg* 2010; **14**: 824-829 [PMID: 20232172 DOI: 10.1007/s11605-010-1167-1]
107 **Talbot C**, Sagar PM, Johnston MJ, Finan PJ, Burke D. Infliximab in the surgical management of complex fistulating anal Crohn's disease. *Colorectal Dis* 2005; **7**: 164-168 [PMID: 15720356 DOI: 10.1111/j.1463-1318.2004.00749.x]
108 **Van Assche G**, Vanbeckevoort D, Bielen D, Coremans G, Aerden I, Noman M, D'Hoore A, Penninckx F, Marchal G, Cornillie F, Rutgeerts P. Magnetic resonance imaging of the effects of infliximab on perianal fistulizing Crohn's disease. *Am J Gastroenterol* 2003; **98**: 332-339 [PMID: 12591051 DOI: 10.1016/S0002-9270(02)05909-9]
109 **Sands BE**, Anderson FH, Bernstein CN, Chey WY, Feagan BG, Fedorak RN, Kamm MA, Korzenik JR, Lashner BA, Onken JE, Rachmilewitz D, Rutgeerts P, Wild G, Wolf DC, Marsters PA, Travers SB, Blank MA, van Deventer SJ. Infliximab maintenance therapy for fistulizing Crohn's disease. *N Engl J Med* 2004; **350**: 876-885 [PMID: 14985485 DOI: 10.1056/NEJMoa030815]
110 **Gaertner WB**, Madoff RD, Spencer MP, Mellgren A, Goldberg SM, Lowry AC. Results of combined medical and surgical treatment of recto-vaginal fistula in Crohn's disease. *Colorectal Dis* 2011; **13**: 678-683 [PMID: 20163426 DOI: 10.1111/j.1463-1318.2010.02234.x]
111 **Rasul I**, Wilson SR, MacRae H, Irwin S, Greenberg GR. Clinical and radiological responses after infliximab treatment for perianal fistulizing Crohn's disease. *Am J Gastroenterol* 2004; **99**: 82-88 [PMID: 14687146 DOI: 10.1046/j.1572-0241.2003.04009.x]
112 **Bell SJ**, Halligan S, Windsor AC, Williams AB, Wiesel P, Kamm MA. Response of fistulating Crohn's disease to infliximab treatment assessed by magnetic resonance imaging. *Aliment Pharmacol Ther* 2003; **17**: 387-393 [PMID: 12562451 DOI: 10.1046/j.1365-2036.2003.01427.x]
113 **Meagher AP,** Farouk R, Dozois RR, Kelly KA, Pemberton JH. J ileal pouch-anal anastomosis for chronic ulcerative colitis: complications and long-term outcome in 1310 patients. *Br J Surg* 1998; **85**: 800-803 [PMID: 9667712 DOI: 10.1046/j.1365-2168.1998.00689.x]
114 **Fleshman JW**, Cohen Z, McLeod RS, Stern H, Blair J. The ileal reservoir and ileoanal anastomosis procedure. Factors affecting technical and functional outcome. *Dis Colon Rectum* 1988; **31**: 10-16 [PMID: 3366021 DOI: 10.1007/BF02552562]
115 **Keighley MR**, Grobler SP. Fistula complicating restorative proctocolectomy. *Br J Surg* 1993; **80**: 1065-1067 [PMID: 8402069 DOI: 10.1002/bjs.1800800849]
116 **Ozuner G**, Hull T, Lee P, Fazio VW. What happens to a pelvic pouch when a fistula develops? *Dis Colon Rectum* 1997; **40**: 543-547 [PMID: 9152180 DOI: 10.1007/BF02055375]
117 **Zinicola R**, Wilkinson KH, Nicholls RJ. Ileal pouch-vaginal fistula treated by abdominoanal advancement of the ileal pouch. *Br J Surg* 2003; **90**: 1434-1435 [PMID: 14598427 DOI: 10.1002/bjs.4314]
118 **Wexner SD**, Rothenberger DA, Jensen L, Goldberg SM, Balcos EG, Belliveau P, Bennett BH, Buls JG, Cohen JM, Kennedy HL. Ileal pouch vaginal fistulas: incidence, etiology, and management. *Dis Colon Rectum* 1989; **32**: 460-465 [PMID: 2676425 DOI: 10.1007/BF02554497]
119 **Tulchinsky H**, Cohen CR, Nicholls RJ. Salvage surgery after restorative proctocolectomy. *Br J Surg* 2003; **90**: 909-921 [PMID: 12905542 DOI: 10.1002/bjs.4278]
120 **Cohen Z**, Smith D, McLeod R. Reconstructive surgery for pelvic pouches. *World J Surg* 1998; **22**: 342-346 [PMID: 9523514 DOI: 10.1007/s002689900394]
121 **Gaertner WB,** Witt J, Madoff RD, Mellgren A, Finne CO, Spencer MP. Ileal pouch fistulas after restorative proctocolectomy: management and outcomes. Poster presentation at the Annual meeting of the American Society of Colon and Rectal Surgeons, Phoenix, Arizona, 2013
122 **Rosser C.** The relation of fistula-in-ano to cancer of the anal canal. *Am Proct Soc* 1934; **35**: 65-71
123 **Korelitz BI**. Carcinoma arising in Crohn's disease fistulae: another concern warranting another type of surveillance. *Am J Gastroenterol* 1999; **94**: 2337-2339 [PMID: 10483989 DOI: 10.1111/j.1572-0241.1999.02337.x]
124 **Anthony T**, Simmang C, Lee EL, Turnage RH. Perianal mucinous adenocarcinoma. *J Surg Oncol* 1997; **64**: 218-221 [PMID: 9121153]
125 **Erhan Y**, Sakarya A, Aydede H, Demir A, Seyhan A, Atici E. A case of large mucinous adenocarcinoma arising in a long-standing fistula-in-ano. *Dig Surg* 2003; **20**: 69-71 [PMID: 12637812 DOI: 10.1159/000068857]
126 **Marti L**, Nussbaumer P, Breitbach T, Hollinger A. [Perianal mucinous adenocarcinoma. A further reason for histological study of anal fistula or anorectal abscess]. *Chirurg* 2001; **72**: 573-577 [PMID: 11383070 DOI: 10.1007/s001040170137]
127 **Navarra G**, Ascanelli S, Turini A, Lanza G, Gafà R, Tonini G. Mucinous adenocarcinoma in chronic anorectal fistula. *Chir Ital* 1999; **51**: 413-416 [PMID: 10738618]
128 **Papapolychroniadis C**, Kaimakis D, Giannoulis K, Berovalis P, Karamanlis E, Haritanti A, Leukopoulos A, Kokkonis G, Masoura OM, Dimitriadis A, Giala M, Harlaftis N. A case of mucinous adenocarcinoma arising in long-standing multiple perianal and presacral fistulas. *Tech Coloproctol* 2004; **8** Suppl 1: s138-s140 [PMID: 15655599 DOI: 10.1007/s10151-004-0136-7]
129 **Patrinou V**, Petrochilos J, Batistatou A, Oneniadum A, Venetsanou-Petrochilou C. Mucinous adenocarcinoma aris-

ing in chronic perianal fistulas. *J Clin Gastroenterol* 2001; **33**: 175-176 [PMID: 11468456 DOI: 10.1097/00004836-200108000-00024]
130 **Schaffzin DM**, Stahl TJ, Smith LE. Perianal mucinous adenocarcinoma: unusual case presentations and review of the literature. *Am Surg* 2003; **69**: 166-169 [PMID: 12641361]
131 **Gaertner WB**, Hagerman GF, Finne CO, Alavi K, Jessurun J, Rothenberger DA, Madoff RD. Fistula-associated anal adenocarcinoma: good results with aggressive therapy. *Dis Colon Rectum* 2008; **51**: 1061-1067 [PMID: 18418652 DOI: 10.1007/s10350-008-9294-4]
132 **Spencer JA**, Ward J, Beckingham IJ, Adams C, Ambrose NS. Dynamic contrast-enhanced MR imaging of perianal fistulas. *AJR Am J Roentgenol* 1996; **167**: 735-741 [PMID: 8751692 DOI: 10.2214/ajr.167.3.8751692]
133 **Lee SH**, Zucker M, Sato T. Primary adenocarcinoma of an anal gland with secondary perianal fistulas. *Hum Pathol* 1981; **12**: 1034-1037 [PMID: 6274783 DOI: 10.1016/S0046-8177(81)80263-8]
134 **Getz SB**, Ough YD, Patterson RB, Kovalcik PJ. Mucinous adenocarcinoma developing in chronic anal fistula: report of two cases and review of the literature. *Dis Colon Rectum* 1981; **24**: 562-566 [PMID: 6271516 DOI: 10.1007/BF02604325]
135 **Tarazi R**, Nelson RL. Anal adenocarcinoma: a comprehensive review. *Semin Surg Oncol* 1994; **10**: 235-240 [PMID: 8085101 DOI: 10.1002/ssu.2980100312]
136 **Zaren HA**, Delone FX, Lerner HJ. Carcinoma of the anal gland: case report and review of the literature. *J Surg Oncol* 1983; **23**: 250-254 [PMID: 6308351 DOI: 10.1002/jso.2930230407]
137 **Basik M**, Rodriguez-Bigas MA, Penetrante R, Petrelli NJ. Prognosis and recurrence patterns of anal adenocarcinoma. *Am J Surg* 1995; **169**: 233-237 [PMID: 7840386 DOI: 10.1016/S0002-9610(99)80143-3]
138 **Nelson RL**, Prasad ML, Abcarian H. Anal carcinoma presenting as a perirectal abscess or fistula. *Arch Surg* 1985; **120**: 632-635 [PMID: 3985803 DOI: 10.1001/archsurg.1985.01390290106019]
139 **Cohen JL**, Stricker JW, Schoetz DJ, Coller JA, Veidenheimer MC. Rectovaginal fistula in Crohn's disease. *Dis Colon Rectum* 1989; **32**: 825-828 [PMID: 2791765 DOI: 10.1007/BF02554548]
140 **Yamamoto T**, Bain IM, Allan RN, Keighley MR. Persistent perineal sinus after proctocolectomy for Crohn's disease. *Dis Colon Rectum* 1999; **42**: 96-101 [PMID: 10211527 DOI: 10.1007/BF02235190]
141 **Sher ME**, Bauer JJ, Gorphine S, Gelernt I. Low Hartmann's procedure for severe anorectal Crohn's disease. *Dis Colon Rectum* 1992; **35**: 975-980 [PMID: 1395986 DOI: 10.1007/BF02253501]
142 **Guillem JG**, Roberts PL, Murray JJ, Coller JA, Veidenheimer MC, Schoetz DJ. Factors predictive of persistent or recurrent Crohn's disease in excluded rectal segments. *Dis Colon Rectum* 1992; **35**: 768-772 [PMID: 1644001 DOI: 10.1007/BF02050327]

P- Reviewer: Liu HM, Perakath B **S- Editor**: Wen LL
L- Editor: A **E- Editor:** Lu YJ

World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4291/wjgp.v5.i3.252

World J Gastrointest Pathophysiol 2014 August 15; 5(3): 252-270
ISSN 2150-5330 (online)

REVIEW

Pancreatitis-imaging approach

Kiran K Busireddy, Mamdoh AlObaidy, Miguel Ramalho, Janaka Kalubowila, Liu Baodong, Ilaria Santagostino, Richard C Semelka

Kiran K Busireddy, Mamdoh AlObaidy, Miguel Ramalho, Janaka Kalubowila, Liu Baodong, Ilaria Santagostino, Richard C Semelka, Department of Radiology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7510, United States

Author contributions: All authors contributed to this paper.

Correspondence to: Richard C Semelka, MD, Department of Radiology, University Of North Carolina at Chapel Hill, CB 7510-2001 Old Clinic Bldg., Chapel Hill, NC 27599-7510, United States. richsem@med.unc.edu

Telephone: +1-919-9669676 Fax: +1-919-8437147

Received: November 5, 2013 Revised: February 13, 2014
Accepted: May 15, 2014
Published online: August 15, 2014

Abstract

Pancreatitis is defined as the inflammation of the pancreas and considered the most common pancreatic disease in children and adults. Imaging plays a significant role in the diagnosis, severity assessment, recognition of complications and guiding therapeutic interventions. In the setting of pancreatitis, wider availability and good image quality make multi-detector contrast-enhanced computed tomography (MD-CECT) the most used imaging technique. However, magnetic resonance imaging (MRI) offers diagnostic capabilities similar to those of CT, with additional intrinsic advantages including lack of ionizing radiation and exquisite soft tissue characterization. This article reviews the proposed definitions of revised Atlanta classification for acute pancreatitis, illustrates a wide range of morphologic pancreatic parenchymal and associated peripancreatic changes for different types of acute pancreatitis. It also describes the spectrum of early and late chronic pancreatitis imaging findings and illustrates some of the less common types of chronic pancreatitis, with special emphasis on the role of CT and MRI.

Key words: Computed tomography; Magnetic resonance imaging; Acute pancreatitis; Chronic pancreatitis; Autoimmune pancreatitis; Chronic pancreatitis; Revised Atlanta classification; Motion-resistant imaging

Core tip: Imaging plays an important role in the diagnosis and staging of acute and chronic pancreatitis. Wider availability and good image quality makes computed tomography (CT) the mostly used imaging technique; however, magnetic resonance imaging (MRI) offers diagnostic capabilities similar to those of CT, with additional intrinsic advantages including lack of ionizing radiation and exquisite soft tissue characterization. This article reviews and illustrates the proposed definitions of the revised Atlanta classification for acute pancreatitis. It also describes the spectrum of early and late chronic pancreatitis imaging findings, with special emphasis on the role of CT and MRI.

Busireddy KK, AlObaidy M, Ramalho M, Kalubowila J, Baodong L, Santagostino I, Semelka RC. Pancreatitis-imaging approach. *World J Gastrointest Pathophysiol* 2014; 5(3): 252-270 Available from: URL: http://www.wjgnet.com/2150-5330/full/v5/i3/252.htm DOI: http://dx.doi.org/10.4291/wjgp.v5.i3.252

INTRODUCTION

Pancreatitis is defined as the inflammation of the pancreas and considered the most common pancreatic disease in children and adults. It can be acute; representing an acute inflammatory process of the pancreas, or chronic; progressing slowly with continued, permanent inflammatory injury to the pancreas.

The incidence of acute pancreatitis is increasing in the United States and worldwide contributing to be one of the major sources of hospitalization. Acute pancreatitis was the most common gastrointestinal diagnosis for hospitalization (with 274119 discharges) in the United

States in 2009[1], usually running a mild clinical course[2]. However, a subset of patients develop severe disease independent of the degree of initial insult or etiology, with high morbidity and mortality up to 45%[3]. Over one-half of cases of acute pancreatitis in adults are related to cholelithiasis or alcohol consumption; whereas trauma, viral infections and systemic diseases account for the majority of cases in children[4].

The incidence of chronic pancreatitis is between five and twelve cases per 100000 persons per year; accounting for more than 120000 outpatient visits and 50000 hospitalizations annually[5]. Alcohol consumption accounts for the majority (80%) of cases of chronic pancreatitis in adults in developed countries; whereas malnutrition is the most common cause worldwide[4].

The purpose of our review is to illustrate the different imaging findings of pancreatitis on computed tomography (CT) and magnetic resonance imaging (MRI); with special emphasis on the revised terminology for acute pancreatitis and substantiate the increasing importance of imaging in the diagnosis, staging and follow-up of acute and chronic pancreatitis[5].

Acute pancreatitis

Acute pancreatitis results from the exudation of fluid containing activated proteolytic enzymes into the interstitium of the pancreas and leakage of this fluid into surrounding tissue.

There is general acceptance that a diagnosis of acute pancreatitis requires two of the following three features: (1) Sudden onset abdominal pain suggestive of acute pancreatitis (epigastric pain radiating to the back); (2) Serum amylase and/or lipase levels at least 3 times greater than the upper limit of normal; and (3) Characteristic imaging findings of acute pancreatitis on contrast-enhanced computerized tomography (CECT), MRI, or transabdominal ultrasonography (US) studies.

If abdominal pain is strongly suggestive of acute pancreatitis but the serum amylase and/or lipase activity is less than 3 times the upper limit of normal, characteristic findings on a CECT or MRI are required to confirm the diagnosis[6].

In order to assess and predict local or systemic effects of pancreatic injury, several disease severity-scoring systems were developed (*e.g.*, Ranson score, APACHE-II). In 1992, the Atlanta classification for acute pancreatitis was introduced to establish international standards of definitions of acute pancreatitis and its complications[7]. This system was designed to facilitate understanding and correlation of findings seen by gastroenterologists, pathologists, radiologists and surgeons; aiding improved communication between clinicians.

This initial Atlanta classification system represented major progress; however, advancing knowledge of the disease process, improved imaging and ever-changing treatment options warranted a revision, which was undertaken in 2012.

The revision of the Atlanta classification focuses heavily on morphologic criteria for defining the various manifestations of acute pancreatitis as outlined principally by means of CT and MRI.

Two distinct phases of acute pancreatitis were introduced: a first, or early, phase that occurs within the 1st wk of onset of disease; and a second, or late, phase that takes place after the 1st week of onset[7].

Early or first phase (less than 1 wk)

During this phase, pancreatic or peripancreatic ischemia or edema may completely resolve, develop fluid collections or progress to permanent necrosis and liquefaction. Severity of the acute pancreatitis in the early phase is entirely based on clinical parameters; mainly determined by the presence and duration of organ failure, but not the morphologic characteristics and its extent in and around the pancreas[8].

Late or second phase (after 1 wk from onset)

This phase occurs mostly in patients with moderate to severe acute pancreatitis and may extend for weeks to months. It is characterized by the presence of local complications, systemic manifestations (due to ongoing inflammation) and/or by transient or persistent organ failure. In this stage, the need for treatment is determined by presence of symptoms or complications, and the type of management is mainly based on the morphologic characteristics of pancreatic and peripancreatic region seen on cross sectional imaging. The severity of acute pancreatitis in late phase is determined by both morphologic criteria and clinical criteria like persistence of organ failure.

Updated terminology of acute pancreatitis

The web based international consensus[7] revised the original Atlanta classification of 1992 and proposed a new classification of acute pancreatitis to avoid the confusion in terminology seen over the last 2 decades. This consensus classification defines criteria for the diagnosis of acute pancreatitis (see above), differentiates the two types of acute pancreatitis (interstitial edematous pancreatitis and necrotizing pancreatitis) classifies the severity of acute pancreatitis into three categories and defines the morphology seen on imaging of pancreatic and peripancreatic collections that arise as complications of acute pancreatitis.

Role of imaging in acute pancreatitis

Imaging plays a significant role in the diagnosis of acute pancreatitis in clinically suspected cases or suggesting alternative diagnoses. It helps determine the causes of pancreatitis: gallstones, biliary duct obstruction or structural abnormalities. It also helps in grading the severity of the disease and identifying pancreatic or peripancreatic complications. Additionally, imaging can be utilized to guide therapeutic interventions.

The choice of appropriate imaging modality depends on the reason for investigation, clinical symptoms,

Table 1 Indications to perform contrast-enhanced computed tomography[58]

Types	Indications
Initial imaging	1 When the diagnosis of acute pancreatitis is uncertain
	2 Patients with hyperamylasemia, severe clinical pancreatitis, abdominal distention and tenderness, fever > 102°, and leukocytosis for the detection of complications
	3 Ranson score > 3 or APACHE score > 8
	4 Patients who fail to improve after 72 h of conservative medical therapy
	5 Acute change in clinical status, such as new fever, pain, and shock after successful initial medical therapy
Followup imaging	1 Acute change in clinical status suggesting complication
	2 7-10 d after presentation if CT severity score is 3-10 at presentation or grade
	3 To determine response to treatment after surgery or interventional radiologic procedures to document response to treatment.
	4 Before discharge of patients with severe acute pancreatitis

time of onset of symptoms and lab findings. However, CECT is the most commonly used modality in the evaluation of acute pancreatitis. In 2010, the ACR committee on appropriateness criteria and its expert panels have developed guidelines for determining the most appropriate imaging examinations for the diagnosis and treatment of acute pancreatitis and have given high score ratings[8,9] to CECT in different clinical scenarios. This is based on it is wide availability and high degree of accuracy. They also stated that MRI appears to offer diagnostic capabilities similar to multi-detector computed tomography (MDCT) with intrinsic advantages including the lack of ionizing radiation and the exquisite soft tissue characterization unmatched by any other imaging modality; allowing better depiction of stones and evaluation of the pancreaticobiliary ductal system.

Ultrasound

Ultrasound is frequently the first investigation performed on admission; although it has little value in the diagnosis of pancreatitis or its complications. Ultrasound is usually reserved to confirm or exclude the presence of stones or biliary dilatation. Early identification and treatment of these calculi may have a significant positive impact on outcome. However, body habitus of patient, operator dependence pose a limitation in detection of distal common bile duct stones accurately compared to CECT or MR imaging[9].

Ultrasound is limited in evaluating the entire pancreatic parenchyma; which is often partially or completely obscured by overlying bowel gas. It can however be helpful in monitoring the evolution of fluid collections, which occur as a result of acute pancreatitis, and in guiding diagnostic and therapeutic interventions.

CECT

CECT can show morphologic characteristic findings that allow for establishing the diagnosis of acute pancreatitis and determining the extent of disease severity. The best time for performing CECT in acute pancreatitis not well established and if performed immediately after the onset of symptoms, the full extent of pancreatic damage and its severity can be easily underestimated[10,11]. Conversely, a CECT obtained more than 5 d after onset of symptoms that reveals a normal aspect of the pancreas or only mild inflammatory changes (fat stranding) surrounding the pancreas virtually excludes a severe form of acute pancreatitis[12].

Not all patients with acute pancreatitis need to undergo contrast-enhanced CT. In general, CT is not indicated in patients who are clinically classified as having mild pancreatitis (no clinical signs of severe pancreatitis) and show rapid improvement with appropriate medical management. CT should be used in patients who are classified as having severe pancreatitis or are at risk of developing severe pancreatitis; ideally after 72 h, to best assess the full extent of the disease[13].

CT should be repeated when the clinical picture drastically changes, such as with sudden onset of fever, decrease in hematocrit or sepsis. CT can also be useful to guide catheter placement for drainage and to assess success of treatment in patients who underwent percutaneous drainage or other interventions.

Furthermore, in patients with their first episode of pancreatitis who are over 40 years of age and have no identifiable cause for pancreatitis, contrast-enhanced CT should be used to exclude a possible neoplasm[13] (Table 1).

The main limiting factors for CECT are ionizing radiation, use of iodinated contrast material; especially in patients in with renal failure or contrast allergy and moderate sensitivity in identifying gallstones and biliary stones[14,15]. The above limiting factors can be overcome by using MRI; which does not use ionizing radiation; allowing it to be used during pregnancy, in patients with recurrent pancreatitis and for patients requiring multiple follow-up exams. Non-enhanced MRI seems to be more accurate and reliable for the early assessment of severity and prognosis of acute pancreatitis than is contrast-enhanced CT[16-18]; thus proving beneficial in patients with renal failure and history of contrast allergy.

MRI

Recent technological developments have dramatically improved the quality of abdominal MRI. Respiration, bowel peristalsis and vascular pulsations are major sources for artifacts affecting the accuracy and reproducibility of MRI. Breathing-independent sequences and respira-

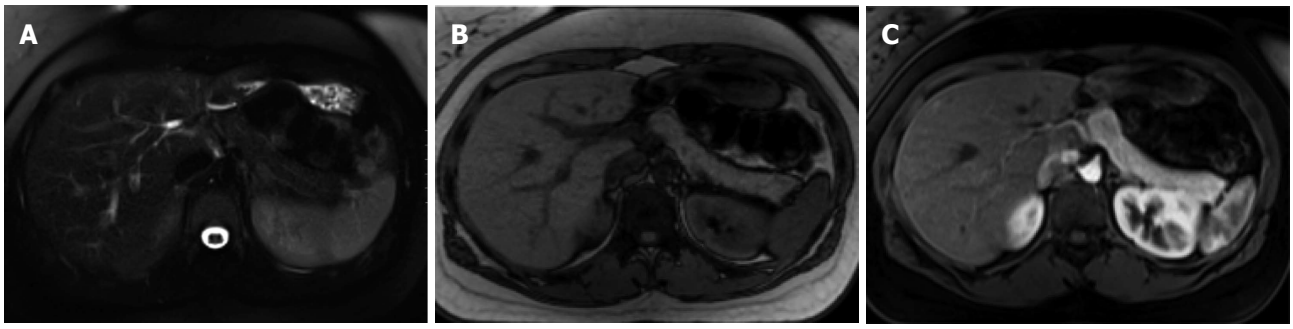

Figure 1 Normal pancreatic appearance on magnetic resonance imaging. A: Axial T2-weighted image with fat-suppression; B: Axial GRE out-of-phase T1-weighted image; C: Axial post-contrast 3D-GRE T1-weighted image with fat-suppression during the late arterial phase. The pancreas demonstrates low T2 signal intensity (A) and high T1 signal intensity on pre-contrast images (B), reflecting high protein content of the exocrine gland. The pancreas demonstrates avid homogenous enhancement on immediate post-contrast images (C), reflecting a normal capillary blush.

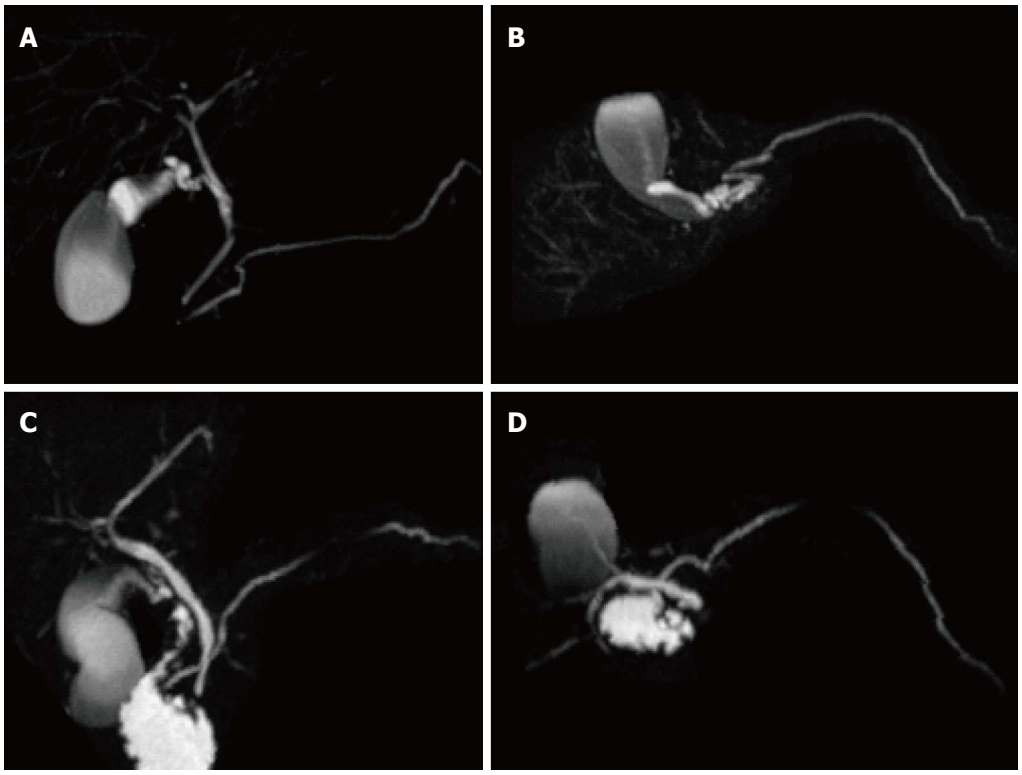

Figure 2 Normal pancreatic duct anatomy and pancreatic divisum. (A and C) Coronal and (B and D) axial post-processed maximum intensity projection 3D-MRCP images from two different patients. In the first patient, the main pancreatic duct courses inferiorly (A) and posteriorly (B), joins the CBD and opens in the major papilla in keeping with normal pancreatic duct anatomy. In the second patient, the main pancreatic duct continues its course superiorly (C) and anteriorly (D), crosses the CBD and opens in the minor papilla in keeping with pancreatic divisum.

tory gating techniques form the foundation of high-quality abdominopelvic MRI. New motion-robust MRI techniques provide promising results even in detection and characterization of pancreatic disease in patients that are not able to cooperate with breath-hold instructions[19].

A variety of pulse sequences are currently used for abdominal MRI including T1- and T2-weighted sequences with or without fat-suppression and post-gadolinium T1-weighted sequences (Figure 1). MR Cholangiopancreatography (MRCP) is routinely added to abdominal protocols to assess ductal obstruction, dilatation or course[20-22] (Figure 2); providing comprehensive evaluation of full range of pancreatic diseases. Due to the increasing incidence of acute pancreatitis due to gallstones in the United States, it is more beneficial to consider MRCP as an initial diagnostic study.

MRI is sensitive for detection of subtle changes of acute pancreatitis; particularly minor peripancreatic inflammatory changes; even in the setting of a morphologically normal pancreas on CT imaging; which may appear normal in up to 15%-30% of patients with clinical features of acute pancreatitis[23]. The sensitivity of MRI exceeds that of CT imaging, emphasizing its role in

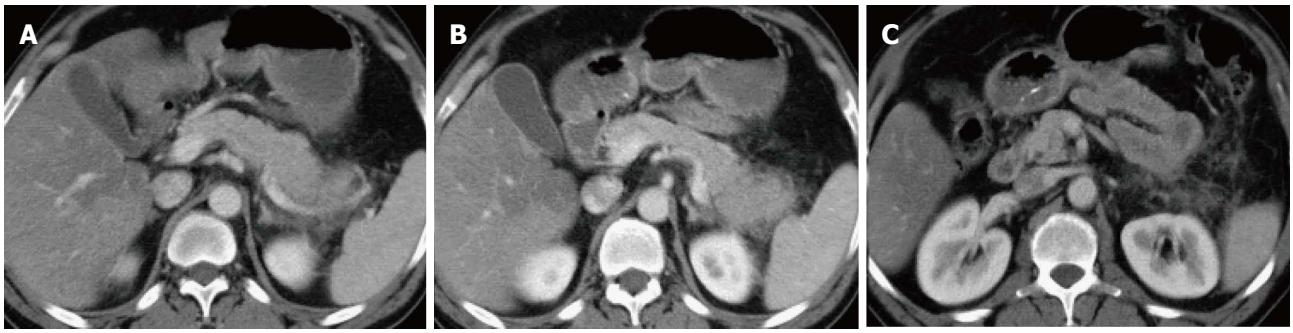

Figure 3 Focal acute edematous pancreatic tail pancreatitis. A-C: Axial CT scan of the pancreas during the late arterial phase. There is evidence of ill-definition and reduced enhancement of the pancreatic tail (A and B), associated with mild peripancreatic fatty stranding extending to the anterior left perinephric space in keeping with focal acute edematous pancreatitis.

the evaluation of patients with clinically suspected acute pancreatitis and negative CT imaging findings.

It should be emphasized that MRI is a non-ionizing cross sectional imaging method and has a safer intravenous contrast profile in comparison to CT. This is particularly important in radiosensitive populations and those requiring repeated imaging follow up. Additionally, patients who present with acute pancreatitis often have a degree of renal impairment.

The factors that make CECT the most frequently applied imaging approach in pancreatitis are related to its universal availability (especially near the emergency room), faster scanning times, and relatively easier interpretability of CT images by physicians and general radiologists. For early presentation of acute pancreatitis, CT might be the preferred method for the reasons stated above. However, the adequate diagnostic performance of MRI along with the mentioned additive advantages favors MRI as the preferred method.

Endoscopic ultrasound

Endoscopic ultrasound (EUS) has shown great utility in providing high-resolution images of the pancreatic duct and parenchyma as well as extra hepatic biliary system; as the probe can be positioned in close proximity to the pancreas. Furthermore, EUS has become an invaluable technique for its ability to obtain targeted biopsies of lesions in and around the pancreas; thus, playing a prominent role in evaluating patients with atypical findings on other imaging studies.

The disadvantages of EUS are the requirement of monitored anesthesia care, need for expert endo-sonographer, modality operator dependence, and interobserver variability.

According to ACR appropriateness criteria, the role of endoscopic US in the evaluation of acute pancreatitis is primarily reserved for assessing and/or confirming choledocolithiasis and subsequent stone removal, as well as for identifying anatomic abnormalities (*e.g.*, pancreas divisum or malignancy) that can lead to acute pancreatitis. However, it has been recently proposed to use EUS in acute pancreatitis, as it is was found to contribute for the detection of causes like cancer, microlithiasis and chronic pancreatitis[24].

IMAGING-BASED MORPHOLOGIC TYPES OF ACUTE PANCREATITIS

Interstitial edematous pancreatitis

Interstitial edematous pancreatitis (IEP) is a milder form of acute pancreatitis that usually resolves over the first week. IEP is characterized by diffuse or localized enlargement of the pancreas secondary to interstitial or inflammatory edema without necrosis.

On CECT, findings include enlarged pancreas with relatively normal enhancement. Peripancreatic fat may be normal or show mild stranding and ground glass opacity due to inflammation, with small to varying amounts of non-enhancing peripancreatic fluid (Figure 3). The characteristic CECT finding that distinguishes IEP is absence of pancreatic parenchymal and peripancreatic necrosis.

On MRI, the signal intensity characteristics of the pancreas in IEP resemble those of normal pancreatic tissue. Enlargement of the pancreas, parenchymal edema and fat stranding are well demonstrated on T1-weighted images (Figure 4). T1-weighted imaging with fat suppression improves the delineation of the pancreas and pancreatic borders[25]. The pancreas demonstrates high signal intensity on pre-contrast fat suppressed T1-weighted images and enhances uniformly on immediate post-gadolinium images, reflecting a normal capillary blush. Fat suppressed T2-weighted sequences are very sensitive for detecting edema or minimal fluid and therefore have a role in detecting even milder forms of pancreatitis[26](Figure 5).

Necrotizing pancreatitis

Necrotizing pancreatitis is the inflammation of the pancreas with obvious pancreatic and peripancreatic tissue necrosis. About 5%-10% of patients develop necrosis; affecting the pancreatic parenchyma in 5%, peripancreatic tissue in 20% and both in 70%. Pancreatic parenchymal necrosis carries a worse prognosis than peripancreatic necrosis[27].

Atlanta classification defines necrotizing pancreatitis as being associated with more than 30% parenchymal necrosis. The presence of less than 30% necrosis demands

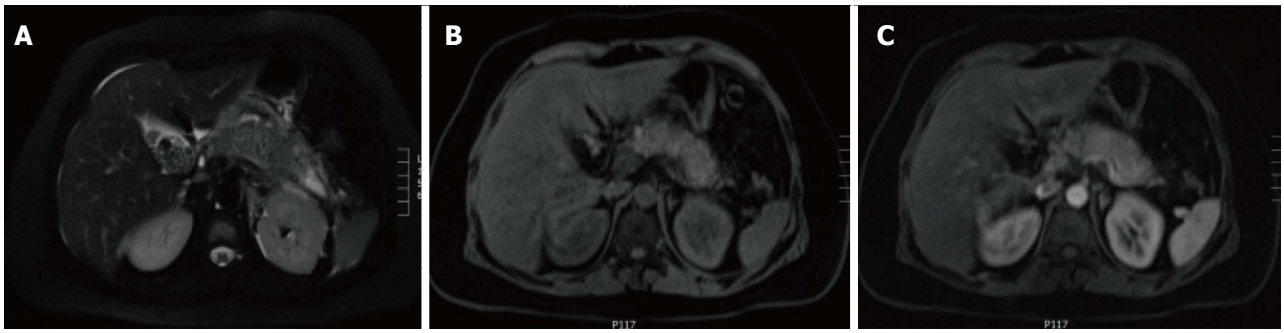

Figure 4 Gallstone acute edematous pancreatic tail pancreatitis. A: Axial fast spin-echo (FSE) T2-weighted image with fat-suppression; B and C: Post-contrast 3D-GRE T1-weighted images with fat-suppression during the late arterial and portal venous phases. There is mild diffuse lace-like increased T2 signal involving the pancreatic parenchyma, associated with a small amount of peripancreatic fluid near the pancreatic tail (A). The pancreas demonstrates diffuse minimal decrease in T1 signal intensity (B) and minimally reduced enhancement on the late arterial phase (C) in keeping with diffuse edematous pancreatitis. There are also innumerable gallstones (A).

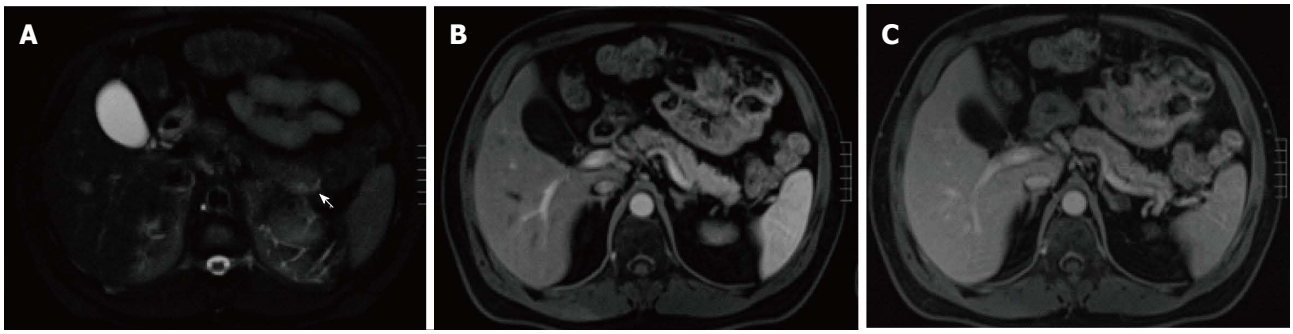

Figure 5 Subtle focal acute edematous pancreatic tail pancreatitis. A: Axial T2 weighted-image with fat-suppression; B and C: Axial post-contrast 3D-GRE T1-weighted images with fat-suppression during the late arterial and portal venous phases. There is a very subtle area of increased T2 signal seen around the pancreatic tail (arrow, A), with fairly normal enhancement of the pancreas on the post-contrast images (B and C) in keeping with subtle focal acute edematous pancreatic tail pancreatitis.

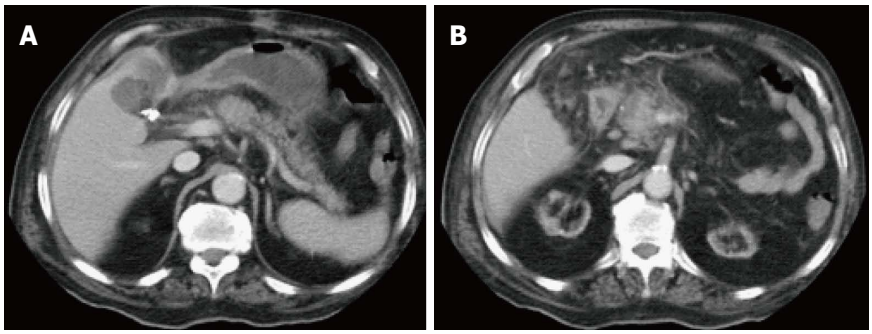

Figure 6 Focal pancreatic head necrotizing pancreatitis confined to the pancreatic parenchyma. A and B: Axial CT scan during the portal venous phase. There is evidence of significantly reduced enhancement of the pancreatic head (B), without peripancreatic extension or necrosis in keeping with focal acute necrotizing pancreatitis.

follow-up scanning in 1 wk to confirm true necrosis *vs* IEP[27].

On CECT, findings include areas of compromised pancreatic parenchymal enhancement on the post-Gadolinium images with or without peripancreatic inhomogeneous fluid collections (Figures 6 and 7). The impairment of pancreatic perfusion and signs of peripancreatic necrosis evolve over several days[28], which explains why an early CECT may underestimate the eventual extent of pancreatic and peripancreatic necrosis.

On MRI, necrosis shows appears as hypointense areas on T1-weighted images corresponding to areas of increased signal on fat-suppressed T2 weighted-images, associated with well defined areas of non-enhancing pancreatic parenchyma on post-Gadolinium sequences[29-31] (Figure 8).

For both CT and MRI, acquisition of an adequate arterial phase is of the utmost importance; as the maximum enhancement of pancreas is reached on the late arterial phase, and higher difference in signal between

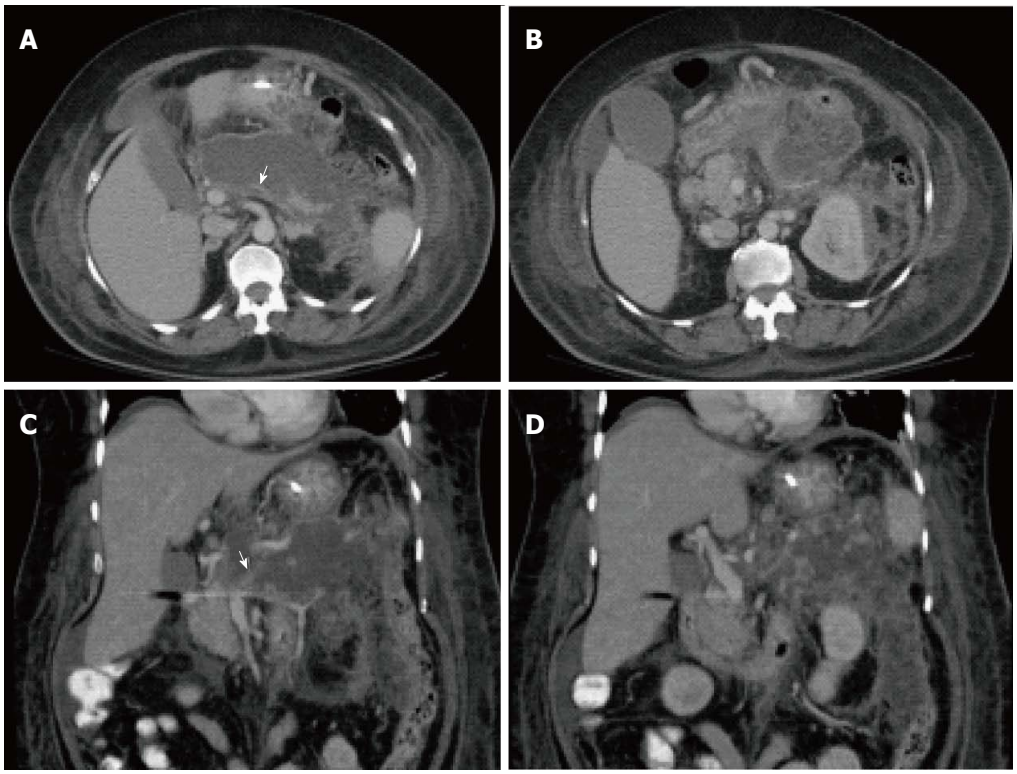

Figure 7 Severe acute necrotizing pancreatitis and peri pancreatitis. A-B: Axial CT scan during the late arterial phase; C-D: Coronal reformatted CT images. There is evidence of lack of arterial enhancement involving the pancreatic body and tail, which are replaced by necrotic tissue, associated with heterogenous peripancreatic tissue inflammation and necrosis extending to left perinephric space (A-B) and paracolic gutter (C-D), in keeping with severe necrotizing pancreatitis and peripancreatitis. There is also evidence of splenic vein thrombosis (arrow, A, C), a known complication of acute pancreatitis.

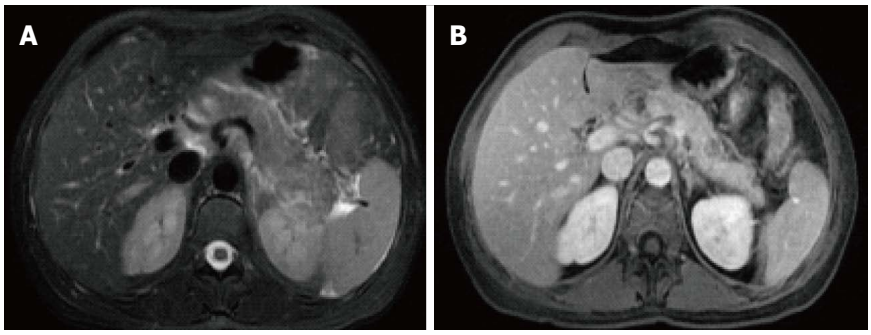

Figure 8 Focal acute necrotizing pancreatitis. A: Axial fast spin-echo T2- weighted image with fat-suppression; B: Axial post-contrast 3D-GRE T1- weighted images with fat-suppression during the venous phase. There is a focal area of low T2 signal involving the proximal part of the pancreatic tail, associated with minimal peripancreatic fat stranding (A). This focal area demonstrates significantly reduced enhancement on the post-contrast images, in keeping with focal necrotizing pancreatitis.

viable and necrotic is achieved on this phase.

Pancreatic duct disruption is an important prognostic factor. It is seen in 30% of the patients of necrotizing pancreatitis[32] when necrosis involves the central gland[33,34]. Drake *et al*[35] study showed that MRCP, a noninvasive imaging method, achieved 95% accuracy in detecting pancreatic duct disruption; thus helping in identifying patients who might benefit from early treatment.

Definition of pancreatic and peripancreatic collections

An important distinction is made between collections that are composed of fluid alone and those that arise from necrosis and contain a solid component (and which may also contain varying amounts of fluid). Below, we define and illustrate the following terms: acute peripancreatic fluid collection; occurring in interstitial edematous pancreatitis, pancreatic pseudocyst as a delayed (usually after 4 wk) complication of interstitial edematous pancreatitis and necrosis; which may be an acute necrotic collection (in the early phase and before demarcation) or walled-off necrosis surrounded by an identifiable capsule on imaging (rarely develops before 4 wk).

Acute peripancreatic fluid collections

Fluid collections less than 4 wk in IEP lacking a discrete

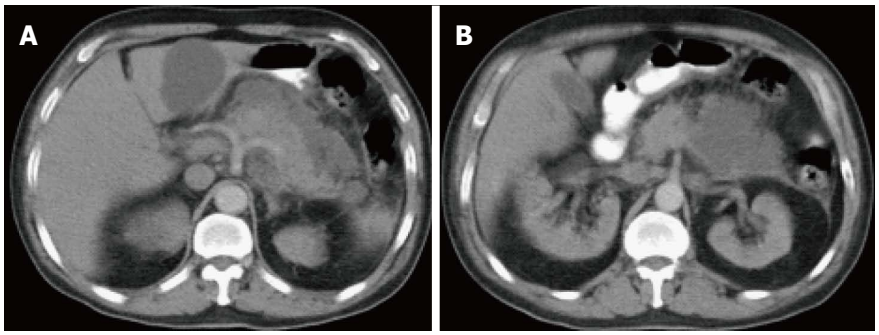

Figure 9 Acute interstitial edematous pancreatitis and acute peripancreatic fluid collections. A-B: Axial CT scan during the portal venous phase. The pancreas is mildly thickened and demonstrates mildly heterogenous enhancement, reflective of edema, in keeping with acute interstitial edematous pancreatitis. There is a peripancreatic fluid with imperceptible wall in keeping with acute peripancreatic fluid collections.

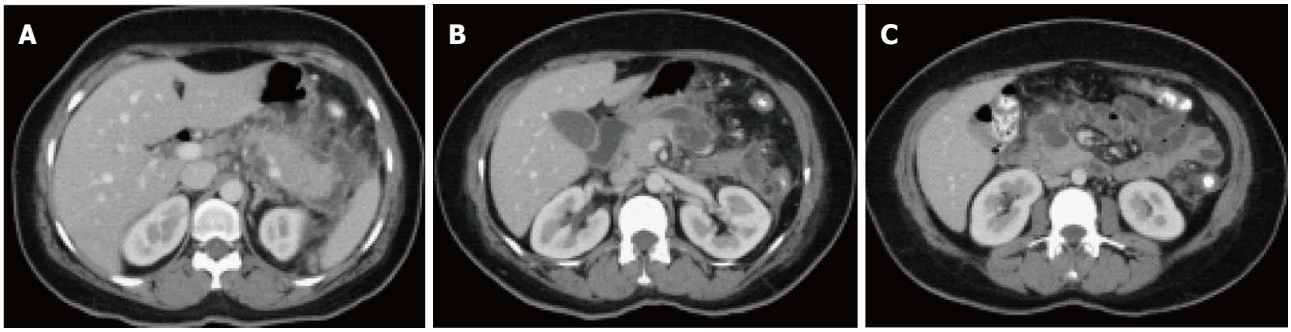

Figure 10 Peripancreatic fluid secondary to multifocal acute necrotizing pancreatitis. A-C: Axial CT images during the late arterial phase. There are two areas of focal necrosis involving the pancreatic body (B) and pancreatic head/uncinate process (C), associated with loculated peripancreatic fluid collection (A).

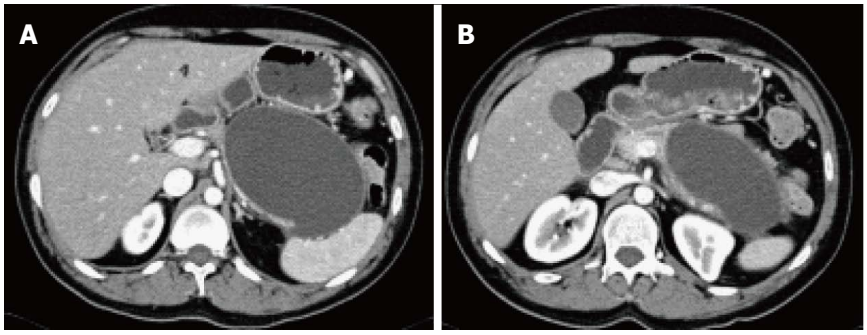

Figure 11 Large pancreatic pseudocyst. A-B: Axial CT scan during the late arterial phase. There is a large oval shaped pancreatic pseudocyst located anterior to the pancreatic body and tail, associated with mass effect on the thinned out pancreatic tissue in keeping with a large pancreatic pseudocyst.

wall, with no internal solid components in the peripancreatic region are called acute peripancreatic fluid collections (APFC). Approximately 50% of APFC's develop within 48 h following the onset of acute pancreatitis[36].

On CT scan, they appear as homogenous collections with low attenuation. They do not have well-defined walls and are confined by normal fascial planes in the retroperitoneum (Figure 9). They can be single or multiple (Figure 10). Most acute fluid collections remain sterile and usually resolve spontaneously without intervention[30].

On MRI, T2-weighted sequences are very sensitive in detecting peripancreatic fluid; which demonstrate high T2 signal intensity. On T1-weighted gradient echo images, APFC's demonstrate low signal intensity in a background of high signal intensity fat. No perceptible enhancement is depicted on post-gadolinium fat-suppressed T1-weighted images. The majority of fluid collections are typically confined to the lesser sac and anterior pararenal space or may track down to the pelvis and superiorly into mediastinum[29]. These collections are usually sterile and are spontaneously reabsorbed.

Pancreatic pseudocysts

Peripancreatic fluid collections that persist more than 4 wk in IEP, with a well-defined wall and no internal solid components in the peripancreatic region are called pseudocysts.

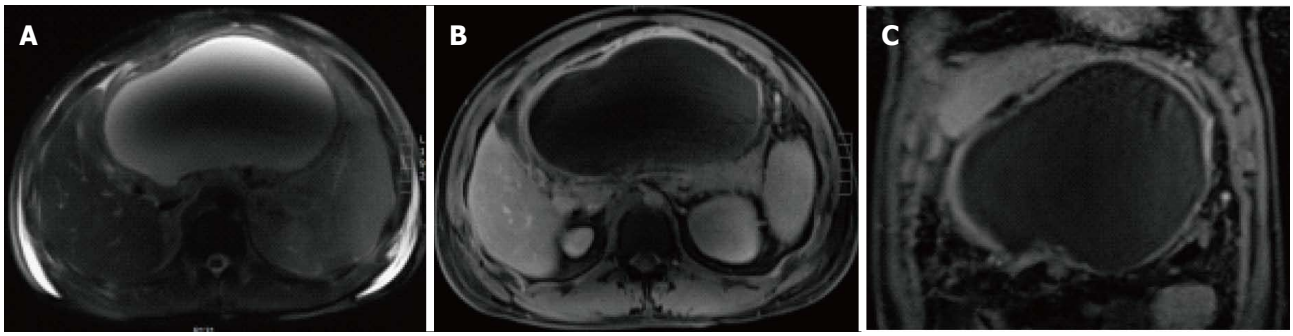

Figure 12 Large pancreatic pseudocyst. A: Axial fast spin-echo T2- weighted image with fat-suppression; B-C: Axial and coronal post-contrast 3D- GRE T1-weighted images with fat-suppression during the portal venous phase. There is a very large thin-walled cyst (A) within the lesser sac; which demonstrates mild uniform wall enhancement (B-C) in keeping with a large pancreatic pseudocyst. The central drop of signal on (A) is related to dielectric shading artifact.

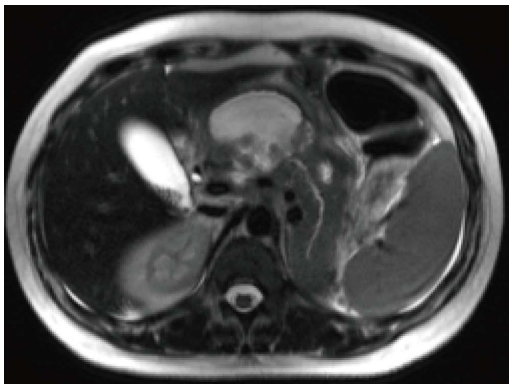

Figure 13 Acute necrotic collection. Axial T2 single-shot turbo spin-echo image. There is a well-defined fluid collection involving the pancreatic neck with peripancreatic extension and communication with the main pancreatic duct. This collection demonstrates well-defined outlines and heterogenous low T2 signal intensity debris within it in keeping with acute necrotic collection. Multiple gallstones are also noted.

On CECT, they appear as homogenous collections of low-attenuation surrounded by a uniform enhancing capsule (Figure 11). Typically, an increase of enhancement is observed in the interstitial phase; reflecting the presence of granulation tissue.

On MRI, pseudocysts demonstrate low in signal intensity on and T1-weighted gradient-echo images and relatively homogeneous high signal intensity on T2-weighted images. Pseudocysts walls enhance minimally on early post-gadolinium images and show progressively intense enhancement on 5-min post-gadolinium images; due to the presence of fibrous tissue (Figure 12).

Pseudocysts may sometime have communication with pancreatic duct and detecting this communication is helpful in the further patients' management. MRCP; a noninvasive imaging modality has an advantage of demonstrating possible communication between pancreatic pseudocyst and pancreatic duct.

The majority of pseudocysts resolve spontaneously. Infection and hemorrhage may complicate simple pseudocysts. Infected pseudocyst may contain gas bubbles on CT. However, absence of these findings on CT may further require confirmation by fine needle aspiration, when there is a strong clinical suspicion.

Acute necrotic collections

During the first 4 wk, a collection containing variable amounts of fluid and necrotic tissue is termed an acute necrotic collection (ANC). Unlike APFCs, ANCs are present within the pancreas and peripancreatic regions. ANC's may often maintain communication with the main pancreatic duct or one of its side-branches; for which, MRI can be useful in delineating this connection.

On CECT, ANC's demonstrate heterogeneous attenuation variably higher that of thin fluid (Figure 7). Follow-up imaging may be useful to characterize acute collections. CECT often shows ANC's as a homogenous non-enhancing area during the first week of necrotizing pancreatitis; making it difficult to be differentiated from APFC's. MRI may be helpful to confirm the presence of solid content in the collection.

On MRI, the necrotic debris may appear as irregularly shaped regions of low signal intensity within the necrotic collections. Breathing-independent T2-weighted sequences such as single-shot echo-train spin echo are useful to evaluate these necrotic collections (Figure 13); not only because of their high sensitivity in demonstrating the complexity of fluid, but also because many of these patients are very debilitated and are unable to co-operate with breath-holding instructions.

An advantage of MRI relative to MDCT in the evaluation of peripancreatic fluid collections is easier appreciation of solid debris with MRI[37]. The sensitivity and specificity of MRI in detecting solid debris of necrosis is 100% when compared to CT; which has a sensitivity of 25% and a specificity of 100%[38]. MRI can help in differentiating fluid collections secondary to pancreatitis from other cystic neoplasms.

Walled-off necrosis

After 4 wk, APFC's mature and develop thick non-epithelialized wall; acquiring the term walled-off necrosis (WON). They commonly occur in the pancreatic body and tail. Management for WON is different from pseudocyst as it contains non-liquefied debris; which needs to be surgically removed. Previously suggested nomenclature for this entity includes: organized pancreatic necrosis, pancreatic sequestration, pseudocyst associated

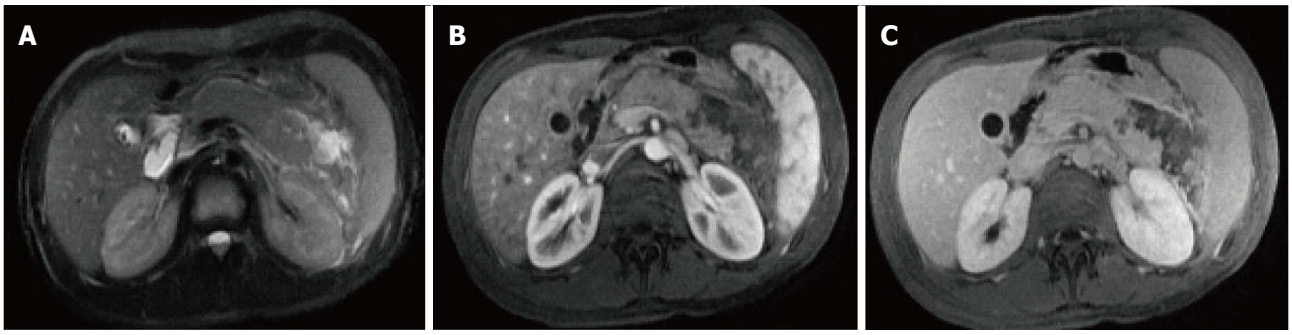

Figure 14 Necrotizing pancreatitis, with peripancreatic walled-off necrosis. A: Axial fast spin-echo T2-weighted image with fat-suppression; B-C: Axial post-contrast 3D-GRE T1-weighted images with fat-suppression during the late arterial and venous phase. There is a focal area of heterogeneous iso to slightly high T2 signal involving the pancreatic body-tail junction (A); which demonstrates lack of enhancement on the post-contrast images (B-C). There is associated sizable peripancreatic fluid collection; which demonstrates heterogeneous T2 signal intensity and thick enhancing wall post-contrast in keeping with walled-off necrosis.

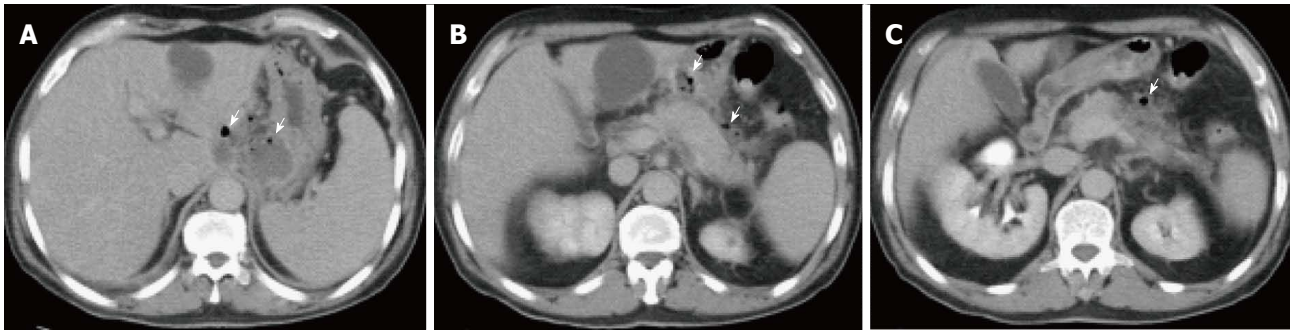

Figure 15 Infected peripancreatic fluid in a patient with acute pancreatitis. A-C: Axial CT scan during the portal venous phase. There are a few gas bubbles seen within a small peripancreatic fluid (arrows, A-C). In the absence of any intervention in keeping with infected peripancreatic fluid.

with necrosis and subacute pancreatic necrosis.

On CECT, walled-off necrosis demonstrates a heterogeneous fluid and non-fluid attenuation with varying degree of loculations surrounded by a well-defined and enhancing encapsulating wall; which may involve both the pancreatic and extrapancreatic tissue. CECT, however, may not readily distinguish solid from fluid contents; as a result, pancreatic and peripancreatic necrosis may be misdiagnosed as a pancreatic pseudocyst. For this purpose, MRI may be required for this distinction (Figure 14).

Infected pancreatic necrosis

Pancreatic and peripancreatic necrosis can remain sterile or become infected. The development of secondary infection in pancreatic necrosis is associated with increased morbidity and mortality[3]. Most studies suggest that there is no absolute correlation between the extent of necrosis and the risk of infection and duration of symptoms[7]. The early diagnosis of infected pancreatic necrosis is very important in the initiation of antibiotic therapy.

The diagnosis of infected ANC or WON can be suspected in the presence of extraluminal gas on CT or MRI. This extraluminal gas is present in areas of necrosis and may or may not form a gas/fluid level depending on the amount of fluid content present at that stage of the disease (Figure 15). The diagnosis may be confirmed by aspiration and analysis including microscopy and culture.

SEVERITY OF ACUTE PANCREATITIS

Clinical vs MCTSI vs MRSI severity index

Several clinical scoring systems like Marshal, SOFA. APACHE or Ranson criteria were designed to accurately correlate the complications like organ failure and mortality in acute pancreatitis. In the last two decades, radiological scoring systems were developed to accurately diagnose and correlate complications in acute pancreatitis.

For the first time in 1990, Balthazar *et al*[28] introduced the CT severity index for assessment of AP; which correlated well with morbidity, mortality and length of hospital stay. CTSI was widely adopted in clinical and research settings; however, a potential limitation was its inability to detect pancreatic necrosis. MCTSI introduced by Mortele *et al*[39] in 2004 to account for the limitations of CTSI (Table 2); which showed improved correlation with severity.

MCTSI incorporated extrapancreatic manifestations and simplified the evaluation of extent of parenchymal necrosis by categorizing into none, less than 30% or more than 30%; in addition to evaluating peripancreatic inflammation by detecting the presence or absence of

Table 2 MCTSI scoring ystem[39]

Prognostic Indicators	Characteristics	MCTSI[1]
Pancreatic inflammation	Normal pancreas	0
	Pancreatic ± peripancreatic inflammatory changes	2
	One or more collection or peripancreatic fat necrosis	4
Pancreatic necrosis	No necrosis	0
	< 30%	2
	> 30%	4
Extrapancreatic complications (pleural effusions, ascites, vascular, gastrointestinal, *etc.*)		2

[1]Scores ⩾ 5 are associated with higher morbidity and mortality.

Table 3 MR severity index scoring system[69]

Prognostic Indicators	Characteristics	MRSI
Pancreatic inflammation	Normal pancreas	0
	Focal or diffuse enlargement of the pancreas	1
	Intrinsic pancreatic abnormalities with inflammatory changes in the peripancreatic fat	2
	Single, poorly defined fluid collection	3
	Two or more poorly defined collection or presence of gas in or adjacent to the pancreas	4
Pancreatic necrosis	No necrosis	0
	< 30%	2
	30%-50%	4
	> 50%	6

peripancreatic fluid. Predictive accuracy of CT scoring systems for severity of AP and comparisons between CTSI and MCTSI were made[40]. They reported that they could not detect any significant differences between CTSI and MCTSI in evaluating the severity of AP. Their study also demonstrated that compared with APACHE II, both CT indexes more accurately diagnosed clinically severe disease and correlated better with the need for intervention and pancreatic infection.

It has been reported that MR severity index (MRSI) significantly correlated with CTSI (Table 3), Ranson score, C-reactive protein levels, appearance of systemic complications, duration of hospitalization and clinical outcome[17,41].

Chronic pancreatitis

Chronic pancreatitis is defined pathologically by continuous or relapsing inflammation of the organ leading to irreversible morphologic injury and typically leading to permanent impairment of both exocrine and endocrine functions. The incidence of chronic pancreatitis ranges from 5-12 per 100000 people in industrialized countries[1].

Table 4 Imaging criteria for chronic pancreatitis[70]

	CT criteria	MRI/S-MRCP criteria
Moderate chronic pancreatitis	⩾ 2 of the following:	Moderate pancreatogram changes
	Main duct enlarged (2-4 mm)	Main duct abnormal and
	Slight gland enlargement (up to 2 × normal)	Abnormal side branches, > 3
	Heterogeneous parenchyma	
	Small cavities (< 10 mm)	
	Irregular ducts	
	Focal acute pancreatitis	
	Increased Density of the main pancreatic duct wall	
	Irregular head/body contour	
Marked chronic pancreatitis	with ⩾ 1 of the following	Main duct abnormal and
	Large cavities (> 10 mm)	Abnormal side branches, > 3
	Gross gland enlargement (2 × normal)	Plus one or more of the following:
	Intraductal filling defects or pancreatic calculi	Large cavity
	Duct obstruction, stricture, or gross irregularity	Obstruction
	Contiguous organ invasion	Filling defects
		Severe dilatation or irregularity

Chronic pancreatitis is a cause of abdominal pain, weight loss, steatorrhea and diabetes mellitus, which may occur as a consequence of multiple factors, including biliary stone disease, alcohol consumption, malignancy, metabolic disorders, and various genetic and environmental insults, including trauma[1].

The histopathological changes in chronic pancreatitis evolve from unevenly distributed fibrosis in early chronic pancreatitis to diffuse fibrosis involving the entire gland in late stages. In advanced disease, large areas of acinar parenchyma are replaced with sclerotic tissue causing atrophy. Ductal irregularities like strictures, dilatation and side branches ectasia occur due to surrounding fibrosis. Other characteristic findings of severe chronic pancreatitis are calcifications and presence of complications like pseudocyst, vascular aneurysms and venous thrombosis.

Role of imaging

Imaging plays a significant role in detecting parenchymal and ductal abnormalities in chronic pancreatitis and helps in differentiating early from advanced phases to a certain extent; which further guides the management of these patients.

Most commonly accepted CT- and MRI-based criteria for diagnosis of chronic pancreatitis are shown in Table 4.

Early chronic pancreatitis

Ultrasound and CT are insensitive in diagnosis of early chronic pancreatitis, as they often show no abnormalities. A recent study showed that parenchymal changes

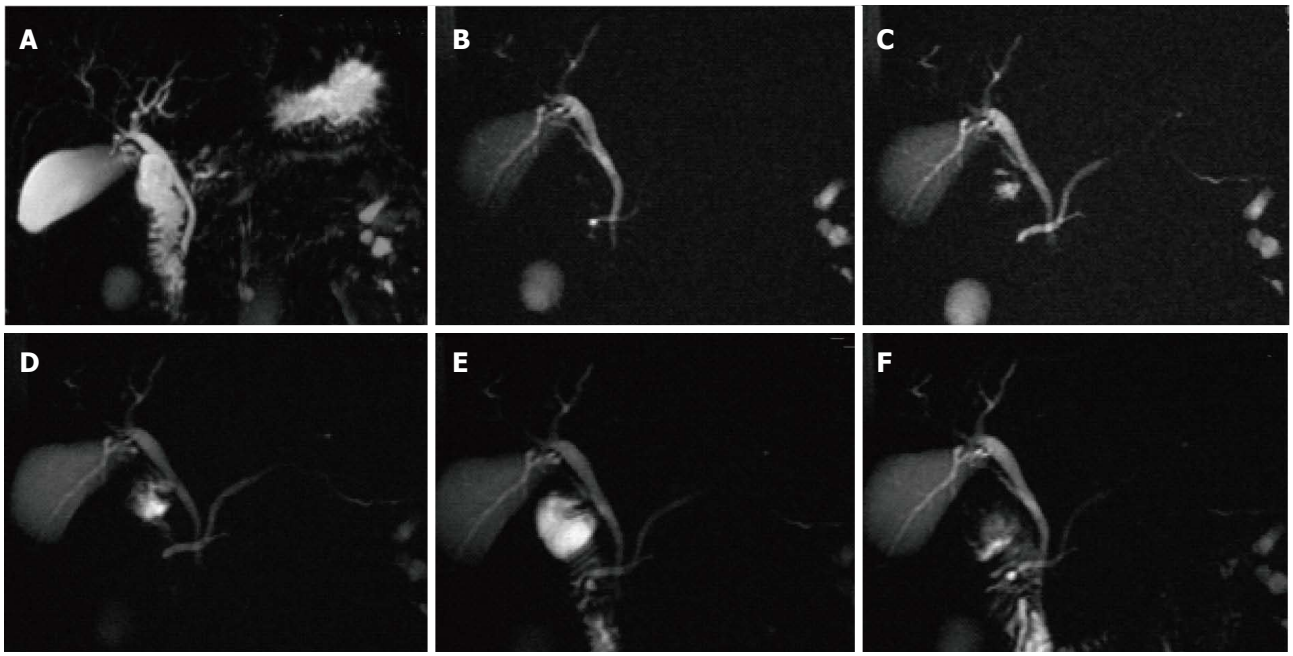

Figure 16 Pancreatic divisum, with a small Santorinicele. A: Coronal 3D- maximum intensity projection MRCP image before administration of secretin; B-F: Selected dynamic secretin thick-slab MRCP images obtained at 30 s (B), 60 s (C), 120 s (D), 4 min (E) and 9 min (F). Prior to administration of secretin, it is difficult to identify the main pancreatic duct (A). After administration of secretin, there is better delineation of the main pancreatic duct (C), with demonstration of pancreatic divisum. There is also enlargement of the accessory pancreatic duct, with demonstration of a small santorinicele (B, F). S-MRCP allows qualitative and quantitative assessment of pancreatic exocrine secretions. In this case, the pancreatic flow output was considered within normal limits; excluding early chronic pancreatitis.

might precede ductal changes in chronic pancreatitis; thus depicting the importance of MRI compared to MRCP in early diagnosis of disease[42].

On MRI, normal pancreas is hyperintense on T1 weighted images and shows uniform enhancement on the late arterial phase (Figure 1). MRI detects not only morphologic characteristics, but also early fibrotic changes. Fibrosis is shown by diminished signal intensity on T1-weighted fat-suppressed images and diminished enhancement on immediate post-Gadolinium gradient-echo images[43]. Low signal intensity on fat-suppressed T1-weighted images reflects loss of the aqueous protein in the acini of the pancreas. Diminished enhancement on capillary phase images reflects disruption of the normal capillary bed and increased chronic inflammation and fibrosis.

MRCP findings in early chronic pancreatitis often demonstrate normal main pancreatic duct with dilated and irregular side duct branches. The limiting factor is the underestimation of ductal size. Some investigators reported that patients with abnormal MR imaging findings but normal MRCP might benefit from dynamic secretin-MRCP (S-MRCP) (Figure 16); which may reveal ductal abnormalities due to improved visualization otherwise not detected on MRCP[42]. Secretin-MRCP has been reported to show ductal changes, like dilatations and strictures in early chronic pancreatitis.

EUS has a prominent role in chronic pancreatitis for its ability to detect early morphologic changes. Endoscopic retrograde cholangiopancreatography (ERCP) is considered to be gold standard test in detecting early changes, but unlike ERCP, EUS is relatively a non-invasive procedure, and also helps in the evaluation of both pancreatic duct and parenchymal changes compared to ERCP that has limitation in evaluating pancreatic side branches and parenchyma[44]. Chong *et al*[45] showed sensitivity of 83% and specificity of 80% of EUS for the diagnosis of chronic pancreatitis.

Late chronic pancreatitis

CT is reported to be 60% to 95% sensitive in diagnosing advanced disease as it can readily detect parenchymal changes associated with advanced chronic pancreatitis[46]. Most common findings on CT include dilatation of main pancreatic duct and its side branches; which can be seen in 68% of patients. The ductal contour may be smooth, beaded or irregular[47].

Other findings include intraductal calcifications, which is the most specific finding and is seen in nearly half of the patients with chronic pancreatitis and parenchymal atrophy (Figure 17). However, parenchymal atrophy is neither specific nor sensitive as it seen normally with aging. Intraductal or parenchymal calcifications are usually seen with alcohol related chronic pancreatitis but not on chronic pancreatitis resultant from other causes.

All patients with late or advanced chronic pancreatitis show diminished signal intensity of the pancreas on T1-weighted fat-suppressed images, an abnormally low percentage of contrast enhancement on immediate post-contrast images, and progressive parenchymal enhancement on the 5-min delayed post-contrast images; reflecting the pattern of enhancement of fibrous tissue.

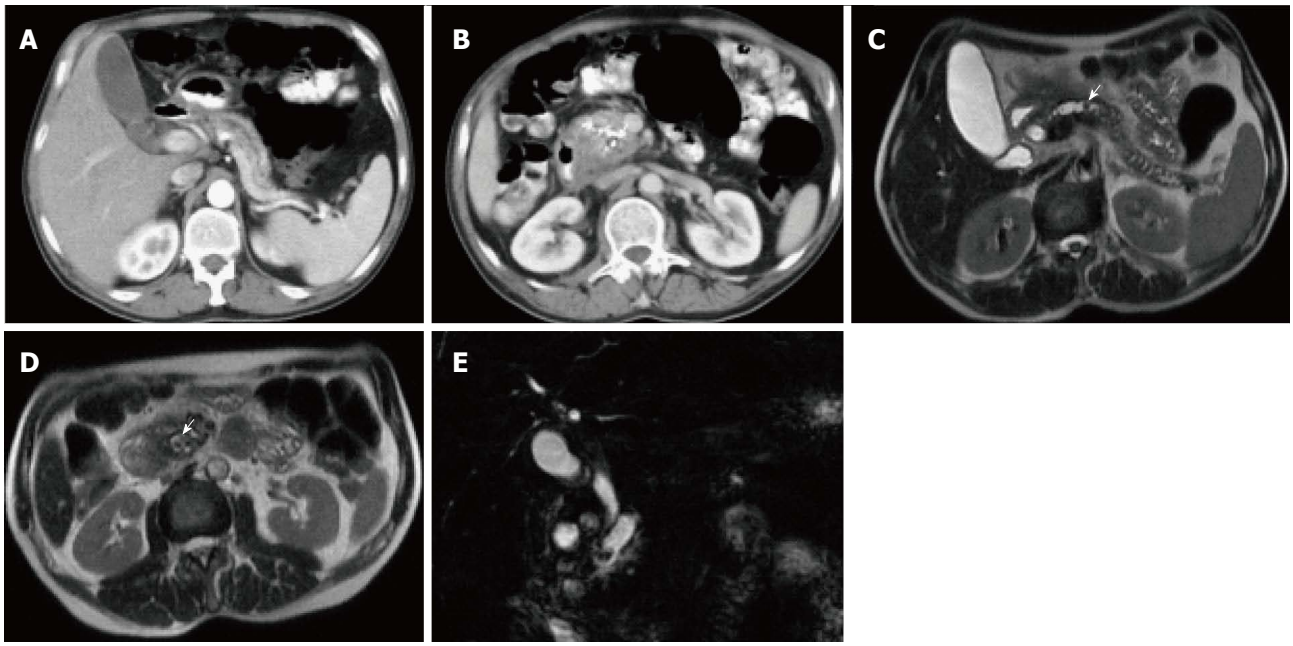

Figure 17 Chronic pancreatitis with pancreatic parenchymal calcifications and pancreatic duct stones. A, B: Axial CT scan during the late arterial phase; C, D: Axial T2 single-shot fast spin-echo images; E: Coronal 3D- Cholangiopancreatogram (MRCP) image. CT shows a markedly dilated and tortuous main pancreatic duct (MPD) (A, B), with foci of thick calcification involving the pancreatic head and uncinate process parenchyma (B). Large the proximal MPD stone was suspected on CT (arrow, B). MRCP shows gross pancreatic ductal dilatation with confirmation of the distal intraductal calculus (arrow, D), and shows an additional mid-pancreatic duct stone, not clearly seen on CT (arrow, C). No pancreatic masses or ductal anomalies are identified.

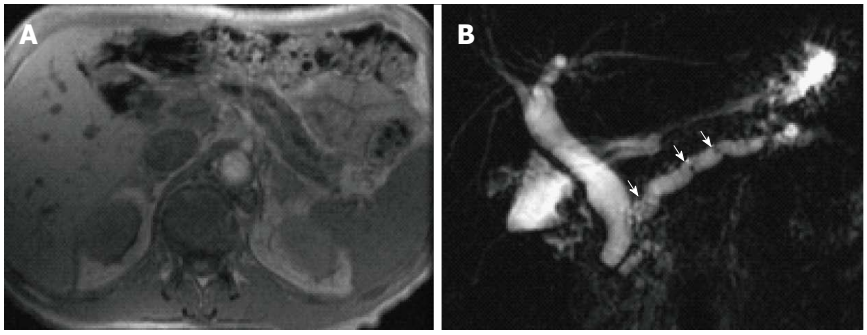

Figure 18 Chronic pancreatitis. A: Axial T1-weighted GRE MRI. B: Coronal-oblique thick-slab MRCP image. There is evidence of diffuse thinning of the pancreatic parenchyma with uniform dilatation of the pancreatic duct and prominence of the pancreatic duct side-branches (A-B), associated with multiple tiny stones at the proximal pancreatic duct (arrows, B) in keeping with chronic pancreatitis. There is also mild uniform dilatation of the CBD, which tapers down to the level of the pancreatic duct (B).

MRCP in advanced phase demonstrates dilatation of the main pancreatic duct with ectasia of the side branches (Figure 18); giving chain of lakes appearance manifested as pancreatic ductal strictures, irregularities and intraductal calculi, appearing as hypointense filling defects.

Enlarged pancreatic head in chronic pancreatitis vs adenocarcinoma

Chronic pancreatitis may involve only the pancreatic head in 30% of patients, resulting in focally enlarged pancreatic head. In these cases, the focus of chronic pancreatitis can simulate the appearance of pancreatic ductal adenocarcinoma.

Both chronic pancreatitis and adenocarcinoma show similar imaging characteristics on CT and MRI due to abundant fibrosis and ductal obstruction; therefore, making the differentiation between these two entities very difficult. Both are generally seen as hypodense lesions on CT, mildly hypointense on T1-weighted images and heterogeneously mildly hyperintense signal on T2-weighted images. However, certain imaging characteristics are helpful in distinguishing enlarged pancreatic head in chronic pancreatitis from adenocarcinoma (Table 5).

Rarely, chronic pancreatitis may involve only the focally enlarged portion of the pancreas, with the reminder of the pancreas having no inflammatory changes. In these cases, the focus of chronic pancreatitis can also simulate the appearance of pancreatic ductal adenocarcinoma. The inflammatory process may also be sufficiently

Table 5 Differentiating imaging features between chronic pancreatitis and pancreatic adenocarcinoma

Chronic pancreatitis	Pancreatic adenocarcinoma
Preserved glandular, feathery or marbled texture similar to that of the remaining pancreas	Definable, circumscribed mass lesion is most often diagnostic for tumor, which disrupts the underlying architecture and results in loss of anatomic detail
Heterogeneous pancreatic enhancement with presence of signal void (cysts and calcifications) on immediate post-gadolinium images	Irregular, heterogeneous, diminished enhancement on postgadolinium images compared to adjacent pancreatic parenchyma
Irregular dilatation of main pancreatic duct with gradual narrowing	Abrupt cut off of the pancreatic duct with significant proximal dilatation +/- presence of double duct sign
Presence of multiple intraductal calcifications (the most specific finding)	Very few ductal calculi compared to chronic pancreatitis
Dilatation of main pancreatic duct with and ectasia of the side branches, giving chain of lakes appearance	Minimal dilatation of side branches
No vascular encasement, significant lymphadenopathy or distant metastasis	Vascular encasement, lymphadenopathy or distant metastasis

destructive that underlying stromal pattern is lost. In these rare cases, diagnosis can only be established by surgical resection and histopathological examination to confirm the absence of malignancy.

Despite the high-resolution images produced by conventional EUS, there are no specific EUS imaging features that can differentiate pancreatic cancer from other common mimics, including lymphoma, focal pancreatitis, neuroendocrine tumors, metastases, and focal AIP[48]. However, one of the strengths of EUS is its ability to allow guided fine needle aspiration (FNA); which may overcome this problem.

In a retrospective analysis by Agarwal *et al*[49], 110 patients with abnormal CT or MRI with an enlarged head of the pancreas or dilated pancreatic duct with or without dilation of the common bile duct underwent EUS or EUS-FNA. The study revealed an accuracy of 99.1% for EUS and/or EUS-FNA in diagnosing pancreatic neoplasm with a sensitivity of 88.8% and specificity of 100%[49]. Given the high accuracy in the evaluation of pancreatic tumors, Eloubeidi *et al*[50] proposed routine EUS-FNA for the differential diagnosis of solid pancreatic masses. Other studies have shown that a negative EUS in ambiguous cases (where a mass is suspected) has a high negative predictive value[51,52].

Positron emission tomography-computed tomography (PET-CT) has an established role in the diagnosis of pancreatic carcinoma, especially when cross sectional imaging or biopsies are equivocal or nondiagnostic. In patients with a suspicion of pancreatic malignancy, a focal increase in ^{18}F-fluorodeoxyglucose (FDG) uptake suggests the diagnosis of malignancy. Nonetheless, the cutoff value of maximum standardized uptake value (SUVmax) is not defined, as it overlaps in benign and malignant pancreatic disease processes[53,54].

Furthermore, FDG-PET's detectability of pancreatic cancer depends on lesion size and degree of FDG uptake and surrounding background uptake. In the setting of chronic pancreatitis, FDG-PET is shown to detect pancreatic adenocarcinoma with a sensitivity of 92% and with a negative predictive value of 87%. In the setting of acute pancreatitis, the specificity can be as low as 50%, as it is known that inflammatory tissue can also demonstrate FDG activity[47].

Complications of chronic pancreatitis

The most common non-neoplastic complications of chronic pancreatitis include pseudocysts, pseudoaneurysms (due to erosion of the arterial wall), splenic vein thrombosis with subsequent development of collaterals, biliary obstruction (due to pseudocysts), and gastrointestinal complications like gastric outlet obstruction or bowel ischemia[6,55]. These complications are well depicted with CT and MRI.

MRI with MRCP may be superior to CT in detecting specific complications like pseudocysts, fistula formation, distal common biliary dilatation and vascular complications associated with higher morbidity and mortality[46].

Special types of chronic pancreatitis autoimmune pancreatitis.

Autoimmune pancreatitis is a distinct form of pancreatitis characterized clinically by obstructive jaundice (with or without pancreatic mass), histologically by a lymphoplasmacytic infiltrate and fibrosis and therapeutically by a dramatic response to steroids[56].

Autoimmune pancreatitis accounts for 2%-6% of chronic pancreatitis[57,58]. It is associated with other autoimmune disorders like Sjogren's syndrome, primary biliary cirrhosis, and primary sclerosing cholangitis[59,60]. Early diagnosis of autoimmune pancreatitis is crucial as it often responds to steroid therapy; thus avoiding complications.

In AIP, affected areas appear enlarged and hypodense on CT. CECT demonstrates diminished enhancement of the involved parenchyma on the late arterial phase and delayed enhancement on the delayed phase (Figure 19). The MR appearance of autoimmune pancreatitis is similar and is characterized by enlarged pancreas with moderately decreased signal intensity on T1-weighted images, mildly high signal intensity on T2-weighted images and delayed post-gadolinium enhancement of the pancreatic

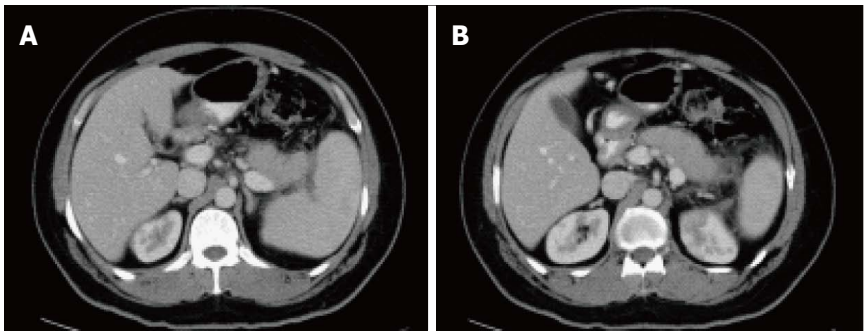

Figure 19 Autoimmune pancreatitis. A, B: Axial CT scan during the late arterial phase. There is evidence of diffuse pancreatic swelling with loss of the normal pancreatic lobulation, obliteration of the pancreatic duct and subtle low attenuating peripancreatic rim (A, B) in keeping with autoimmune pancreatitis. Patient had high IgG4 level (> 0.500 g/L).

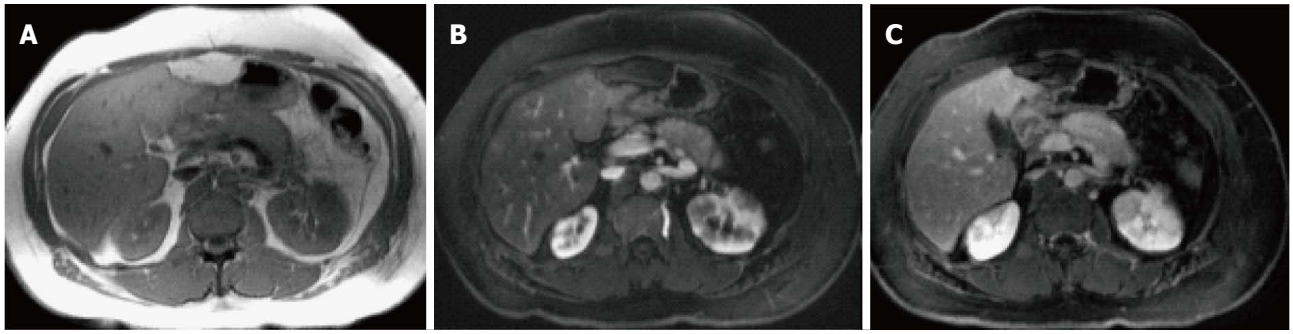

Figure 20 Autoimmune pancreatitis. A: GRE T1-weighted image; B, C: Post- contrast 3D-GRE T1-weighted images with fat-suppression during the late arterial and portal venous phases. There is evidence of diffuse pancreatic swelling with reduced T1 signal, loss of the normal pancreatic lobulation and obliteration of the pancreatic duct, associated with a rim of low T1 signal (A). The pancreas demonstrates diffuse reduced enhancement on the late arterial phase and progression of enhancement on the portal venous phase in keeping with autoimmune pancreatitis. The patient had significant biliary tree irregularities in keeping with primary sclerosing cholangitis (not shown). Additionally, there are a few bilateral wedge-shaped areas of renal hypo- enhancement in keeping with segmental infarcts.

parenchyma (Figure 20). Additional findings that may be observed in autoimmune pancreatitis include: (1) capsule like rim surrounding the diseased parenchyma, that is hypointense on T2-weighted images and may show delayed post-gadolinium enhancement[59]; (2) absence of parenchymal atrophy; (3) ductal dilatation proximal to the site of stenosis; (4) absence of peripancreatic fluid; and (5) clear demarcation of the abnormality[60].

MRCP depicts diffuse or segmental narrowing and irregularity of the main pancreatic duct as characteristic findings. The most commonly involved segment is the intrapancreatic common bile duct, and less frequently multifocal intrahepatic biliary strictures are noted.

Autoimmune pancreatitis is has 3 types based on morphologic patterns: diffuse, focal, and multifocal. Diffuse disease is the most common type. CT and MRI commonly show a swollen, sausage-like pancreas with poorly demonstrated borders and a capsule-like rim of low-density/intensity[61].

The diffuse form of AIP may mimic diffuse disorders like lymphoma, metastases or other diffuse infiltrative processes. In most of these disorders, unlike AIP, the parenchyma is heterogeneous and shows irregular contours.

Focal disease is less common and manifests as a well-defined hypodense mass, often involving the head and mimicking pancreatic adenocarcinoma. In patients who underwent pancreatic resection for suspected malignancy, 2.5%-8% were ultimately diagnosed with AIP without malignancy[58,62]. However, the probability of AIP *vs* pancreatic cancer in patients with obstructive jaundice can be predicted based on CT/MRI findings.

Diffusely enlarged pancreas showing low density mass with enhancement on delayed phases on CT/MRI, especially with a capsule-like rim, and no pancreatic ductal cutoff is highly likely to have AIP. Low-density mass on CECT, pancreatic ductal cutoff in presence or absence of pancreatic atrophy mostly suggests pancreatic cancer.

Groove/paraduodenal pancreatitis

Groove pancreatitis is a rare form of focal chronic pancreatitis involving the anatomic groove between the pancreatic head, duodenum and common bile duct. Groove pancreatitis is categorized into 2 forms: pure, involving exclusively the groove; and segmental, involving the groove and extending in to the pancreatic head[63] (Figure 21).

Pathogenesis remains controversial but may result from obstruction of the accessory pancreatic duct as it drains into the second portion of the duodenum through the

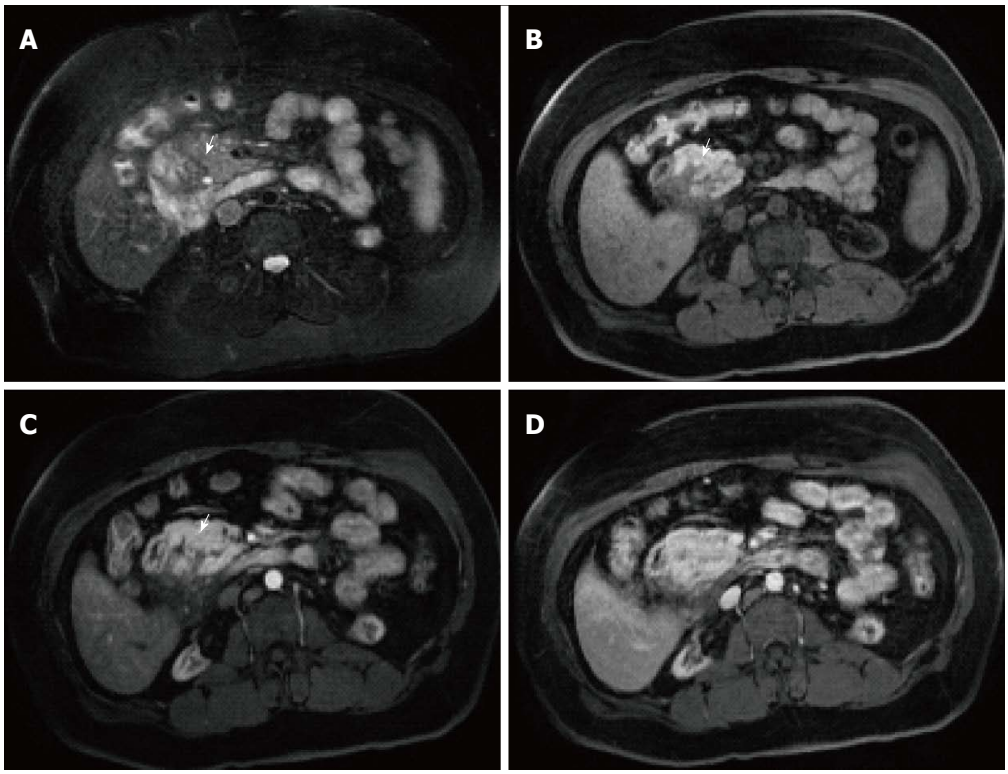

Figure 21 Groove pancreatitis. A: Axial T2-weighted single-shot fast spin-echo (SS-FSE) images with fat-suppression; (B) Pre- and (C, D) Post-contrast 3D-GRE T1- weighted images with fat-suppression during the late arterial and portal venous phases. There is a slightly low T2 signal sheet-like mass in the pancreaticoduodenal groove, with tiny cystic changes (arrow, A). The mass shows low T1 signal with extension into the pancreatic head (arrow, B). Imperceptible enhancement is depicted on the immediate post-contrast image (arrow, C), with progressive enhancement on the subsequent delayed images (D) in keeping with groove pancreatitis.

minor ampulla[64]. Presence of cystic changes, frequently located in the expected region of the pancreatic accessory duct, is considered a prominent feature of this process, likely related to accessory duct obstruction[65]. It is commonly seen in patients with history of alcohol abuse[64].

The classic MDCT features in the pure form can range from ill-defined fat stranding to frank soft tissue within the pancreaticoduodenal groove with increased delayed enhancement due to fibrosis. Thickening of medial duodenal wall on coronal images and presence of cysts can be appreciated sometimes[66]. On MRI, groove pancreatitis is characterized by a sheet-like mass in the groove that shows low signal on T1-weighted images, slightly high signal on T2-weighted images relative to the pancreas and may show delayed enhancement. Cystic lesions are well shown on T2-weighted images in the groove or duodenal wall[63].

It may be challenging to differentiate groove pancreatitis from pancreatic head duct adenocarcinoma. Recently, it was shown that by using three strict diagnostic criteria for groove pancreatitis: (1) focal thickening of the second portion of the duodenum; (2) abnormal increased enhancement of the second portion of the duodenum; and (3) cystic changes in the region of the pancreatic accessory duct, distinction from pancreatic duct adenocarcinoma could be achieved with high diagnostic accuracy (87.2% of patients), and a diagnosis of cancer could be excluded with a negative predictive value of 92.9%[67].

Hereditary pancreatitis

Hereditary pancreatitis is an autosomal dominant disease presenting as multiple episodes of pancreatitis in the absence of any predisposing factors. Imaging findings include parenchymal and intraductal calcifications and parenchymal atrophy. However, in hereditary pancreatitis, imaging plays an important role to rule out structural causes of pancreatitis and to closely monitor the development of pancreatic cancer, the risk of which is increased by many folds in these patients.

CONCLUSION

In summary, imaging plays an important role in the diagnosis and staging of acute and chronic pancreatitis. Both CT and MRI are widely used and represent the best cross sectional techniques in the setting of pancreatitis. Wider availability and good image quality make CT the mostly used imaging technique; however, due to its nonionizing nature, unmatched soft tissue contrast and higher safety profile of intravascular contrast media make MRI particularly valuable in pregnant patients, patients with recurrent pancreatitis and patients requiring multiple follow up examinations. Also, early form of chronic pancreatitis and some specific types of chronic pancreatitis benefit from being imaged with MRI.

REFERENCES

1 **Peery AF**, Dellon ES, Lund J, Crockett SD, McGowan CE, Bulsiewicz WJ, Gangarosa LM, Thiny MT, Stizenberg K, Morgan DR, Ringel Y, Kim HP, Dibonaventura MD, Carroll

CF, Allen JK, Cook SF, Sandler RS, Kappelman MD, Shaheen NJ. Burden of gastrointestinal disease in the United States: 2012 update. *Gastroenterology* 2012; **143**: 1179-1187.e1-3 [PMID: 22885331 DOI: 10.1053/j.gastro.2012.08.002]
2 **Bradley EL**. A clinically based classification system for acute pancreatitis. Summary of the International Symposium on Acute Pancreatitis, Atlanta, Ga, September 11 through 13, 1992. *Arch Surg* 1993; **128**: 586-590 [PMID: 8489394]
3 **Petrov MS**, Shanbhag S, Chakraborty M, Phillips AR, Windsor JA. Organ failure and infection of pancreatic necrosis as determinants of mortality in patients with acute pancreatitis. *Gastroenterology* 2010; **139**: 813-820 [PMID: 20540942 DOI: 10.1053/j.gastro.2010.06.010]
4 **Shanbhogue AK**, Fasih N, Surabhi VR, Doherty GP, Shanbhogue DK, Sethi SK. A clinical and radiologic review of uncommon types and causes of pancreatitis. *Radiographics* 2009; **29**: 1003-1026 [PMID: 19605653 DOI: 10.1148/rg.294085748]
5 **Etemad B**, Whitcomb DC. Chronic pancreatitis: diagnosis, classification, and new genetic developments. *Gastroenterology* 2001; **120**: 682-707 [PMID: 11179244]
6 **Bollen TL**. Imaging of acute pancreatitis: update of the revised Atlanta classification. *Radiol Clin North Am* 2012; **50**: 429-445 [PMID: 22560690 DOI: 10.1016/j.rcl.2012.03.015]
7 **Banks PA**, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG, Tsiotos GG, Vege SS. Classification of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus. *Gut* 2013; **62**: 102-111 [PMID: 23100216 DOI: 10.1136/gutjnl-2012-302779]
8 **Perez A**, Whang EE, Brooks DC, Moore FD, Hughes MD, Sica GT, Zinner MJ, Ashley SW, Banks PA. Is severity of necrotizing pancreatitis increased in extended necrosis and infected necrosis? *Pancreas* 2002; **25**: 229-233 [PMID: 12370532]
9 **Megibow AJ**, Ralls PW, Balfe DM, Bree RL, DiSantis DJ, Glick SN, Kidd R, Levine MS, Mezwa DG, Saini S, Shuman WP, Greene FL, Laine LA, Lillemoe K. Acute pancreatitis. American College of Radiology. ACR Appropriateness Criteria. *Radiology* 2000; **215** Suppl: 203-207 [PMID: 11037427]
10 **Banks PA**, Freeman ML. Practice guidelines in acute pancreatitis. *Am J Gastroenterol* 2006; **101**: 2379-2400 [PMID: 17032204 DOI: 10.1111/j.1572-0241.2006.00856.x]
11 **Morgan DE**. Imaging of acute pancreatitis and its complications. *Clin Gastroenterol Hepatol* 2008; **6**: 1077-1085 [PMID: 18928934 DOI: 10.1016/j.cgh.2008.07.012]
12 **Moulton JS**. The radiologic assessment of acute pancreatitis and its complications. *Pancreas* 1991; **6** Suppl 1: S13-S22 [PMID: 1788247]
13 **Thoeni RF**. The revised Atlanta classification of acute pancreatitis: its importance for the radiologist and its effect on treatment. *Radiology* 2012; **262**: 751-764 [PMID: 22357880 DOI: 10.1148/radiol.11110947]
14 **Anderson SW**, Lucey BC, Varghese JC, Soto JA. Accuracy of MDCT in the diagnosis of choledocholithiasis. *AJR Am J Roentgenol* 2006; **187**: 174-180 [PMID: 16794173 DOI: 10.2214/ajr.05.0459]
15 **Anderson SW**, Rho E, Soto JA. Detection of biliary duct narrowing and choledocholithiasis: accuracy of portal venous phase multidetector CT. *Radiology* 2008; **247**: 418-427 [PMID: 18372450 DOI: 10.1148/radiol.2472070473]
16 **Kim YK**, Kim CS, Han YM. Role of fat-suppressed t1-weighted magnetic resonance imaging in predicting severity and prognosis of acute pancreatitis: an intraindividual comparison with multidetector computed tomography. *J Comput Assist Tomogr* 2009; **33**: 651-656 [PMID: 19820487 DOI: 10.1097/RCT.0b013e3181979282]
17 **Stimac D**, Miletić D, Radić M, Krznarić I, Mazur-Grbac M, Perković D, Milić S, Golubović V. The role of nonenhanced magnetic resonance imaging in the early assessment of acute pancreatitis. *Am J Gastroenterol* 2007; **102**: 997-1004 [PMID: 17378903 DOI: 10.1111/j.1572-0241.2007.01164.x]
18 **Viremouneix L**, Monneuse O, Gautier G, Gruner L, Giorgi R, Allaouchiche B, Pilleul F. Prospective evaluation of non-enhanced MR imaging in acute pancreatitis. *J Magn Reson Imaging* 2007; **26**: 331-338 [PMID: 17654731 DOI: 10.1002/jmri.21037]
19 **Azevedo RM**, de Campos RO, Ramalho M, Herédia V, Dale BM, Semelka RC. Free-breathing 3D T1-weighted gradient-echo sequence with radial data sampling in abdominal MRI: preliminary observations. *AJR Am J Roentgenol* 2011; **197**: 650-657 [PMID: 21862807 DOI: 10.2214/ajr.10.5881]
20 **Bret PM**, Reinhold C, Taourel P, Guibaud L, Atri M, Barkun AN. Pancreas divisum: evaluation with MR cholangiopancreatography. *Radiology* 1996; **199**: 99-103 [PMID: 8633179]
21 **Soto JA**, Barish MA, Yucel EK, Clarke P, Siegenberg D, Chuttani R, Ferrucci JT. Pancreatic duct: MR cholangiopancreatography with a three-dimensional fast spin-echo technique. *Radiology* 1995; **196**: 459-464 [PMID: 7617861]
22 **Takehara Y**, Ichijo K, Tooyama N, Kodaira N, Yamamoto H, Tatami M, Saito M, Watahiki H, Takahashi M. Breath-hold MR cholangiopancreatography with a long-echo-train fast spin-echo sequence and a surface coil in chronic pancreatitis. *Radiology* 1994; **192**: 73-78 [PMID: 8208969]
23 **Balthazar EJ**. CT diagnosis and staging of acute pancreatitis. *Radiol Clin North Am* 1989; **27**: 19-37 [PMID: 2642273]
24 **Scaglione M**, Casciani E, Pinto A, Andreoli C, De Vargas M, Gualdi GF. Imaging assessment of acute pancreatitis: a review. *Semin Ultrasound CT MR* 2008; **29**: 322-340 [PMID: 18853839]
25 **Miller FH**, Keppke AL, Dalal K, Ly JN, Kamler VA, Sica GT. MRI of pancreatitis and its complications: part 1, acute pancreatitis. *AJR Am J Roentgenol* 2004; **183**: 1637-1644 [PMID: 15547203 DOI: 10.2214/ajr.183.6.01831637]
26 **Kim YK**, Ko SW, Kim CS, Hwang SB. Effectiveness of MR imaging for diagnosing the mild forms of acute pancreatitis: comparison with MDCT. *J Magn Reson Imaging* 2006; **24**: 1342-1349 [PMID: 17083111 DOI: 10.1002/jmri.20801]
27 **Ashley SW**, Perez A, Pierce EA, Brooks DC, Moore FD, Whang EE, Banks PA, Zinner MJ. Necrotizing pancreatitis: contemporary analysis of 99 consecutive cases. *Ann Surg* 2001; **234**: 572-579; discussion 572-579 [PMID: 11573050]
28 **Balthazar EJ**, Robinson DL, Megibow AJ, Ranson JH. Acute pancreatitis: value of CT in establishing prognosis. *Radiology* 1990; **174**: 331-336 [PMID: 2296641]
29 **Balthazar EJ**, Freeny PC, vanSonnenberg E. Imaging and intervention in acute pancreatitis. *Radiology* 1994; **193**: 297-306 [PMID: 7972730 DOI: 10.1148/radiology.193.2.7972730]
30 **Lenhart DK**, Balthazar EJ. MDCT of acute mild (nonnecrotizing) pancreatitis: abdominal complications and fate of fluid collections. *AJR Am J Roentgenol* 2008; **190**: 643-649 [PMID: 18287434 DOI: 10.2214/ajr.07.2761]
31 **Matos C**, Cappeliez O, Winant C, Coppens E, Devière J, Metens T. MR imaging of the pancreas: a pictorial tour. *Radiographics* 2002; **22**: e2 [PMID: 11796914 DOI: 10.1148/radiographics.22.1.g02jae2e2]
32 **Lau ST**, Simchuk EJ, Kozarek RA, Traverso LW. A pancreatic ductal leak should be sought to direct treatment in patients with acute pancreatitis. *Am J Surg* 2001; **181**: 411-415 [PMID: 11448431]
33 **Sandrasegaran K**, Tann M, Jennings SG, Maglinte DD, Peter SD, Sherman S, Howard TJ. Disconnection of the pancreatic duct: an important but overlooked complication of severe acute pancreatitis. *Radiographics* 2007; **27**: 1389-1400 [PMID: 17848698 DOI: 10.1148/rg.275065163]
34 **Tann M**, Maglinte D, Howard TJ, Sherman S, Fogel E, Madura JA, Lehman GA. Disconnected pancreatic duct syndrome: imaging findings and therapeutic implications in 26 surgically corrected patients. *J Comput Assist Tomogr* 2003; **27**: 577-582 [PMID: 12886147]
35 **Drake LM**, Anis M, Lawrence C. Accuracy of magnetic resonance cholangiopancreatography in identifying pancreatic duct disruption. *J Clin Gastroenterol* 2012; **46**: 696-699 [PMID:

22565603 DOI: 10.1097/MCG.0b013e31825003b3]
36 **Zaheer A**, Singh VK, Qureshi RO, Fishman EK. The revised Atlanta classification for acute pancreatitis: updates in imaging terminology and guidelines. *Abdom Imaging* 2013; **38**: 125-136 [PMID: 22584543 DOI: 10.1007/s00261-012-9908-0]
37 **Macari M**, Finn ME, Bennett GL, Cho KC, Newman E, Hajdu CH, Babb JS. Differentiating pancreatic cystic neoplasms from pancreatic pseudocysts at MR imaging: value of perceived internal debris. *Radiology* 2009; **251**: 77-84 [PMID: 19332847 DOI: 10.1148/radiol.2511081286]
38 **Morgan DE**, Baron TH, Smith JK, Robbin ML, Kenney PJ. Pancreatic fluid collections prior to intervention: evaluation with MR imaging compared with CT and US. *Radiology* 1997; **203**: 773-778 [PMID: 9169703]
39 **Mortele KJ**, Wiesner W, Intriere L, Shankar S, Zou KH, Kalantari BN, Perez A, vanSonnenberg E, Ros PR, Banks PA, Silverman SG. A modified CT severity index for evaluating acute pancreatitis: improved correlation with patient outcome. *AJR Am J Roentgenol* 2004; **183**: 1261-1265 [PMID: 15505289 DOI: 10.2214/ajr.183.5.1831261]
40 **Bollen TL**, Singh VK, Maurer R, Repas K, van Es HW, Banks PA, Mortele KJ. Comparative evaluation of the modified CT severity index and CT severity index in assessing severity of acute pancreatitis. *AJR Am J Roentgenol* 2011; **197**: 386-392 [PMID: 21785084 DOI: 10.2214/AJR.09.4025]
41 **Arvanitakis M**, Koustiani G, Gantzarou A, Grollios G, Tsitouridis I, Haritandi-Kouridou A, Dimitriadis A, Arvanitakis C. Staging of severity and prognosis of acute pancreatitis by computed tomography and magnetic resonance imaging-a comparative study. *Dig Liver Dis* 2007; **39**: 473-482 [PMID: 17363349 DOI: 10.1016/j.dld.2007.01.015]
42 **Balci NC**, Alkaade S, Magas L, Momtahen AJ, Burton FR. Suspected chronic pancreatitis with normal MRCP: findings on MRI in correlation with secretin MRCP. *J Magn Reson Imaging* 2008; **27**: 125-131 [PMID: 18058927 DOI: 10.1002/jmri.21241]
43 **Semelka RC**, Shoenut JP, Kroeker MA, Micflikier AB. Chronic pancreatitis: MR imaging features before and after administration of gadopentetate dimeglumine. *J Magn Reson Imaging* 1993; **3**: 79-82 [PMID: 8428105]
44 **Raman SP**, Fishman EK, Lennon AM. Endoscopic ultrasound and pancreatic applications: what the radiologist needs to know. *Abdom Imaging* 2013; **38**: 1360-1372 [PMID: 23334660 DOI: 10.1007/s00261-013-9979-6]
45 **Chong AK**, Hawes RH, Hoffman BJ, Adams DB, Lewin DN, Romagnuolo J. Diagnostic performance of EUS for chronic pancreatitis: a comparison with histopathology. *Gastrointest Endosc* 2007; **65**: 808-814 [PMID: 17466199 DOI: 10.1016/j.gie.2006.09.026]
46 **Choueiri NE**, Balci NC, Alkaade S, Burton FR. Advanced imaging of chronic pancreatitis. *Curr Gastroenterol Rep* 2010; **12**: 114-120 [PMID: 20424983 DOI: 10.1007/s11894-010-0093-4]
47 **Perez-Johnston R**, Sainani NI, Sahani DV. Imaging of chronic pancreatitis (including groove and autoimmune pancreatitis). *Radiol Clin North Am* 2012; **50**: 447-466 [PMID: 22560691 DOI: 10.1016/j.rcl.2012.03.005]
48 **Papanikolaou IS**, Adler A, Neumann U, Neuhaus P, Rösch T. Endoscopic ultrasound in pancreatic disease--its influence on surgical decision-making. An update 2008. *Pancreatology* 2009; **9**: 55-65 [PMID: 19077455 DOI: 10.1159/000178875]
49 **Agarwal B**, Krishna NB, Labundy JL, Safdar R, Akduman EI. EUS and/or EUS-guided FNA in patients with CT and/or magnetic resonance imaging findings of enlarged pancreatic head or dilated pancreatic duct with or without a dilated common bile duct. *Gastrointest Endosc* 2008; **68**: 237-42; quiz 334, 335 [PMID: 18423464 DOI: 10.1016/j.gie.2008.01.026]
50 **Eloubeidi MA**, Varadarajulu S, Desai S, Shirley R, Heslin MJ, Mehra M, Arnoletti JP, Eltoum I, Wilcox CM, Vickers SM. A prospective evaluation of an algorithm incorporating routine preoperative endoscopic ultrasound-guided fine needle aspiration in suspected pancreatic cancer. *J Gastrointest Surg* 2007; **11**: 813-819 [PMID: 17440790 DOI: 10.1007/s11605-007-0151-x]
51 **Voss M**, Hammel P, Molas G, Palazzo L, Dancour A, O' Toole D, Terris B, Degott C, Bernades P, Ruszniewski P. Value of endoscopic ultrasound guided fine needle aspiration biopsy in the diagnosis of solid pancreatic masses. *Gut* 2000; **46**: 244-249 [PMID: 10644320]
52 **Williams DB**, Sahai AV, Aabakken L, Penman ID, van Velse A, Webb J, Wilson M, Hoffman BJ, Hawes RH. Endoscopic ultrasound guided fine needle aspiration biopsy: a large single centre experience. *Gut* 1999; **44**: 720-726 [PMID: 10205212]
53 **Santhosh S**, Mittal BR, Bhasin D, Srinivasan R, Rana S, Das A, Nada R, Bhattacharya A, Gupta R, Kapoor R. Role of (18)F-fluorodeoxyglucose positron emission tomography/computed tomography in the characterization of pancreatic masses: experience from tropics. *J Gastroenterol Hepatol* 2013; **28**: 255-261 [PMID: 23278193 DOI: 10.1111/jgh.12068]
54 **Asagi A**, Ohta K, Nasu J, Tanada M, Nadano S, Nishimura R, Teramoto N, Yamamoto K, Inoue T, Iguchi H. Utility of contrast-enhanced FDG-PET/CT in the clinical management of pancreatic cancer: impact on diagnosis, staging, evaluation of treatment response, and detection of recurrence. *Pancreas* 2013; **42**: 11-19 [PMID: 22699206 DOI: 10.1097/MPA.0b013e3182550d77]
55 **Remer EM**, Baker ME. Imaging of chronic pancreatitis. *Radiol Clin North Am* 2002; **40**: 1229-1242, v [PMID: 12479708]
56 **Shimosegawa T**, Chari ST, Frulloni L, Kamisawa T, Kawa S, Mino-Kenudson M, Kim MH, Klöppel G, Lerch MM, Löhr M, Notohara K, Okazaki K, Schneider A, Zhang L. International consensus diagnostic criteria for autoimmune pancreatitis: guidelines of the International Association of Pancreatology. *Pancreas* 2011; **40**: 352-358 [PMID: 21412117 DOI: 10.1097/MPA.0b013e3182142fd2]
57 **Sah RP**, Pannala R, Chari ST, Sugumar A, Clain JE, Levy MJ, Pearson RK, Smyrk TC, Petersen BT, Topazian MD, Takahashi N, Vege SS. Prevalence, diagnosis, and profile of autoimmune pancreatitis presenting with features of acute or chronic pancreatitis. *Clin Gastroenterol Hepatol* 2010; **8**: 91-96 [PMID: 19800984 DOI: 10.1016/j.cgh.2009.09.024]
58 **Nishimori I**, Tamakoshi A, Otsuki M. Prevalence of autoimmune pancreatitis in Japan from a nationwide survey in 2002. *J Gastroenterol* 2007; **42** Suppl 18: 6-8 [PMID: 17520216 DOI: 10.1007/s00535-007-2043-y]
59 **Irie H**, Honda H, Baba S, Kuroiwa T, Yoshimitsu K, Tajima T, Jimi M, Sumii T, Masuda K. Autoimmune pancreatitis: CT and MR characteristics. *AJR Am J Roentgenol* 1998; **170**: 1323-1327 [PMID: 9574610 DOI: 10.2214/ajr.170.5.9574610]
60 **Van Hoe L**, Gryspeerdt S, Ectors N, Van Steenbergen W, Aerts R, Baert AL, Marchal G. Nonalcoholic duct-destructive chronic pancreatitis: imaging findings. *AJR Am J Roentgenol* 1998; **170**: 643-647 [PMID: 9490945 DOI: 10.2214/ajr.170.3.9490945]
61 **Takahashi N**, Fletcher JG, Fidler JL, Hough DM, Kawashima A, Chari ST. Dual-phase CT of autoimmune pancreatitis: a multireader study. *AJR Am J Roentgenol* 2008; **190**: 280-286 [PMID: 18212210 DOI: 10.2214/ajr.07.2309]
62 **Hardacre JM**, Iacobuzio-Donahue CA, Sohn TA, Abraham SC, Yeo CJ, Lillemoe KD, Choti MA, Campbell KA, Schulick RD, Hruban RH, Cameron JL, Leach SD. Results of pancreaticoduodenectomy for lymphoplasmacytic sclerosing pancreatitis. *Ann Surg* 2003; **237**: 853-858; discussion 853-858 [PMID: 12796582 DOI: 10.1097/01.sla.0000071516.54864.c1]
63 **Blasbalg R**, Baroni RH, Costa DN, Machado MC. MRI features of groove pancreatitis. *AJR Am J Roentgenol* 2007; **189**: 73-80 [PMID: 17579155 DOI: 10.2214/ajr.06.1244]
64 **Chatelain D**, Vibert E, Yzet T, Geslin G, Bartoli E, Manaouil D, Delcenserie R, Brevet M, Dupas JL, Regimbeau JM. Groove pancreatitis and pancreatic heterotopia in the minor duodenal papilla. *Pancreas* 2005; **30**: e92-e95 [PMID: 15841034]

65 **Triantopoulou C**, Dervenis C, Giannakou N, Papailiou J, Prassopoulos P. Groove pancreatitis: a diagnostic challenge. *Eur Radiol* 2009; **19**: 1736-1743 [PMID: 19238393 DOI: 10.1007/s00330-009-1332-7]
66 **Raman SP**, Salaria SN, Hruban RH, Fishman EK. Groove pancreatitis: spectrum of imaging findings and radiology-pathology correlation. *AJR Am J Roentgenol* 2013; **201**: W29-W39 [PMID: 23789694 DOI: 10.2214/AJR.12.9956]
67 **Kalb B**, Martin DR, Sarmiento JM, Erickson SH, Gober D, Tapper EB, Chen Z, Adsay NV. Paraduodenal pancreatitis: clinical performance of MR imaging in distinguishing from carcinoma. *Radiology* 2013; **269**: 475-481 [PMID: 23847255 DOI: 10.1148/radiol.13112056]
68 **O'Connor OJ**, McWilliams S, Maher MM. Imaging of acute pancreatitis. *AJR Am J Roentgenol* 2011; **197**: W221-W225 [PMID: 21785045 DOI: 10.2214/AJR.10.4338]
69 **Yang R**, Jing ZL, Zhang XM, Tang W, Xiao B, Huang XH, Yang L, Feng ZS. MR imaging of acute pancreatitis: correlation of abdominal wall edema with severity scores. *Eur J Radiol* 2012; **81**: 3041-3047 [PMID: 22571930 DOI: 10.1016/j.ejrad.2012.04.005]
70 **Conwell DL**, Wu BU. Chronic pancreatitis: making the diagnosis. *Clin Gastroenterol Hepatol* 2012; **10**: 1088-1095 [PMID: 22642958 DOI: 10.1016/j.cgh.2012.05.015]

P- Reviewer: Clark CJ, Demir IE **S- Editor**: Ji FF **L- Editor**: A
E- Editor: Lu YJ

World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4291/wjgp.v5.i3.271

World J Gastrointest Pathophysiol 2014 August 15; 5(3): 271-283
ISSN 2150-5330 (online)

REVIEW

New insights to occult gastrointestinal bleeding: From pathophysiology to therapeutics

Antonio Damián Sánchez-Capilla, Paloma De La Torre-Rubio, Eduardo Redondo-Cerezo

Antonio Damián Sánchez-Capilla, Paloma De La Torre-Rubio, Department of Gastroenterology, University Hospital Virgen de, Las Nieves, 18014 Granada, Spain
Eduardo Redondo-Cerezo, Endoscopy Unit, Department of Gastroenterology, University Hospital Virgen de Las Nieves, 18014 Granada, Spain
Author contributions: Sánchez-Capilla AD and De La Torre-Rubio P reviewed the bibliography and wrote the first draft; Redondo-Cerezo E overviewed the paper and wrote the final paper in English.
Correspondence to: Eduardo Redondo-Cerezo, MD, PhD, Endoscopy Unit, Department of Gastroenterology, University Hospital Virgen de Las Nieves, Avenida de las Fuerzas Armadas 2, 18014 Granada, Spain. eredondoc@gmail.com
Telephone: +34-958-020146 Fax: +34-958-120169
Received: March 11, 2014 Revised: June 1, 2014
Accepted: June 18, 2014
Published online: August 15, 2014

Abstract

Obscure gastrointestinal bleeding is still a clinical challenge for gastroenterologists. The recent development of novel technologies for the diagnosis and treatment of different bleeding causes has allowed a better management of patients, but it also determines the need of a deeper comprehension of pathophysiology and the analysis of local expertise in order to develop a rational management algorithm. Obscure gastrointestinal bleeding can be divided in occult, when a positive occult blood fecal test is the main manifestation, and overt, when external sings of bleeding are visible. In this paper we are going to focus on overt gastrointestinal bleeding, describing the physiopathology of the most usual causes, analyzing the diagnostic procedures available, from the most classical to the novel ones, and establishing a standard algorithm which can be adapted depending on the local expertise or availability. Finally, we will review the main therapeutic options for this complex and not so uncommon clinical problem.

Key words: Obscure gastrointestinal bleeding; Angiodysplasia; Wireless capsule endoscopy; Double balloon enteroscopy

Core tip: This is an invited in depth review of occult gastrointestinal bleeding, addressing its pathophysiology, diagnosis and treatment. Our paper tries to unify in one single manuscript all what a general gastroenterologist should know about those items. From the essentials of pathophysiology, we have tried to build a rational approach to those patients' management depending on the severity of the condition, proposing an evidence-based management algorithm.

Sánchez-Capilla AD, De La Torre-Rubio P, Redondo-Cerezo E. New insights to occult gastrointestinal bleeding: From pathophysiology to therapeutics. *World J Gastrointest Pathophysiol* 2014; 5(3): 271-283 Available from: URL: http://www.wjgnet.com/2150-5330/full/v5/i3/271.htm DOI: http://dx.doi.org/10.4291/wjgp.v5.i3.271

INTRODUCTION

Gastrointestinal bleeding is a term that includes any bleeding originating from the esophagus to the anus. Classically, it has been classified in upper or lower depending on the location of the bleeding source, proximal or distal to the angle of Treitz.

The usual management of gastrointestinal bleeding (GIB) involves an upper endoscopy and colonoscopy in a first attempt to find the bleeding lesion. If those are unsuccessful, and there's a bleeding persistence or recurrence, the entity is called gastrointestinal bleeding of obscure origin or obscure gastrointestinal bleeding (OGIB), being the source of bleeding usually located in the small bowel. This seg-

Table 1 Causes of obscure gastrointestinal bleeding (in order of frequency)

Overlooked lesions in the upper GI tract or in the colon
Upper GI tract (proximal to the angle of Treitz)
Cameron ulcers
Fundic varices
Peptic ulcer
Angiectasia
Dieulafoy lesion
Gastric antral vascular ectasia
Colorectal lesions
Angiectasia
Polyps
Neoplasms
Anal disease
Dieulafoy lesion
Mid-GI tract lesions
< 40 yr
Meckel diverticulum
Dieulafoy lesion
Tumors (GIST, Lymphoma, Carcinoids, *etc.*)
Inflammatory bowel disease
Celiac disease
40-60 yr
Small bowel tumors
Angiodysplasia
Celiac disease
NSAID's related lesions
> 60 yr
Angiodysplasia
Small bowel tumors
NSAID's related lesions
Rare causes (< 1%)
Haemobilia
Aortoenteric fistula
Hemosuccus pancreaticus

GI: Gastrointestinal; NSAID: Non-steroidal anti-inflammatory drug.

ment of the gastrointestinal tract has been impossible to endoscopic exploration for a long time. It has been studied with suboptimal procedures such as small bowel series or enteroclysis in mild cases, or with more aggressive methods in severe cases, such as intraoperative enteroscopy (IE).

But the development of new endoscopic procedures like wireless capsule endoscopy or therapeutic procedures like the new enteroscopes, with different modalities of overtubes and balloons, has allowed an accurate exploration of this part of the GI tract, modifying significantly OGIB patients' management.

From 2006 a new OGIB classification has been proposed, based on the segment of the GI tract where the bleeding source is located, which determines the needed procedures for its diagnosis and treatment. Indeed, upper gastrointestinal bleeding is defined as the one with a bleeding source proximal to the ampulla, therefore accessible to upper endoscopy; mid GI bleeding is established when the causative lesion is between the ampulla and the ileocecal valve. Finally, lower GI bleeding has a colorectal bleeding source accessible to colonoscopy[1].

Therefore, obscure OGIB can be defined as a persistent or recurrent GI bleeding without a bleeding source found after performing upper endoscopy and colonoscopy. OGIB comprises 5% of all GI bleeding cases, constituting a diagnostic and a therapeutic challenge, either because of the morbidity and mortality associated, as well as for the high consumption of health resources for its diagnosis and treatment[2].

In most of OGIB patients (75%), the bleeding source is located in the small bowel[3], being normally a mid-GI bleeding[4]. The rest of the lesions are usually in areas accessible to conventional endoscopy, but overlooked in previous endoscopic procedures.

OGIB refers to two different clinical situations, regarding the onset of the GI bleeding: (1) Obscure-occult GI bleeding refers to the patient with a GI bleeding detected by a positive occult blood fecal test, with or without iron depletion; (2) Obscure-overt GI bleeding, in which an evident GI bleeding is seen, in the form of melena or hematochezia[5]. This review addresses the diagnosis and management of patients with obscure-overt GI bleeding, with a special interest in the different available procedures, establishing a management algorithm.

ETHIOLOGY

Causes of OGIB include overlooked bleeding lesions by upper endoscopy or colonoscopy, as well as the ones that, after an exhaustive endoscopic study, are classified as mid-GI bleeding[6]. The causative condition of OGIB is highly determined by age, being tumors as lymphoma, carcinoids, and GIST more likely in patients of less than 40 years, and vascular lesions as angiodysplasia more usual in elder patients, comprising 40% of all cases[7]. Table 1 contains the main recognized causes of OGIB[5].

PATHOPHYSIOLOGY OF THE MOST USUAL CAUSES OF OGIB

Angiodysplasia

Angiodysplasia is one of the most usual causes of over OGIB in patients older than 40 years, and the most frequent cause in patients older than 60 years[7]. They are also known as arteriovenous malformations or vascular ectasia, more frequently found in the stomach, duodenum, cecum and ascending colon. Most of them are acquired but some may be present at birth, or as a part of some hereditary syndromes[8]. Pathogenesis is uncertain and four theories have been proposed: (1) Some attribute angiodysplasia to a mild chronic venous obstruction. This hypothesis is concordant with the observation of a higher number of these lesions in the right colon, where the wall tension is higher; (2) They could be a complication of mucosal chronic ischemia, which could appear in episodes of bowel obstruction or after tough straining when defecating; (3) Some authors think they could be a complication of local ischemia in patients with heart, vascular or lung disease[9]; (4) Some of them, usually in younger patients, could be congenital or associated to hereditary syndromes; (5) It has also been suggested a pathogenic relation between aortic ste-

nosis and angiodysplasia, caused by the haemodinamic abnormalities determined by the valvular disease (Heyde Syndrome)[10]. Therapy is controversial, but some studies have shown a reduction in bleeding episodes after valvular replacement; and (6) In terminal cardiac failure, left ventricular assisted devices have been associated with increased bleeding episodes from angiodysplasia. In these cases, pathogenesis seems related with anticoagulant therapy, vascular malformations, loss of activity of Von Willebrand factor and mucosal ischemia[11].

Small bowel tumors

Despite infrequent, GI bleeding is the usual clinical onset, being more frequent in benign tumors as leyomioma than in malignant lesions as leyomiosarcoma[12]. Bleeding is caused by erosion of the tumor surface or by the rupture of aberrant vascular structures within the lesion.

Meckel diverticulum

This is a relevant condition in patients of less than 25 years old. Despite rare, it is the most frequent congenital abnormality in the GI tract. They are caused by the incomplete obliteration of the vitelin duct during embryogenesis, which leads to the formation of a true small bowel diverticulum[13]. Meckel diverticulum has all the bowel wall layers, and in 12%-21% of cases it may contain ectopic tissues (gastric or duodenal mucosa or even pancreatic ducts). They are usually asymptomatic, but may also cause abdominal pain or OGIB. Bleeding is caused by an ulceration of the small bowel due to acid secretion by heterotopic gastric mucosa contained within the diverticular layers. Bleeding can be chronic and insidious, or acute and massive, but transfusion is hardly ever required. The main anatomical risk factor that makes bleeding more likely is diverticula size of more than 2 cm[14].

Dieulafoy's lesion

Etiology is unknown. Lesions are normally found in the proximal stomach, in the lesser curvature, near de esophago-gastric junction. It is usually a submucosal, dilated, aberrant vessel that erodes the overlaying mucosa without a previous ulcer[15]. This is caused by the lack of ramification of the submucosal artery which makes its diameter ten times the normal diameter of a mucosal capillary. Triggering causes are unclear and it usually appears in male patients with comorbidities such as cardiovascular diseases, arterial hypertension, chronic kidney disease, diabetes or alcohol abuse. It is important to mention that this lesion can be overlooked in an endoscopic exam[16], given that quite frequently the aberrant vessel cannot be seen unless it bleeds actively.

Celiac disease and inflammatory bowel disease

GI bleeding is usually associated to complications in both conditions, which can be ulcers or tumors like adenocarcinoma or lymphoma.

At lasts, we would like to emphasize three rare OGIB causes, associated to a high mortality and a difficult diagnosis[17].

Haemobilia: It consists in the bleeding from the biliary tree caused by a communication with vascular structures. The most frequent causes are a closed traumatisms, hepatic artery or portal vein aneurisms, liver abscesses, neoplasms or secondary to procedures such as liver biopsy or bile duct stones extraction[18]. Diagnosis is always difficult[19]. It should be suspected in the anamnesis, when the patient presents upper right quadrant pain, jaundice and OGIB, but this is an unusual form of presentation. Diagnosis can be confirmed by direct endoscopic visualization of blood passing through the papilla. Angiography is a therapeutic option but, despite a successful embolization or surgical treatment of the originating vessel, mortality is high.

Aortoenteric fistula: It is an exceptional but severe cause of OGIB, usually related to a previous aortoiliac surgery. The most common cause of primary aortoenteric fistula is an arteriosclerotic aneurism, infectious aortitis or tuberculosis[20]. The most common cause of secondary aortoenteric fistula is an abdominal vascular graft infected, usually some years after its positioning. Pathophysiology involves a graft and surrounding tissue infection of low aggressiveness, usually caused by *S. aureus* or *E. coli*, with causes erosion and communication between the graft and the lumen of the GI tract[21]. Other secondary causes are penetrating ulcers, tumor invasion of the aorta, trauma or radiation. The onset is usually a self-limited premonitory bleeding episode followed, days or weeks later, by a second episode typically massive and life-threatening.

Pancreatic hemosuccus: It is usually caused by the erosion of the splenic artery by a pseudocyst which causes a pseudoaneurysm communicated with the pancreatic duct. Suspicion is arisen by the observation of blood emerging from the ampulla, in a plausible clinical scenario. Angio-CT scan can be diagnostic. Angiography can help to establish a diagnosis, and it can be also therapeutic, but frequently surgery is needed for bleeding control[21].

OGIB AND ANTICOAGULATION

Oral hypercoagulation therapy has been described as a factor increasing OGIB incidence, worsening prognosis and changing management. In a 2014 study the risk of a severe bleeding episode increased up to 4%-23%, being higher when INR was above 4[22]. Despite this, anticoagulants did not seem to modify the type of lesion that caused the bleeding[23,24].

Risk factors associated with a higher bleeding risk in patients under oral anticoagulants therapy are: (1) Age: In patients older than 70 years annual bleeding risk is 3%; (2) A previous episode of GI bleeding or peptic ulcer increases the risk in up to 2.1% to 6.5%; (3) Co-

morbidities: Chronic kidney failure, diabetes, cardiac disease, alcohol abuse; and (4) Association with antiplatelet drugs.

Recently, some new anticoagulants have been developed with lower rates of intracranial bleedings[25] but with a likely increase in GI bleeding[26].

DIAGNOSTIC AND THERAPEUTIC PROCEDURES IN THE PATIENT WITH AN OVERT OGIB

For the evaluation of OGIB, particularly mid GI bleeding, angiography, gamma praphy and intraoperative enteroscopy have been classically performed. But the technological improvements with capsule endoscopy, CT-angiography and balloon assisted enteroscopy (BAE) have relegated the classical techniques to a second step and are nowadays used only in selected patients. Moreover, the diagnostic procedure selected in each case depends largely on different factors, as patient's symptoms, bleeding severity, as well as local expertise and availability, or the need of therapeutic procedures.

Repeated upper and lower endoscopy

Bleeding lesions within reach of upper endoscopy have been indentified in 10%-64% of patients who underwent push enteroscopy and in 24%-25% of patients who underwent BAE because of a suspected OGIB. Nevertheless, few missing lesions are found in lower enteroscopy, with about 7% of findings within the reach of a conventional colonoscope, usually in patients with a previous poor bowel cleansing or with profuse bleeding. In the previously mentioned study, repeated endoscopy (upper or lower) revealed overlooked lesions in 15% of patients[27-32]. However, in another Australian paper, only 4% of 50 patients submitted for enteroscopy had overlooked lesions by upper or lower endoscopy, concluding that repeated endoscopy is not cost-effective[33].

Therefore, despite lesions within the reach of conventional endoscopy might be overlooked, it is not recommended to repeat these procedures in all cases, because this would raise the costs, delaying the definitive diagnosis and overloading endoscopy units. So, it is only recommended to repeat these procedures in selected cases, as in those with previous suboptimal results due to a bad bowel cleansing or with a recurrent GI bleeding with a high suspicion of an upper GI tract origin. If hemobilia or hemosuccus are suspected, upper endoscopy with a duodenoscope is mandatory.

Some authors recommend that, if needed, the second conventional endoscopy should be performed with a push enteroscope, which would allow a deeper exploration in case no other lesions are found in the upper GI tract[34,35].

Small bowel series

Neither small bowel series nor enteroclysis have a diagnostic accuracy of more than 5% (22) and 21% respectively[36], with a particular lack of accuracy in flat mucosal lesions, as angiodysplasia, a frequent cause of bleeding cause in the small bowel. The development of capsule endoscopy and enteroscopy has limited its use to a few situations[25,37,38].

The development of other radiologic methods as CT or MRI enteroclysis with new multidetector equipment, offers higher diagnostic capabilities, even for flat vascular lesions[39].

CT angiography

It has been recently added to the diagnostic armamentarium for OGIB, with a reported sensibility and specificity of 79%-90% and 95%-99% respectively[40,41]. It detects bleedings of 0.3-0.5 mL/min with a diagnostic accuracy near 100%, having the advantages of its availability and non-invasiveness. Nevertheless it lacks therapeutic capabilities, requires radiation exposure and need intravenous contrast with a known association with nephropathy and allergic reactions.

For all those reasons, CT angiography should be considered as the first diagnostic procedure in patients with active bleeding and hemodynamic impairment, instead of other procedures with a longer duration as gammagraphy, or more invasive as arteriography, which should be reserved for therapeutic purposes in patients with an active bleeding in CT angiography.

Furthermore, CT angiography has shown its usefulness in patients with an intermittent OGIB and a normal endoscopic study, leading to the detection of unusual cases of OGIB, like stromal tumors up to 1-2 cm. It is also the first option in diverticular disease with an excellent accuracy when studying vascular abnormalities causing GI bleeding, like aortoenteric fistulae.

Gammagraphy

Gammagraphy consists in the injection of patient's red cells tagged with Tc99 that survive in the bloodstream up to 24 h, leading to the detection of GI bleedings even of a very low rate (> 0.1 mL/min)[42]. Both properties make the procedure highly sensitive but with poor specificity, finding positive results in around 45% of patients in different published series[43]. The use of colloidal-sulphur Tc99 determines a quicker exploration because there is no need to tag red cells, but it has a lower accuracy because of the quicker dilution of the isotope in the bloodstream.

The main drawback of gammagraphy is its low precision when locating the bleeding source in the bowel, which can be mistaken in up to 25% of patients[44]. For these reasons, as well as for the high rate of false positive and negative results and the lack of therapeutic abilities, this procedure has a very limited role in OGIB, sometimes only as a previous step to angiography[45].

Angiography

It has diagnostic and therapeutic capabilities. It needs higher bleeding rate than angiography (> 0.5 mL/min),

with a better performance in severe cases. However, it allows the diagnosis of non-bleeding lesions as angiodysplasia or tumors and, for this reason, its sensibility shifts between 30% and 47%[38,46], while its specificity is usually near 100%. Nevertheless, angiography is an invasive procedure with associated risks and complications in up to 9.3% of patients[47]. Therefore, it is considered a second line procedure, limited to clinical pictures in which a lesion is likely.

A provocative test, giving to the patient hypo coagulants drugs, fibrinolytic agents or vasodilators, has not shown an improvement on angiography accuracy[48].

As a therapeutic method, it allows the administration of vasoactive agents inside the responsible vessel or to perform an embolization with different substances, leading to bleeding resolution in up to 70%-100% of patients[49,50].

Wireless capsule endoscopy

Wireless capsule endoscopy (WCE) has been a great progress in small bowel examination, representing a safe and minimally invasive method for the diagnosis of OGIB.

In a 2010 systematic review, 66% of WCE indications were OGIB, with a diagnostic yield of 60.5%, being angiodysplasia the most frequent finding (50%), followed by ulcers (26.8%) and neoplasms (8.8%)[51]. Different studies have shown that WCE is more useful in overt OGIB than in occult OGIB[51-55]. Other factors related to an increase in WCE yield are[56]: (1) Performance within two weeks after the bleeding episode; (2) Hemoglobin< 10 g/dL; (3) Persistence of GI bleeding for more than 6 mo; and (4) More than one overt bleeding episode.

But WCE has other potential roles, as the detection of overlooked lesions on conventional procedures like upper endoscopy[56] or colonoscopy[57], assessing number, size and location of lesions for a better planning of the therapeutic procedure. Indeed, in a 2008 study[58] from our group, 30 patients with angiodysplasia found on CE were followed for one year, observing that patients with bigger angiodysplasia (> 1 cm) have a higher clinical impact (lower hemoglobin rates, higher transfusion requirements) and therefore higher needs of therapeutic interventions after WCE (75% *vs* 18.2%), which lead to lower rates of rebleeding. In conclusion, this paper found that angiodysplasia size (> 1 cm) and number (> 10) is related with a higher mortality (20% *vs* 4% and 25% *vs* 0% respectively).

When compared with push enteroscopy or small bowel series, WCE has proved to be superior in OGIB: In a metaanalysis published by Triester in 2005, diagnostic yield of WCE was 63% compared to 28% and 6% of push enteroscopy and small bowel series respectively. Another meta-analysis of the same year showed similar results[59-61].

Regarding other procedures, CE has shown a higher yield than angiography or CT-angiography but very similar to BAE, with the differences of its invasiveness and its ability to explore the whole small bowel in a single procedure[62-64].

This higher yield has shown to have a direct impact on management of two thirds patients with OGIB[65,66], as well as a high negative predictive value, with a rebleeding rate after a normal CE in the following 19 mo of 5.6%[67].

Therefore, CE is a first line procedure for OGIB management, although it is far from the ideal. Important disadvantages, like biopsy sampling, lack of therapeutic abilities, lack of a remote motion control, battery limitations etc. imply the need of other methods to manage those patients[68,69].

Anyway, significant research is being conducted in this field, with devices that will likely allow biopsy sampling, therapeutic interventions, real time motion control from outside the patient by means of magnetic fields control or articulated arms (Spider Pill), improvements in batteries durability etc. Some of those advances have already been incorporated, as bleeding lesions detection improvements by pattern differences in color wavelength (FICE-CE, Given Imagin)[70].

Enteroscopy

Enteroscopy allows the endoscopic observation of the small bowel beyond the angle of Treitz by means of an enteroscope.

Push enteroscopy: Until recently, push enteroscopy (PE) has been the standard of care in patients with OGIB, after a normal upper endoscopy and colonoscopy. It consists in the passage of an enteroscope by mouth, which makes possible the exploration of a variable length of the small bowel, ranging from 30-160 cm beyond the angle of Treitz[71]. PE permits only a partial vision of the small bowel, but its main indication is still OGIB, with a global diagnostic yield of 12%-80% and better results in overt OGIB.

In conclusion, PE has the advantage of its therapeutic capabilities but also the important drawbacks of a partial exploration of the GI tract and its invasiveness. Thus, it should be carefully used for previously identified lesions which are likely within the reach of this enteroscope[25,71-75].

Double balloon enteroscopy: Double balloon enteroscopy (DBE) has been a great improvement in small bowel exploration, because it provides a complete examination of the bowel lumen, as well as biopsy sampling and therapeutic abilities[76].

First described in 2001, it was widely available in 2004, consisting in an enteroscope with a balloon attached to its tip, as well as another balloon over an overtube. The alternative inflation and progression with the overtube and the balloon-enteroscope system provides a deeper progression through the small bowel, with a significantly increased mean bowel length explored as compared to PE[77,78].

The combination of lower and upper DBE grants

a visualization of the whole length of the small bowel, which is not always needed[79].

Diagnostic yield of DBE in OGIB ranges between 47%-80%[5], similar to that of WCE[58]. Its yield is increased when the procedure is performed within one month after the bleeding event.

Keeping in sight the similar diagnostic yield of WCE and DBE, they are actually considered complementary procedures[80], being WCE the first tool to be used, because of its lower cost, less invasiveness and higher availability. Information from WCE examination is helpful to decide between an upper or lower enteroscopy. In case we don't have a previous WCE, or if an upper and lower enteroscopy is needed, it is usually recommended to begin the endoscopic examination with the upper enteroscopy, because it is technically easier and has an increased likelihood of finding the causative lesion[81,82].

The main drawback of DBE is that a complete small bowel examination is not feasible in up to 29%[5], it needs sedation (usually under general anesthesia), its availability is limited to referral centers, and it has a prolonged examination time and other difficulties usually found in the lower approach due to poor bowel preparation, abdominal adhesions etc.

Nevertheless, DBE is a safe procedure, with less than 1% complications, being the most usual perforation (0.4%), pancreatitis (0.3%), and ileus[83,84]. Complications are not related to age, but with therapeutic maneuvers or anatomical abnormalities of the bowel (*i.e.,* previous surgeries, abdominal radiotherapy or intestinal lymphoma treated with chemotherapy)[6].

Other enteroscopies: Other enteroscopes used with overtube and balloons are: (1) Single balloon enteroscopy (Olympus Inc.): Exploration times and depth of insertion in small bowel enteroscopy are similar to these of DBE. In a 2010 paper[85] 100 patients were studied (50 patients with DBE and 50 with SBE) achieving DBE a higher number of complete enteroscopies when compared with SBE (66% *vs* 22%) and a higher number of findings. However, in this study, a system different from the original SBE (Olympus®) was used (Fujifilm®), having a different flexibility and balloon pressure. Later, Takano *et al*[86] had to stop prematurely a study comparing DBE with SBE, because of the differences in complete enteroscopies between both systems (57.1% *vs* 0%), finding no differences with regards to findings or complications[86]. Finally, in 2011 and 2012 two studies with 130 and 107 patients respectively[87,88] showed no differences between both systems regarding depth of insertion, complete bowel examination, complications and findings; (2) Spiral enteroscopy (Endo-Ease Discovery SB, Spirus Medical Inc.): The device includes an overtube with a helical portion which grasps the bowel folds, reaching as far as 200 cm beyond the angle of Treitz; and (3) Shapelock (USGI Medical Inc.): It consists in an overtube with multiple titanium rings, joined by four titanium wires and covered by a detachable sheath. When tension is applied on the wires, the overtube is fixed, allowing the passage of the enteroscope. Today, it has more applicability in patients with altered anatomy due to previous surgeries, in incomplete colonoscopy and in NOTES[89-92].

Intraoperative enteroscopy: It has been considered the gold standard for small bowel examination for long time[4], and although balloon assisted enteroscopy (BAE) is preferred because it is less invasive and have similar results in the diagnosis and management of small bowel disorders, the IE is an important reserve tool. It consists in the insertion of the endoscope through an enterotomy, exploring the mucosa while the surgeon facilitates the advance of the endoscope and observes the serosal surface. Palpation and transilumination play an important role in this procedure, which allows the whole bowel examination in more than 90% of patients.

Intraoperative enteroscopy (IE) has a diagnostic yield of 50%-100%[4,93], with therapeutic possibilities, but it is invasive. 12%-33%[77,78] of complications and 8%[94-96] of mortality limit its use to cases in which the other diagnostic methods are contraindicated or impossible, and always in patients with clinically significant OGIB[93-98].

PROPOSAL OF A DIAGNOSTIC ALGORITHM

In a patient with OGIB, after conventional upper endoscopy and colonoscopy, we should consider to repeat colonoscopy when a poor bowel cleansing is reported, or when we suspect an incomplete colonic evaluation. Upper endoscopy should be repeated if a strong suspicion of an upper GI tract bleeding lesion is still a concern despite a previous normal upper endoscopy.

Once a bleeding cause within reach to conventional endoscopy has been ruled out, depending on patient' s situation, an evaluation of the small bowel by WCE and BAE (balloon assisted enteroscopy, DBE or SBE) should be the next step, beginning with the less invasive one, which is WCE[5,67,75,99].

After normal WCE, if the bleeding stops spontaneously, a conservative attitude is recommended, with a clinical follow-up of the patient. If there is a strong suspicion of small bowel disease, even with a previous normal WCE, BAE should be performed[100].

Nevertheless, some authors think that if pretest likelihood of a correct diagnosis and treatment with BAE is higher than 25%-30%, we should proceed directly with BAE, because it is the most cost-effective option[101,102]. Moreover, in centers with a high number of patients and experienced endoscopists, DBE can be considered as a first step procedure in some clinical settings[102].

After rebleeding, repeated WCE finds lesions in up to 20%-35% of patients. Those lesions can be found and treated afterwards with BAE, which can also detect up to 30% of lesions previously overlooked by WCE[103-105].

If a patient presents hemodynamic instability and an active bleeding, angiography and IE should be the first

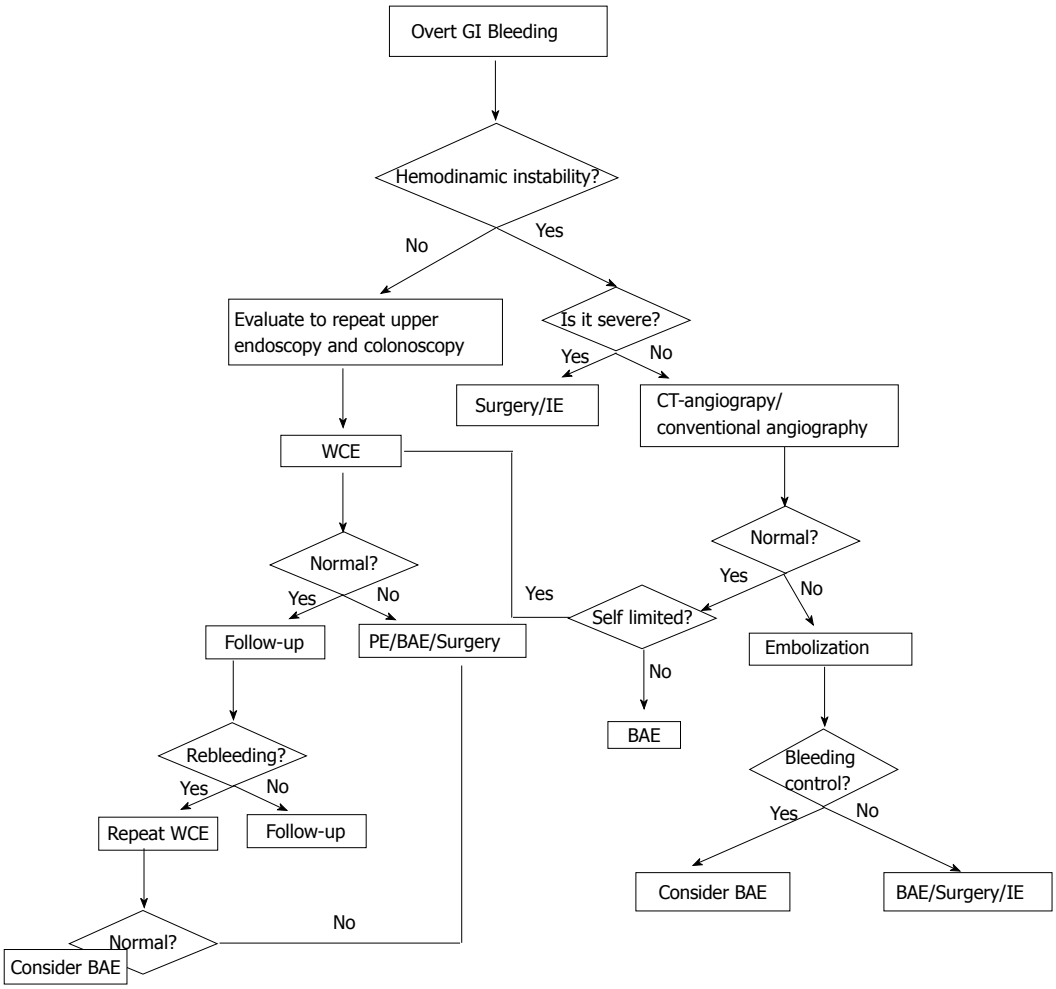

Figure 1 Proposal of a diagnostic algorithm. OGIB: Obscure gastrointestinal bleeding; WCE: Wireless capsule endoscopy; DBE: Double balloon enteroscopy; PE: Push enteroscopy; IE: Intraoperative enteroscopy.

diagnostic procedures. CT angiography is increasingly being used in this setting, because it offers an accurate diagnosis in many patients, it is less invasive, widely available and quick. Anatomical location of the lesion is usually accurate with few complications. After detecting a lesion by CT-angiography, conventional angiography or surgery can be used to apply the specific therapy[106] (Figure 1).

OGIB THERAPY

Therapy is directed by the type of lesion and its location. There are three major types of available therapies.

Pharmacological therapy[107]

Hormonal therapy (estrogens and progesterone) was initially explored by Koch et al in 1952 after observing that a patients with hereditary hemorrhagic theleangiectasia (HHT) whose bleeding varied depending on her menstrual cycle. The mechanism of action is not well understood, but there are several theories: (1) Estrogen and progesterone receptors have been detected in nasal and epidermal telangiectatic lesions in patients with HHT, and the hormone-receptor binding improved endothelial integrity in patients with HHT; (2) In animals this treatment improved vascular stasis within the mesenteric microcirculation and decreased the mucosal blood flow; (3) In patients on dialysis, estrogens shorten bleeding time by the reduction of endothelial prostacyclin production; and (4) Finally hormones may also decrease vascular endothelial growth factor.

Estrogens and progesterone therapy has been widely used in OGIB, with contradictory results, although some reports have observed a significant reduction in transfusion requirements, and even a complete resolution of bleeding.

A study of 43 patients, 38 of which were treated with hormonal therapy and followed for a mean time of 535 d (range 25-1551 d), reported benefits in patients with bleeding from sporadic angiodysplasia[108]. However, this has not been confirmed in other studies. The best data come from a multicenter, placebo-controlled trial involving 72 noncirrhotic patients which had bleedings from documented angiodysplasia; there was no benefit from hormonal therapy[109]. Based on these findings, hormonal therapy seems to have poor therapeutic advantages in

patients with sporadic angiodysplasia.

Some other papers do not recommend their use because of their lack of beneficial effects on OGIB and their adverse events (thrombosis, gynecomastia, loss of libido in males, metrorragia…). In general, their efficacy has not been proved, except for the treatment of hereditary hemorrhagic telangiectasia, von Willebrand disease, chronic kidney failure and gastric antral vascular ectasia (GAVE), in which hormonal therapy reduces transfusion requirements but not the size or number of lesions[98-100].

Somatostatin analogs: Octeotride reduces splacnic arterial flow by inhibiting angiogenesis and endothelial related growth factors[101-103]. Also, octeotride can inhibit angiogenesis by inhibiting endothelial cell proliferation. It has shown efficacy in acute and chronic GI bleeding, and can be used in patients with contraindications or a poor response to hormonal therapy. In Rossini et al study[110], treatment with octreotide in 3 patients decreased the need for blood transfusions during the follow-up period (8 to 17 mo). Other authors have published similar results[111], and have observed comparable side effects including diarrhea, steatorrhea, or changes in glucose metabolism.

A 2010 meta-analysis[112] analyzed 3 studies with a total of 62 patients, observing that 76% of patients responded to this therapy, achieving a significant reduction in transfusion requirements.

Depot formulations like LAR-Octeotride, which allow intramuscular administration once a month, have gained acceptance in selected cases[113,114]. In a study with 15 patients[115] treated with LAR-Octeotride for a recurrent bleeding from gastrointestinal angiodysplasia, the proportion of patients who experienced a bleeding event was lower during treatment than prior to treatment (20 *vs* 73), median transfusion requirements were reduced (2 *vs* 10 units), and median hemoglobin levels were higher during therapy (10 *vs* 7 g/dL).

Non-selective beta-blockers: They reduce splacnic flow, pulse and cardiac output. They are usually used in portal hypertension related OGIB and monotherapy or in association with LAR-Octeotride.

Thalidomide: It was retired in the 60s because of its teratogenic effect. However, thalidomide has recently shown to be an effective anti-inflammatory treatment in Crohn's disease. In addition to its anti-inflammatory effects, it also displays antiangiogenic activity, which may be useful for the treatment of GI bleeding. It can be taken orally and it could be used in patients with contraindications to other therapies. Obviously it is forbidden in childbearing aged women and in patients with peripheral neuropathy. It must be used cautiously in patients with cardiovascular or neurologic diseases, chronic kidney or liver failure and in immunosuppressed patients.

Some reports show promising outcomes in bleeding control[112]. In a randomized trial in 2011[116] patients treated with thalidomide were more likely than those treated with iron supplements to experience a positive clinical outcome (71% *vs* 4%).

Other drugs: (1) Antifibrinolitics: Tranexamic acid is an antifibrinolytic agent whose haemostatic effect is due to the inhibition of plasminogen activation in body fluids and tissues. Epsilon-aminocaproic acid has controlled chronic bleeding in patients suffering from HHT. These drugs have a prothrombotic activity and, for this reason, coagulation abnormalities or thrombophilia have to be ruled out before initiating the therapy; (2) Danazol: There are two single reports with positive results after hormonal therapy failure in patients with hereditary hemorrhagic teleangiectasia; (3) Desmopresin; and (4) Recombinant factor Ⅶ: Reserved to cases of massive overt OGIB.

Endoscopic therapy

There are different methods, injection therapies, thermal methods or mechanical devices which can be used with different endoscopes, depending on the location of the bleeding cause.

Argon plasma coagulation: It is safe and the most common and successful method used to treat angiodysplasia because of its easy application (especially for large superficial lesions), low cost, and reported limited depth of coagulation. Argon plasma coagulation (APC) has been used for a variety of bleeding lesions, including angiodysplasia, in these lesions submucosal saline injection prior to treatment with APC may protect against deep wall injury.

In a study of 50 patients with small bowel lesions, 44 patients were treated with APC for angiodysplasia[117]. After a mean follow-up of 55 mo, hemoglobin levels increased from a mean of 7.6 g/dL prior to treatment to 11.0 g/dL following it, and there was a significant decrease in the number of patients requiring blood transfusions. However, small bowel bleeding recurred in 21 of the patients treated with APC. A later study with 98 patients[118] reported similar results. The risk factors associated with rebleeding were the number of lesions and the presence of valvular and or arrhythmic cardiac disease.

Electrocoagulation: Bipolar or heater probe coagulation is effective for treatment of angiodysplasia in the colon or upper gastrointestinal tract. The risk of perforation with heater probe coagulation may be increased in the colon and small bowel, beyond the duodenum. Monopolar coagulation may be less effective and is associated with an increased rate of complications.

Mechanical hemostasis: Mechanical hemostatic methods such as endoscopic clips have the advantage of avoiding tissue injury, which may be particularly desirable in patients taking anticoagulants and/or antiplatelet agents, or in patients with coagulation defects.

Another mechanical method that has been described in some case reports is band ligation[119], that is safe and

effective for the treatment of acutely bleeding small bowel vascular lesions with similar results to APC (recurrent bleeding in 43%) and which can be a definitive treatment for Dieulafoy's lesion.

Angiography

Angiography is indicated in patients with GI bleeding who fail to respond to medical and/or endoscopic therapy, as an alternative to surgery in hemodynamically unstable patients with severe bleeding or for patients with ongoing or recurrent bleeding following attempts to control the bleeding endoscopically. Angiographic therapies include the infusion of vasoactive drugs (vasopressin) or the delivery of agents to mechanically occlude the vascular supply of the bleeding lesion (embolization).

Vasopressin causes generalized vasoconstriction via a direct action upon vessel walls, especially the arterioles, capillaries, and venules. It should be used with caution in patients with coronary artery disease, congestive cardiomyopathy, severe hypertension, or severe peripheral vascular disease. Other side effects are arrhythmias and water retention leading to hyponatremia.

Agents used for embolization include biodegradable gelatin sponge, polyvinyl alcohol particles, liquid agents, and metallic coils. Microcoils have become the preferred agent for embolizing bleeding vessels and can be deployed by means of a microcatheter to the site of bleeding. The complications of embolization include those associated with arteriography itself (*e.g.*, hematomas, arterial thrombosis, dissection, embolism, and pseudoaneurysm formation), and bowel infarction.

The choice between vasopressin and embolization should be individualized for each patient, taking into account angiography experience. Embolization with microcoils may be more successful than vasopressin infusion (95% *vs* 80%-90%)[120,121]) but it is associated with a higher rate of complications.

Initial hemostasis may be achieved in up to 80%-95% of patients in whom angiographic therapy is technically feasible, but rebleeding is a common problem (9%-56% in embolization and 5%-50% in intra-arterial vasopressin infusion).

Surgery

Surgical therapy is reserved for patients with a known bleeding cause, found with other methods, patients with increasing transfusion requirements or life-threatening bleedings from clearly identified origins, or for cases in which haemodinamic instability does not allow the clinicians to complete the diagnostic algorithm and an IE is mandatory. In this last situation, rebleeding is usual[86,87].

CONCLUSION

Despite technological advances, OGIB is still a diagnostic challenge for gastroenterologists, with important hospital resources consumption and delayed diagnoses. WCE is the most cost-effective diagnostic procedure to identify the bleeding source and its location. In selected cases, with an outstanding severity, CT-angiography is an alternative.

Although therapy depends on the bleeding cause, BAE plays an important role in the management of lesions found in WCE. It is less aggressive than intraoperative enteroscopy and has a high index of success. A pharmacological alternative to surgery or endoscopy are depot formulations of somatostatin analogs.

REFERENCES

1 **Ell C**, May A. Mid-gastrointestinal bleeding: capsule endoscopy and push-and-pull enteroscopy give rise to a new medical term. *Endoscopy* 2006; **38**: 73-75 [PMID: 16429358 DOI: 10.1055/s-2005-921131]

2 **Prakash C**, Zuckerman GR. Acute small bowel bleeding: a distinct entity with significantly different economic implications compared with GI bleeding from other locations. *Gastrointest Endosc* 2003; **58**: 330-335 [PMID: 14528203]

3 **Moawad FJ**, Veerappan GR, Wong RK. Small bowel is the primary source of obscure gastrointestinal bleeding. *Gastroenterology* 2008; **135**: 1016 [PMID: 18694750 DOI: 10.1053/j.gastro.2008.05.083]

4 **Gralnek IM**. Obscure-overt gastrointestinal bleeding. *Gastroenterology* 2005; **128**: 1424-1430 [PMID: 15887123 DOI: 10.1053/j.gastro.2005.03.067]

5 **Raju GS**, Gerson L, Das A, Lewis B. American Gastroenterological Association (AGA) Institute technical review on obscure gastrointestinal bleeding. *Gastroenterology* 2007; **133**: 1697-1717 [PMID: 17983812 DOI: 10.1053/j.gastro.2007.06.007]

6 **Mujica VR**, Barkin JS. Occult gastrointestinal bleeding. General overview and approach. *Gastrointest Endosc Clin N Am* 1996; **6**: 833-845 [PMID: 8899413]

7 **Meyer CT**, Troncale FJ, Galloway S, Sheahan DG. Arteriovenous malformations of the bowel: an analysis of 22 cases and a review of the literature. *Medicine* (Baltimore) 1981; **60**: 36-48 [PMID: 6969839 DOI: 10.1097/00005792-198101000-00004]

8 **Sami SS**, Al-Araji SA, Ragunath K. Review article: gastrointestinal angiodysplasia - pathogenesis, diagnosis and management. *Aliment Pharmacol Ther* 2014; **39**: 15-34 [PMID: 24138285 DOI: 10.1111/apt.12527]

9 **Foutch PG**. Angiodysplasia of the gastrointestinal tract. *Am J Gastroenterol* 1993; **88**: 807-818 [PMID: 8389094]

10 **Scheffer SM**, Leatherman LL. Resolution of Heyde's syndrome of aortic stenosis and gastrointestinal bleeding after aortic valve replacement. *Ann Thorac Surg* 1986; **42**: 477-480 [PMID: 3490235]

11 **Islam S**, Cevik C, Madonna R, Frandah W, Islam E, Islam S, Nugent K. Left ventricular assist devices and gastrointestinal bleeding: a narrative review of case reports and case series. *Clin Cardiol* 2013; **36**: 190-200 [PMID: 23378047 DOI: 10.1002/clc.22096]

12 **Soulellis CA**, Carpentier S, Chen YI, Fallone CA, Barkun AN. Lower GI hemorrhage controlled with endoscopically applied TC-325 (with videos). *Gastrointest Endosc* 2013; **77**: 504-507 [PMID: 23290722 DOI: 10.1016/j.gie.2012.10.014]

13 **Sagar J**, Kumar V, Shah DK. Meckel's diverticulum: a systematic review. *J R Soc Med* 2006; **99**: 501-505 [PMID: 17021300 DOI: 10.1258/jrsm.99.10.501]

14 **Yahchouchy EK**, Marano AF, Etienne JC, Fingerhut AL. Meckel's diverticulum. *J Am Coll Surg* 2001; **192**: 658-662 [PMID: 11333103 DOI: 10.1016/S1072-7515(01)00817-1]

15 **Lee YT**, Walmsley RS, Leong RW, Sung JJ. Dieulafoy's lesion. *Gastrointest Endosc* 2003; **58**: 236-243 [PMID: 12872092

DOI: 10.1067/mge.2003.126]
16 **Lara LF**, Sreenarasimhaiah J, Tang SJ, Afonso BB, Rockey DC. Dieulafoy lesions of the GI tract: localization and therapeutic outcomes. *Dig Dis Sci* 2010; **55**: 3436-3441 [PMID: 20848205 DOI: 10.1007/s10620-010-1385-0]
17 **Ferguson A**, Arranz E, O'Mahony S. Clinical and pathological spectrum of coeliac disease--active, silent, latent, potential. *Gut* 1993; **34**: 150-151 [PMID: 8432463 DOI: 10.1136/gut.34.2.150]
18 **Moreno RD**, Harris M, Bryk HB, Pachter HL, Miglietta MA. Late presentation of a hepatic pseudoaneurysm with hemobilia after angioembolization for blunt hepatic trauma. *J Trauma* 2007; **62**: 1048-1050 [PMID: 17426568 DOI: 10.1097/01.ta.0000235295.02414.d7]
19 **Bloechle C**, Izbicki JR, Rashed MY, el-Sefi T, Hosch SB, Knoefel WT, Rogiers X, Broelsch CE. Hemobilia: presentation, diagnosis, and management. *Am J Gastroenterol* 1994; **89**: 1537-1540 [PMID: 8079933]
20 **Saers SJ**, Scheltinga MR. Primary aortoenteric fistula. *Br J Surg* 2005; **92**: 143-152 [PMID: 15685700 DOI: 10.1002/bjs.4928]
21 **Suter M**, Doenz F, Chapuis G, Gillet M, Sandblom P. Haemorrhage into the pancreatic duct (Hemosuccus pancreaticus): recognition and management. *Eur J Surg* 1995; **161**: 887-892 [PMID: 8775630]
22 **Nantes O**, Zozaya JM, Montes R, Hermida J. Gastrointestinal lesions and characteristics of acute gastrointestinal bleeding in acenocoumarol-treated patients. *Gastroenterol Hepatol* 2014: S0210-5705(14)00024-7 [PMID: 24582763 DOI: 10.1016/j.gastrohep.2013.12.008]
23 **Barada K**, Abdul-Baki H, El Hajj II, Hashash JG, Green PH. Gastrointestinal bleeding in the setting of anticoagulation and antiplatelet therapy. *J Clin Gastroenterol* 2009; **43**: 5-12 [PMID: 18607297 DOI: 10.1097/MCG.0b013e31811edd13]
24 **Thomopoulos KC**, Mimidis KP, Theocharis GJ, Gatopoulou AG, Kartalis GN, Nikolopoulou VN. Acute upper gastrointestinal bleeding in patients on long-term oral anticoagulation therapy: endoscopic findings, clinical management and outcome. *World J Gastroenterol* 2005; **11**: 1365-1368 [PMID: 15761977]
25 **Rasmussen LH**, Larsen TB, Graungaard T, Skjøth F, Lip GY. Primary and secondary prevention with new oral anticoagulant drugs for stroke prevention in atrial fibrillation: indirect comparison analysis. *BMJ* 2012; **345**: e7097 [PMID: 23129490 DOI: 10.1136/bmj.e7097]
26 **Holster IL**, Valkhoff VE, Kuipers EJ, Tjwa ET. New oral anticoagulants increase risk for gastrointestinal bleeding: a systematic review and meta-analysis. *Gastroenterology* 2013; **145**: 105-112.e15 [PMID: 23470618 DOI: 10.1053/j.gastro.2013.02.041]
27 **Zaman A**, Katon RM. Push enteroscopy for obscure gastrointestinal bleeding yields a high incidence of proximal lesions within reach of a standard endoscope. *Gastrointest Endosc* 1998; **47**: 372-376 [PMID: 9609429 DOI: 10.1016/S0016-5107(98)70221-4]
28 **Descamps C**, Schmit A, Van Gossum A. "Missed" upper gastrointestinal tract lesions may explain "occult" bleeding. *Endoscopy* 1999; **31**: 452-455 [PMID: 10494684 DOI: 10.1055/s-1999-151]
29 **Chak A**, Cooper GS, Canto MI, Pollack BJ, Sivak MV. Enteroscopy for the initial evaluation of iron deficiency. *Gastrointest Endosc* 1998; **47**: 144-148 [PMID: 9512279 DOI: 10.1016/S0016-5107(98)70347-5]
30 **Fry LC**, Bellutti M, Neumann H, Malfertheiner P, Mönkemüller K. Incidence of bleeding lesions within reach of conventional upper and lower endoscopes in patients undergoing double-balloon enteroscopy for obscure gastrointestinal bleeding. *Aliment Pharmacol Ther* 2009; **29**: 342-349 [PMID: 19035975 DOI: 10.1111/j.1365-2036.2008.03888.x]
31 **Tee HP**, Kaffes AJ. Non-small-bowel lesions encountered during double-balloon enteroscopy performed for obscure gastrointestinal bleeding. *World J Gastroenterol* 2010; **16**: 1885-1889 [PMID: 20397267 DOI: 10.3748/wjg.v16.i15.1885]
32 **Leaper M**, Johnston MJ, Barclay M, Dobbs BR, Frizelle FA. Reasons for failure to diagnose colorectal carcinoma at colonoscopy. *Endoscopy* 2004; **36**: 499-503 [PMID: 15202045 DOI: 10.1055/s-2004-814399]
33 **Gilbert D**, O'Malley S, Selby W. Are repeat upper gastrointestinal endoscopy and colonoscopy necessary within six months of capsule endoscopy in patients with obscure gastrointestinal bleeding? *J Gastroenterol Hepatol* 2008; **23**: 1806-1809 [PMID: 19032448]
34 **Lin S**, Branch MS, Shetzline M. The importance of indication in the diagnostic value of push enteroscopy. *Endoscopy* 2003; **35**: 315-321 [PMID: 12664388 DOI: 10.1055/s-2003-38144]
35 **Rabe FE**, Becker GJ, Besozzi MJ, Miller RE. Efficacy study of the small-bowel examination. *Radiology* 1981; **140**: 47-50 [PMID: 7244242]
36 **Moch A**, Herlinger H, Kochman ML, Levine MS, Rubesin SE, Laufer I. Enteroclysis in the evaluation of obscure gastrointestinal bleeding. *AJR Am J Roentgenol* 1994; **163**: 1381-1384 [PMID: 7992733 DOI: 10.2214/ajr.163.6.7992733]
37 **Jonnalagadda S**, Prakash C. Intestinal strictures can impede wireless capsule enteroscopy. *Gastrointest Endosc* 2003; **57**: 418-420 [PMID: 12612534 DOI: 10.1067/mge.2003.128]
38 **Graça BM**, Freire PA, Brito JB, Ilharco JM, Carvalheiro VM, Caseiro-Alves F. Gastroenterologic and radiologic approach to obscure gastrointestinal bleeding: how, why, and when? *Radiographics* 2010; **30**: 235-252 [PMID: 20083596 DOI: 10.1148/rg.301095091]
39 **Huprich JE**, Fletcher JG, Alexander JA, Fidler JL, Burton SS, McCullough CH. Obscure gastrointestinal bleeding: evaluation with 64-section multiphase CT enterography--initial experience. *Radiology* 2008; **246**: 562-571 [PMID: 18227546 DOI: 10.1148/radiol.2462061920]
40 **Kennedy DW**, Laing CJ, Tseng LH, Rosenblum DI, Tamarkin SW. Detection of active gastrointestinal hemorrhage with CT angiography: a 4(1/2)-year retrospective review. *J Vasc Interv Radiol* 2010; **21**: 848-855 [PMID: 20400333 DOI: 10.1016/j.jvir.2010.01.039]
41 **Yoon W**, Jeong YY, Shin SS, Lim HS, Song SG, Jang NG, Kim JK, Kang HK. Acute massive gastrointestinal bleeding: detection and localization with arterial phase multidetector row helical CT. *Radiology* 2006; **239**: 160-167 [PMID: 16484350 DOI: 10.1148/radiol.2383050175]
42 **Som P**, Oster ZH, Atkins HL, Goldman AG, Sacker DF, Harold WH, Fairchild RG, Richards P, Brill AB. Detection of gastrointestinal blood loss with 99mTc-labeled, heat-treated red blood cells. *Radiology* 1981; **138**: 207-209 [PMID: 6969900]
43 **Zuckerman GR**, Prakash C, Askin MP, Lewis BS. AGA technical review on the evaluation and management of occult and obscure gastrointestinal bleeding. *Gastroenterology* 2000; **118**: 201-221 [PMID: 10611170 DOI: 10.1016/S0016-5085(00)70430-6]
44 **Hunter JM**, Pezim ME. Limited value of technetium 99m-labeled red cell scintigraphy in localization of lower gastrointestinal bleeding. *Am J Surg* 1990; **159**: 504-506 [PMID: 2334015 DOI: 10.1016/S0002-9610(05)81256-5]
45 **Ng DA**, Opelka FG, Beck DE, Milburn JM, Witherspoon LR, Hicks TC, Timmcke AE, Gathright JB. Predictive value of technetium Tc 99m-labeled red blood cell scintigraphy for positive angiogram in massive lower gastrointestinal hemorrhage. *Dis Colon Rectum* 1997; **40**: 471-477 [PMID: 9106699 DOI: 10.1007/BF02258395]
46 **Fiorito JJ**, Brandt LJ, Kozicky O, Grosman IM, Sprayragen S. The diagnostic yield of superior mesenteric angiography: correlation with the pattern of gastrointestinal bleeding. *Am J Gastroenterol* 1989; **84**: 878-881 [PMID: 2667335]
47 **Egglin TK**, O'Moore PV, Feinstein AR, Waltman AC. Complications of peripheral arteriography: a new system to iden-

tify patients at increased risk. *J Vasc Surg* 1995; **22**: 787-794 [PMID: 8523614 DOI: 10.1016/S0741-5214(95)70070-6]
48 **Bloomfeld RS**, Smith TP, Schneider AM, Rockey DC. Provocative angiography in patients with gastrointestinal hemorrhage of obscure origin. *Am J Gastroenterol* 2000; **95**: 2807-2812 [PMID: 11051352 DOI: 10.1111/j.1572-0241.2000.03191.x]
49 **Miller M**, Smith TP. Angiographic diagnosis and endovascular management of nonvariceal gastrointestinal hemorrhage. *Gastroenterol Clin North Am* 2005; **34**: 735-752 [PMID: 16303580 DOI: 10.1016/j.gtc.2005.09.001]
50 **Busch OR**, van Delden OM, Gouma DJ. Therapeutic options for endoscopic haemostatic failures: the place of the surgeon and radiologist in gastrointestinal tract bleeding. *Best Pract Res Clin Gastroenterol* 2008; **22**: 341-354 [PMID: 18346688 DOI: 10.1016/j.bpg.2007.10.018]
51 **Liao Z**, Gao R, Xu C, Li ZS. Indications and detection, completion, and retention rates of small-bowel capsule endoscopy: a systematic review. *Gastrointest Endosc* 2010; **71**: 280-286 [PMID: 20152309 DOI: 10.1016/j.gie.2009.09.031]
52 **Fireman Z**, Kopelman Y. The role of video capsule endoscopy in the evaluation of iron deficiency anaemia. *Dig Liver Dis* 2004; **36**: 97-102 [PMID: 15002814 DOI: 10.1016/j.dld.2003.10.009]
53 **Rastogi A**, Schoen RE, Slivka A. Diagnostic yield and clinical outcomes of capsule endoscopy. *Gastrointest Endosc* 2004; **60**: 959-964 [PMID: 15605012 DOI: 10.1016/S0016-5107(04)02226-6]
54 **May A**, Wardak A, Nachbar L, Remke S, Ell C. Influence of patient selection on the outcome of capsule endoscopy in patients with chronic gastrointestinal bleeding. *J Clin Gastroenterol* 2005; **39**: 684-688 [PMID: 16082277 DOI: 10.1097/01.mcg.0000173857.22933.3b]
55 **Bresci G**, Parisi G, Bertoni M, Tumino E, Capria A. The role of video capsule endoscopy for evaluating obscure gastrointestinal bleeding: usefulness of early use. *J Gastroenterol* 2005; **40**: 256-259 [PMID: 15830284 DOI: 10.1007/s00535-004-1532-5]
56 **Sidhu R**, Sanders DS, McAlindon ME. Does capsule endoscopy recognise gastric antral vascular ectasia more frequently than conventional endoscopy? *J Gastrointestin Liver Dis* 2006; **15**: 375-377 [PMID: 17205150]
57 **Estévez E**, González-Conde B, Vázquez-Iglesias JL, Alonso PA, Vázquez-Millán Mde L, Pardeiro R. Incidence of tumoral pathology according to study using capsule endoscopy for patients with obscure gastrointestinal bleeding. *Surg Endosc* 2007; **21**: 1776-1780 [PMID: 17356941]
58 **Redondo-Cerezo E**, Gómez-Ruiz CJ, Sánchez-Manjavacas N, Viñuelas M, Jimeno C, Pérez-Vigara G, Morillas J, Pérez-García JI, García-Cano J, Pérez-Sola A. Long-term follow-up of patients with small-bowel angiodysplasia on capsule endoscopy. Determinants of a higher clinical impact and rebleeding rate. *Rev Esp Enferm Dig* 2008; **100**: 202-207 [PMID: 18563976 DOI: 10.4321/S1130-01082008000400002]
59 **Marmo R**, Rotondano G, Piscopo R, Bianco MA, Cipolletta L. Meta-analysis: capsule enteroscopy vs. conventional modalities in diagnosis of small bowel diseases. *Aliment Pharmacol Ther* 2005; **22**: 595-604 [PMID: 16181299 DOI: 10.1111/j.1365-2036.2005.02625.x]
60 **Lewis BS**, Eisen GM, Friedman S. A pooled analysis to evaluate results of capsule endoscopy trials. *Endoscopy* 2005; **37**: 960-965 [PMID: 16189768 DOI: 10.1055/s-2005-870353]
61 **Triester SL**, Leighton JA, Leontiadis GI, Fleischer DE, Hara AK, Heigh RI, Shiff AD, Sharma VK. A meta-analysis of the yield of capsule endoscopy compared to other diagnostic modalities in patients with obscure gastrointestinal bleeding. *Am J Gastroenterol* 2005; **100**: 2407-2418 [PMID: 16279893 DOI: 10.1111/j.1572-0241.2005.00274.x]
62 **Saperas E**, Dot J, Videla S, Alvarez-Castells A, Perez-Lafuente M, Armengol JR, Malagelada JR. Capsule endoscopy versus computed tomographic or standard angiography for the diagnosis of obscure gastrointestinal bleeding. *Am J Gastroenterol* 2007; **102**: 731-737 [PMID: 17397406 DOI: 10.1111/j.1572-0241.2007.01058.x]
63 **Pasha SF**, Leighton JA, Das A, Harrison ME, Decker GA, Fleischer DE, Sharma VK. Double-balloon enteroscopy and capsule endoscopy have comparable diagnostic yield in small-bowel disease: a meta-analysis. *Clin Gastroenterol Hepatol* 2008; **6**: 671-676 [PMID: 18356113 DOI: 10.1016/j.cgh.2008.01.005]
64 **Hartmann D**, Schmidt H, Bolz G, Schilling D, Kinzel F, Eickhoff, Huschner W, Möller K, Jakobs R, Reitzig P, Weickert U, Gellert K, Schultz H, Guenther K, Hollerbuhl H, Schoenleben K, Schulz HJ, Riemann JF. A prospective two-center study comparing wireless capsule endoscopy with intraoperative enteroscopy in patients with obscure GI bleeding. *Gastrointest Endosc* 2005; **61**: 826-832 [DOI: 10.1016/S0016-5107(05)00372-X]
65 **Redondo-Cerezo E**, Pérez-Vigara G, Pérez-Sola A, Gómez-Ruiz CJ, Chicano MV, Sánchez-Manjavacas N, Morillas J, Pérez-García JI, García-Cano J. Diagnostic yield and impact of capsule endoscopy on management of patients with gastrointestinal bleeding of obscure origin. *Dig Dis Sci* 2007; **52**: 1376-1381 [PMID: 17356913 DOI: 10.1007/s10620-006-9605-3]
66 **Mylonaki M**, Fritscher-Ravens A, Swain P. Wireless capsule endoscopy: a comparison with push enteroscopy in patients with gastroscopy and colonoscopy negative gastrointestinal bleeding. *Gut* 2003; **52**: 1122-1126 [PMID: 12865269 DOI: 10.1136/gut.52.8.1122]
67 **Lai LH**, Wong GL, Chow DK, Lau JY, Sung JJ, Leung WK. Long-term follow-up of patients with obscure gastrointestinal bleeding after negative capsule endoscopy. *Am J Gastroenterol* 2006; **101**: 1224-1228 [PMID: 16771942 DOI: 10.1111/j.1572-0241.2006.00565.x]
68 **Swain P**, Fritscher-Ravens A. Role of video endoscopy in managing small bowel disease. *Gut* 2004; **53**: 1866-1875 [PMID: 15542530 DOI: 10.1136/gut.2003.035576]
69 **Ben-Soussan E**, Savoye G, Antonietti M, Ramirez S, Lerebours E, Ducrotté P. Factors that affect gastric passage of video capsule. *Gastrointest Endosc* 2005; **62**: 785-790 [PMID: 16246700 DOI: 10.1016/j.gie.2005.07.040]
70 **Nogales Rincón O**, Merino Rodríguez B, González Asanza C, Fernández-Pacheco PM. [Utility of capsule endoscopy with flexible spectral imaging color enhancement in the diagnosis of small bowel lesions]. *Gastroenterol Hepatol* 2013; **36**: 63-68 [PMID: 23140757 DOI: 10.1016/j.gastrohep.2012.08.004]
71 **Sidhu R**, Sanders DS, Morris AJ, McAlindon ME. Guidelines on small bowel enteroscopy and capsule endoscopy in adults. *Gut* 2008; **57**: 125-136 [PMID: 18094205 DOI: 10.1136/gut.2007.129999]
72 **Benz C**, Jakobs R, Riemann JF. Do we need the overtube for push-enteroscopy? *Endoscopy* 2001; **33**: 658-661 [PMID: 11490380 DOI: 10.1055/s-2001-16208]
73 **Taylor AC**, Chen RY, Desmond PV. Use of an overtube for enteroscopy--does it increase depth of insertion? A prospective study of enteroscopy with and without an overtube. *Endoscopy* 2001; **33**: 227-230 [PMID: 11293754 DOI: 10.1055/s-2001-12799]
74 **Carey EJ**, Fleischer DE. Investigation of the small bowel in gastrointestinal bleeding--enteroscopy and capsule endoscopy. *Gastroenterol Clin North Am* 2005; **34**: 719-734 [PMID: 16303579 DOI: 10.1016/j.gtc.2005.08.009]
75 **Mergener K**, Ponchon T, Gralnek I, Pennazio M, Gay G, Selby W, Seidman EG, Cellier C, Murray J, de Franchis R, Rösch T, Lewis BS. Literature review and recommendations for clinical application of small-bowel capsule endoscopy, based on a panel discussion by international experts. Consensus statements for small-bowel capsule endoscopy, 2006/2007. *Endoscopy* 2007; **39**: 895-909 [PMID: 17968807 DOI: 10.1055/s-2007-966930]
76 **May A**, Nachbar L, Pohl J, Ell C. Endoscopic interventions

in the small bowel using double balloon enteroscopy: feasibility and limitations. *Am J Gastroenterol* 2007; **102**: 527-535 [PMID: 17222315 DOI: 10.1111/j.1572-0241.2007.01063.x]
77 **May A**, Nachbar L, Schneider M, Ell C. Prospective comparison of push enteroscopy and push-and-pull enteroscopy in patients with suspected small-bowel bleeding. *Am J Gastroenterol* 2006; **101**: 2016-2024 [PMID: 16968508 DOI: 10.1111/j.1572-0241.2006.00745.x]
78 **Matsumoto T**, Moriyama T, Esaki M, Nakamura S, Iida M. Performance of antegrade double-balloon enteroscopy: comparison with push enteroscopy. *Gastrointest Endosc* 2005; **62**: 392-398 [PMID: 16111958 DOI: 10.1016/j.gie.2005.04.052]
79 **Yamamoto H**, Sekine Y, Sato Y, Higashizawa T, Miyata T, Iino S, Ido K, Sugano K. Total enteroscopy with a nonsurgical steerable double-balloon method. *Gastrointest Endosc* 2001; **53**: 216-220 [PMID: 11174299 DOI: 10.1067/mge.2001.112181]
80 **Alexander JA**, Leighton JA. Capsule endoscopy and balloon-assisted endoscopy: competing or complementary technologies in the evaluation of small bowel disease? *Curr Opin Gastroenterol* 2009; **25**: 433-437 [PMID: 19461511 DOI: 10.1097/MOG.0b013e32832d641e]
81 **Gay G**, Delvaux M, Fassler I. Outcome of capsule endoscopy in determining indication and route for push-and-pull enteroscopy. *Endoscopy* 2006; **38**: 49-58 [PMID: 16429355 DOI: 10.1055/s-2005-921176]
82 **Gerson LB**. Double-balloon enteroscopy: the new gold standard for small-bowel imaging? *Gastrointest Endosc* 2005; **62**: 71-75 [PMID: 15990822 DOI: 10.1016/S0016-5107(05)00507-9]
83 **Mensink PB**, Haringsma J, Kucharzik T, Cellier C, Pérez-Cuadrado E, Mönkemüller K, Gasbarrini A, Kaffes AJ, Nakamura K, Yen HH, Yamamoto H. Complications of double balloon enteroscopy: a multicenter survey. *Endoscopy* 2007; **39**: 613-615 [PMID: 17516287 DOI: 10.1055/s-2007-966444]
84 **Gerson LB**, Tokar J, Chiorean M, Lo S, Decker GA, Cave D, Bouhaidar D, Mishkin D, Dye C, Haluszka O, Leighton JA, Zfass A, Semrad C. Complications associated with double balloon enteroscopy at nine US centers. *Clin Gastroenterol Hepatol* 2009; **7**: 1177-1182, 1177-1182 [PMID: 19602453 DOI: 10.1016/j.cgh.2009.07.005]
85 **May A**, Färber M, Aschmoneit I, Pohl J, Manner H, Lotterer E, Möschler O, Kunz J, Gossner L, Mönkemüller K, Ell C. Prospective multicenter trial comparing push-and-pull enteroscopy with the single- and double-balloon techniques in patients with small-bowel disorders. *Am J Gastroenterol* 2010; **105**: 575-581 [PMID: 20051942 DOI: 10.1038/ajg.2009.712]
86 **Takano N**, Yamada A, Watabe H, Togo G, Yamaji Y, Yoshida H, Kawabe T, Omata M, Koike K. Single-balloon versus double-balloon endoscopy for achieving total enteroscopy: a randomized, controlled trial. *Gastrointest Endosc* 2011; **73**: 734-739 [PMID: 21272875 DOI: 10.1016/j.gie.2010.10.047]
87 **Domagk D**, Mensink P, Aktas H, Lenz P, Meister T, Luegering A, Ullerich H, Aabakken L, Heinecke A, Domschke W, Kuipers E, Bretthauer M. Single- vs. double-balloon enteroscopy in small-bowel diagnostics: a randomized multicenter trial. *Endoscopy* 2011; **43**: 472-476 [PMID: 21384320 DOI: 10.1055/s-0030-1256247]
88 **Efthymiou M**, Desmond PV, Brown G, La Nauze R, Kaffes A, Chua TJ, Taylor AC. SINGLE-01: a randomized, controlled trial comparing the efficacy and depth of insertion of single- and double-balloon enteroscopy by using a novel method to determine insertion depth. *Gastrointest Endosc* 2012; **76**: 972-980 [PMID: 22980289 DOI: 10.1016/j.gie.2012.06.033]
89 **Pai RD**, Carr-Locke DL, Thompson CC. Endoscopic evaluation of the defunctionalized stomach by using ShapeLock technology (with video). *Gastrointest Endosc* 2007; **66**: 578-581 [PMID: 17725949 DOI: 10.1016/j.gie.2007.02.062]
90 **Kuga R**, Furuya CK, Hondo FY, Ide E, Ishioka S, Sakai P. ERCP using double-balloon enteroscopy in patients with Roux-en-Y anatomy. *Dig Dis* 2008; **26**: 330-335 [PMID: 19188724 DOI: 10.1159/000177018]
91 **Rex DK**, Khashab M, Raju GS, Pasricha J, Kozarek R. Insertability and safety of a shape-locking device for colonoscopy. *Am J Gastroenterol* 2005; **100**: 817-820 [PMID: 15784024 DOI: 10.1111/j.1572-0241.2005.40801.x]
92 **Haber GWL**. A prospective study to evaluate the ShapeLock guide to enable complete colonoscopy in previously failed cases. *Gastrointest Endosc* 2006; **63**: AB226 [DOI: 10.1016/j.gie.2006.03.573]
93 **Schulz HJ**, Schmidt H. Intraoperative enteroscopy. *Gastrointest Endosc Clin N Am* 2009; **19**: 371-379 [PMID: 19647646 DOI: 10.1016/j.giec.2009.04.011]
94 **Douard R**, Wind P, Berger A, Maniere T, Landi B, Cellier C, Cugnenc PH. Role of intraoperative enteroscopy in the management of obscure gastointestinal bleeding at the time of video-capsule endoscopy. *Am J Surg* 2009; **198**: 6-11 [PMID: 19393986 DOI: 10.1016/j.amjsurg.2008.06.036]
95 **Zaman A**, Sheppard B, Katon RM. Total peroral intraoperative enteroscopy for obscure GI bleeding using a dedicated push enteroscope: diagnostic yield and patient outcome. *Gastrointest Endosc* 1999; **50**: 506-510 [PMID: 10502171 DOI: 10.1016/S0016-5107(99)70073-8]
96 **Lewis MP**, Khoo DE, Spencer J. Value of laparotomy in the diagnosis of obscure gastrointestinal haemorrhage. *Gut* 1995; **37**: 187-190 [PMID: 7557565 DOI: 10.1136/gut.37.2.187]
97 **Douard R**, Wind P, Panis Y, Marteau P, Bouhnik Y, Cellier C, Cugnenc P, Valleur P. Intraoperative enteroscopy for diagnosis and management of unexplained gastrointestinal bleeding. *Am J Surg* 2000; **180**: 181-184 [PMID: 11084125 DOI: 10.1016/S0002-9610(00)00447-5]
98 **Kovacs TO**, Jensen DM. Recent advances in the endoscopic diagnosis and therapy of upper gastrointestinal, small intestinal, and colonic bleeding. *Med Clin North Am* 2002; **86**: 1319-1356 [PMID: 12510456 DOI: 10.1016/S0025-7125(02)00079-2]
99 **Apostolopoulos P**, Liatsos C, Gralnek IM, Kalantzis C, Giannakoulopoulou E, Alexandrakis G, Tsibouris P, Kalafatis E, Kalantzis N. Evaluation of capsule endoscopy in active, mild-to-moderate, overt, obscure GI bleeding. *Gastrointest Endosc* 2007; **66**: 1174-1181 [PMID: 18061718 DOI: 10.1016/j.gie.2007.06.058]
100 **Cellier C**. Obscure gastrointestinal bleeding: role of videocapsule and double-balloon enteroscopy. *Best Pract Res Clin Gastroenterol* 2008; **22**: 329-340 [PMID: 18346687 DOI: 10.1016/j.bpg.2007.12.006]
101 **Somsouk M**, Gralnek IM, Inadomi JM. Management of obscure occult gastrointestinal bleeding: a cost-minimization analysis. *Clin Gastroenterol Hepatol* 2008; **6**: 661-670 [PMID: 18550005 DOI: 10.1016/j.cgh.2008.02.033]
102 **Albert JG**, Nachtigall F, Wiedbrauck F, Dollinger MM, Gittinger FS, Hollerbach S, Wienke A. Minimizing procedural cost in diagnosing small bowel bleeding: comparison of a strategy based on initial capsule endoscopy versus initial double-balloon enteroscopy. *Eur J Gastroenterol Hepatol* 2010; **22**: 679-688 [PMID: 20446352]
103 **Viazis N**, Papaxoinis K, Vlachogiannakos J, Efthymiou A, Theodoropoulos I, Karamanolis DG. Is there a role for second-look capsule endoscopy in patients with obscure GI bleeding after a nondiagnostic first test? *Gastrointest Endosc* 2009; **69**: 850-856 [PMID: 18950762 DOI: 10.1016/j.gie.2008.05.053]
104 **Bar-Meir S**. Video capsule endoscopy or double-balloon enteroscopy: are they equivalent? *Gastrointest Endosc* 2009; **69**: 875-876 [PMID: 19327474 DOI: 10.1016/j.gie.2008.07.051]
105 **Kaffes AJ**, Siah C, Koo JH. Clinical outcomes after double-balloon enteroscopy in patients with obscure GI bleeding and a positive capsule endoscopy. *Gastrointest Endosc* 2007; **66**: 304-309 [PMID: 17643704 DOI: 10.1016/j.gie.2007.02.044]
106 **González-Galilea A,** Gálvez-Calderón C, García Sánchez V, de Dios-Vega J.F. Hemorragia digestiva de origen oscuro. Orientación diagnóstica y terapéutica. Publicación electróni-

ca. Available from: URL: http: //www.sapd.es/revista/article.php?file=vol34_n1/03

107 **van Cutsem E**, Rutgeerts P, Vantrappen G. Treatment of bleeding gastrointestinal vascular malformations with oestrogen-progesterone. *Lancet* 1990; **335**: 953-955 [PMID: 1970032 DOI: 10.1016/0140-6736(90)91010-8]

108 **Barkin JS**, Ross BS. Medical therapy for chronic gastrointestinal bleeding of obscure origin. *Am J Gastroenterol* 1998; **93**: 1250-1254 [PMID: 9707046 DOI: 10.1111/j.1572-0241.1998.404_i.x]

109 **Junquera F**, Feu F, Papo M, Videla S, Armengol JR, Bordas JM, Saperas E, Piqué JM, Malagelada JR. A multicenter, randomized, clinical trial of hormonal therapy in the prevention of rebleeding from gastrointestinal angiodysplasia. *Gastroenterology* 2001; **121**: 1073-1079 [PMID: 11677198 DOI: 10.1053/gast.2001.28650]

110 **Blich M**, Fruchter O, Edelstein S, Edoute Y. Somatostatin therapy ameliorates chronic and refractory gastrointestinal bleeding caused by diffuse angiodysplasia in a patient on anticoagulation therapy. *Scand J Gastroenterol* 2003; **38**: 801-803 [PMID: 12889571 DOI: 10.1080/00365520310001969]

111 **Rossini FP**, Arrigoni A, Pennazio M. Octreotide in the treatment of bleeding due to angiodysplasia of the small intestine. *Am J Gastroenterol* 1993; **88**: 1424-1427 [PMID: 8362842]

112 **Bauditz J**, Wedel S, Lochs H. Thalidomide reduces tumour necrosis factor alpha and interleukin 12 production in patients with chronic active Crohn's disease. *Gut* 2002; **50**: 196-200 [PMID: 11788559 DOI: 10.1136/gut.50.2.196]

113 **Krikis N**, Tziomalos K, Perifanis V, Vakalopoulou S, Karagiannis A, Garipidou V, Harsoulis F. Treatment of recurrent gastrointestinal haemorrhage in a patient with von Willebrand's disease with octreotide LAR and propranolol. *Gut* 2005; **54**: 171-172 [PMID: 15591529 DOI: 10.1136/gut.2004.049031]

114 **Molina-Infante J**, Perez-Gallardo B, Gonzalez-Garcia G, Fernandez-Bermejo M, Mateos-Rodriguez JM, Robledo-Andres P. Octreotide LAR for severe obscure-overt gastrointestinal haemorrhage in high-risk patients on anticoagulation therapy. *Gut* 2007; **56**: 447 [PMID: 17339258 DOI: 10.1136/gut.2006.113878]

115 **Bon C**, Aparicio T, Vincent M, Mavros M, Bejou B, Raynaud JJ, Zampeli E, Airinei G, Sautereau D, Benamouzig R, Michopoulos S. Long-acting somatostatin analogues decrease blood transfusion requirements in patients with refractory gastrointestinal bleeding associated with angiodysplasia. *Aliment Pharmacol Ther* 2012; **36**: 587-593 [PMID: 22831465 DOI: 10.1111/apt.12000]

116 **Ge ZZ**, Chen HM, Gao YJ, Liu WZ, Xu CH, Tan HH, Chen HY, Wei W, Fang JY, Xiao SD. Efficacy of thalidomide for refractory gastrointestinal bleeding from vascular malformation. *Gastroenterology* 2011; **141**: 1629-37.e1-4 [PMID: 21784047 DOI: 10.1053/j.gastro.2011.07.018]

117 **May A**, Friesing-Sosnik T, Manner H, Pohl J, Ell C. Long-term outcome after argon plasma coagulation of small-bowel lesions using double-balloon enteroscopy in patients with mid-gastrointestinal bleeding. *Endoscopy* 2011; **43**: 759-765 [PMID: 21544778 DOI: 10.1055/s-0030-1256388]

118 **Samaha E**, Rahmi G, Landi B, Lorenceau-Savale C, Malamut G, Canard JM, Bloch F, Jian R, Chatellier G, Cellier C. Long-term outcome of patients treated with double balloon enteroscopy for small bowel vascular lesions. *Am J Gastroenterol* 2012; **107**: 240-246 [PMID: 21946281 DOI: 10.1038/ajg.2011.325]

119 **Junquera F**, Brullet E, Campo R, Calvet X, Puig-Diví V, Vergara M. Usefulness of endoscopic band ligation for bleeding small bowel vascular lesions. *Gastrointest Endosc* 2003; **58**: 274-279 [PMID: 12872104 DOI: 10.1067/mge.2003.357]

120 **Cherian MP**, Mehta P, Kalyanpur TM, Hedgire SS, Narsinghpura KS. Arterial interventions in gastrointestinal bleeding. *Semin Intervent Radiol* 2009; **26**: 184-196 [PMID: 21326563 DOI: 10.1055/s-0029-1225661]

121 **Mirsadraee S**, Tirukonda P, Nicholson A, Everett SM, McPherson SJ. Embolization for non-variceal upper gastrointestinal tract haemorrhage: a systematic review. *Clin Radiol* 2011; **66**: 500-509 [PMID: 21371695 DOI: 10.1016/j.crad.2010.11.016]

P- Reviewer: Calabrese C, Moeschler O, Misra SP, Sung J, Yang MH **S- Editor**: Ji FF **L- Editor**: A **E- Editor:** Lu YJ

World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4291/wjgp.v5.i3.284

World J Gastrointest Pathophysiol 2014 August 15; 5(3): 284-292
ISSN 2150-5330 (online)

REVIEW

Role of hemostatic powders in the endoscopic management of gastrointestinal bleeding

Marco Bustamante-Balén, Gema Plumé

Marco Bustamante-Balén, Digestive Endoscopy Unit, Gastroenterology Department, La Fe University Hospital, Valencia 46026, Spain
Gema Plumé, Valencian Institute of Pathology, Universidad Católica de Valencia, Calle Quevedo 2, Valencia 46001, Spain
Author contributions: Bustamante-Balén M and Plumé G designed the study, reviewed the literature, drafted and revised the manuscript and gave final approval of the version to be published; both authors contributed equally to this work.
Correspondence to: Marco Bustamante, MD, PhD, Digestive Endoscopy Unit, Gastroenterology Department, La Fe University Hospital, Avinguda Fernando Abril Martorell, no. 106, Valencia 46026, Spain. bustamante_mar@gva.es
Telephone: +34-67-6092456 Fax: +34-96-1622410
Received: April 1, 2014 Revised: May 16, 2014
Accepted: June 18, 2014
Published online: August 15, 2014

Abstract

Acute gastrointestinal bleeding (AGIB) is a prevalent condition with significant influence on healthcare costs. Endoscopy is essential for the management of AGIB with a pivotal role in diagnosis, risk stratification and management. Recently, hemostatic powders have been added to our endoscopic armamentarium to treat gastrointestinal (GI) bleeding. These substances are intended to control active bleeding by delivering a powdered product over the bleeding site that forms a solid matrix with a tamponade function. Local activation of platelet aggregation and coagulation cascade may be also boosted. There are currently three powders commercially available: hemostatic agent TC-325 (Hemospray®), EndoClot™ polysaccharide hemostatic system, and Ankaferd Bloodstopper®. Although the available evidence is based on short series of cases and there is no randomized controlled trial yet, these powders seem to be effective in controlling GI bleeding from a variety of origins with a very favorable side effects profile. They can be used either as a primary therapy or a second-line treatment, and they seem to be especially indicated in cases of cancer-related bleeding and lesions with difficult access. In this review, we will comment on the mechanism of action, efficacy, safety and technical challenges of the use of powders in several clinical scenarios and we will try to define the main current indications of use and propose new lines of research in this area.

Key words: Gastrointestinal hemorrhage; Endoscopy; Powders; Endoscopic hemostasis

Core tip: Hemostatic powders are a new endoscopic therapeutic modality for gastrointestinal bleeding. Based on their characteristics and mechanism of action, they may be very useful in controlling bleeding in some situations. In the last two years, a large number of studies, mainly short series of cases, have been published on this topic but their role in the management algorithm is not yet defined. In this review, we will comment on the efficacy and safety of the use of powders in several clinical scenarios and we will try to define the main current indications of use and propose new lines of research in this area.

Bustamante-Balén M, Plumé G. Role of hemostatic powders in the endoscopic management of gastrointestinal bleeding. *World J Gastrointest Pathophysiol* 2014; 5(3): 284-292 Available from: URL: http://www.wjgnet.com/2150-5330/full/v5/i3/284.htm DOI: http://dx.doi.org/10.4291/wjgp.v5.i3.284

INTRODUCTION

Acute gastrointestinal bleeding is a prevalent condition with significant influence on healthcare costs. The annual rate of hospitalizations from acute upper GI bleeding (AUGIB) in the United States is around 160 hospital

Table 1 Hemostatic powders currently available

Name	Composition	Mechanism of action	Regulatory clearance
Hemospray™	Mineral	Absorption of water Concentration of platelets and clotting factors Mechanical tamponade	Approved in Europe and Canada[1] Under evaluation in United States
EndoClot™ PHS	Absorbable hemostatic polysaccharides	Absorption of water Concentration of platelets and clotting factors Mechanical tamponade	Approved in Turkey, Europe, Malaysia and Australia
Ankaferd® Blood Stopper	Mixture of plants	Encapsulated protein network that provides focal points for erythrocyte aggregation	Approved in Turkey

[1]For non-variceal upper gastrointestinal bleeding.

admissions per 100000 population[1], leading to approximately 300000 hospitalizations annually. Between 36% and 50% of AUGIB episodes in most published series are due to non-variceal causes, mainly peptic ulcer[2,3]. Despite improvements in medical and endoscopic therapy, mortality from AUGIB remains around 10%, with higher rates for variceal bleeding and malignancy[2]. On the other hand, severe acute lower GI bleeding (ALGIB), mainly caused by diverticular disease, vascular lesions and ischemic colitis, is an emerging cause of hospital admission[4]. In one study, the ratio of hospitalization rates between upper and lower GI complications decreased from 4.3 to 1.4 in 10 years[5].

Endoscopy plays a pivotal role in the management of both types of GI bleeding, allowing diagnosis, risk stratification and treatment[6-8]. Endoscopic hemostatic therapy is the basis of treatment in patients with active bleeding or with endoscopic features that predict an increased risk of further hemorrhage. However, endoscopic therapy in clinical practice has some drawbacks that limit its efficacy. For instance, despite being highly effective in achieving hemostasis in UAGIB, in 5%-10% of patients this bleeding will not be initially controlled or they will experience a recurrence[9]. In patients with severe acute bleeding and a difficult anatomy (*e.g.*, posterior duodenal wall or the upper region of the lesser gastric curvatures), endoscopic therapy can be challenging, often requiring a high level of technical expertise. Finally, this life-threatening condition can also present outside normal working hours when a less skilled endoscopist is on call. Therefore, a simple and effective hemostatic tool might have a significant impact on endoscopic therapy efficacy of AGIB.

Recently, hemostatic powders have been added to our endoscopic armamentarium to treat GI bleeding. They are intended to control active bleeding by delivering a substance over the bleeding site using a catheter through the working channel of the endoscope. Perhaps the main advantage of this technology is that less precision is needed, allowing for treatment of lesions with difficult access and refractory to standard therapy[10]. There are three hemostatic powders currently available for endoscopic usage (Table 1): hemostatic agent TC-325 (Hemospray™), EndoClot™ polysaccharide hemostatic system (PHS), and Ankaferd Bloodstopper® (ABS). In this review, we will describe the mechanism of action, efficacy in different clinical scenarios, safety of the hemostatic powders, and will comment on the possible role of this tool in the endoscopic treatment of GI bleeding.

MECHANISM OF ACTION

All three powders are designed to be applied through the working channel of the endoscope over the bleeding area. Their components, in contact with moisture, form a stable mechanical barrier that covers the bleeding site, inducing hemostasis. Therefore, they should only be applied if there is an active bleeding. Slight differences are found because of their different chemical composition.

Hemospray™ (TC-325)

TC-325 (Hemospray™, Cook Medical Inc, Winston-Salem, NC, United States) is a proprietary inorganic powder containing no human or animal proteins, botanicals or allergens. It is neither absorbed nor metabolized, therefore it is considered metabolically inert and nontoxic (information provided by the manufacturer). The precise mechanism of action is unknown but it is hypothesized that the powder, in contact with water, forms an adhesive covering that seals the tissue, producing a mechanical tamponade (Table 1). In 24-72 h, this adherent coat sloughs off into the lumen and is completely eliminated from the GI tract[11]. Water absorption also leads to concentration of platelets and clotting factors with activation of platelets and the coagulation cascade[12]. The *in vitro* effects of TC-325 on standardized coagulation and platelet function have been studied, showing that both prothrombin time and activated partial thromboplastin are reduced in a dose-dependent way in the presence of the powder[13]. These results suggest that Hemospray™ may facilitate local hemostasis.

EndoClot™ PHS

EndoClot™ PHS (EndoClot Plus Inc, Santa Clara, California, United States) is a starch-derived compound that consists of biocompatible absorbable hemostatic polysaccharides. In contact with blood, it rapidly absorbs water, causing a high concentration of platelets, red blood cells and coagulation proteins at the bleeding site, thus accelerating the physiological clotting cascade

(Table 1). A gelled matrix is formed that adheres to and seals the bleeding tissue. This matrix is cleared from the bleeding site in a few hours or days[14]. When applied to skin wounds, it seems to improve healing by activating fibroblasts and transforming growth factor (TGF)-β1 release[15], but this effect has not been studied in the GI mucosa.

ABS

This is a mixture of plants, including *Thymus vulgaris, Glycyrrhiza glabra, Vitis vinifera, Alpinia officinarum* and *Urtica dioica*. *In vitro* and ultrastructural studies suggest that ABS rapidly forms an encapsulated protein network that provides focal points for erythrocyte and activated leukocyte aggregation (Table 1)[16,17]. This network stems from interactions between ABS and blood proteins, such as fibrinogen, inducing protein agglutination. Total protein, albumin and globulin levels also decrease in serum, probably *via* agglutination of these molecules in the growing protein network. However, most coagulation factors are not affected by the addition of ABS to fresh normal plasma or serum[18].

ABS also has functional properties, inhibiting fibrinolysis and some natural anticoagulant pathways via interaction with the protein C anticoagulation pathway. On one hand, it enhances the expression of plasminogen activator inhibitor 1 (PAI-1), one of the major inhibitors of fibrinolysis, thus increasing clot stability. On the other hand, ABS has also been shown to down-regulate the endothelial cell protein C receptor (EPCR), a natural enhancer of protein C activity, therefore taking part in inactivation of factors Va and VIIIa[19].

EVIDENCE SUPPORTING THE ROLE OF POWDERS IN ENDOSCOPIC HEMOSTASIS

The evidence supporting the role of powders in GI bleeding is of moderate quality and based mainly on short series of cases and retrospective studies without a control group.

Hemospray™ (TC-325)

Animal models: This powder has been tested in animal models of arterial gastrointestinal bleeding. Giday *et al*[10] performed a randomized controlled trial on 10 pigs allocated to treatment with TC-325 or sham after surgical creation of an arterial bleeding from a gastroepiploic vessel opened up to the gastric lumen. The endpoint of the study was the proportion of animals in which hemostasis was achieved at 1 h. In the treatment group, acute hemostasis was achieved in the whole group with no rebleeding in the first 6 h compared to 0% of animals in the sham group. Mean time to hemostasis was 13.8 min.

Ulcer bleeding: Sung *et al*[11] carried out a pilot study on the efficacy of TC-325 as the primary hemostatic method in 20 patients with active peptic ulcer bleeding. Hemostasis was achieved in all but one (95%), a patient with a Forrest Ia ulcer who ultimately needed embolization to stop bleeding. Two patients met the criteria for rebleeding during follow-up, but no active bleeding was detected in the second-look endoscopy. However, it must be pointed out that most treated bleedings were moderate and the only patient with a high risk lesion had a worse outcome.

Cancer-related GI bleeding: Conventional therapy in this kind of bleeding has moderate success and high rates of rebleeding. Chen *et al*[20] reported on a short series of 5 patients with upper GI bleeding secondary to gastroduodenal tumors. The authors reported control of bleeding in all cases with only one case of rebleeding in a patient with disseminated intravascular coagulation. Leblanc *et al*[21] treated 5 patients with bleeding from GI neoplasms (2 esophageal, 2 gastric and 1 pancreatic) with TC-325. Successful hemostasis was achieved in all patients. Two patients showed recurrent bleeding, again successfully treated with TC-325.

Patients on antithrombotic therapy: Hostel *et al*[22] evaluated 16 patients with upper GI bleeding treated with TC-325 either as a monotherapy or as salvage therapy. In 9 patients, the source of bleeding was a peptic ulcer and in 2, a neoplasm. Eight patients were on antithrombotic therapy (ATT), including patients on antiplatelet agents, NSAIDs or VKA/heparin. Initial hemostasis was achieved in 5/8 patients on ATT and in all patients of the non-ATT group (P = 0.2). The source of bleeding was a spurting arterial vessel in two of the three failures of TC-325 in patients of the ATT group. Rates of rebleeding were similar in both groups (around 25%) and in most cases bleeding was retreated with TC-325.

Bleeding secondary to a therapeutic intervention: Leblanc *et al*[21] used TC-325 to control bleeding after a therapeutic endoscopic intervention in 13 patients (5 esophageal EMR, 4 duodenal EMR, 2 ampullary resections and 1 biliary sphincterotomy). The powder achieved complete hemostasis in all patients, either as a first-line treatment or a rescue therapy, including 2 cases with spurting arterial vessels. There were no rebleedings. Very recently, TC-325 has been successfully applied to a severe bleeding after endoscopic ultrasound-guided pseudocyst drainage which had been refractory to adrenaline and fibrin glue injection[23], and in a case of bleeding after a rectal submucosal endoscopic dissection[24].

Bleeding related to portal hypertension: TC-325 has been used in cases of both esophageal and gastric variceal bleeding with good short-term results[25-27]. Smith *et al*[28] controlled acute bleeding from severe portal hypertensive gastropathy in 3 patients. However, it is only able to control the initial bleeding and cannot prevent further bleed-

ings.

Lower GI bleeding: TC-325 has also been used for lower GI bleeding[29] which is currently not a licensed use of the powder. In the largest series published to date, 9 patients with lower GI bleeding were treated with TC-325, 4 of them with post-polypectomy bleedings. Successful initial hemostasis was achieved in all patients, with 2 cases (22%) of rebleeding[30]. Smith *et al*[28] treated one patient with a portal hypertensive colopathy with TC-325, achieving a decrease in transfusion requirements. Very recently, Kraft *et al*[31] described the use of TC-325 for the treatment of a lower GI bleeding from diffuse colonic ulcers secondary to diclofenac.

Larger case series: A multicenter European trial has been published on the use of Hemospray™ in non-variceal upper GI bleeding[32]. In this trial, 63 patients with a variety of indications, including ulcers, tumors and post-therapeutic bleeding, were treated with Hemospray™ as either monotherapy or second-line therapy. Primary hemostasis was achieved in 85% of patients when Hemospray was used as monotherapy. Seven patients rebled by the 7th day, therefore 15 patients (27%) failed to achieve sustained hemostasis. The 3 patients who rebled from a peptic ulcer had a Forrest Ia lesion. Hemospray was used as a second-line therapy in 8 patients, with two early rebleedings.

Very recently, a case series from two Swiss hospitals evaluated the performance of Hemospray™ on 16 patients with bleeding from different sources. In most cases, the powder was used as a rescue therapy with an initial hemostasis rate of 93%. Two patients rebled (12.5%), both presenting with oozing bleeding in the previous endoscopy[33].

EndoClot

There is only one publication in a peer reviewed journal reporting on EndoClot in control and prevention of EMR-related bleeding. EndoClot was applied to mucosal defects after resection of 181 lesions (82 patients) regardless of if there was immediate post-resection bleeding. Among them, 20 lesions in 18 patients had early bleeding (five of them showing spurting bleeding). Bleeding was controlled with a single round of spray in 18 lesions (90%) and two cases needed hot biopsy forceps applied to achieve hemostasis. Bleeding recurred in three of these 18 patients but no therapy was needed. The authors concluded that EndoClot effectively achieves hemostasis in controlling and preventing EMR-related bleeding[34]. Two trials on the prevention of bleeding after endoscopic mucosal resection (NCT01496781 and NCT01735786) are ongoing but there are no data available yet[35,36]. Finally, there are some studies presented only in abstract form on short series of patients with a variety of bleeding lesions, reporting a success rate of around 80%, including some with coagulation disorders[37-39].

ABS

This agent has been approved in Turkey (Table 1) for clinical hemorrhages refractory to conventional hemostatic measures. There are several reports on the mechanism of action and clinical efficacy of ABS, almost all from the same Turkish groups.

Animal studies: Several authors have shown that ABS has a clinically meaningful hemostatic effect in rats and swine models with arterial sections, skin lacerations and liver puncture wounds, even if they were treated with warfarin[40-43].

Peptic ulcer bleeding: ABS has shown efficacy in peptic ulcer bleeding in some reports with a very low number of patients, including a child and a patient with thrombocytopenia[44,45].

Cancer-related GI bleeding: Several studies have assessed ABS efficacy on malignant GI bleeding, showing a good performance[46,47]. Clinical observation suggests that the hemostatic effect of ABS on malignant bleeding persists for a long time after its delivery. Some authors have suggested that this may be due to an inhibitory effect of ABS on tumor angiogenesis. A decrease in microvessel density (MVD) in tumoral sections stained with CD34 after treatment with Ankaferd has been described[48].

Other indications: Case reports on the treatment with ABS of post-sphincterotomy bleeding[49], Mallory-Weiss syndrome[44,50] and gastric post-polypectomy bleeding[51] have been reported. Two reports on the use of ABS to control esophageal variceal bleeding have also been published[52,53].

Lower GI bleeding: ABS has been applied in patients with radiation proctitis, with transient control of bleeding, but with no effect on telangiectasias[54]. There are also anecdotal cases of ABS use on post-polypectomy bleeding[50,51] and solitary rectal ulcer[55].

Larger case series: The most recent series on the use of ABS to control upper GI bleeding included 27 patients with active non-variceal UGIB[56]. Bleeding lesions were not described. In one patient, bleeding ceased after spraying isotonic saline and in the other, 26 ABS was applied. Bleeding stopped in 19 cases (73%). In 6 of the remaining 7 patients, ABS was sprayed again plus another endoscopic hemostatic method (clip, injection, APC), achieving an adequate control in all cases. The overall rate of rebleeding was 20%. Bleeding control with ABS was more difficult in patients with a coagulopathy or who were taking AAS.

TECHNICAL ISSUES

Hemospray™

The Hemospray™ package includes a delivering device

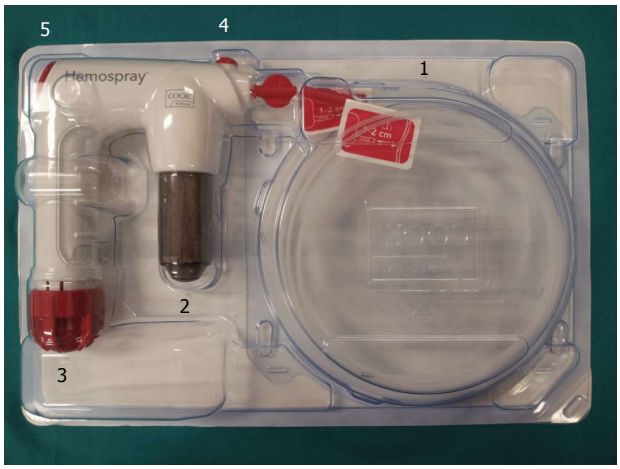

Figure 1 Hemospray™ package. 1: Spray catheters; 2: Powder cartridge; 3: Activation knob; 4: Security valve; 5: Trigger.

with a powder syringe (20 g each), two catheters (7 and 10 F, suitable for a working channel of 2.8 and 3.7 respectively) and a CO_2 cartridge (Figure 1). The latter is activated by turning a red knob placed at the base of the handle until it stops. Before inserting the catheter in the working channel of the endoscope, blood must be removed as much as possible and the bleeding site must be identified. Then, air is flushed through the accessory channel and the catheter is slowly advanced through it until the catheter tip is visualized. Care must be taken in not placing the catheter directly in contact with blood or the mucosa to avoid occlusion. It is advisable to maintain a 1-2 cm distance from the bleeding site during the procedure. Then, after turning the red valve placed at the top of the delivery device to the open position, TC-325 is ready to be delivered by depressing the red trigger button in 1-2 s pulses. Following the manufacturer´s instructions, no more than 3 devices (60 g) should be applied per patient. However, some authors have used up to 7 syringes in one patient without any secondary effect[11].

In a large trial, 7 of 63 patients (11%) treated with Hemospray suffered technical-related complications[32]. There were 3 blockages of the application catheter, 2 cases of the endoscope transiently adhering to the esophageal mucosa after use with the endoscope in retroflexion, 1 occlusion of the working channel of the endoscope and 1 malfunction of the CO_2 cartridge. In spite of this, most of the examiners felt that Hemospray was easier to use than conventional hemostatic methods[32].

Special indications suppose some technical challenges. Powder application is feasible with a duodenoscope, but caution must be taken with the use of the elevator to prevent plication of the catheter[21,57]. Hemospray cannot be used to control bleeding during EMR or ESD because it would obscure the resection field. However, Hemospray can be used at the end of the procedure if indicated.

EndoClot

The EndoClot™ PHS consists of a canister containing 1, 2 or 3 g of the powder, an air compressor that propels air down the catheter and a powder-gas mixing chamber attached to a delivery catheter that is introduced through the working channel of the endoscope[14]. After spraying, the bleeding site must be observed for 5 min. If bleeding recurs, the powder can be reapplied[34].

ABS

ABS can be delivered through the working channel of the endoscope by injecting 50-mL vials through a disposable catheter. Topical application of ABS must completely cover the bleeding surface. Following the author´s recommendations, a spray catheter or a wash pipe should be used. The amount of powder to be applied is dependent on the extent of bleeding. During administration of ABS, a local discoloration may be observed that together with the network formation may hamper the detection of the bleeding point. Therefore, the application of ABS should be performed only after precise location of source of bleeding.

SAFETY

The main theoretical concerns of using powders on an active bleeding site are local damage because of foreign body reactions and systemic embolization because of the introduction of particles into the blood stream. Embolization is of concern, especially in the case of Hemospray™ and Endoclot™ in which the powder is delivered by means of a system of positive outflow pressure. Another theoretical problem for the three powders may be bowel obstruction, caused by the formed matrix itself when it is sloughed off from the gastrointestinal mucosa a few days after its application. These secondary effects have been more extensively studied for Hemospray™, while there are very few available data about secondary effects of Endoclot and Ankaferd.

Preclinical studies

Studies with TC-325 carried out on animal models with open wounds showed endothelial and transmural damage in transected vessels in pigs, along with small clots and powder residues in lungs[58]. However, these studies referred to external wounds and more severe vessel injuries than the standard vessel defect in a GI bleeding. In the animal study by Giday *et al*[10] on gastric arterial bleeding, necropsy of the animals treated with TC-325 showed no foreign body granuloma and no signs of embolization in brain or lungs. The same group, in a study designed to identify local and systemic secondary effects following endoscopic application of TC-325, showed no local or regional particulate effects and no distance embolic effects[59]. In similar studies using Ankaferd, these secondary effects have also not been described[42]. Bowel obstruction has not been described in animals[59].

Table 2 Possible indications for the use of hemostatic powders

Primary hemostatic method	Adjuvant therapy
Lesions with a difficult endoscopic access	Failure of conventional methods
Less experienced examiner	
Malignant gastrointestinal bleeding	
Massive bleeding as a mean to achieve an initial hemostasis	

Clinical studies

There are no trials specifically designed to address secondary effects of powders. However, a recent European multicenter study has shown no secondary effects when using TC-325 for a variety of indications, including peptic ulcers, vascular lesions, malignancies and post-therapeutic bleedings[32]. No secondary effects of Ankaferd and EndoClot have been described in the scarce literature available. Bowel obstruction has not been described with TC-325, even when maximum doses were delivered[11]. Intestinal blockage seems to be rare with EndoClot because in most cases bleeding is controlled only with 3 g of powder and starch particles are rapidly degraded in the GI tract.

Regarding Hemospray™, specific concerns have been raised for some indications. For instance, when treating bleeding from esophageal or gastric varices, thromboembolism may be an issue because particles might enter the vascular system. In fact, its use in this setting is contraindicated by the manufacturer. However, the Hemospray™ outflow pressure is less than the intravariceal pressure of a bleeding varix when applied from a distance of 1-2 cm and no embolism has been shown in this indication[25,27]. *In vitro* coagulation time modifications caused by TC-325 do not seem to pose any clinical problem in cirrhotic patients[25]. A case of biliary blockage has been described when TC-325 was applied in a patient with post-sphincterotomy bleeding[57].

The application of a pressure spray on the resection area after EMR could theoretically cause a perforation. However, no perforation was detected in a small series[21]. Only one case of bowel perforation after treatment of a severe portal hypertensive gastropathy with TC-325 has been reported[28] but it was not clear if the perforation was related to the procedure. Following the manufacturer´s instructions, Hemospray™ use is contraindicated in patients with suspected GI perforation or those at high risk of perforation during the endoscopic procedure (information provided by the manufacturer). Some secondary effects of TC-325 when applied in the large bowel have been described. A case of abdominal cramps after each application of Hemospray™ on a resection area in the rectum was described. This patient did not show any long-term secondary effect[22].

CONCLUSION

Randomized controlled trials comparing powders with standard endoscopic methods are not yet available, thus the current evidence must be considered as moderate at best. The precise role of this technology in the therapeutic algorithm or GI bleeding is yet to be defined but from the present review some practical conclusions can be drawn.

Topical hemostatic powders seem to be effective to control both upper and lower gastrointestinal bleeding from a variety of sources. They can be used as a primary method or a second-line therapy and in combination with standard hemostatic methods. However, there is a substantial proportion of patients who fail to achieve primary hemostasis, mainly Forrest Ia peptic ulcer bleedings. In case of a primary failure, an adjuvant conventional endoscopic method can be applied after removing the adherent matrix with water flushing. There is some risk of rebleeding in the first week after the initial hemorrhagic episode, probably because the mineral matrix sloughs off from the mucosa after 24-72 h. A second-look endoscopy may be appropriate in this subset of patients with special risk of rebleeding.

Perhaps the most specific indication for the use of powders in GI bleeding is hemorrhage from a neoplastic lesion, which may have several bleeding points. Powders may be very useful in this setting because, when applied, they cover a large area of mucosa. Failure to achieve hemostasis with conventional methods is the other main indication for powders (Table 2).

However, active research is needed to clarify grey areas, like secondary effects and long-term efficacy. Future areas of research should be the development of well-designed randomized trials to assess efficacy of powders *vs* conventional endoscopic treatment as a primary therapeutic option, paying special attention to safety issues. Possible outcomes would be rates of rebleeding, need for adjuvant endoscopic therapy, and transfusion requirements. Sample size should be large enough to evaluate the efficacy of powders in the management of high risk bleeding lesions (*e.g.*, Forrest Ia). Rebleeding may be an underreported event in the literature; therefore, long-term efficacy must be addressed in the incoming trials. Long-term secondary effects on GI mucosa should also be addressed. Finally, since many conventional hemostatic methods are considerably cheaper, an economic analysis of the use of powders on GI bleeding should also be carried out. Larger trials, which may give response to some of these answers, are eagerly awaited.

REFERENCES

1 **Lewis JD**, Bilker WB, Brensinger C, Farrar JT, Strom BL. Hospitalization and mortality rates from peptic ulcer disease and GI bleeding in the 1990s: relationship to sales of nonsteroidal anti-inflammatory drugs and acid suppression medications. *Am J Gastroenterol* 2002; **97**: 2540-2549 [PMID: 12385436 DOI: 10.1111/j.1572-0241.2002.06037.x]

2 **Hearnshaw SA**, Logan RF, Lowe D, Travis SP, Murphy MF, Palmer KR. Acute upper gastrointestinal bleeding in the UK: patient characteristics, diagnoses and outcomes in the 2007 UK audit. *Gut* 2011; **60**: 1327-1335 [PMID: 21490373

DOI: 10.1136/gut.2010.228437]
3 **Pérez Aisa A**, Nuevo J, López Morante AA, González Galilea A, Martin de Argila C, Aviñoa Arreal D, Feu F, Borda Celaya F, Gisbert JP, Pérez Roldan F, Gonzalvo Sorribes JM, Palazón Azorín JM, Ponce Romero M, Castro Fernández M, Catalina Rodriguez MV, Gallego Montañés S, Calvet X, Rodrigo Saez L, Montoro Huguet M, González Méndez Y, Sierra Hernández A, Sánchez Hernández E, Dominguez Muñoz E, Pérez Cuadrado E, Muñoz M, Lanas A. [Current management of nonvariceal upper gastrointestinal bleeding in Spain]. *Gastroenterol Hepatol* 2012; **35**: 468-475 [PMID: 22542917 DOI: 10.1016/j.gastrohep.2012.02.007]
4 **Hreinsson JP**, Gumundsson S, Kalaitzakis E, Björnsson ES. Lower gastrointestinal bleeding: incidence, etiology, and outcomes in a population-based setting. *Eur J Gastroenterol Hepatol* 2013; **25**: 37-43 [PMID: 23013623 DOI: 10.1097/MEG.0b013e32835948e3]
5 **Lanas A**, García-Rodríguez LA, Polo-Tomás M, Ponce M, Alonso-Abreu I, Perez-Aisa MA, Perez-Gisbert J, Bujanda L, Castro M, Muñoz M, Rodrigo L, Calvet X, Del-Pino D, Garcia S. Time trends and impact of upper and lower gastrointestinal bleeding and perforation in clinical practice. *Am J Gastroenterol* 2009; **104**: 1633-1641 [PMID: 19574968 DOI: 10.1038/ajg.2009.164]
6 **Rockey DC**. Lower gastrointestinal bleeding. *Gastroenterology* 2006; **130**: 165-171 [PMID: 16401479 DOI: 10.1053/j.gastro.2005.11.042]
7 **Laine L**, McQuaid KR. Endoscopic therapy for bleeding ulcers: an evidence-based approach based on meta-analyses of randomized controlled trials. *Clin Gastroenterol Hepatol* 2009; **7**: 33-47; quiz 1-2 [PMID: 18986845 DOI: 10.1016/j.cgh.2008.08.016]
8 **Green BT**, Rockey DC. Lower gastrointestinal bleeding--management. *Gastroenterol Clin North Am* 2005; **34**: 665-678 [PMID: 16303576 DOI: 10.1016/j.gtc.2005.10.001]
9 **Gralnek IM**, Barkun AN, Bardou M. Management of acute bleeding from a peptic ulcer. *N Engl J Med* 2008; **359**: 928-937 [PMID: 18753649 DOI: 10.1056/NEJMra0706113]
10 **Giday SA**, Kim Y, Krishnamurty DM, Ducharme R, Liang DB, Shin EJ, Dray X, Hutcheon D, Moskowitz K, Donatelli G, Rueben D, Canto MI, Okolo PI, Kalloo AN. Long-term randomized controlled trial of a novel nanopowder hemostatic agent (TC-325) for control of severe arterial upper gastrointestinal bleeding in a porcine model. *Endoscopy* 2011; **43**: 296-299 [PMID: 21384319 DOI: 10.1055/s-0030-1256125]
11 **Sung JJ**, Luo D, Wu JC, Ching JY, Chan FK, Lau JY, Mack S, Ducharme R, Okolo P, Canto M, Kalloo A, Giday SA. Early clinical experience of the safety and effectiveness of Hemospray in achieving hemostasis in patients with acute peptic ulcer bleeding. *Endoscopy* 2011; **43**: 291-295 [PMID: 21455870 DOI: 10.1055/s-0030-1256311]
12 **Cox ED**, Schreiber MA, McManus J, Wade CE, Holcomb JB. New hemostatic agents in the combat setting. *Transfusion* 2009; **49** Suppl 5: 248S-255S [PMID: 19954487 DOI: 10.1111/j.1537-2995.2008.01988.x]
13 **Holster ILDM MP**, Ducharme R, Kuipers EJ, Tjwa ET. In vivo examination of the effects of the hemostatic powder (HemosprayTM) on coagulation and thrombus formation in humans. *Gastrointest Endosc* 2012: AB240
14 **AMP technology**. Polymer Solution for hemostasis. 2011. Available from: URL: http: //endoclot.com/technology.html. Last accessed: May 2014
15 **Wang Y**, Xu M, Dong H, Liu Y, Zhao P, Niu W, Xu D, Ji X, Xing C, Lu D, Li Z. Effects of PerClot® on the healing of full-thickness skin wounds in rats. *Acta Histochem* 2012; **114**: 311-317 [PMID: 21782216 DOI: 10.1016/j.acthis.2011.06.012]
16 **Beyazit Y**, Kurt M, Kekilli M, Goker H, Haznedaroglu IC. Evaluation of hemostatic effects of Ankaferd as an alternative medicine. *Altern Med Rev* 2010; **15**: 329-336 [PMID: 21194248]
17 **Haznedaroglu BZ**, Haznedaroglu IC, Walker SL, Bilgili H, Goker H, Kosar A, Aktas A, Captug O, Kurt M, Ozdemir O, Kirazli S, Firat HC. Ultrastructural and morphological analyses of the in vitro and in vivo hemostatic effects of Ankaferd Blood Stopper. *Clin Appl Thromb Hemost* 2010; **16**: 446-453 [PMID: 19833624 DOI: 10.1177/1076029609343706]
18 **Goker H**, Haznedaroglu IC, Ercetin S, Kirazli S, Akman U, Ozturk Y, Firat HC. Haemostatic actions of the folkloric medicinal plant extract Ankaferd Blood Stopper. *J Int Med Res* 2008; **36**: 163-170 [PMID: 18304416]
19 **Esmon CT**, Esmon NL. Regulatory mechanisms in hemostasis. In: Hoffman RBEJ, Silberstein LE, Heslop HE, Wietz JI, Anastasi J, editors. Hematology: basic principles and practice. 6th ed. Eselvier, 2013
20 **Chen YI**, Barkun AN, Soulellis C, Mayrand S, Ghali P. Use of the endoscopically applied hemostatic powder TC-325 in cancer-related upper GI hemorrhage: preliminary experience (with video). *Gastrointest Endosc* 2012; **75**: 1278-1281 [PMID: 22482923 DOI: 10.1016/j.gie.2012.02.009]
21 **Leblanc S**, Vienne A, Dhooge M, Coriat R, Chaussade S, Prat F. Early experience with a novel hemostatic powder used to treat upper GI bleeding related to malignancies or after therapeutic interventions (with videos). *Gastrointest Endosc* 2013; **78**: 169-175 [PMID: 23622976 DOI: 10.1016/j.gie.2013.03.006]
22 **Holster IL**, Kuipers EJ, Tjwa ET. Hemospray in the treatment of upper gastrointestinal hemorrhage in patients on antithrombotic therapy. *Endoscopy* 2013; **45**: 63-66 [PMID: 23208778 DOI: 10.1055/s-0032-1325793]
23 **Tarantino I**, Barresi L, Granata A, Curcio G, Traina M. Hemospray for arterial hemorrhage following endoscopic ultrasound-guided pseudocyst drainage. *Endoscopy* 2014; **46** Suppl 1: E71 [PMID: 24523191 DOI: 10.1055/s-0033-1359164]
24 **Curcio G,** Granata A, Traina M. Hemospray for multifocal bleeding following ultra-low rectal endoscopic submucosal dissection. *Dig Endosc* 2014; **26**: 606-607 [PMID: 24734986 DOI: 10.1111/den.12301]
25 **Holster IL**, Poley JW, Kuipers EJ, Tjwa ET. Controlling gastric variceal bleeding with endoscopically applied hemostatic powder (Hemospray™). *J Hepatol* 2012; **57**: 1397-1398 [PMID: 22864337 DOI: 10.1016/j.jhep.2012.07.024]
26 **Ibrahim M**, El-Mikkawy A, Mostafa I, Devière J. Endoscopic treatment of acute variceal hemorrhage by using hemostatic powder TC-325: a prospective pilot study. *Gastrointest Endosc* 2013; **78**: 769-773 [PMID: 24120338 DOI: 10.1016/j.gie.2013.07.037]
27 **Stanley AJ**, Smith LA, Morris AJ. Use of hemostatic powder (Hemospray) in the management of refractory gastric variceal hemorrhage. *Endoscopy* 2013; **45** Suppl 2: E86-E87 [PMID: 23526533 DOI: 10.1055/s-0032-1326258]
28 **Smith LA**, Morris AJ, Stanley AJ. The use of hemospray in portal hypertensive bleeding; a case series. *J Hepatol* 2014; **60**: 457-460 [PMID: 24140803 DOI: 10.1016/j.jhep.2013.10.008]
29 **Granata A**, Curcio G, Azzopardi N, Barresi L, Tarantino I, Traina M. Hemostatic powder as rescue therapy in a patient with H1N1 influenza with uncontrolled colon bleeding. *Gastrointest Endosc* 2013; **78**: 451 [PMID: 23948196 DOI: 10.1016/j.gie.2013.07.010]
30 **Holster IL**, Brullet E, Kuipers EJ, Campo R, Fernández-Atutxa A, Tjwa ET. Hemospray treatment is effective for lower gastrointestinal bleeding. *Endoscopy* 2014; **46**: 75-78 [PMID: 24218304 DOI: 10.1055/s-0033-1344988]
31 **Kratt T**, Lange J, Königsrainer A, Malek N, Adam P, Bösmüller H, Goetz M. Successful Hemospray treatment for recurrent diclofenac-induced severe diffuse lower gastrointestinal bleeding avoiding the need for colectomy. *Endoscopy* 2014; **46** Suppl 1: E173-E174 [PMID: 24756280 DOI: 10.1055/s-0034-1365100]

32 **Smith LA**, Stanley AJ, Bergman JJ, Kiesslich R, Hoffman A, Tjwa ET, Kuipers EJ, von Holstein CS, Oberg S, Brullet E, Schmidt PN, Iqbal T, Mangiavillano B, Masci E, Prat F, Morris AJ. Hemospray Application in Nonvariceal Upper Gastrointestinal Bleeding: Results of the Survey to Evaluate the Application of Hemospray in the Luminal Tract. *J Clin Gastroenterol* 2013 Dec 10; Epub ahead of print [PMID: 24326829 DOI: 10.1097/MCG.0000000000000054]

33 **Sulz MC**, Frei R, Meyenberger C, Bauerfeind P, Semadeni GM, Gubler C. Routine use of Hemospray for gastrointestinal bleeding: prospective two-center experience in Switzerland. *Endoscopy* 2014; **46**: 619-624 [PMID: 24770964 DOI: 10.1055/s-0034-1365505]

34 **Huang R**, Pan Y, Hui N, Guo X, Zhang L, Wang X, Zhang R, Luo H, Zhou X, Tao Q, Liu Z, Wu K. Polysaccharide hemostatic system for hemostasis management in colorectal endoscopic mucosal resection. *Dig Endosc* 2014; **26**: 63-68 [PMID: 23551344 DOI: 10.1111/den.12054]

35 **Liu Z**. EndoClot for preventing rebleeding after endoscopic mucosal resection (EMR). 2013. Available from: URL: http://clinicaltrials.gov/show/NCT01735786. Last accessed: may 2014

36 **Liu Z**. EndoClot for Hemostasis and preventing post-procedure bleeding after endoscopic mucosal resection (EMR). 2013. Available from: URL: http://clinicaltrials.gov/show/NCT01496781. Accessed on May 2014

37 **Halkerston K**, Evans J, Ismail D, Catnach S, Chaudhary R, Fullard M, King A, Leahy A. Early clinical experience of EndoclotTM in the treatment of acute gastro-intestinal bleeding. *Gut* 2013; **62** (Suppl 1): A149 [DOI: 10.1136/gutnl-2013-304907.335]

38 **Müller-Gerbes D**, Beek A, Dormann A. Erfahrungen mit EndoClotTM PHS bei blutungen im oberen gastrointestinaltrakt. *Z Gastroenterol* 2013; **51**: K457 [DOI: 10.1055/s-0033-1353097]

39 **Sörensen AS**, Kalmar G, Schmidt A, Caca K. Endoskopische blutstillung mit absorbierbaren modifizierten polymeren (AMP; EndoClotTM)-erste Erfahrungen im oberen gastrointestinaltrakt. *Z Gastroenterol* 2013; **51**: K270 [DOI: 10.1055/s-0033-1352910]

40 **Bilgili H**, Kosar A, Kurt M, Onal IK, Goker H, Captug O, Shorbagi A, Turgut M, Kekilli M, Kurt OK, Kirazli S, Aksu S, Haznedaroglu IC. Hemostatic efficacy of Ankaferd Blood Stopper in a swine bleeding model. *Med Princ Pract* 2009; **18**: 165-169 [PMID: 19349716 DOI: 10.1159/000204344]

41 **Cipil HS**, Kosar A, Kaya A, Uz B, Haznedaroglu IC, Goker H, Ozdemir O, Koroglu M, Kirazli S, Firat HC. In vivo hemostatic effect of the medicinal plant extract Ankaferd Blood Stopper in rats pretreated with warfarin. *Clin Appl Thromb Hemost* 2009; **15**: 270-276 [PMID: 19117967 DOI: 10.1177/1076029608329578]

42 **Kandemir O**, Buyukates M, Kandemir NO, Aktunc E, Gul AE, Gul S, Turan SA. Demonstration of the histopathological and immunohistochemical effects of a novel hemostatic agent, Ankaferd Blood Stopper, on vascular tissue in a rat aortic bleeding model. *J Cardiothorac Surg* 2010; **5**: 110 [PMID: 21073754 DOI: 10.1186/1749-8090-5-110]

43 **Kosar A**, Cipil HS, Kaya A, Uz B, Haznedaroglu IC, Goker H, Ozdemir O, Ercetin S, Kirazli S, Firat HC. The efficacy of Ankaferd Blood Stopper in antithrombotic drug-induced primary and secondary hemostatic abnormalities of a rat-bleeding model. *Blood Coagul Fibrinolysis* 2009; **20**: 185-190 [PMID: 19657315 DOI: 10.1097/MBC.0b013e32831c4cb0]

44 **Ozaslan E**, Purnak T, Yildiz A, Haznedaroglu IC. The effect of a new hemostatic agent for difficult cases of non-variceal gastrointestinal bleeding: Ankaferd blood stopper. *Hepatogastroenterology* 2010; **57**: 191-194 [PMID: 20583410]

45 **Yarali N**, Oruc M, Bay A, Dalgic B, Bozkaya IO, Arıkoglu T, Kara A, Tunc B. A new hemostatic agent--Ankaferd blood stopper: management of gastrointestinal bleeding in an infant and other experiences in children. *Pediatr Hematol Oncol* 2010; **27**: 592-596 [PMID: 20863156 DOI: 10.3109/08880018.2010.503337]

46 **Kurt M**, Akdogan M, Onal IK, Kekilli M, Arhan M, Shorbagi A, Aksu S, Kurt OK, Haznedaroglu IC. Endoscopic topical application of Ankaferd Blood Stopper for neoplastic gastrointestinal bleeding: A retrospective analysis. *Dig Liver Dis* 2010; **42**: 196-199 [PMID: 19540818 DOI: 10.1016/j.dld.2009.05.006]

47 **Zulfikar OB**, Emiroglu HH, Kebudi R. Nasogastric application of topical Ankaferd Blood Stopper for bleeding from primary esophageal adenocarcinoma in a child with disseminated intravascular coagulation. *Dig Liver Dis* 2011; **43**: 247-248 [PMID: 21177146 DOI: 10.1016/j.dld.2010.10.002]

48 **Turhan N**, Kurt M, Shorbagi A, Akdogan M, Haznedaroglu IC. Topical Ankaferd Blood Stopper administration to bleeding gastrointestinal carcinomas decreases tumor vascularization. *Am J Gastroenterol* 2009; **104**: 2874-2877 [PMID: 19888263 DOI: 10.1038/ajg.2009.431]

49 **Beyazit Y**, Köklü S, Akbal E, Kurt M, Kekilli M, Haznedaroglu IC. Successful treatment of endoscopic sphincterotomy-induced early hemorrhage with application of Ankaferd Blood Stopper. *Gastrointest Endosc* 2010; **72**: 1325-1326 [PMID: 21111880 DOI: 10.1016/j.gie.2010.03.1119]

50 **Kurt M**, Onal I, Akdogan M, Kekilli M, Arhan M, Sayilir A, Oztas E, Haznedaroglu I. Ankaferd Blood Stopper for controlling gastrointestinal bleeding due to distinct benign lesions refractory to conventional antihemorrhagic measures. *Can J Gastroenterol* 2010; **24**: 380-384 [PMID: 20559581]

51 **Karaman A**, Torun E, Gürsoy S, Yurci A, Ozbakir O. Efficacy of Ankaferd Blood Stopper in postpolypectomy bleeding. *J Altern Complement Med* 2010; **16**: 1027-1028 [PMID: 20954959 DOI: 10.1089/acm.2010.0089]

52 **Tuncer I**, Doganay L, Ozturk O. Instant control of fundal variceal bleeding with a folkloric medicinal plant extract: Ankaferd Blood Stopper. *Gastrointest Endosc* 2010; **71**: 873-875 [PMID: 19922917 DOI: 10.1016/j.gie.2009.08.021]

53 **Ozaslan E**, Purnak T, Yildiz A, Haznedaroglu IC. Bleeding due to slippage of elastic band during variceal ligation: successful use of Ankaferd blood stopper. *Indian J Gastroenterol* 2010; **29**: 166-168 [PMID: 20814774 DOI: 10.1007/s12664-010-0043-y]

54 **Ozaslan E**, Purnak T, Ozyigit G, Akyol F, Yildiz A, Haznedaroglu IC. No prolonged effect of Ankaferd Blood Stopper on chronic radiation proctitis. *Endoscopy* 2010; **42** Suppl 2: E271-E272 [PMID: 20931479 DOI: 10.1055/s-0030-1255773]

55 **Ibis M**, Kurt M, Onal IK, Haznedaroglu IC. Successful management of bleeding due to solitary rectal ulcer via topical application of Ankaferd blood stopper. *J Altern Complement Med* 2008; **14**: 1073-1074 [PMID: 19055332 DOI: 10.1089/acm.2008.0314]

56 **Gungor G**, Goktepe MH, Biyik M, Polat I, Tuna T, Ataseven H, Demir A. Efficacy of ankaferd blood stopper application on non-variceal upper gastrointestinal bleeding. *World J Gastrointest Endosc* 2012; **4**: 556-560 [PMID: 23293725 DOI: 10.4253/wjge.v4.i12.556]

57 **Moosavi S**, Chen YI, Barkun AN. TC-325 application leading to transient obstruction of a post-sphincterotomy biliary orifice. *Endoscopy* 2013; **45** Suppl 2: E130 [PMID: 23716095 DOI: 10.1055/s-0032-1326370]

58 **Kheirabadi BS**, Mace JE, Terrazas IB, Fedyk CG, Estep JS, Dubick MA, Blackbourne LH. Safety evaluation of new hemostatic agents, smectite granules, and kaolin-coated gauze in a vascular injury wound model in swine. *J Trauma* 2010; **68**: 269-278 [PMID: 20154537 DOI: 10.1097/TA.0b013e3181c97ef1]

59 **Giday S**, Van Alstine W, Van Vleet J, Ducharme R, Brandner E, Florea M, Johnston K, Negron-Garcia J, Ringenberger

K. Safety analysis of a hemostatic powder in a porcine model of acute severe gastric bleeding. *Dig Dis Sci* 2013; **58**: 3422-3428 [PMID: 23982209 DOI: 10.1007/s10620-013-2846-z]

P- Reviewer: Fallone CA, Hokama A, Kate V, Li YY, Thomopoulos KC **S- Editor**: Ji FF **L- Editor**: Roemmele A **E- Editor:** Lu YJ

World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4291/wjgp.v5.i3.293

World J Gastrointest Pathophysiol 2014 August 15; 5(3): 293-303
ISSN 2150-5330 (online)

REVIEW

Predictors of response to anti-tumor necrosis factor therapy in ulcerative colitis

Evanthia Zampeli, Michalis Gizis, Spyros I Siakavellas, Giorgos Bamias

Evanthia Zampeli, Gastroenterology Department, Alexandra General Hospital, 11528 Athens, Greece
Michalis Gizis, Spyros I Siakavellas, Giorgos Bamias, Academic Department of Gastroenterology, Ethnikon and Kapodistriakon University of Athens, Laikon Hospital, 15235 Athens, Greece
Author contributions: Zampeli E, Gizis M and Siakavellas SI reviewed the literature; Zampeli E, Gizis M, Siakavellas SI and Bamias G analyzed the data; Zampeli E and Bamias G designed the structure of the review; Zampeli E and Bamias G wrote the paper.
Correspondence to: Giorgos Bamias, Consultant in Gastroenterology, Academic Department of Gastroenterology, Ethnikon and Kapodistriakon University of Athens, 17 Agiou Thoma st., 15235 Athens, Greece. gbamias@gmail.com
Telephone: +30-21-06456504 Fax: +30-21-07791839
Received: January 4, 2014 Revised: March 7, 2014
Accepted: June 10, 2014
Published online: August 15, 2014

Abstract

Ulcerative colitis (UC) is an immune-mediated, chronic inflammatory disease of the large intestine. Its course is characterized by flares of acute inflammation and periods of low-grade chronic inflammatory activity or remission. Monoclonal antibodies against tumor necrosis factor (anti-TNF) are part of the therapeutic armamentarium and are used in cases of moderate to severe UC that is refractory to conventional treatment with corticosteroids and/or immunosuppressants. Therapeutic response to these agents is not uniform and a large percentage of patients either fail to improve (primary non-response) or lose response after a period of improvement (secondary non-response/loss of response). In addition, the use of anti-TNF agents has been related to uncommon but potentially serious adverse effects that preclude their administration or lead to their discontinuation. Finally, use of these medications is associated with a considerable cost for the health system. The identification of parameters that may predict response to anti-TNF drugs in UC would help to better select for patients with a high probability to respond and minimize risk and costs for those who will not respond. Analysis of the major clinical trials and the accumulated experience with the use of anti-TNF drugs in UC has resulted to the report of such prognostic factors. Included are clinical and epidemiological characteristics, laboratory markers, endoscopic indicators and molecular (immunological/genetic) signatures. Such predictive parameters of long-term outcomes may either be present at the commencement of treatment or determined during the early period of therapy. Validation of these prognostic markers in large cohorts of patients with variable characteristics will facilitate their introduction into clinical practice and the best selection of UC patients who will benefit from anti-TNF therapy.

Key words: Ulcerative colitis; Infliximab; Adalimumab; Anti-tumor necrosis factor; Predictors of response; Personalized treatment

Core tip: The use of anti-tumor necrosis factor (TNF) monoclonal antibodies for the treatment of ulcerative colitis has been associated with high rates of primary and secondary non-response, important safety issues and considerable cost. Selection of patients with the highest probability to response to anti-TNF treatment would overcome these problems. Analysis of the pivotal trials and accumulated experience from clinical practice has led to the identification of certain prognostic factors for favorable or adverse outcomes. These include clinical and epidemiological parameters, biological markers of inflammation, endoscopic findings, molecular signatures and pharmacological factors. Incorporation of such predictors into the current therapeutic protocols may lead to the optimization of anti-TNF treatment in ulcerative colitis.

Zampeli E, Gizis M, Siakavellas SI, Bamias G. Predictors of

response to anti-tumor necrosis factor therapy in ulcerative colitis. *World J Gastrointest Pathophysiol* 2014; 5(3): 293-303 Available from: URL: http://www.wjgnet.com/2150-5330/full/v5/i3/293.htm DOI: http://dx.doi.org/10.4291/wjgp.v5.i3.293

INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory disease of the colon, which affects almost 0.1% of the Western population[1]. Its natural history is dominated by chronic, relapsing intestinal inflammation, extra-intestinal involvement, and the development of long-term complications, which lead a considerable percentage of patients to colectomy.

Treatment for UC has been traditionally aimed against controlling acute and chronic inflammation[2]. Conventional therapy consists of 5-aminosalicylic acid (5-ASA) compounds, whereas more severe cases are handled with steroids during the acute phase and immunosuppressants (thiopurines) as the maintenance regimen. Despite the proven efficacy of these drugs, a significant number of patients do not accomplish durable remission and/or experience side effects. Furthermore, there has been a change in the therapeutic goals in UC in recent years. Traditionally, such goals have been considered the achievement of clinical remission and the avoidance of colectomy. Nowadays, however, it has become clear that treatment should include the complete elimination of active inflammation in the colon without long-term use of corticosteroids. In this context, mucosal healing and deep remission which both indicate the absence of endoscopically and biologically (*i.e.,* serological and/or fecal inflammatory markers) evident inflammation may be the ultimate endpoint. The accomplishment of such demanding endpoints has been linked to better long-term outcomes including colectomy and cancer prevention[3].

In recent years, treatment of UC has been revolutionized by the therapeutic application of monoclonal antibodies against tumor necrosis factor (TNF) as these agents offer effective long-term treatment for the most difficult cases.

ANTI-TNF TREATMENT IN UC

There are currently three monoclonal antibodies against human TNF that are licensed for the treatment of UC, infliximab (IFX), adalimumab (ADA), and Golimumab[4]. The data regarding Golimumab are limited. Therefore, our review will focus on IFX and ADA. IFX is a chimeric mouse/human IgG1 antibody that is administered intravenously. On the other hand, ADA is a humanized IgG1 antibody administered as subcutaneous injection. The two clinical scenarios for anti-TNF therapy in UC are: firstly, outpatient cases with moderate to severe UC who are refractory or intolerant to first-line treatment; and, secondly, patients with acute severe disease refractory to intravenous steroids[4]. In regards to the latter scenario, data exist only for IFX.

Recent clinical trials have established the efficacy of anti-TNF treatment in UC. In the two pivotal IFX trials, ACT 1/2, the primary short-term (8 wk) response of moderate to severe UC to IFX has been reported to be 65.5%/69.4% for clinical response and 33.9%/39% for remission, respectively (dose regimen 5 mg/kg at 0-2-6)[5]. Among patients who responded to the induction regimen nearly 50% maintained their response at week 30. Similarly, in the definitive clinical trial (ULTRA) for ADA, short-term response at week 8 was achieved in nearly 50% of patients, whereas long-term remission rate at week 52 was 17%[6].

Despite these encouraging results, the use of anti-TNF monoclonal antibodies is compromised in clinical practice by certain issues of efficacy and safety. Anti-TNF failure is an intriguing issue as it may be attributed to both disease characteristics and the drugs' interference with the immune system. Primary non-response is characterized by lack of response to induction therapy. The incidence ranges between 20%-40% for both anti-TNF agents. Switching to another drug is common practice, with a success rate of more than 50%[7,8]. On the other hand, loss of response is defined as the recurrence of the patient's symptoms following successful induction of remission. In the case of CD it has been estimated between 23%-46%[9], whereas no solid data exists for UC. It is believed that immunogenicity underlies secondary failure, as antibodies against anti-TNF drugs and reduced trough levels have been implicated in the majority of studies[10-12]. Optimization of treatment (dose increase and/or shortening of the administration interval) leads to recovery of response in 60%-90% of patients[10].

The use of anti-TNF has also been associated with safety concerns. Among the most fearful ones are: severe infectious including reactivation of latent tuberculosis, neurological manifestations and risk of neoplasia. In addition, infusion reactions and delayed hypersensitivity to IFX occurred in 10% and 1% of patients, respectively, in the ACT trials. The most significant side effects are probably associated with long-term administration and combination with other immunomodulatory medications. It should be noted that in the ACT and ULTRA studies there were no differences between active drug and placebo.

Taken together, it is currently evident that fine-tuning of the use of anti-TNF therapy in UC is required. The ultimate goal should be to achieve maximum efficacy with a minimum risk for side effects. When therapeutic strategies are designed the following parameters should be taken into consideration: (1) the patients who receive anti-TNF therapy are the ones with the most difficult-to-treat disease; (2) the drugs' efficiencies are far from perfect with high rates of primary and secondary failures; (3) the potential for serious side effects especially with chronic use; and (4) the high cost of these medications. One significant way to address these problems and optimize the clinical use of anti-TNF agents would be

Table 1 Prognostic indicators of response to anti-tumor necrosis factor treatment in ulcerative colitis

At initiation of treatment	During treatment
Clinical and epidemiological parameters	
Severity of the disease	Early clinical response
Younger age	
Duration of colitis < 3 yr	
Extensive colitis	
Laboratory indicators	
CRP	Low CRP at week 12
Hemoglobin	Drop of serum CRP
Serum albumin	Fecal calprotectin
Immunological and genetic markers	
p-ANCA	Gene expression profiling
Pre-treatment mucosal TNF-α expression	Percentages of regulatory T cells
Mucosal expression of IL-17 and IFN-γ	
Genetic polymorphisms	
Endoscopic findings	
	Mucosal healing
Treatment-related factors	
Pharmacological history	Number of IFX infusions
Exposure to immunosuppressants	Co-administration of immunosuppressants
Response to prior treatment with infliximab	Escalation of anti-TNF therapy
	IFX trough levels
	Antibodies against anti-TNF

CRP: C-reactive protein; p-ANCA: Perinuclear antineutrophil cytoplasmatic antibodies; TNF:Tumor necrosis factor; IL: Interleukin; INF: Interferon; IFX: Infliximab.

to carefully select patients in whom there is decreased probability for primary or secondary non-response. Such an approach will ensure that the patients who receive the medications are those who will most probably benefit. As almost ten years have passed since the initial application of anti-TNF therapies in UC, analyses of the pivotal clinical trials and accumulation of clinical experience has allowed the identification of such factors that signify a better response to these treatments (Tables 1 and 2). It is the purpose of the current review to summarize information regarding prognostic markers for response to anti-TNF monoclonal antibodies in patients with UC.

PREDICTORS OF RESPONSE

Prognostic factors at the initiation of anti-TNF treatment

Clinical and epidemiological parameters: Several studies have looked into the effect that the severity of the UC episode may have on the response to anti-TNF administration. In a study by Jürgens *et al*[13], 90 UC outpatients were treated with IFX and followed for 14 wk. Disease activity was quantified by use of the Colitis Activity Index (CAI). Nearly half of the patients achieved early remission at week 14. Overall, the mean CAI dropped from 10.4 points at baseline to 5.0 at week 14 ($P < 0.001$). The authors reported a significant positive association between UC activity and response to treatment with IFX. It should be noted, however, that only a small number of severe cases were included in this study.

In a second report, 191 UC patients who received at least one infusion of IFX between 2000 and 2009 were analyzed with the aim to identify predictors of response[14]. Mean follow-up was 18 mo. Failure outcomes included primary-non response, dose-escalation, colectomy and hospitalization, which were noted in 22%, 45%, 19% and 36% of patients, respectively. In contrast to the study by Jurgens, administration of IFX for the indication of acute severe colitis was associated with a 3-fold risk for unfavorable outcome.

Park *et al*[15] studied 89 Korean patients with moderate to severe UC who were treated with IFX. Following induction, 59 patients exhibited clinical response at week 8 (66.3%). None had a colectomy within one year, in contrast to 11/30 of those who did not respond. Predictors of primary non-response to the drug were the severity of the disease before initiation as well as prior cytomegalovirus (CMV) infection of the colon. Patients with a pre-treatment Mayo score ⩾ 11 had an increased risk of colectomy (OR = 5.05, $P = 0.007$).

Analysis of the large clinical trials ACT 1 and 2- offers additional information regarding prognostic factors for colectomy (*i.e.,* failure of IFX) in patients with moderate to severe UC[16]. As reported by Sandborn *et al*[16], 630 patients who participated in the ACT trials had a complete follow-up for colectomy. A baseline Mayo score of ⩾ 10 strongly increased the risk for colectomy (HR = 1.84, $P = 0.01$).

Prognostic indicators for response to ADA in UC have also been reported recently. A placebo controlled trial of ADA for UC patients with refractory disease who were naïve to biologics evaluated the short-term efficacy of the drug[17]. At week 8, 18.5% were in remission ($P = 0.031$ *vs* placebo). Study analysis identified a trend towards less efficacy in cases of more severe disease at baseline. Patients with Mayo score ⩾ 10, CRP ⩾ 10 mg/L and extensive disease responded less favorably

Table 2 Clinical trials that reported prognostic indicators for response to anti- tumor necrosis factor treatment in Ulcerative Colitis

Ref.	Type of study	No. of patients	Anti-TNF drug	Response endpoints	Predictor of response
Arijs *et al*[26]	Cohort		IFX	Endoscopic and histological healing	Mucosal gene expression signature
Armuzzi *et al*[31]	Retrospective	88 (78.4% IFX experienced)	ADA	Clinical remission (4-54 wk)	Short-term clinical remission Low CRP at week 12 (remission at week 54) [1]*Previous immunosupressant use (lower long-term remission rates)*
Armuzzi *et al*[27]	Prospective	126	IFX	Steroid–free clinical remission Mucosal healing Colectomy (12 mo)	Thiopurine-naïve status Combination treatment CRP drop to normal
Ben–Horin *et al*[10]	Retrospective	62 (CD/UC)	IFX	Loss or response	[1]*Low trough levels* *Anti-infliximab antibodies*
Cesarini *et al*[39]	Retrospective	41 (secondary loss of response)	IFX	Clinical remission Colectomy–free (52 wk)	Rapid clinical response to optimization
Colombel *et al*[3]	Prospective (ACT trials)	728	IFX	Clinical remission Clinical response Colectomy	Mucosal healing at week 8 (predictive of long-term outcome)
De Vos *et al*[32]	Prospective	53	IFX	Mayo clinical score Endoscopic remission	Fecal Calprotectin
Fasanmade *et al*[23]	Retrospective	728	IFX	Trough levels Clinical response	[1]*Serum albumin concentration*
Ferrante *et al*[21]	Cohort	121	IFX	Colectomy-free survival (33 mo)	Short term clinical response CRP > 5 mg/L [1]*Previous iv treatment with steroids/cyclosporin*
Ferrante *et al*[18]	Cohort	100	IFX	Early clinical response	Younger age pANCA-/ACSA+
Garcia-Bosch *et al*[28]	Retrospective	48	ADA	Clinical response (partial Mayo score) Colectomy (week 54)	Response to prior treatment with infliximab Early response to adalimumab
Gonzalez-Lama *et al*[20]	Retrospective	47	IFX	Clinical response Steroid-free remission Colectomy	[1]*Disease extent*
Gustavsson *et al*[35]	Placebo controlled trial	45	IFX	Colectomy (3 yr f-up)	Mucosal healing at 3 mo
Jakobovits *et al*[19]	Retrospective	30	IFX (not standard induction regimen 0-2-6)	Colectomy	[1]*Younger age at diagnosis*
Jürgens *et al*[13]	Retrospective	90	IFX	Clinical response Clinical remission (week 14)	CAI-disease activity ANCA seronegativity IL23R genotype
Lee *et al*[22]	Retrospective	134	IFX	Clinical response Clinical remission	Haemoglobin > 11.5 CRP > 3 Immunomodulator-naïve status Response at week 2 Mucosal healing
Kohn *et al*[36]	Open label	83 severe colitis	IFX	Colectomy/Death > 2 mo after first infusion (median f-up 23 mo)	[1]*Single infusion*
Li *et al*[34]	Prospective?	17 24	IFX	CRP Clinical response Endoscopic healing	Changes in percentages of Foxp3(+) Tregs (mucosal and systemic)
McDermott *et al*[30]	Retrospective	23 (86% infliximab experienced)	ADA	Failure (discontinuation of ADA) Colectomy (follow-up 22 mo)	[1]*Short–term failure (increased risk for colectomy)*
Olsen *et al*[24]	Retrospective	59	IFX	UCDAI	Mucosal TNF-a mRNA expression
Oussalah *et al*[14]	Retrospective	191	IFX (≥1 infusion)	Primary non-response Colectomy Infliximab optimization Hospitalization (median 18 mo)	[1]*Indication for acute severe colitis* *Hb ⩽ 9.4 g/dL* *Non-response*
Park *et al*[15]	Retrospective	89	IFX	Clinical response Clinical remission Colectomy	[1]*Mayo score ⩾ 11)* *CMV infection (within prior 3 mo)*
Reinisch *et al*[17]	Prospective (ULTRA 1)	390 (anti-TNF naïve)	ADA	Clinical remission at week 8	[1]*Mayo score ⩾ 10* *CRP = 10 mg/L*
Rismo *et al*[25]	Prospective	74	IFX	UCDAI	Mucosal gene expression signature (Th1 and Th17 related cytokines)

Rostholder *et al*[38]	Retrospective observational	56	IFX	Clinical remission	Escalation of infliximab therapy
Sandborn *et al*[16]	Prospective (ACT1&2)	630	IFX	Colectomy (54 wk)	[1]*Concomitant steroids* *CRP ⩾ 2 mg/dL* *Disease duration < 3 yr* *Mayo ⩾ 10*
Seow *et al*[40]	Cohort	115	IFX	Clinical remission Endoscopic improvement Colectomy	Trough levels
Steenholdt *et al*[41]	Retrospective	106 (CD/UC)	IFX	Loss of response	[1]*Trough levels* *Anti-infliximab antibodies*
Taxonera *et al*[29]	Retrospective	30 (IFX experienced)	ADA	Clinical response at week12 Colectomy (follow-up 48 wk)	Short–term response at week-12 (Associated with less withdrawal and colectomy rates)
Toedter *et al*[33]	Prospective (ACT-1)	48	IFX	Clinical response	Mucosal gene expression signature

[1]Italics correspond to prognostic factors for adverse outcome. IFX: Infliximab; ADA: Adalimumab; UCDAI: Ulcerative colitis disease activity index; HACA: Human anti-chimeric antibodies; CRP: C-reactive protein.

to ADA in the short-term. It should be noted, however, that these parameters did not strongly affect the result and their consideration as predictive factors must be cautious.

In all, the majority of studies appear to support the notion that severe UC demonstrates a less favorable response to treatment with anti-TNF monoclonal antibodies. From the pure clinical standpoint, the best candidate for anti-TNF administration may be an outpatient with moderate to severe UC but not severe disease requiring hospitalization, as defined by the criteria of Truelove and Witts.

In addition to disease severity, other clinical parameters may also affect the response to anti-TNF in UC. Ferrante *et al*[18] studied a cohort of 100 UC patients who were treated with IFX. More than half had extensive disease, were on immunosuppressants and received a single infusion as opposed to the standard induction scheme. Early clinical response was accomplished in 65% of patients. Younger age was associated with a higher percentage of early clinical response (responders: median age 35.7 years *vs* non-responders: 41.6, $P = 0.049$). Different results were obtained by Jakobovits *et al*[19] who reviewed the records of 30 patients with refractory UC who had received a single IFX infusion over the period 2000-2006. Half of the patients underwent colectomy over a median follow-up period of 140 d. In this cohort, younger age at diagnosis correlated with increased risk of surgery (colectomy: mean age 27.5 years *vs* non-colectomy 38.7 years, $P = 0.016$). In contrast, the indication before starting IFX was not relevant to colectomy rates. The number of patients in this study was too small for definitive conclusions to be drawn. In the analysis of the ACT trials duration of colitis ⩽ 3 years strongly increased the risk for colectomy (hazard ratio = 0.36, $P < 0.001$, respectively)[16]. Finally, disease extent may also affect response to treatment. Gonzalez-Lama *et al*[20] studied 47 UC patients who were treated with IFX and were followed for a mean duration of 8 mo. Pre-treatment predictive factors were sought: extent of the disease was the only factor that was related to higher response rates to IFX ($P = 0.02$). Extensive colitis appeared to respond less favorably in the short term in the aforementioned study of ADA as well[17].

Laboratory indicators: Among the various laboratory biomarkers of inflammation, C-reactive protein (CRP) has been the most extensively applied to clinical practice. The association between CRP and inflammatory activity in UC has not been equally strong as it is for Crohn's disease. Nevertheless, its relevance increases when cases of severe UC are studied. As these are the patients that usually require administration of anti-TNF agents, the predictive value of CRP for treatment efficacy/failure may be increased in this population. Ferrante *et al*[21] reported on a cohort of 121 UC outpatients treated with IFX and followed for a median of 33 mo. Eighty-one patients (67%) exhibited short-term response and 21 (17%) underwent colectomy. A value of pre-treatment CRP ⩾ 5 mg/L was an independent predictor for colectomy (HR = 14.5, $P = 0.006$). Similar results were presented in a study of 134 Korean patients with UC who had received at least one infusion of IFX[22]. At week 8, 87% and 45% achieved response and remission, respectively. A pre-treatment CRP ⩾ 3 mg/dL was predictive of clinical remission at week 8 (OR = 4.77, $P = 0.01$). The association between elevated CRP and less favorable response to anti-TNF was also confirmed in the analysis of the ACT trials[16]. A baseline CRP ⩾ 2 mg/L was significantly associated with increased colectomy risk (HR = 1.73, $P = 0.04$). Of note, several studies found an association between elevated CRP and colectomy[21]. Therefore, increased CRP may represent a strong marker of inflammation that requires potent treatment and will respond optimally to anti-TNF. Alternatively, CRP may be an indicator of refractory disease.

In the previous Korean study, high pre-treatment hemoglobin **w**as also a predictor of good response to IFX[22]. Baseline haemoglobin of ⩾ 11.5 g/dL was associated with higher probability for remission at week 8 (OR = 4.47, $P = 0008$). This is in accordance with the study by Oussalah[14] who reported that pre-treatment hemo-

globin ≤ 9.4 g/dL predicted primary non-response to IFX (OR = 4.35). This occurred in 22% of 191 treated patients who were included in the study. According to Truelove criteria low hemoglobin is an indicator of severe disease, which increases the risk of non-response to IFX. High pre-treatment hemoglobulin may reflect the presence of milder disease that responds better to anti-TNF treatment.

Serum albumin concentration may also have prognostic value. A study by Fasanmade *et al*[23] focused on the association between serum IFX and albumin concentration. Data from 728 patients who participated in two clinical trials were analyzed. A value of serum albumin that was outside the normal range was directly related to trough IFX levels and clinical response. Patients with low serum albumin had reduced IFX concentration and worse clinical outcomes. This correlation may reflect a common clearance pathway for albumin and anti-TNF antibodies that belong to the IgG class of immunoglobulins. In all, measurement of albumin before commencement of treatment may serve as a predictive marker of the drug's pharmacokinetics.

Immunological and genetic markers: In recent years significant advances have taken place in our understanding of the immunopathogenesis of UC. In addition, genome wide association studies have discovered polymorphisms which confer susceptibility to or protect from developing UC. These studies led to the identification of several immunological markers with may serve as indicators of disease activity and severity. The possibility that such markers may also serve as predictors of response to treatment, in particular to therapy with anti-TNF monoclonal antibodies, has been increasingly explored.

One of the classical immunological markers that are associated with UC is the presence of perinuclear antineutrophil cytoplasmatic antibodies (p-ANCA). In two recent studies absence of this marker was strongly associated with better response to IFX. In a retrospective study of 90 patients who were evaluated up to week 14 on scheduled IFX infusions, negativity for p-ANCA (along with disease severity and IL23R genotype) was predictive of IFX efficacy[13]. Similar results were obtained in the study by Ferrante *et al*[18]. The authors followed 100 UC patients treated with IFX (84 patients received a single infusion). ANCA seronegativity served as predictor of good response. Notably, a serological phenotype of ANCA+/ASCA- status was particularly correlated with lower rates of response ($P = 0.049$).

During acute flares of UC an abundance of inflammatory mediators are upregulated at the intestinal mucosa and can be detected at both the mRNA and the protein level, whereas, anti-inflammatory treatment is paralleled by a decrease or even disappearance of these markers. Therefore, such markers may hold predictive value for the response to anti-TNF treatment. A first obvious target has been TNF itself. Olsen *et al*[24] looked for predictive factors of response to induction treatment (weeks 0, 2, 6) with IFX in a cohort of 59 patients with moderate to severe disease. The outcome was assessed based on UC disease activity index (UCDAI). Among various parameters elevated pre-treatment mucosal TNF-a expression was the only independent predictive factor of clinical and endoscopic remission ($P = 0.01$ and $P = 0.003$, OR = 2.5 and 4.8, respectively).

UC-related intestinal inflammation has been characterized by upregulation of several components of the major adaptive immunity pathways (Th1, Th2, Th17). A recent study looked at the expression of the pivotal Th1 (IFN-γ) and Th17 (IL-17) cytokines before and after treatment with IFX[25]. Mucosal cytokine profile was determined by PCR and confirmed by immunohistochemistry in biopsies of 74 UC patients. Efficacy was evaluated after 3 infusions and was based on UCDAI. High pre-treatment mucosal expression of IL-17 and IFN-γ significantly correlated with remission after induction therapy (OR = 5.4, $P = 0.013$ and OR = 5.5, $P = 0.011$, respectively).

In a much broader approach, Arijs *et al*[26] performed a gene-array study in mRNA from colonic mucosal biopsies obtained from UC patients who received induction therapy with IFX. Analysis of the arrays revealed genes that were differentially expressed among responders and non-responders. Genes that showed a highly differential expression were osteoprotegerin, stanniocalcin-1, prostaglandin-endoperoxide synthase 2, interleukin 13 receptor alpha-2 and interleukin 11. The sensitivity and specificity in predicting response to IFX based on this gene profiling was 95% and 85%, respectively.

The effect of genetic polymorphisms to response to treatment remains unknown. In the aforementioned study by Jurgens the effect of UC-associated, IL-23R variants on the efficacy of IFX was reported[13]. In this study of 90 patients, homozygosity for the IBD-risk-increasing IL23R variants was associated with higher probability to respond to IFX than homozygosity for IBD-risk-decreasing IL23R variants (74.1% *vs* 34.6%; $P = 0.001$).

Treatment-related factors: Several studies have shown that the pharmacological history plays an important role in the response to anti-TNF treatment. In the study by Ferrante *et al*[21], 121 UC patients received IFX and were followed-up for a median of 33 mo. Colectomy was performed in 21 patients (21%). Previous *iv* treatment with steroids and/or cyclosporine significantly increased the risk for colectomy (HR = 2.4, $P = 0.033$). A similar association was seen in the study by Oussalah *et al*[14]. Previous use of cyclosporine was a positive predictive factor for colectomy (hazard ratio = 2.53). Finally, in the analysis of the colectomy rates in the context of the ACT-trials patients who were on steroids when IFX was started had an increased risk for surgery (HR = 1.84, $P = 0.01$)[16]. However, caution is required for the interpretation of these associations, which should take into consideration the severity of the disease. Indeed, in all of these studies

more severe disease was associated to adverse outcomes and less favorable response to anti-TNF. Therefore, the use of *iv* steroids and/or cyclosporine may simply reflect severe disease.

The association between exposure to immunosuppressants and efficacy of anti-TNF therapy merits special attention. Converging lines of evidence indicate that immunosuppressant-naïve patients respond better to anti-TNF. The efficacy of IFX was evaluated in a cohort of 126 steroid-dependent patients[27]. Approximately half of the patients achieved steroid-free remission, whereas mucosal healing at 12 mo was accomplished in one third. Thiopurine-naïve status was positively associated to steroid-free remission as well as mucosal healing at 12 mo (HR = 2.8 and OR = 3.6, respectively). In the aforementioned Korean study[22] immunomodulator-naïve status was an independent predictors for early clinical remission (OR = 4.89, $P = 0.01$). This consistent finding is in agreement with the growing evidence regarding earlier introduction of biologics in patients with moderate disease, as patients who never received thiopurines may had suffered a shorter disease course.

Finally, for patients who receive ADA as a second anti-TNF monoclonal antibody, the treatment efficacy is affected by the response to prior treatment with IFX. This was shown in a recent retrospective study that evaluated the clinical response and colectomy rate in a cohort of 48 UC patients treated with ADA[28]. The majority (81.3%) was previously exposed to IFX. Early response to ADA at week 12 was significantly more frequent in patients who achieved remission on prior treatment with IFX ($P = 0.01$).

Prognostic factors during anti-TNF treatment

Several recent studies have provided evidence to support the notion that patients with early response to anti-TNF (*i.e.,* within 3 mo) are the ones who will also benefit in the long-term. Early response was defined by a variety of clinical and biological markers in these publications.

Clinical parameters: A Spanish study evaluated the efficacy of ADA in 48 UC patients who were followed-up to week 54[28]. In this cohort the only predictive factor for colectomy was the absence of early clinical response, which was determined by partial Mayo score at week 12 (colectomy: 14.7% *vs* no colectomy: 42.9%, $P = 0.035$).

These results were replicated in a cohort of 121 UC outpatients[21] Eighty-one patients initially responded to IFX with 2/3 maintaining clinical response throughout follow-up. Twenty-one patients ended up with colectomy after a median follow-up of 33 mo. No predictors for durable response were identified. Colectomy on the other hand strongly correlated with early non-response to IFX (HR = 10.8, $P < 0.001$).

In the study by Lee *et al*[22], 45% of 134 patients with UC who received at least a single IFX infusion, achieved remission at week 8. Short-term remission rates were higher in patients who responded very early, at week 2 (OR = 20.54, $P = 0.006$).

The value of ADA in 30 UC patients who had failed IFX was studied retrospectively[29]. Response and remission rates were assessed at weeks 4 and 12 and colectomy rates over a mean follow-up of 48 mo. In the long-term 50% were still on ADA and 20% underwent colectomy. The risk of surgery was higher for patients who did not achieve response at week 12 ($P = 0.001$).

Similarly, Mc Dermott *et al*[30] studied 23 patients who received ADA induction and maintenance treatment. Of note, 86% had previously failed IFX. Discontinuation of ADA over a follow-up period of 22 mo was the primary endpoint and occurred in 70% of patients. Colectomy-free survival at 24 mo was 59%. The only factor associated with increased risk for surgery was the absence of early response to ADA. Among patients who underwent colectomy, 55% had failed ADA at week 12.

Armuzzi *et al*[31] evaluated the short- and long-term effects of ADA in 88 UC patients out of whom 78% had previously received IFX. The rates of clinical remission increased from 17% to 43% at weeks 4 and 54, respectively. Interestingly, achievement of early remission as well as low CRP at week 12 predicted remission at week 54 (OR = 4.17 and 2.63, respectively).

Laboratory indicators: The same conclusion regarding the predictive value of early response was obtained when laboratory markers of inflammation were studied. We already mentioned the predictive value of low CRP at week 12 in the study by Arnuzzi[31]. In another publication from the same group regarding 126 steroid-dependent patients who received IFX[27] drop of serum CRP value to normal after the induction-regimen predicted steroid-free remission and mucosal healing at 12 mo (HR = 4.6, OR = 6.0, respectively). Similar results were reported in a study that used fecal calprotectin as an inflammatory marker. Serial weekly measurements of fecal calprotectin were performed in a cohort of 53 patients who received IFX[32]. Two thirds of patients achieved endoscopic remission at week 10, whereas the median calprotectin level significantly drop from baseline ($P < 0.001$). Early reduction of calprotectin at week 2 predicted endoscopic remission. At week 10, clinical and endoscopic remission strongly correlated to fecal calprotectin concentration.

Immunological markers: Early post-IFX changes of the mucosal and peripheral immunophenotype of UC patients showed strong correlation with clinical response to the drug. Toedter *et al*[33] studied 113 colonic biopsies from 48 patients who participated in the ACT-1 trial. Biopsies were taken before and after treatment with IFX up to week 30. Gene expression profiling was performed. The investigators were able to identify certain genes that demonstrated significant alterations in patients that responded to treatment with IFX but not in non-responders.

In a study that included both Crohn's and UC, the

effect of IFX on the percentages of regulatory T cells (Treg) was investigated[34]. Flow cytometry, PCR and immunohistochemistry were applied to quantify the expression of Forkhead box protein3 (Foxp3)-positive T cells in both peripheral blood samples and mucosal biopsies before and after IFX treatment. Responders to IFX were characterized by significantly increased numbers of CD4(+) CD25(+) Foxp3(+)Treg and CD4(+) CD25(-) Foxp3(+) Tregs in blood ($P < 0.05$) and a significant down-regulation in the tissue ($P < 0.001$). The duration of clinical response to IFX correlated to a sustainable peripheral increase of Foxp3 (+) Treg cells.

Although such individual molecular characterization is far from being clinically applicable, it shows that personalized therapy which will be based on the particular immunophenotype may guide the therapeutic approach in the future.

Endoscopic findings: In recent years, mucosal healing (*i.e.,* the disappearance of visible active inflammatory lesions in endoscopy) has emerged as a definitive endpoint in the natural history of UC and an indispensable therapeutic target both in clinical trials and "real-life" practice. This is because mucosal healing has been shown to be associated with sustained long-term remission in patients with UC[3].

In the pivotal ACT trials endoscopic evaluations were performed at various time points and mucosal healing was defined as Mayo subscore of 0 (normal) or 1 (mild). Early endoscopic improvement at week 8 was associated to improved clinical outcomes[3]. Accordingly, low endoscopy subscores at week 8 predicted reduced risk of colectomy through week 54 ($P = 0.0004$) as well as higher remission and steroid-free remission rates ($P < 0.0001$).

A single IFX infusion or placebo was administered to 45 patients with acute, steroid-refractory UC[35]. Three years later the beneficial effect of the drug persisted as less patients in the IFX group underwent operation (50% *vs* 76%, $P = 0.012$). Endoscopic remission at month 3 strongly predicted a reduced long-term risk for colectomy ($P = 0.02$).

Mucosal healing was also a positive predictive factor for long-term remission in the study by Lee *et al*[22]. A variety of predictors for short-term outcome were identified whereas the only parameter associated with sustained long-term benefit was endoscopic remission (OR = 4.66, $P = 0.04$).

Treatment-related factors: The number of IFX infusions was associated with improved sustained response to anti-TNF treatment. Kohn *et al*[36] studied the effect of IFX treatment in 83 patients with severe steroid-refractory UC.Patients received ⩾ 1 infusions and were followed for a median of 23 mo. Twelve out of 83 patients (15%) had a colectomy within 2 mo. The risk for a prime adverse event was significantly higher among patients who received a single IFX infusion as opposed to those who were given two or more doses (OR = 9.53, $P = 0.001$).

The combined administration with immunosuppressants appears to have an advantage in comparison to single IFX therapy. This was shown in the study by Armuzzi *et al*[27]. In this cohort of 126 steroid-dependent UC patients combination treatment with IFX and thiopurines was a predictor of steroid-free remission (HR = 2.2). In another prospective trial Panaccione studied 231 patients with moderate disease who were biologics-naïve and had not received azathioprine over the 3 mo before enrollment. Patients were offered IFX monotherapy, azathioprine monotherapy or combination treatment. Steroid-free remission at week 16 was significantly more common in the combination arm of the study ($P < 0.05$ compared to both monotherapies)[37].

The need for escalation of anti-TNF therapy is also a poor prognostic factor for long-term outcome. In a cohort of 56 patients with moderate colitis who were treated with IFX, 89% proceeded to maintenance treatment[38]. During a mean follow-up of 38 mo, clinical remission was achieved in 36% of patients at 12 mo, whereas 54% required escalation of treatment. Intensification of IFX treatment was a negative predictive factor of remission at 12 mo ($P = 0.01$). In accordance, colectomy was performed more often in the "escalation" group (33% *vs* 21%).

In a related study, Cesarini *et al*[39] showed that rapid response to escalation treatment has a favorable effect on long-term outcome. They studied the records of 41 UC patients with loss of response to IFX who were treated with either dose doubling or interval shortening. The primary outcome was rapid response which was evaluated at the follow-up visit after treatment escalation. Remission and colectomy were evaluated by week 52. The majority (90%) responded rapidly and 46% achieved rapid remission. Only 4 patients (9.8%) underwent colectomy by week 52. The main predictor for avoidance of colectomy was initial response to intensification treatment ($P = 0.002$).

Recent developments emphasize the importance of serum trough levels of IFX and ADA and the formation of antibodies against anti-TNF monoclonal antibodies for the pharmacokinetics as well as the therapeutic efficacy of these drugs. In a study of 115 UC patients on maintenance treatment, clinical outcomes were associated to IFX trough levels[40]. Detectable drug in serum predicted clinical remission and endoscopic improvement at week 54 ($P < 0.001$ for both parameters). Reduced trough levels correlated with increased risk of colectomy in this cohort ($P < 0.001$). Interestingly, antibody-status was not predictive of response to IFX treatment.

Steenholdt *et al*[41] retrospectively studied 106 IBD patients on IFX, who either maintained or lost their response. Significantly higher IFX levels and lower antibodies titer were measured in patients with sustained response to IFX ($P < 0.0001$). Moreover, the authors suggested threshold values for the two parameters to ac-

curately predict and/or explain loss of response to IFX.

Similarly, Ben-Horin *et al*[10] tested the samples of 62 mixed IBD patients for anti- IFX antibodies and serum trough levels. Low trough levels and high antibodies titer were found in 83% of patients with loss of response and in 8% of patients who maintained remission ($P < 0.001$).

Critique of available markers

As the number of UC patients who have been exposed to anti-TNF monoclonal antibodies steadily increases, more factors will be reported that may be associated with better or worse response to these medications. Before, however, their use is recommended for the selection of patients in clinical practice, careful analysis of the specifics of each marker should be performed and inherent problems with the interpretation of the results from clinical trials should be kept in mind.

Clinical markers have the advantage to be readily available and identifiable in a straightforward fashion. They are easy to use, replicable, non-invasive and, overall, convenient for use in clinical practice. Caution, however, is needed when data from clinical trials are analyzed as the definition of a certain parameter may vary between different studies. In particular, clinical response and remission may be related to a variety of activity scoring systems or arbitrarily defined clinical criteria. In addition, the time point in which a certain clinical marker is reported is of pivotal significance. This is so because UC is a lifelong condition and, therefore, only time points with significant length are relevant to a true remission. Criticism also occurs regarding RCTs in the means that they may not always include patients that reflect 'real-life' IBD populations[42].

Endoscopic markers such as mucosal healing are of significance as recent studies have shown that they are indeed associated with better disease outcomes. It should be noted, however, that the major clinical trials have defined mucosal healing as Endoscopy Mayo score of 0 or 1. Whether the latter score truly represents absolute and complete elimination of inflammation is questionable. In addition, such markers require the performance of an invasive procedure (colonoscopy) soon after the commencement of treatment ($\leqslant$ 3 mo), which may not be easily acceptable from a patient, in particular when clinical remission has taken place.

Serological markers such as CRP are also easy to obtain. Nevertheless, there has not been good correlation between CRP and clinical activity of UC with the exception of severe cases. In addition, its prognostic value has only been reported in a minority of trials, given the fact that CRP is usually determined in every case of UC. Fecal calprotectin is a good indicator of ongoing acute (neutrophilic) inflammation in the colon. However, no studies have indicated that the magnitude of pre-treatment fecal calprotectin predicts the response to anti-TNF. In addition, the measurement of fecal calprotectin is not widely applied in practice and technical issues exist regarding the standardization of methodology. It should be noted, however, that both serum CRP and fecal calprotectin may be more useful when their short-term change in response to anti-TNF is considered rather that their absolute pre-treatment values.

Immunological and genetic markers are important as they hold promise for individualized therapy based on the specific characteristics of each individual patient. The major drawbacks for the application of such markers are technical challenges and lack of replication for most results. An additional problem is the redundancy of the immunological pathways that underlie inflammation in UC. Therefore, a single marker may not be sufficient enough to cover the whole mechanism of injury. Similarly, UC is a polygenetic trait and single gene polymorphisms do not usually lead to the manifestation of the disease phenotype. Nonetheless, as additional biological drugs will become available for the treatment of UC, selection of patients according to the predominant immunogenetic pathway may become the most cost-effective approach.

CONCLUSION

Currently, no single marker fulfils all criteria for being an appropriate prognostic indicator for response to anti-TNF treatment in UC. The ideal predictor should be clearly defined, simple and easy to obtain, as well as of repetitive association between different trials. Alternatively, a predictive model which includes clinical, laboratory and even genetic and/or immunological parameters may be more difficult to develop but more accurate in its predictive value. In that context, and whilst our experience with anti-TNF therapy in UC expands, it is important to continue the search for optimal predictive factors of response or failure. Each of the proposed prognostic parameters should be validated in large populations of patients and across clinical trials of different ethnicities. Eventually, personalized treatment may be the best, safest and most cost-effective strategy in diseases with such a complex pathogenetic background.

REFERENCES

1 **Ordás I**, Eckmann L, Talamini M, Baumgart DC, Sandborn WJ. Ulcerative colitis. *Lancet* 2012; **380**: 1606-1619 [PMID: 22914296 DOI: 10.1016/S0140-6736(12)60150-0]

2 **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]

3 **Colombel JF**, Rutgeerts P, Reinisch W, Esser D, Wang Y, Lang Y, Marano CW, Strauss R, Oddens BJ, Feagan BG, Hanauer SB, Lichtenstein GR, Present D, Sands BE, Sandborn WJ. Early mucosal healing with infliximab is associated with improved long-term clinical outcomes in ulcerative colitis. *Gastroenterology* 2011; **141**: 1194-1201 [PMID:

21723220 DOI: 10.1053/j.gastro.2011.06.054]
4 **Blonski W**, Buchner AM, Lichtenstein GR. Treatment of ulcerative colitis. *Curr Opin Gastroenterol* 2014; **30**: 84-96 [PMID: 24285003 DOI: 10.1097/MOG.0000000000000031]
5 **Rutgeerts P**, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J, Travers S, Rachmilewitz D, Hanauer SB, Lichtenstein GR, de Villiers WJ, Present D, Sands BE, Colombel JF. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2005; **353**: 2462-2476 [PMID: 16339095]
6 **Sandborn WJ**, van Assche G, Reinisch W, Colombel JF, D' Haens G, Wolf DC, Kron M, Tighe MB, Lazar A, Thakkar RB. Adalimumab induces and maintains clinical remission in patients with moderate-to-severe ulcerative colitis. *Gastroenterology* 2012; **142**: 257-65.e1-3 [PMID: 22062358 DOI: 10.1053/j.gastro.2011.10.032]
7 **Ho GT**, Smith L, Aitken S, Lee HM, Ting T, Fennell J, Lees CW, Palmer KR, Penman ID, Shand AG, Arnott ID, Satsangi J. The use of adalimumab in the management of refractory Crohn's disease. *Aliment Pharmacol Ther* 2008; **27**: 308-315 [PMID: 18081730]
8 **Swaminath A**, Ullman T, Rosen M, Mayer L, Lichtiger S, Abreu MT. Early clinical experience with adalimumab in treatment of inflammatory bowel disease with infliximab-treated and naïve patients. *Aliment Pharmacol Ther* 2009; **29**: 273-278 [PMID: 19006540 DOI: 10.1111/j.1365-2036.2008.03878.x]
9 **Siegel CA**, Melmed GY. Predicting response to Anti-TNF Agents for the treatment of crohn's disease. *Therap Adv Gastroenterol* 2009; **2**: 245-251 [PMID: 21180547 DOI: 10.1177/1756283X09336364]
10 **Ben-Horin S**, Yavzori M, Katz L, Kopylov U, Picard O, Fudim E, Coscas D, Bar-Meir S, Goldstein I, Chowers Y. The immunogenic part of infliximab is the F(ab')2, but measuring antibodies to the intact infliximab molecule is more clinically useful. *Gut* 2011; **60**: 41-48 [PMID: 20519742 DOI: 10.1136/gut.2009.201533]
11 **Maser EA**, Villela R, Silverberg MS, Greenberg GR. Association of trough serum infliximab to clinical outcome after scheduled maintenance treatment for Crohn's disease. *Clin Gastroenterol Hepatol* 2006; **4**: 1248-1254 [PMID: 16931170]
12 **Karmiris K**, Paintaud G, Noman M, Magdelaine-Beuzelin C, Ferrante M, Degenne D, Claes K, Coopman T, Van Schuerbeek N, Van Assche G, Vermeire S, Rutgeerts P. Influence of trough serum levels and immunogenicity on long-term outcome of adalimumab therapy in Crohn's disease. *Gastroenterology* 2009; **137**: 1628-1640 [PMID: 19664627 DOI: 10.1053/j.gastro.2009.07.062]
13 **Jürgens M**, Laubender RP, Hartl F, Weidinger M, Seiderer J, Wagner J, Wetzke M, Beigel F, Pfennig S, Stallhofer J, Schnitzler F, Tillack C, Lohse P, Göke B, Glas J, Ochsenkühn T, Brand S. Disease activity, ANCA, and IL23R genotype status determine early response to infliximab in patients with ulcerative colitis. *Am J Gastroenterol* 2010; **105**: 1811-1819 [PMID: 20197757 DOI: 10.1038/ajg.2010.95]
14 **Oussalah A**, Evesque L, Laharie D, Roblin X, Boschetti G, Nancey S, Filippi J, Flourié B, Hebuterne X, Bigard MA, Peyrin-Biroulet L. A multicenter experience with infliximab for ulcerative colitis: outcomes and predictors of response, optimization, colectomy, and hospitalization. *Am J Gastroenterol* 2010; **105**: 2617-2625 [PMID: 20736936 DOI: 10.1038/ajg.2010.345]
15 **Park SH**, Yang SK, Hong SM, Park SK, Kim JW, Lee HJ, Yang DH, Jung KW, Kim KJ, Ye BD, Byeon JS, Myung SJ, Kim JH. Severe disease activity and cytomegalovirus colitis are predictive of a nonresponse to infliximab in patients with ulcerative colitis. *Dig Dis Sci* 2013; **58**: 3592-3599 [PMID: 23979435 DOI: 10.1007/s10620-013-2828-1]
16 **Sandborn WJ**, Rutgeerts P, Feagan BG, Reinisch W, Olson A, Johanns J, Lu J, Horgan K, Rachmilewitz D, Hanauer SB, Lichtenstein GR, de Villiers WJ, Present D, Sands BE, Colombel JF. Colectomy rate comparison after treatment of ulcerative colitis with placebo or infliximab. *Gastroenterology* 2009; **137**: 1250-160; quiz 1520 [PMID: 19596014 DOI: 10.1053/j.gastro.2009.06.061]
17 **Reinisch W**, Sandborn WJ, Hommes DW, D'Haens G, Hanauer S, Schreiber S, Panaccione R, Fedorak RN, Tighe MB, Huang B, Kampman W, Lazar A, Thakkar R. Adalimumab for induction of clinical remission in moderately to severely active ulcerative colitis: results of a randomised controlled trial. *Gut* 2011; **60**: 780-787 [PMID: 21209123 DOI: 10.1136/gut.2010.221127]
18 **Ferrante M**, Vermeire S, Katsanos KH, Noman M, Van Assche G, Schnitzler F, Arijs I, De Hertogh G, Hoffman I, Geboes JK, Rutgeerts P. Predictors of early response to infliximab in patients with ulcerative colitis. *Inflamm Bowel Dis* 2007; **13**: 123-128 [PMID: 17206703]
19 **Jakobovits SL**, Jewell DP, Travis SP. Infliximab for the treatment of ulcerative colitis: outcomes in Oxford from 2000 to 2006. *Aliment Pharmacol Ther* 2007; **25**: 1055-1060 [PMID: 17439506]
20 **Gonzalez-Lama Y**, Fernandez-Blanco I, Lopez-SanRoman A, Taxonera C, Casis B, Tabernero S, Bermejo F, Martinez-Silva F, Mendoza JL, Martinez-Montiel P, Carneros JA, Sanchez F, Maté J, Gisbert JP. Open-label infliximab therapy in ulcerative colitis: a multicenter survey of results and predictors of response. *Hepatogastroenterology* 2008; **55**: 1609-1614 [PMID: 19102352]
21 **Ferrante M**, Vermeire S, Fidder H, Schnitzler F, Noman M, Van Assche G, De Hertogh G, Hoffman I, D'Hoore A, Van Steen K, Geboes K, Penninckx F, Rutgeerts P. Long-term outcome after infliximab for refractory ulcerative colitis. *J Crohns Colitis* 2008; **2**: 219-225 [PMID: 21172214 DOI: 10.1016/j.crohns.2008.03.004]
22 **Lee KM**, Jeen YT, Cho JY, Lee CK, Koo JS, Park DI, Im JP, Park SJ, Kim YS, Kim TO, Lee SH, Jang BI, Kim JW, Park YS, Kim ES, Choi CH, Kim HJ. Efficacy, safety, and predictors of response to infliximab therapy for ulcerative colitis: a Korean multicenter retrospective study. *J Gastroenterol Hepatol* 2013; **28**: 1829-1833 [PMID: 23829336 DOI: 10.1111/jgh.12324]
23 **Fasanmade AA**, Adedokun OJ, Olson A, Strauss R, Davis HM. Serum albumin concentration: a predictive factor of infliximab pharmacokinetics and clinical response in patients with ulcerative colitis. *Int J Clin Pharmacol Ther* 2010; **48**: 297-308 [PMID: 20420786]
24 **Olsen T**, Goll R, Cui G, Christiansen I, Florholmen J. TNF-alpha gene expression in colorectal mucosa as a predictor of remission after induction therapy with infliximab in ulcerative colitis. *Cytokine* 2009; **46**: 222-227 [PMID: 19286392 DOI: 10.1016/j.cyto.2009.02.001]
25 **Rismo R**, Olsen T, Cui G, Christiansen I, Florholmen J, Goll R. Mucosal cytokine gene expression profiles as biomarkers of response to infliximab in ulcerative colitis. *Scand J Gastroenterol* 2012; **47**: 538-547 [PMID: 22486187 DOI: 10.3109/00365521.2012.667146]
26 **Arijs I**, Li K, Toedter G, Quintens R, Van Lommel L, Van Steen K, Leemans P, De Hertogh G, Lemaire K, Ferrante M, Schnitzler F, Thorrez L, Ma K, Song XY, Marano C, Van Assche G, Vermeire S, Geboes K, Schuit F, Baribaud F, Rutgeerts P. Mucosal gene signatures to predict response to infliximab in patients with ulcerative colitis. *Gut* 2009; **58**: 1612-1619 [PMID: 19700435 DOI: 10.1136/gut.2009.178665]
27 **Armuzzi A**, Pugliese D, Danese S, Rizzo G, Felice C, Marzo M, Andrisani G, Fiorino G, Sociale O, Papa A, De Vitis I, Rapaccini GL, Guidi L. Infliximab in steroid-dependent ulcerative colitis: effectiveness and predictors of clinical and endoscopic remission. *Inflamm Bowel Dis* 2013; **19**: 1065-1072 [PMID: 23448790 DOI: 10.1097/MIB.0b013e3182802909]
28 **García-Bosch O**, Gisbert JP, Cañas-Ventura A, Merino O,

Cabriada JL, García-Sánchez V, Gutiérrez A, Nos P, Peñalva M, Hinojosa J, García-Planella E, Muñoz F, Calvet X, Panés J. Observational study on the efficacy of adalimumab for the treatment of ulcerative colitis and predictors of outcome. *J Crohns Colitis* 2013; **7**: 717-722 [PMID: 23142005 DOI: 10.1016/j.crohns.2012.10.004]

29 **Taxonera C**, Estellés J, Fernández-Blanco I, Merino O, Marín-Jiménez I, Barreiro-de Acosta M, Saro C, García-Sánchez V, Gento E, Bastida G, Gisbert JP, Vera I, Martinez-Montiel P, Garcia-Morán S, Sánchez MC, Mendoza JL. Adalimumab induction and maintenance therapy for patients with ulcerative colitis previously treated with infliximab. *Aliment Pharmacol Ther* 2011; **33**: 340-348 [PMID: 21133961 DOI: 10.1111/j.1365-2036.2010.04531.x]

30 **McDermott E**, Murphy S, Keegan D, O'Donoghue D, Mulcahy H, Doherty G. Efficacy of Adalimumab as a long term maintenance therapy in ulcerative colitis. *J Crohns Colitis* 2013; **7**: 150-153 [PMID: 22520592 DOI: 10.1016/j.crohns.2012.03.016]

31 **Armuzzi A**, Biancone L, Daperno M, Coli A, Pugliese D, Annese V, Aratari A, Ardizzone S, Balestrieri P, Bossa F, Cappello M, Castiglione F, Cicala M, Danese S, D'Incà R, Dulbecco P, Feliciangeli G, Fries W, Genise S, Gionchetti P, Gozzi S, Kohn A, Lorenzetti R, Milla M, Onali S, Orlando A, Papparella LG, Renna S, Ricci C, Rizzello F, Sostegni R, Guidi L, Papi C. Adalimumab in active ulcerative colitis: a "real-life" observational study. *Dig Liver Dis* 2013; **45**: 738-743 [PMID: 23683530 DOI: 10.1016/j.dld.2013.03.018]

32 **De Vos M**, Dewit O, D'Haens G, Baert F, Fontaine F, Vermeire S, Franchimont D, Moreels T, Staessen D, Terriere L, Vander Cruyssen B, Louis E. Fast and sharp decrease in calprotectin predicts remission by infliximab in anti-TNF naïve patients with ulcerative colitis. *J Crohns Colitis* 2012; **6**: 557-562 [PMID: 22398050 DOI: 10.1016/j.crohns.2011.11.002]

33 **Toedter G**, Li K, Marano C, Ma K, Sague S, Huang CC, Song XY, Rutgeerts P, Baribaud F. Gene expression profiling and response signatures associated with differential responses to infliximab treatment in ulcerative colitis. *Am J Gastroenterol* 2011; **106**: 1272-1280 [PMID: 21448149 DOI: 10.1038/ajg.2011.83]

34 **Li Z**, Arijs I, De Hertogh G, Vermeire S, Noman M, Bullens D, Coorevits L, Sagaert X, Schuit F, Rutgeerts P, Ceuppens JL, Van Assche G. Reciprocal changes of Foxp3 expression in blood and intestinal mucosa in IBD patients responding to infliximab. *Inflamm Bowel Dis* 2010; **16**: 1299-1310 [PMID: 20196149 DOI: 10.1002/ibd.21229]

35 **Gustavsson A**, Järnerot G, Hertervig E, Friis-Liby I, Blomquist L, Karlén P, Grännö C, Vilien M, Ström M, Verbaan H, Hellström PM, Magnuson A, Halfvarson J, Tysk C. Clinical trial: colectomy after rescue therapy in ulcerative colitis - 3-year follow-up of the Swedish-Danish controlled infliximab study. *Aliment Pharmacol Ther* 2010; **32**: 984-989 [PMID: 20937043 DOI: 10.1111/j.1365-2036.2010.04435.x]

36 **Kohn A**, Daperno M, Armuzzi A, Cappello M, Biancone L, Orlando A, Viscido A, Annese V, Riegler G, Meucci G, Marrollo M, Sostegni R, Gasbarrini A, Peralta S, Prantera C. Infliximab in severe ulcerative colitis: short-term results of different infusion regimens and long-term follow-up. *Aliment Pharmacol Ther* 2007; **26**: 747-756 [PMID: 17697208]

37 **Panaccione R,** Sandborn WJ, Gordon G. Infliximab, azathioprine, or infliximab azathioprine for treatment of moderate to severe ulcerative colitis: The UC SUCCESS trial. *J Crohns Colitis* 2011; **5**: 2

38 **Rostholder E**, Ahmed A, Cheifetz AS, Moss AC. Outcomes after escalation of infliximab therapy in ambulatory patients with moderately active ulcerative colitis. *Aliment Pharmacol Ther* 2012; **35**: 562-567 [PMID: 22239070 DOI: 10.1111/j.1365-2036.2011.04986.x]

39 **Cesarini M**, Katsanos K, Papamichael K, Ellul P, Lakatos PL, Caprioli F, Kopylov U, Tsianos E, Mantzaris GJ, Ben-Horin S, Danese S, Fiorino G. Dose optimization is effective in ulcerative colitis patients losing response to infliximab: a collaborative multicentre retrospective study. *Dig Liver Dis* 2014; **46**: 135-139 [PMID: 24246151 DOI: 10.1016/j.dld.2013.10.007]

40 **Seow CH**, Newman A, Irwin SP, Steinhart AH, Silverberg MS, Greenberg GR. Trough serum infliximab: a predictive factor of clinical outcome for infliximab treatment in acute ulcerative colitis. *Gut* 2010; **59**: 49-54 [PMID: 19651627 DOI: 10.1136/gut.2009.183095]

41 **Steenholdt C**, Bendtzen K, Brynskov J, Thomsen OØ, Ainsworth MA. Cut-off levels and diagnostic accuracy of infliximab trough levels and anti-infliximab antibodies in Crohn's disease. *Scand J Gastroenterol* 2011; **46**: 310-318 [PMID: 21087119 DOI: 10.3109/00365521.2010.536254]

42 **Ha C**, Ullman TA, Siegel CA, Kornbluth A. Patients enrolled in randomized controlled trials do not represent the inflammatory bowel disease patient population. *Clin Gastroenterol Hepatol* 2012; **10**: 1002-1007; quiz e78 [PMID: 22343692 DOI: 10.1016/j.cgh.2012.02.004]

P- Reviewer: Akiho H, Zoller M **S- Editor**: Wen LL
L- Editor: A **E- Editor:** Lu YJ

World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4291/wjgp.v5.i3.304

World J Gastrointest Pathophysiol 2014 August 15; 5(3): 304-321
ISSN 2150-5330 (online)

REVIEW

Genetic update on inflammatory factors in ulcerative colitis: Review of the current literature

Patricia Sarlos, Erzsebet Kovesdi, Lili Magyari, Zsolt Banfai, Andras Szabo, Andras Javorhazy, Bela Melegh

Patricia Sarlos, 1st Department of Internal Medicine, University of Pecs, 7623 Pecs, Hungary
Erzsebet Kovesdi, Lili Magyari, Zsolt Banfai, Andras Szabo, Bela Melegh, Department of Medical Genetics, University of Pecs, 7624 Pecs, Hungary
Erzsebet Kovesdi, Lili Magyari, Zsolt Banfai, Andras Szabo, Bela Melegh, Szentagothai Research Centre, 7624 Pecs, Hungary
Andras Javorhazy, Department of Urology, University of Pecs, 7621 Pecs, Hungary
Author contributions: Sarlos P, Kovesdi E, Magyari L, Banfai Z, Szabo A, Javorhazy A, Melegh B contributed equally to this work; Melegh B conceived the study; Kovesdi E, Magyari L, Banfai Z wrote the genetic part of the study; Sarlos P, Szabo A wrote the clinical section of the review; Javorhazy A prepared the Figure.
Supported by The grant of the Hungarian Science Foundation, No. OTKA K103983
Correspondence to: Bela Melegh, MD, PhD, DSc, Department of Medical Genetics, University of Pecs, Szigeti 12, 7624 Pecs, Hungary. bela.melegh@aok.pte.hu
Telephone: +36-72-536427 Fax: +36-72-536032
Received: January 28, 2014 Revised: March 19, 2014
Accepted: July 12, 2014
Published online: August 15, 2014

Abstract

Ulcerative colitis (UC) is one of the main types of inflammatory bowel disease, which is caused by dysregulated immune responses in genetically predisposed individuals. Several genetic factors, including interleukin and interleukin receptor gene polymorphisms and other inflammation-related genes play central role in mediating and modulating the inflammation in the human body, thereby these can be the main cause of development of the disease. It is clear these data are very important for understanding the base of the disease, especially in terms of clinical utility and validity, but summarized literature is exiguous for challenge health specialist that can used in the clinical practice nowadays. This review summarizes the current literature on inflammation-related genetic polymorphisms which are associated with UC. We performed an electronic search of Pubmed Database among publications of the last 10 years, using the following medical subject heading terms: UC, ulcerative colitis, inflammation, genes, polymorphisms, and susceptibility.

Key words: Ulcerative colitis; Inflammatory factors; Genes; Polymorphisms; Susceptibility

Core tip: Ulcerative colitis (UC) is a disorder of the idiopathic and chronic inflammation of the colonic mucosa. Several genetics factors influence the development of the disease, especially interleukin and interleukin receptor gene polymorphisms and other inflammation-related genes. In this review we collected the current literature on PubMed Database about those genetic markers that are associated with UC, we focused on the following terminology: UC, inflammation, genes, polymorphisms, susceptibility.

Sarlos P, Kovesdi E, Magyari L, Banfai Z, Szabo A, Javorhazy A, Melegh B. Genetic update on inflammatory factors in ulcerative colitis: Review of the current literature. *World J Gastrointest Pathophysiol* 2014; 5(3): 304-321 Available from: URL: http://www.wjgnet.com/2150-5330/full/v5/i3/304.htm DOI: http://dx.doi.org/10.4291/wjgp.v5.i3.304

INTRODUCTION

Ulcerative colitis (UC; MIM 191390) and Crohn's disease (CD; MIM 26600) are the two main, related forms of inflammatory bowel disease (IBD) which are chronic, relapsing inflammatory disorders of the gastrointestinal tract[1]. The highest annual incidence rate of UC was re-

ported in Europe (24.3/100000) and in North America (19.2/100000). However, in Asia and in the Middle East the rate is much lower (6.3/100000) believed to be associated with the different level of industrialization[2]. UC has a bimodal pattern of incidence, with the mean age diagnosis between ages 15 and 30 years, and a second smaller peak between ages 50 and 70 years[3]. Clinically, UC is characterized by superficial, continuous mucosal inflammation and ulcers restricted to the colon, whereas CD is a segmental, transmural disorder involving any part of the gastrointestinal tract[4].

Although the precise etiology of IBD still remains obscure, the accepted hypothesis is that in genetically susceptible individuals the commensal luminal flora trigger an inappropriate, overactive mucosal immune response causing intestinal tissue damage that is further modified by specific environmental factors (*e.g.,* smoking)[5].

At first, observational family studies and twin studies directed the interest to genetic components in the pathogenesis of IBD[6, 7]. Recently, genome-wide association studies (GWAS) have resulted in the identification of many novel single nucleotide polymorphisms (SNPs) for CD initially and latterly for UC which is thought to be more genetically heterogeneous than CD. To date, the number of known risk loci has expanded to 163, of which 110 confer common susceptibility to IBD, whereas 30 seem to be specific to CD and 23 to UC[8].

Immunologically, CD is associated with a T helper type 1 (Th1)[9] and T helper type 17 (Th17)[10] immune response, thus interferon gamma/interleukin-12 (IFNγ/IL-12) and interleukin-23/interleukin-17 (IL-23/IL-17) cytokines assign the downstream release of complex network of further pro-inflammatory cytokines (*e.g.,* IL-18, IL-2, IL-1, IL-21, IL-22, IL-17A, IL-17F, IL-26). However, UC is thought to be the result of a Th17 (IL-17) and a modified Th2 response (IL-13, IL-5 and IL-9). In addition, interleukin-6 (IL-6) and tumor necrosis factor alpha (TNFα) are produced by both T helper type 2 (Th2) cells and Th1 cells.

The IBD-associated loci encode for genes involved in maintenance of epithelial barrier integrity, antigen pattern recognition, autophagy, innate immunological response, coordination of adaptive immune responses and leukocyte recruitment (Figure 1).

Most of the difference at molecular level between UC and CD is found in human leukocyte antigen (*HLA)* Class Ⅱ genes and in genes related to pattern recognition [*e.g.,* nucleotide-binding oligomerization domains *(NODs), toll-like receptors (TLRs)*], innate immunity (*e.g., IL-23R*) or autophagy pathways (*e.g., ATG16L1, IRGM*). The *HLA* class Ⅱ genes *DR2*, *DR9*, and *DRB1*0103* were identified as susceptibility genes for UC, whereas *DR4* was a protective gene[11,12]. *HLA* haplotype *DRB1*0103* is significantly associated with disease susceptibility, extensive disease, and an increased risk of colectomy[13]. While several genes involved in bacterial sensing [nucleotide-binding oligomerization domain 2/caspase activation recruitment domain 15 (*NOD2/CARD15*)] and processing mechanisms (autophagy related genes *ATG16L1* and *IRGM*) are defective only in CD, the Th17/IL-23 axis related cytokines [*e.g.,* IL-23R, IL-12B and their downstream components signal transducer and activator of transcription 3 (STAT3), janus kinase 2 (JAK2)] have been associated with both CD and UC.

Dysfunction of the barrier integrity, enhanced permeability is also a main feature in UC. Recently, in a large review epithelial barrier genes were discussed in detail, namely, extracellular matrix protein 1 (*ECM1*), cadherin type 1 (*CDH1*), hepatocyte nuclear factor 4, alpha (*HNF4α*), and laminin beta 1 (*LAMB1*).

These genes were found not to be associated with CD, implying they may confer susceptibility specifically to UC[14]. Interestingly, the *CDH1* locus represents the first genetic association also identified in a GWAS for colorectal cancer susceptibility[15, 16].

In our review we focus on inflammation-related genes and polymorphisms including interleukin and interleukin receptor gene polymorphisms which are involved in the pathogenesis of UC.

INFLAMMATION-RELATED GENETIC FACTORS

Cytotoxic T-lymphocyte antigen 4

Cytotoxic T-lymphocyte antigen 4 (CTLA4) is an inhibitory receptor expressed by activated T cells. It is an important downregulator of the T cell activation and might contribute to peripheral tolerance. CTLA4 is a good candidate gene for susceptibility to UC, because it acts as a negative regulator of T cell activation and T/B, T/monocyte-macrophage cognate interaction. The localization of *CTLA4* gene is on chromosome 2q33. Several genetic polymorphisms have been reported in the human *CTLA4* gene[17, 18].

In a Tunisian population study, where A+49G was analyzed comparing the UC patients with the control subjects, the frequencies of the +49A allele and the homozygous +49 A/A genotype were higher in UC patients than in controls, but those differences were not statistically significant[17].

In a Dutch Caucasian and in a Han Chinese UC cohort studies the C-318T and A+49G polymorphisms of *CTLA4* gene were examined. No significant differences were observed in distribution of allele, genotype and haplotype frequencies between UC and control group[19].

A Hungarian cohort was examined for the same polymorphisms and no association was found between heterozygous AG genotype, homozygous GG variant, and G allele frequency of the *CTLA4* gene A+49G polymorphisms comparing the UC (IBD) group to the healthy controls. The A+49G does not represent an obligatory susceptibility factor for UC[20].

The A-1661G and the T-1722C two other SNPs in the non-exonic region were investigated in the Han

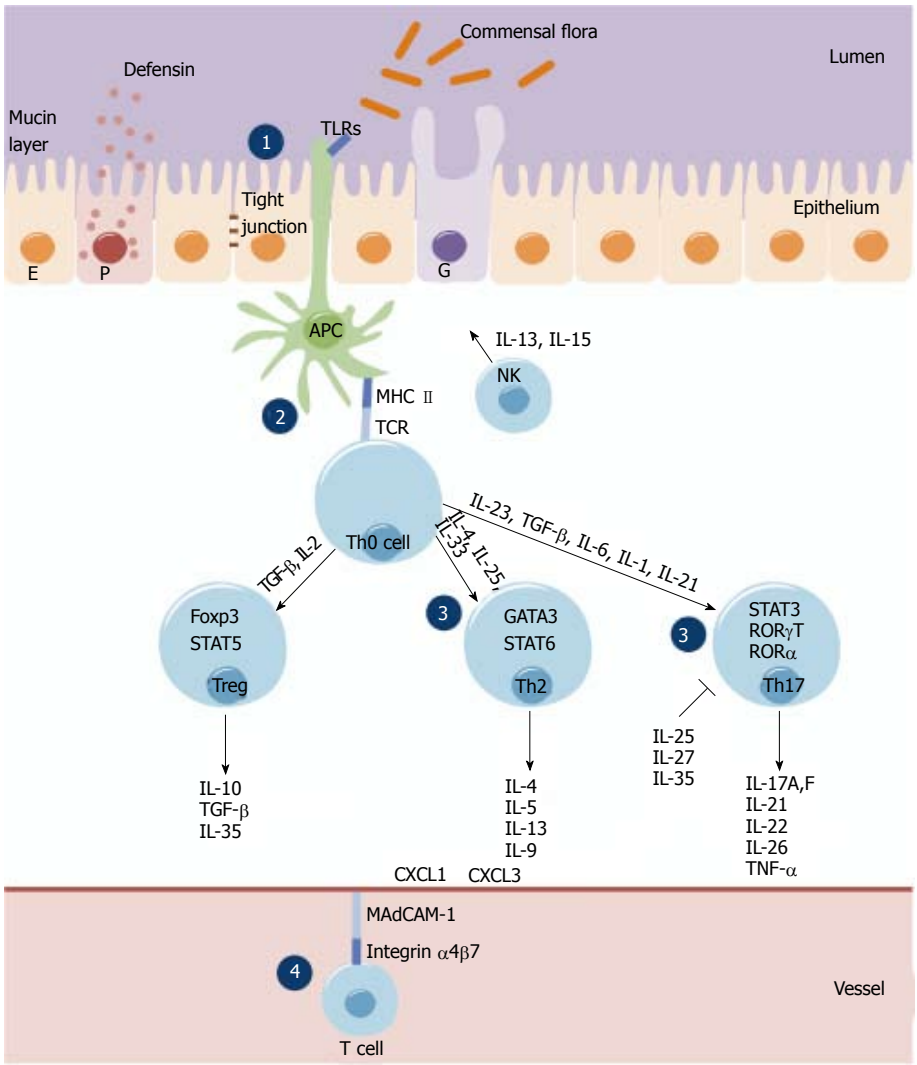

Figure 1 The Ulcerative colitis-associated risk loci. Damage of the epithelial barrier (E), the mucinous biofilm layer secreted from goblet cells (G), the antibacterial peptides (*e.g.*, defensines) produced by Paneth cells (P) and the tight junctions leads to increased permeability in ulcerative colitis (UC). Antigen presenting cells (APCs) (*i.e.*, macrophages and dendritic cells) in the lamina propria are increased in absolute number in UC, they bind microbial products through detection molecules of the innate immune system, including Toll like receptors (TLRs) on the cell surface and on the cytoplasmatic NOD-like receptors (NLRs). Stimulation of these receptors induces intracellular signaling cascades, resulting in secretion of large number of cytokines, chemokines, and immunomodulatory factors. The development of the Th2, Th17 and Treg subsets from naïve, Th0 cells during primary immune response is mainly determined by cytokines and chemokines, and is under the control of certain transcription factors: T-bet (T-box expressed in T cells), GATA3 (GATA binding protein), RORγt (retinoid-related orphan receptor γt), RORα, STATs (signal transducer and activator of transcription) and FoxP3 (forkhead box P3). Leukocyte migration and recruitment from vessels is mediated by selectins, integrins, ICAMs and chemokines (*i.e.*, c-c motif chemokine ligand, CXCL). The UC-associated loci encode genes involved in: (1) maintenance of epithelial barrier integrity (*e.g.*, ECM1, CDH1, HNF4A, LAMB1, PTGER4, SLC22A4/SLC22A5, MYO9B, MDR1); (2) antigen pattern recognition (*e.g.*, NLRs, TLRs); (3) innate and adaptive immunological responses (*e.g.*, IL-23R, IL-12B, TNFα, IL10R, JAK2, STAT3, HLA-region); and (4) leukocyte recruitment (integrin α4β7, ICAM-1, MAdCAM-1, CXCLs, CCRs).

Chinese population. The frequency of A/G + G/G genotype at position -1661 was statistically higher in UC patients than in healthy controls. The G allele frequency was also significantly increased in UC patients than in the controls. The A-1661G polymorphism of the *CTLA4* is a risk factor for UC in Han Chinese of central China. They found no association between T-1722C polymorphism and UC[18].

Janus kinase 2

Janus kinase 2 (JAK2) is a member of a family of tyrosine kinases involved in a specific subset of cytokine receptor signaling pathways. JAK2 has been found to be constitutively associated with the prolactin receptor and required for responses to gamma interferon[21-23].

In GWAS studies several UC loci were identified. The rs10758669 and the rs10974944 SNPs in the *JAK2* locus were found to be strongly associated with UC in the population from the United Kingdom[14] and Netherlands[24].

In a Korean population two SNPs (rs10758669 and rs10975003) were investigated. The rs10758669 showed no significant differences in genotype and allele distribution between UC patients and controls, while it was significant on level of genotype and allele frequencies in case of rs10975003. The rs10975003 SNP plays role in

the pathogenesis of UC in Koreans[25].

Signal transducer and activator of transcription 3

This protein is a member of the signal transducer and activator of transcription (STAT) protein family. It is encoded by the signal transducer and activator of transcription 3 (*STAT3*) gene. In response to cytokines and growth factors, STAT family members are phosphorylated by the receptor associated kinases, and then form homo- or heterodimers that translocate to the cell nucleus where they act as transcription activators. This protein is activated by phosphorylation in response to various cytokines and growth factors including interferons (IFNs), epidermal growth factor (EGF), interleukin-5 (IL-5), IL-6, hepatocyte growth factor (HGF), leukemia inhibitory factor (LIF) and bone morphogenetic protein 2 (BMP2). STAT3 relays the expression of a variety of genes in response to cell stimuli, and thus plays pivotal role in several cellular processes, such as cell growth and apoptosis[26-28].

In a large GWAS study the rs12948909 SNPs in the *STAT3* locus was identified and found to be strongly associated with UC in the United Kingdom population[14].

In the Hungarian population the *STAT3* rs744166 was investigated. *STAT3* rs744166 TT genotype and T allele frequencies were significantly higher in patients with UC than in controls. Logistic regression analysis revealed that the TT genotype confers as an increased risk for the development of UC[23].

In a North American study the same polymorphisms were tested, but no significant differences were found between the UC group and healthy controls[29].

Tumor necrosis factor alpha

The pro-inflammatory cytokine, tumor necrosis factor alpha (TNFα) has important pathogenic role both in CD and in UC[30,31]. Through its ability to cause epithelial barrier disruption in colonic epithelial cells[32,33] it is responsible for tissue damage. The *TNFα* gene can be found at the inflammatory bowel disease 3 *(IBD3)* locus within the major histocompatibility complex (MHC) region. In several studies it has been found as a susceptibility locus for IBD[34-36]. Level of TNFα is elevated in serum, stools, and inflamed bowel mucosa of patients with IBD[37-41].

The polymorphism at position -308 is a point mutation, where the presence of G defines the common variant *TNF1*, and A the less common variant *TNF2*. Susceptibility to UC has been positively[42] and negatively[43] associated with carriage of *TNF2* allele. Some studies suggested that this allele might have a small but significant association with greater levels of TNF transcription[44, 45]. However other authors did not find any influence of *TNFα* bi-allelic polymorphism on UC susceptibility, although they reported a higher frequency of the *TNF2* allele in women with extensive disease compared with those with distal colitis[46]. In Mexican Mestizo UC patients increased frequency of *TNF2* allele and *TNF* 1/2 genotype was found, suggesting this could be an additional genetic marker for the susceptibility to UC[47]. Similar findings have been reported in patients with UC with Caucasian origin[42, 46, 48, 49].

The *TNFα* polymorphisms A-308G and T-857C increase the TNFα production, raising the possibility of correlation with different disease course or response to therapy[50]. The A-238G and A-308G in *TNFα* promoter region have been found as a susceptibility factor in different autoimmune disorders, including asthma[51,52], psoriasis[53] and rheumatoid arthritis[54]. The polymorphism A-238G was associated with lower production of TNFα in Caucasian UC patients[46].

In a New Zealand Caucasian UC cohort was found, that carriers of the *TNFα* -308A allele may give higher risk for pancolitis and the necessity for bowel resection[55]. In Israeli Jewish patients having CD or UC, the allele and carrier frequencies of -857T allele did not differ between IBD patients and controls, suggesting this SNP in Ashkenazi Jewish patients neither determines the susceptibility, nor influences the clinical phenotype of CD or UC[56].

Different studies supported that *TNFa* -308 in UC may be an ethnic population-specific risk factor. Studies from East Asia suggested strong association of the *TNFα* -308 gene promoter polymorphism for UC in East Asians. Allele frequency of *TNFα* -308A was significantly higher in Han Chinese UC patients than in healthy controls. Haplotype analysis revealed 6 haplotypes including H5 (*TNF* 1031T/863C/857C/380G/308A/238G/) and H3 (*TNF* 1031C/863C/857C/380G/308A/238G/). Haplotype frequency of H5 was significantly higher in UC patients, suggesting that H5 is associated with UC and the *TNFα* gene may be a susceptibility gene to UC[57]. Interestingly, the meta-analysis did not reveal any association of the *TNFα* -308 gene promoter polymorphism with UC in Europeans[58]. In a Caucasoid population from the North of Spain the *TNFα*-308 alleles did not influence the appearance of steroid dependency either in UC or in CD[59]. In Italian pediatric patients the *TNFα* -308 was significantly increased in patients with UC[60].

In Czech pediatric IBD patients significant differences in TNFα -308 A polymorphism were found between UC group and controls, but no differences were noted between this polymorphism and the clinical characteristics of UC[61].

Significant correlation of the *TNFα* -863A variant was demonstrated with colonic disease and greater height at diagnosis[62], but in this study they could not find any significant difference for the -857 allele. In patients with UC only a trend toward an increased frequency of steroid resistance was found in carriers of the *TNFα* risk genotype compared to non-carriers[60].

In the Han Chinese population the *TNFα* C-1031T, A-863C and T-857C allele/carrier frequencies were analyzed between UC patients and healthy controls. They did not find any significant difference of the tested al-

lele/carrier frequencies between UC patients and controls, only the *TNFα* -857T was increased in UC patients but did not reach statistical significance[57].

Organic cation transporter 1/2

OCTN1 *(SLC22A4)* and OCTN2 (*SLC22A5)* are widely expressed [63-66], but specifically expressed in principal intestinal cell types affected by CD: epithelial cells, CD68+ macrophages and CD43+ T cells. *SLC22A4* and *SLC22A5* encode the polytopic transmembrane sodium-dependent carnitine and sodium-independent organic cation transporters OCTN1 and OCTN2[67]. OCTNs have important role in the maintenance of intracellular homeostasis and in the energy production of the cell [68]. Both OCTNs play important role in the maintenance of gastrointestinal health and in the prevention of gut inflammation[69, 70].

CD associated variants, the *OCTN1* T1672C and *OCTN2* -207G were as strongly associated with UC in unrelated Caucasian subjects[71].

Homozygous patients for the *OCTN1* 1672T variant were significantly associated with UC in a study cohort from Italy, suggesting that OCTN1 could have a role in modulating the severity of chronic inflammation in UC[72].

The mutation that leads to L503F substitution in the OCTN1 protein can alter the transporter's activity[73, 74]. Only a weak gender-specific effect of L503F was observed at male UC patients in a cohort of familial and sporadic IBD from the central Pennsylvania, United States[75].

Multidrug-resistant transporter-1

The multidrug-resistant transporter-1 (*MDR1*) gene encodes the transmembrane protein, P-glycoprotein 170 (Pgp)[76]. This gene is an excellent candidate gene for the pathogenesis of IBD[77]. Pgp functions as an ATP-dependent efflux transporter pump and is expressed in many normal tissues like in the epithelial surfaces of the intestine, biliary ductules, proximal tubules of kidneys and central nervous system[78-80].

One of the most significant *MDR1* gene mutations is the C3435T polymorphism. Decreased expression of the *MDR1* gene and lower Pgp activity has been associated with this variant. However, studies showed conflicting results. In a German study the T allele and TT genotype frequencies of C3435T polymorphism were significantly increased in UC patients[81]. Glas *et al*[82] found in a small group of UC patients partial accordance with a trend towards an increased frequency of T allele compared to controls, but a statistical difference was detected only in one of two different control groups. In a meta-analysis significant association of the 3435T allele and the 3435TT genotype has been found with UC[83]. The triallelic G2677T/A and the C3435T have been shown to correlate with Pgp expression[84-87]. Significant association of C3435T and G2677T was detected with UC: UC patients had significantly higher frequency of 2677T allele and of the 3435TT genotype. Haplotype analysis revealed that carriers of 3435T/2677T haplotype have significantly higher risk of having UC[88]. In a Japanese UC cohort the C3435T was predictive of susceptibility to later onset UC, but not for the early onset of UC[89].

Large study with German and British UC and CD patients failed to demonstrate association. It was confirmed, that this SNP is associated with UC especially in patients with extensive colitis[90]. In addition completely negative findings have been reported in large studies from North America[7], Slovenia[91] and Italy[92]. Similarly to these results, UC patients with Caucasian origin from central Poland were found that *MDR1* C3435T polymorphism is not a risk factor for IBD, including both UC and CD[93].

A study with New Zealand IBD patients supported the role of *MDR1* as a candidate gene for UC. Heterozygous carriers for the variants C1236T, rs2235046 and G2677T/A showed a lower risk of developing UC compared with homozygotes. Subgroup analysis revealed that C1236T and rs3789243 are associated with IBD when stratified for age of onset. The *MDR1* variant rs3789243 was found to be associated with pancolitis in UC patients[94]. In the genetically heterogeneous North Indian UC cohort was found that this SNP is significantly overrepresented in UC patients.

When German IBD patients were genotyped for the two *MDR1* SNPs in positions 2677G>T/A and 3435C>T it was found that the combined genotypes derived from these positions are possibly associated with young age onset of UC and severe course of disease[95]. The 2677T allele was significantly increased in British UC cases compared with controls. The TT genotype was significantly associated with severe UC. No significant association was seen with C3435T and UC or any clinical subgroup. A meta-analysis of 9 association studies of C3435T showed a significant association of the 3435T allele with UC, but not with CD. These results indicated that *MDR1* sequence variants are associated with a small increase in the risk of developing UC and may influence disease behavior[96]. The *MDR1* gene polymorphism G2677T/A showed significant association with CD, and the C3435T with Spanish UC patients[97]. The *MDR1* 3435 TT genotype and T-allelic frequencies were significantly higher in patients with UC compared with controls. The association was strongest with extensive UC, and this was also confirmed with multivariate analysis. However G2677T was not associated with UC or CD. Two-locus haplotypes showed both positive (3435T/G2677 haplotype) and negative (C3435/2677T haplotype) associations with UC. Homozygotes for the haplotype 3435T/G2677 were significantly increased in UC. Allelic variations of the *MDR1* gene determined the disease extent as well as susceptibility to UC in the Scottish population[90].

Nucleotide-binding oligomerization domain1/ caspase activation recruitment domain 4

Nucleotide-binding oligomerization domain1/ caspase

activation recruitment domain 4 *(NOD1/CARD4)* is a member of the Nod-like receptor family, which is phylogenetically conserved[5, 98]. It is constitutively expressed in epithelial cells throughout the gastrointestinal tract[99]. *NOD1/CARD4* contains leucine-rich repeat (LRR) domain and NOD domains and has only one CARD domain[100].

Polymorphism in LRR domain of the *NOD1/CARD4* gene showed association with disease severity of UC in North Indian patients. This might be due to disruption of the LRR region critical for NOD1-mediated bacterial sensing. Haplotype-based approach showed that GTTG haplotype carriers were over represented in UC patients which could increase the risk of the disease[101].

Initially, it was suggested that there is association of the deletion variant of *NOD1/CARD4* +32656 (complex intronic insertion-deletion polymorphism) with susceptibility to IBD using a combination of transmission disequilibrium testing (TDT) and case-control analysis[102]. However this variant was not associated with a strong effect on susceptibility to IBD in children and adults in a Northern Europe study cohort[103]. Similar results have been found in the East Anglia IBD cohort, where no association was found between *NOD1* +32656 and IBD and also no heterogeneity between UC and CD[104].

Toll-like receptors

Essential components of innate immunity are the Toll-like receptors (TLRs). These are transmembrane receptors which recognize the microbial compounds from different bacteria, fungi and viruses[105-107]. TLRs are expressed by intestinal epithelial cells and immune cells in IBD patients[108,109]. TLR signaling in the intestinal sites of the colon can inhibit the inflammatory responses and maintains the colonic homeostasis[110-112]. TLRs can be found on the cell membrane (TLR1, 2, 4, 5 and 9) or on intracellular organelles (TLR3, 7 and 8)[113]. From the 10 human TLRs we review only three members regarding to their association to UC.

TLR2 is localized on the cell's surface. With its cofactors (TLR1 and TLR6) it binds lipoproteins, which are important surface antigen of the Gram-negative outer membrane[114], TLR4 consists of a leucine-rich repeat region (LRR) and an intracellular domain homologous to IL-1 receptor[115]. It recognizes conserved pathogenic motifs of Gram-negative bacteria, mainly lipopolysaccharides (LPS). Signaling through TLR4 results in the activation of the transcriptional activator, known as nuclear factor κB (NF-κB)[116]. Similarly to TLR2, TLR9 is localized on the cell's surface. It recognizes unmethylated CpG DNA in bacteria and viruses[117,118].

The allele and carrier frequencies of the Thr399Ile mutation in the *TLR4* gene were significantly associated with UC in a Caucasian population[119]. Association of *TLR4* Asp299Gly polymorphism with UC was reported first in Caucasian UC patients[120]. In a study, mentioned before[119], increased frequency of this polymorphism was observed, but it did not reach statistical significance. Similarly to Török *et al* study, the Asp299Gly and Thr399Ile mutations in *TLR4* gene were associated with UC in Greek and in North Indian patients[121,122], but not in Dutch or Italian patients[123,124]. Interestingly, the *TLR4* Asp299Gly did not show association with UC in different Asian UC populations[125-128].

The *TLR2* Arg677Trp and Arg753Glu, *TLR4* Asp299Gly and Thr399Ile, and *TLR9* gene C1237T polymorphisms were genotyped in Chinese Han IBD patients; however none of these polymorphisms was associated with IBD. In Caucasians, both TLR4 299Gly and 399Ile conferred as a significant risk factor for developing UC and CD[127].

Three SNPs of *TLR9* (C-1486T, G1174A, A2848G) were genotyped. These variations were associated with an increased risk of UC in the Japanese population. *TLR9* -1486CC, 1174GG and 2848AA showed increased risk for UC, but *TLR9* -1486TT, 1174AA and 2848GG decreased the risk of UC, although there were no correlations between SNPs and disease phenotype or *TLR9* mRNA expression[129]. Possible associations between genetic variations in *TLR9* and IBD in the German population were investigated, but no associations were detected between *TLR9* gene variations and UC susceptibility[130].

Cell adhesion molecules

Cell adhesion molecules (CAM) mediate the extravasation of leukocytes and their accumulation in inflamed intestinal mucosa. This process is controlled by a family of CAM including the intercellular cell adhesion molecule (ICAM-1), the platelet endothelial cell adhesion molecule (PECAM-1), the selectins (E, L, and P selectin) and the integrins[131,132].

ICAM-1 (CD54) is a cell surface glycoprotein belonging to the immunoglobulin superfamily. It plays key role in transendothelial migration of leukocytes, and lymphocyte activation. The membrane glycoprotein PECAM-1 (CD31) is expressed on vascular endothelial cells, platelets, some lymphocyte subsets, and monocytes[133-135]. It has important role in transendothelial migration of circulating leukocytes during inflammatory process[136], apoptosis[137] and integrin regulation[138]. The E-selectin (CD62E) is a glycoprotein, which is expressed on endothelial cells in response to pro-inflammatory cytokines (IL-1, TNF). It supports rolling of leukocytes at sites of inflammation and tissue injury. E-selectin expression is upregulated both in CD and in UC patients playing an important role in mediating of the inflammatory process in IBD[139]. L-selectin (CD62L) is expressed on normal naive T and B cells, leukocytes and on natural killer (NK) cells. It is involved in the adhesion of T cells to endothelial cells, which are regarded as crucial in the selective migration of lymphocytes to inflamed tissue sites during an inflammatory response[140].

Several mutations in *ICAM1* (G241R and K469E), *PECAM-1* (V125L), *PECAM-1* (G98T and S128R),

E-selectin (L554F) and *L-selectin* (F206L) were analyzed in Tunisian IBD patients and controls. A significant increase in allele frequencies of 206L of *L-selectin* and the associated genotype F/L was observed both in UC and in CD patients. In the subgroup analysis the L206 allele and F/L206 genotype frequencies were significantly increased in UC patients with left-sided type. No significant differences in allele or genotype frequencies were observed for *ICAM-1*, *E-selectin*, and *PECAM-1* polymorphisms between UC patients, CD patients, and controls[141].

INTERLEUKINS IN UC

Interleukin 1

Interleukin 1 (IL-1) is pro-inflammatory cytokine, which affects cell proliferation, differentiation, and the function of many innate and specific immunocompetent cells, and acts as an endogenous pyrogen. It broadcast many inflammatory diseases by initiating and potentiating immune and inflammatory responses[142].

IL-1 is composed of two main proteins the IL-1A and the IL-1B[143]. IL-1B has major role in initiating and amplifying the inflammatory response[144]. The IL-1 receptor antagonist (IL-1RN) is an anti-inflammatory cytokine, which lacks the IL-1 receptor accessory protein (IL-1RAP) interacting domain[142].

In Mexican Mestizo UC patients five SNPs were analyzed; the rs419598, the rs315951 and the rs315952 in the *IL-1RN* gene, the rs16955 in the *IL-1B* gene and the 3811058 in the *IL-1F10* gene. Significant increased frequencies of *IL-1RN6*/1TC (rs315952), *IL-1RN6*/2CC (rs315951) and decreased frequency of *IL-1B*-511 TC (rs16944) genotypes were found in UC patients. The patients group showed increased frequencies of *IL-1RN* CTC and TCG haplotypes, whereas TTG and CTG haplotypes frequencies were decreased[145].

IL-2

IL-2 functions as a T cell growth factor, furthermore it supports the proliferation and differentiation of NK cells to increase their cytolytic functions. This IL plays important role in the development of Th1, Th2, Treg, and Th17 differentiation[146].

In the *IL-2/IL-21* region several polymorphisms (rs6822844, rs13151961, rs13119723 and rs6840978) were studies. In a Dutch population the minor alleles of the examined SNPs were associated with IBD. The strongest association of these SNPs was found in the UC patients. In an Italian UC cohort the same strong association of the minor alleles was observed with UC. Similarly to this results, in the North American study was demonstrated, that these alleles have the strongest effect among the IBD patients in the UC subgroup [147].

IL-6

IL-6 is a multifunctional, pleiotropic cytokine that is responsible for regulation of immune responses, acute-phase responses, hematopoiesis, and inflammation[148].

An Irish population study the *IL-6* -174 genotype frequency showed significant difference between CD and UC group[149]. In the Caucasian population the same polymorphism was examined in CD and UC patients and found significant difference UC and CD susceptibility[150].

IL-8

IL-8 is member of the CXC chemokine family[151]. Its has two receptors the CXCR1 (IL-8RA) and the CXCR2 (IL-8RB)[152]. It exerts effect mainly on the chemotaxis and migration of neutrophils, monocytes, lymphocytes, and fibroblasts[153].

IL-8 T-251A was analyzed in a Polish population. The allele frequency showed significant difference comparing the UC group to the controls[154], however this association was not observable in a Chinese UC cohort[155]. Additional polymorphisms were also tested and their effect on the serum level of IL-18. Haplotype frequency of the -353A/ -251A/ +678T haplotype was considerably higher in UC group compared to controls, suggesting this haplotype is likely to be more common in severe UC patients than in mild to moderate cases[155].

IL-10

The anti-inflammatory cytokine IL-10 is produced by many cells like monocytes, T cells, B cells, NK cells, macrophages, and dendritic cells (DCs). It prevents the antigen presentation and also the subsequent release of pro-inflammatory cytokines, so it alleviates the activated immune system[156].

In a GWAS, the polymorphism rs3024505 demonstrated the most meaningful association in the combined verification UC samples, suggesting that defective IL-10 function plays important role in the pathogenesis of the UC[157]. The same polymorphisms was investigated in Australian population[158] and Danish cohort[159] and found that the rs3024505 was associated with the risk of UC.

Three promoter polymorphisms of the *IL-10* gene G-1082A, C-819T, and C-592A were studied in many population but the results are contrary. In an Italian cohort the G-1082A and the C-819T SNPs were investigated. The -1082 genotype frequencies were significantly different between UC patients and controls. The frequency of the -1082A allele was also significantly higher in the UC patients than in controls. Allele and genotype frequencies of T-819C were not significantly associated with the disease. Furthermore, the frequencies of haplotypes -1082A/-819C and -1082A/-819T, which have been described to have a decreased promoter activity, were significantly increased in UC patients than in controls[160]. In a North-Eastern Mexican population the G-1082A and the C-592A SNPs were examined. The -1082 AA and -592 AA genotypes showed significantly lower frequencies in UC compared to healthy controls, while individuals heterozygous at *IL-10*-1082 have sig-

nificantly increased occurrence of UC[161]. In a Tunisian group the A-627C and the G-1117A polymorphisms were examined and found that these two variants influencing the UC susceptibility and phenotype[162]. In the Asian population the association was confirmed between A-1082G polymorphism and UC[125, 163].

IL-12

IL-12 is an interleukin that is naturally produced by dendritic cells, macrophages and human B-lymphoblastoid cells in response to antigenic stimulation. It participates in the differentiation of naive T cells into Th1 cells and involved in the activities of natural killer cells and T lymphocytes. IL-12 mediates gradation of the cytotoxic activity of NK cells and CD8+ cytotoxic T lymphocytes[164]. IL-12 consist of two subunit p35 (IL-12A) and p40 (IL-12B), which is shared by IL-12 and IL-23 cytokines. The IL-12 receptor has two subunits: IL-12RB1 and IL-12RB2[165, 166].

In a German population four SNPs (rs3212227, rs17860508, rs10045431 and rs6887695) of the *IL-12B* were investigated. Two SNPs, the rs10045431 and rs6887695 showed association with increased UC susceptibility[167]. From these SNPs the rs6887695 was investigated in a Japanese population where significant association was manifested between UC patients and controls[168].

IL-17

The interleukin 17 (IL-17 or IL-17A) is a pro-inflammatory cytokine secreted by activated T cells its main tasks is inducing and mediating pro-inflammatory responses. It induces the production of many other cytokines, chemokines and prostaglandins from fibroblasts, endothelial cells, epithelial cells, keratinocytes, and macrophages[169-171].

In a Japanese population the rs2275913 SNP in the *IL-17A* gene and the rs763780 SNP in the *IL-17F* gene were investigated and found significant differences between UC group and healthy controls on level of -197A/A and 7488T/T genotype frequencies[172].

Even more recently, a GWAS in a very large European UC cohort identified an association between another IL-17 pathway gene (*IL-17REL*) and UC[173].

IL-18

IL-18 is produced by macrophages and other cells. It functions by binding to the interleukin-18 receptor, and together with IL-12 it induces cell-mediated immunity following infection. IL-18 induces gene expression and synthesis of TNF, IL-1, Fas ligand, and several chemokines[174].

In the *IL-18* gene several polymorphisms were examined. The G-137C, the C-607A and the G-656T are located in the promoter region, while the A105C, the T113G and the C127T are coding variants. In a Japanese study the G allele at 113 and the T allele at 127 were significantly higher in patients with UC compared to the control[175]. In another Japanese study allele and genotype frequency of G-137C were significantly higher in the proctitis-type UC patients than in controls[176]. The frequency of haplotype 2 (-607A, -137C), which have lower promoter activity and IFNγ-mRNA level was significantly increased in the proctitis-type patients than in the control group[176]. The C-607A and the G-137C SNPs were associated with the development of UC in Tunisian patients. The -137GG genotype frequency was significantly higher in UC than in controls and statistically significant association was found between -607AA genotype in UC patients and the distal localization of the lesions[177].

IL-23

IL-23 main functions are very important in innate and adaptive immunity to regulate Th17 function and expansion[178]. This cytokine induces CD8+ memory T cells to proliferate and produce IL-17. IL-23 binds to its receptor IL-23R, which polymorphisms' play the main role in the autoimmune diseases[179-181] especially in IBD[182].

Several independent functional SNPs of the *IL-23R* gene and its neighboring region were determined; several were found susceptible to (rs10889677, rs11209032, rs11465804, rs11805303, rs1495965, rs2201841, rs1004819) CD and UC in non-Jewish subjects[183].

In a Chinese cohort the rs7530511 and rs11805303 SNPs were studied and positive association was found between these variants and UC susceptibility[184]. In the Jiangsu Han population the rs11805303 was found as a susceptibility factor to UC[185].

In a Swedish population the rs10889677, the rs11209032, the rs11465804, the rs2201841 and the rs1004819 polymorphisms were investigated, and found that the rs11465804G, rs2201841C and rs1004819T allele frequencies showed significant differences between UC patient group and control. These genetic variants are individual risk factor for developing the disease[186].

In Hungarian UC patients for the *IL-23R* rs1004819A allele we found significantly higher allele frequency compared to control subjects and the SNP rs2201841 showed significant association with UC risk for homozygotes[187].

IL-26

Expression of IL-26 seems to be restricted to memory T cells, NK cells, and Th17 cells. Thereby it could have pro-inflammatory effects in IBD[188].

Only a few markers were investigated, from these the rs2870946-G and the rs1558744-A showed association with UC[189]. Further meta-analysis study confirmed the association of rs1558744-A with UC[190].

CONCLUSION

This review shows that substantial progress has been made in the past 10 years in to find inflammatory related genetic factors and cytokines in UC. We reviewed different genes and gene polymorphisms which play role in the inflammatory process of UC. These genes could be

potential targets of novel treatment strategies.

From the reviewed genes contributing to inflammation *TNFα*, *MDR1* and *TLRs* were the most investigated genes. *TNFα* has been found as a susceptibility locus for both UC and CD[34-36]. The *TNF2* allele and *TNF 1/2* could be good candidate markers for the susceptibility to UC. Based on the different studies with different populations (East Asians, Han Chinese, Spanish Caucasoid, Italian, and Czech), the *TNFα* -308 is the most studied SNP and this may be an ethnic population-specific risk factor for UC especially for Asian populations but not for Europeans. It should be noted this polymorphism is also a susceptibility factor in other autoimmune disorders (asthma, psoriasis, and rheumatoid arthritis) too.

The *MDR1* C3435T is one of the most tested SNP in UC, but with conflicting results. Some studies showed significantly increased 3435T allele and 3435TT genotype frequencies of C3435T[81,83,97], or only a trend towards an increased frequency of T allele[82], or the T allele was predictive of susceptibility to later onset UC, but not for the early onset of UC[89]. But some studies failed to demonstrate association with UC[91-93]. Other SNPs of *MDR1* (C1236T and rs3789243) were associated with IBD when stratified for age of onset. The rs3789243 was found to be significantly overrepresented in genetically heterogeneous North Indian UC patients.

We reviewed 3 members from the 10 human TLRs regarding to their association to UC. Allele and carrier frequencies of the *TLR4* Asp299Gly and Thr399Ile were significantly associated with UC in Caucasian[119,120], Greek and in North Indian patients[121,122], but not with Dutch, Italian[123,124] or Asian patients[125-128]. Interestingly the *TLR9* polymorphisms (C-1486T, G1174A, A2848G) were associated with an increased risk of UC in the Japanese population. *TLR9* -1486CC, 1174GG and 2848AA polymorphisms show increased risk for UC, but *TLR9* -1486TT, 1174AA and 2848GG decrease the risk of UC[129].

From the reviewed cytokines *IL-10, IL-18* and *IL-23* were the most investigated genes. The IL-10 is a major anti-inflammatory cytokine, which attenuates the activated immune system with inhibiting both the antigen presentation and subsequent release of pro-inflammatory cytokines. IL-10 is a shared risk gene for CD and UC too. The promoter polymorphisms of this gene (G-1082A, C-819T, and C-592A) which are in tight linkage disequilibrium were extensively studied in many populations but with contradictory results. In the Caucasian population the carriers of G-1082A SNP were more susceptible to UC, whereas in another study carriers were associated with lower UC incidence[160,161]. In the Asian population the results strengthened the positive relationship between this SNP and UC susceptibility[125,163]. In a North-Eastern Mexican population the -592AA genotypes showed significantly decreased frequency in UC compared the results to the healthy controls[161]. In a Tunisian group the A-627C and the G-1117A variants influencing the UC susceptibility and phenotype[162]. Several other studies handle with these non-coding SNP in CD too and determine susceptibility to the disease or not.

The G-137C, the C-607A and the G-656T promoter SNPs and several others in the coding regions of *IL-18* gene (A105C, the T113G and the C127T) were examined. The Japanese population is the most studies for these SNPs, significant difference was found in the allele frequency of the A105C between CD patients and controls, while this correlation could not be detected in UC patients. The 113G and 127T allele frequencies were significantly increased in patients with UC compared the results to the healthy controls[175]. In case of promoter polymorphisms, the -137CC genotype frequency was significantly increased in proctitis-type UC patients than in controls, while the other two C-607A and G-137C SNPs were associated with the development of UC in Tunisian patients[177].

The *IL23R* gene was identified as a CD susceptibility gene in North American non-Jewish subjects. Several independent functional SNPs in the gene and its neighboring region were determined[183]. After the primary publications, several studies have been published these SNPs in IBD and other autoimmune disease too (ankylosing spondylitis, psoriasis, Sjögren syndrome, systemic lupus erythematosus). From these SNPs (rs10889677, rs11209032, rs11465804, rs11805303, rs1495965, rs2201841, rs100481) several are risk factor to IBD both in European and Asian populations[185-187].

It can be established that these interleukin gene variants are strongly population dependent but in the given population they can be predictors for CD or UC. Despite the advances in the field of UC/IBD genetics, testing for these genetic variants is currently not recommended for clinical purposes[191].

Understanding of the detailed pathogenesis of IBD and identifying new disease associated SNPs led to the development of selective inhibitors for ILs, chemokines and their receptors. This strategies can optimize treatment efficacy and lead to personalized medicine based on the patient's genotype.

Biological agents are used in patients with moderate to severe disease activity who have failed conventional therapy with glucocorticoids and thiopurines. Today, the most effective and best studied anti-cytokine agents in IBD are the anti-TNFα antibodies. The mechanism of action of TNFα antagonists is based on the neutralization of both soluble TNFα and membrane TNFα and has a more global effect on inflammation than the blockade of other cytokines. Currently, three TNFα inhibitors are approved by the United States Food and Drug Administration (FDA) for inducing and maintaining clinical remission in UC: the chimeric (25% murine and 75% human sequence) monoclonal full-length IgG1 mAb infliximab[192], the fully human mAbs adalimumab[193,194] and golimumab[195]. The pegylated humanised antibody certolizumab pegol is approved only for CD (beside rheumatoid arthritis, RA and psoriatic arthritis). Etanercept, a

dimeric fusion protein consisting of soluble p75-TNFR2 and the Fc portion of human IgG1, used in rheumatoid arthritis therapy, is not efficient for the treatment of intestinal inflammation[196].

Despite the expeditious development of newer biological therapies, only few have shown benefit in clinical trials in UC. Targeting of IL-23 or the IL-23 receptor or IL-23 axis is a potential therapeutic approach for autoimmune diseases including psoriasis, IBD, RA and multiple sclerosis[197]. Recently, testing of anti-IL-12/23 treatment in patients with CD has been performed. In Phase Ⅱ trial, patients with moderate to severe CD that was resistant to TNF antagonists had an increased rate of response to induction with the fully human mAb ustekinumab directed against the p40, as compared with placebo[198]. However, due to the common p40 subunit and IL-12RB1 chain, the major drawback of anti-IL-23 treatment can be the simultaneous inhibition of IL-12 and a possible shutdown of the immune system. Nevertheless, it would be much more useful to design drugs that target the IL-23p19 or IL-23RA itself, so inhibiting IL-23 without modifying the effects of IL-12[197].

Treatment of CD patients with the IL-17 blocker secukinumab (anti-IL-17A) was ineffective and higher rates of adverse events were noted compared with placebo[199].

One new additional treatment for UC may be tofacitinib, an inhibitor of Janus kinases 1, 2, and 3 with *in vitro* functional specificity for kinases 1 and 3 over kinase 2, which is expected to block signaling involving gamma chain-containing cytokines including ILs-2, 4, 7, 9, 15, and 21. Tofacitinib, was approved for the treatment of RA in the USA, Japan and Russia in April 2013. In a double-blind, placebo-controlled, Phase Ⅱ trial, patients with moderately to severely active UC treated with tofacitinib were more likely to have clinical response and remission than those receiving placebo[200].

Targeting leukocyte recruitment and cell adhesion molecules could be also an option for IBD therapy. Natalisumab, a recombinant humanised monoclonal IgG4 antibody, targets both the α4β1 heterodimer located in the central nervous system and the α4β7 integrin in the gut. The FDA approved natalizumab for both induction of remission and maintenance of remission for moderate to severe CD, though it has not been approved for this use in the European Union due to concerns over its risk/benefit ratio (risk of progressive multifocal leukoencephalopathy)[201]. Vedolizumab is a humanized mAb that specifically recognizes the α4β7 heterodimer, selectively blocks gut lymphocyte trafficking without interfering with trafficking to the central nervous system. In the Phase Ⅲ study, vedolizumab was more effective than placebo as induction and maintenance therapy for UC suggesting that blockade of T cell homing in the gut may favor mucosal healing in UC[202, 203].

The exact positioning of these promising new therapies in the management of UC remains uncertain currently. Additional long-term safety data and clinical experience will be needed to determine an overall benefit/harm ratio of newly developed biological agents.

The identified separate loci in IBD research individually have only modest effects on IBD susceptibility. They account together for only 20%-25% of the heritability, suggesting that gene-gene interactions as well as gene-environmental interactions could play a key role in IBD pathogenesis and fill the so called "genetic vacuum" of polygenic diseases[204]. More complete understanding of the immunopathogenic role of the various genes and ILs in intestinal inflammation will help in the development of more effective novel therapeutic strategies in UC. Next generation techniques in combination with the data analysis by systems-biology approach hopefully will contribute to the personalized therapy of the patients in the near future.

REFERENCES

1 **Podolsky DK**. Inflammatory bowel disease. *N Engl J Med* 2002; **347**: 417-429 [PMID: 12167685]

2 **Molodecky NA**, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, Benchimol EI, Panaccione R, Ghosh S, Barkema HW, Kaplan GG. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology* 2012; **142**: 46-54.e42; quiz e30 [PMID: 22001864 DOI: 10.1053/j.gastro.2011.10.001]

3 **Loftus EV**. Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. *Gastroenterology* 2004; **126**: 1504-1517 [PMID: 15168363]

4 **Silverberg MS**, Satsangi J, Ahmad T, Arnott ID, Bernstein CN, Brant SR, Caprilli R, Colombel JF, Gasche C, Geboes K, Jewell DP, Karban A, Loftus EV, Peña AS, Riddell RH, Sachar DB, Schreiber S, Steinhart AH, Targan SR, Vermeire S, Warren BF. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. *Can J Gastroenterol* 2005; **19 Suppl A**: 5A-36A [PMID: 16151544]

5 **Van Limbergen J**, Russell RK, Nimmo ER, Ho GT, Arnott ID, Wilson DC, Satsangi J. Genetics of the innate immune response in inflammatory bowel disease. *Inflamm Bowel Dis* 2007; **13**: 338-355 [PMID: 17206667]

6 **Orholm M**, Munkholm P, Langholz E, Nielsen OH, Sørensen TI, Binder V. Familial occurrence of inflammatory bowel disease. *N Engl J Med* 1991; **324**: 84-88 [PMID: 1984188]

7 **Brant SR**, Panhuysen CI, Nicolae D, Reddy DM, Bonen DK, Karaliukas R, Zhang L, Swanson E, Datta LW, Moran T, Ravenhill G, Duerr RH, Achkar JP, Karban AS, Cho JH. MDR1 Ala893 polymorphism is associated with inflammatory bowel disease. *Am J Hum Genet* 2003; **73**: 1282-1292 [PMID: 14610718]

8 **Jostins L**, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY, Lee JC, Schumm LP, Sharma Y, Anderson CA, Essers J, Mitrovic M, Ning K, Cleynen I, Theatre E, Spain SL, Raychaudhuri S, Goyette P, Wei Z, Abraham C, Achkar JP, Ahmad T, Amininejad L, Ananthakrishnan AN, Andersen V, Andrews JM, Baidoo L, Balschun T, Bampton PA, Bitton A, Boucher G, Brand S, Büning C, Cohain A, Cichon S, D'Amato M, De Jong D, Devaney KL, Dubinsky M, Edwards C, Ellinghaus D, Ferguson LR, Franchimont D, Fransen K, Gearry R, Georges M, Gieger C, Glas J, Haritunians T, Hart A, Hawkey C, Hedl M, Hu X, Karlsen TH, Kupcinskas L, Kugathasan S, Latiano A, Laukens D, Lawrance IC, Lees CW, Louis E, Mahy G, Mansfield J, Morgan AR, Mowat C,

Newman W, Palmieri O, Ponsioen CY, Potocnik U, Prescott NJ, Regueiro M, Rotter JI, Russell RK, Sanderson JD, Sans M, Satsangi J, Schreiber S, Simms LA, Sventoraityte J, Targan SR, Taylor KD, Tremelling M, Verspaget HW, De Vos M, Wijmenga C, Wilson DC, Winkelmann J, Xavier RJ, Zeissig S, Zhang B, Zhang CK, Zhao H, Silverberg MS, Annese V, Hakonarson H, Brant SR, Radford-Smith G, Mathew CG, Rioux JD, Schadt EE, Daly MJ, Franke A, Parkes M, Vermeire S, Barrett JC, Cho JH. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* 2012; **491**: 119-124 [PMID: 23128233]

9 **Fuss IJ**, Neurath M, Boirivant M, Klein JS, de la Motte C, Strong SA, Fiocchi C, Strober W. Disparate CD4+ lamina propria (LP) lymphokine secretion profiles in inflammatory bowel disease. Crohn's disease LP cells manifest increased secretion of IFN-gamma, whereas ulcerative colitis LP cells manifest increased secretion of IL-5. *J Immunol* 1996; **157**: 1261-1270 [PMID: 8757634]

10 **Fujino S**, Andoh A, Bamba S, Ogawa A, Hata K, Araki Y, Bamba T, Fujiyama Y. Increased expression of interleukin 17 in inflammatory bowel disease. *Gut* 2003; **52**: 65-70 [PMID: 12477762]

11 **Ferguson LR**, Shelling AN, Browning BL, Huebner C, Petermann I. Genes, diet and inflammatory bowel disease. *Mutat Res* 2007; **622**: 70-83 [PMID: 17628615]

12 **Stokkers PC**, van Aken BE, Basoski N, Reitsma PH, Tytgat GN, van Deventer SJ. Five genetic markers in the interleukin 1 family in relation to inflammatory bowel disease. *Gut* 1998; **43**: 33-39 [PMID: 9771403]

13 **Bouma G,** Crusius JB, Garcia-Gonzalez MA, Meijer BU, Hellemans HP, Hakvoort RJ, Schreuder GM, Kostense PJ, Meuwissen SG, Pena AS. Genetic markers in clinically well defined patients with ulcerative colitis (UC). *Clin Exp Immunol* 1999; **115**: 294-300

14 **Anderson CA**, Massey DC, Barrett JC, Prescott NJ, Tremelling M, Fisher SA, Gwilliam R, Jacob J, Nimmo ER, Drummond H, Lees CW, Onnie CM, Hanson C, Blaszczyk K, Ravindrarajah R, Hunt S, Varma D, Hammond N, Lewis G, Attlesey H, Watkins N, Ouwehand W, Strachan D, McArdle W, Lewis CM, Lobo A, Sanderson J, Jewell DP, Deloukas P, Mansfield JC, Mathew CG, Satsangi J, Parkes M. Investigation of Crohn's disease risk loci in ulcerative colitis further defines their molecular relationship. *Gastroenterology* 2009; **136**: 523-9.e3 [PMID: 19068216]

15 **Houlston RS**, Webb E, Broderick P, Pittman AM, Di Bernardo MC, Lubbe S, Chandler I, Vijayakrishnan J, Sullivan K, Penegar S, Carvajal-Carmona L, Howarth K, Jaeger E, Spain SL, Walther A, Barclay E, Martin L, Gorman M, Domingo E, Teixeira AS, Kerr D, Cazier JB, Niittymäki I, Tuupanen S, Karhu A, Aaltonen LA, Tomlinson IP, Farrington SM, Tenesa A, Prendergast JG, Barnetson RA, Cetnarskyj R, Porteous ME, Pharoah PD, Koessler T, Hampe J, Buch S, Schafmayer C, Tepel J, Schreiber S, Völzke H, Chang-Claude J, Hoffmeister M, Brenner H, Zanke BW, Montpetit A, Hudson TJ, Gallinger S, Campbell H, Dunlop MG. Meta-analysis of genome-wide association data identifies four new susceptibility loci for colorectal cancer. *Nat Genet* 2008; **40**: 1426-1435 [PMID: 19011631]

16 **Barrett JC**, Lee JC, Lees CW, Prescott NJ, Anderson CA, Phillips A, Wesley E, Parnell K, Zhang H, Drummond H, Nimmo ER, Massey D, Blaszczyk K, Elliott T, Cotterill L, Dallal H, Lobo AJ, Mowat C, Sanderson JD, Jewell DP, Newman WG, Edwards C, Ahmad T, Mansfield JC, Satsangi J, Parkes M, Mathew CG, Donnelly P, Peltonen L, Blackwell JM, Bramon E, Brown MA, Casas JP, Corvin A, Craddock N, Deloukas P, Duncanson A, Jankowski J, Markus HS, Mathew CG, McCarthy MI, Palmer CN, Plomin R, Rautanen A, Sawcer SJ, Samani N, Trembath RC, Viswanathan AC, Wood N, Spencer CC, Barrett JC, Bellenguez C, Davison D, Freeman C, Strange A, Donnelly P, Langford C, Hunt SE, Edkins S, Gwilliam R, Blackburn H, Bumpstead SJ, Dronov S, Gillman M, Gray E, Hammond N, Jayakumar A, McCann OT, Liddle J, Perez ML, Potter SC, Ravindrarajah R, Ricketts M, Waller M, Weston P, Widaa S, Whittaker P, Deloukas P, Peltonen L, Mathew CG, Blackwell JM, Brown MA, Corvin A, McCarthy MI, Spencer CC, Attwood AP, Stephens J, Sambrook J, Ouwehand WH, McArdle WL, Ring SM, Strachan DP. Genome-wide association study of ulcerative colitis identifies three new susceptibility loci, including the HNF4A region. *Nat Genet* 2009; **41**: 1330-1334 [PMID: 19915572 DOI: 10.1038/ng.483]

17 **Ben Alaya W**, Sfar I, Aouadi H, Jendoubi S, Najjar T, Filali A, Gorgi Y, Ben Abdallah T, Mouelhi L, Matri S, Ayed K. Association between CTLA-4 gene promoter (49 A/G) in exon 1 polymorphisms and inflammatory bowel disease in the Tunisian population. *Saudi J Gastroenterol* 2009; **15**: 29-34 [PMID: 19568552 DOI: 10.4103/1319-3767.43285]

18 **Zhou F**, Xia B, Guo QS, Wang Q, Li L, Jiang L, Cheng H. [Cytotoxic T lymphocyte antigen-4 promoter gene polymorphism is significantly associated with ulcerative colitis]. *Zhonghua Nei Ke Za Zhi* 2006; **45**: 478-481 [PMID: 16831326]

19 **Xia B**, Crusius JB, Wu J, Zwiers A, van Bodegraven AA, Peña AS. CTLA4 gene polymorphisms in Dutch and Chinese patients with inflammatory bowel disease. *Scand J Gastroenterol* 2002; **37**: 1296-1300 [PMID: 12465728]

20 **Magyari L**, Faragó B, Bene J, Horvatovich K, Lakner L, Varga M, Figler M, Gasztonyi B, Mózsik G, Melegh B. No association of the cytotoxic T-lymphocyte associated gene CTLA4 +49A/G polymorphisms with Crohn's disease and ulcerative colitis in Hungarian population samples. *World J Gastroenterol* 2007; **13**: 2205-2208 [PMID: 17465502]

21 **Ghoreschi K**, Laurence A, O'Shea JJ. Janus kinases in immune cell signaling. *Immunol Rev* 2009; **228**: 273-287 [PMID: 19290934 DOI: 10.1111/j.1600-065X.2008.00754.x]

22 **Vera-Lastra O,** Jara LJ, Espinoza LR. Prolactin and autoimmunity. *Autoimmun Rev* 2002; **1**: 360-364

23 **Polgar N,** Csongei V, Szabo M, Zambo V, Melegh BI, Sumegi K, Nagy G, Tulassay Z, Melegh B. Investigation of JAK2, STAT3 and CCR6 polymorphisms and their gene-gene interactions in inflammatory bowel disease. *Int J Immunogenet* 2012; **39**: 247-252 [DOI: 10.1111/j.1744-313X.2012.01084.x]

24 **Festen EA**, Stokkers PC, van Diemen CC, van Bodegraven AA, Boezen HM, Crusius BJ, Hommes DW, van der Woude CJ, Balschun T, Verspaget HW, Schreiber S, de Jong DJ, Franke A, Dijkstra G, Wijmenga C, Weersma RK. Genetic analysis in a Dutch study sample identifies more ulcerative colitis susceptibility loci and shows their additive role in disease risk. *Am J Gastroenterol* 2010; **105**: 395-402 [PMID: 19861958 DOI: 10.1038/ajg.2009.576]

25 **Yang SK**, Jung Y, Kim H, Hong M, Ye BD, Song K. Association of FCGR2A, JAK2 or HNF4A variants with ulcerative colitis in Koreans. *Dig Liver Dis* 2011; **43**: 856-861 [PMID: 21831733 DOI: 10.1016/j.dld.2011.07.006]

26 **Jabalameli MR**. Role of STAT3 Locus in autoimmune diseases. *Genes Immun* 2011; **12**: 155; author reply 156 [PMID: 21209620 DOI: 10.1038/gene.2010.55]

27 **Cénit MC**, Alcina A, Márquez A, Mendoza JL, Díaz-Rubio M, de las Heras V, Izquierdo G, Arroyo R, Fernández O, de la Concha EG, Matesanz F, Urcelay E. STAT3 locus in inflammatory bowel disease and multiple sclerosis susceptibility. *Genes Immun* 2010; **11**: 264-268 [PMID: 20200543 DOI: 10.1038/gene.2010.10]

28 **Schultz KR**. STAT3 mutations and persistence of autoimmunity. *Blood* 2013; **122**: 2295-2296 [PMID: 24092924 DOI: 10.1182/blood-2013-08-521138]

29 **Plevy S**, Silverberg MS, Lockton S, Stockfisch T, Croner L, Stachelski J, Brown M, Triggs C, Chuang E, Princen F, Singh S. Combined serological, genetic, and inflammatory markers differentiate non-IBD, Crohn's disease, and ulcerative colitis patients. *Inflamm Bowel Dis* 2013; **19**: 1139-1148 [PMID:

23518807 DOI: 10.1097/MIB.0b013e318280b19e]
30 **Nikolaus S**, Bauditz J, Gionchetti P, Witt C, Lochs H, Schreiber S. Increased secretion of pro-inflammatory cytokines by circulating polymorphonuclear neutrophils and regulation by interleukin 10 during intestinal inflammation. *Gut* 1998; **42**: 470-476 [PMID: 9616306]
31 **Van Deventer SJ**. Tumour necrosis factor and Crohn's disease. *Gut* 1997; **40**: 443-448 [PMID: 9176068]
32 **Deem RL**, Shanahan F, Targan SR. Triggered human mucosal T cells release tumour necrosis factor-alpha and interferon-gamma which kill human colonic epithelial cells. *Clin Exp Immunol* 1991; **83**: 79-84 [PMID: 1899066]
33 **Bruewer M**, Luegering A, Kucharzik T, Parkos CA, Madara JL, Hopkins AM, Nusrat A. Proinflammatory cytokines disrupt epithelial barrier function by apoptosis-independent mechanisms. *J Immunol* 2003; **171**: 6164-6172 [PMID: 14634132]
34 **Hampe J**, Shaw SH, Saiz R, Leysens N, Lantermann A, Mascheretti S, Lynch NJ, MacPherson AJ, Bridger S, van Deventer S, Stokkers P, Morin P, Mirza MM, Forbes A, Lennard-Jones JE, Mathew CG, Curran ME, Schreiber S. Linkage of inflammatory bowel disease to human chromosome 6p. *Am J Hum Genet* 1999; **65**: 1647-1655 [PMID: 10577918 DOI: 10.1086/302677]
35 **Dechairo B**, Dimon C, van Heel D, Mackay I, Edwards M, Scambler P, Jewell D, Cardon L, Lench N, Carey A. Replication and extension studies of inflammatory bowel disease susceptibility regions confirm linkage to chromosome 6p (IBD3). *Eur J Hum Genet* 2001; **9**: 627-633 [PMID: 11528509 DOI: 10.1038/sj.ejhg.5200687]
36 **Yang H**, Plevy SE, Taylor K, Tyan D, Fischel-Ghodsian N, McElree C, Targan SR, Rotter JI. Linkage of Crohn's disease to the major histocompatibility complex region is detected by multiple non-parametric analyses. *Gut* 1999; **44**: 519-526 [PMID: 10075959]
37 **Borruel N**, Carol M, Casellas F, Antolín M, de Lara F, Espín E, Naval J, Guarner F, Malagelada JR. Increased mucosal tumour necrosis factor alpha production in Crohn's disease can be downregulated ex vivo by probiotic bacteria. *Gut* 2002; **51**: 659-664 [PMID: 12377803]
38 **Komatsu M**, Kobayashi D, Saito K, Furuya D, Yagihashi A, Araake H, Tsuji N, Sakamaki S, Niitsu Y, Watanabe N. Tumor necrosis factor-alpha in serum of patients with inflammatory bowel disease as measured by a highly sensitive immuno-PCR. *Clin Chem* 2001; **47**: 1297-1301 [PMID: 11427462]
39 **Braegger CP**, Nicholls S, Murch SH, Stephens S, MacDonald TT. Tumour necrosis factor alpha in stool as a marker of intestinal inflammation. *Lancet* 1992; **339**: 89-91 [PMID: 1345871]
40 **Murch SH**, Braegger CP, Walker-Smith JA, MacDonald TT. Location of tumour necrosis factor alpha by immunohistochemistry in chronic inflammatory bowel disease. *Gut* 1993; **34**: 1705-1709 [PMID: 8031350]
41 **Breese EJ**, Michie CA, Nicholls SW, Murch SH, Williams CB, Domizio P, Walker-Smith JA, MacDonald TT. Tumor necrosis factor alpha-producing cells in the intestinal mucosa of children with inflammatory bowel disease. *Gastroenterology* 1994; **106**: 1455-1466 [PMID: 8194690]
42 **Sashio H**, Tamura K, Ito R, Yamamoto Y, Bamba H, Kosaka T, Fukui S, Sawada K, Fukuda Y, Tamura K, Satomi M, Shimoyama T, Furuyama J. Polymorphisms of the TNF gene and the TNF receptor superfamily member 1B gene are associated with susceptibility to ulcerative colitis and Crohn's disease, respectively. *Immunogenetics* 2002; **53**: 1020-1027 [PMID: 11904678 DOI: 10.1007/s00251-001-0423-7]
43 **Vatay A**, Bene L, Kovács A, Prohászka Z, Szalai C, Romics L, Fekete B, Karádi I, Füst G. Relationship between the tumor necrosis factor alpha polymorphism and the serum C-reactive protein levels in inflammatory bowel disease. *Immunogenetics* 2003; **55**: 247-252 [PMID: 12811429 DOI: 10.1007/s00251-003-0575-8]
44 **Louis E**, Franchimont D, Piron A, Gevaert Y, Schaaf-Lafontaine N, Roland S, Mahieu P, Malaise M, De Groote D, Louis R, Belaiche J. Tumour necrosis factor (TNF) gene polymorphism influences TNF-alpha production in lipopolysaccharide (LPS)-stimulated whole blood cell culture in healthy humans. *Clin Exp Immunol* 1998; **113**: 401-406 [PMID: 9737669]
45 **Bouma G**, Crusius JB, Oudkerk Pool M, Kolkman JJ, von Blomberg BM, Kostense PJ, Giphart MJ, Schreuder GM, Meuwissen SG, Peña AS. Secretion of tumour necrosis factor alpha and lymphotoxin alpha in relation to polymorphisms in the TNF genes and HLA-DR alleles. Relevance for inflammatory bowel disease. *Scand J Immunol* 1996; **43**: 456-463 [PMID: 8668926]
46 **Koss K**, Satsangi J, Fanning GC, Welsh KI, Jewell DP. Cytokine (TNF alpha, LT alpha and IL-10) polymorphisms in inflammatory bowel diseases and normal controls: differential effects on production and allele frequencies. *Genes Immun* 2000; **1**: 185-190 [PMID: 11196710]
47 **Yamamoto-Furusho JK**, Uscanga LF, Vargas-Alarcón G, Rodríguez-Pérez JM, Zuñiga J, Granados J. Polymorphisms in the promoter region of tumor necrosis factor alpha (TNF-alpha) and the HLA-DRB1 locus in Mexican mestizo patients with ulcerative colitis. *Immunol Lett* 2004; **95**: 31-35 [PMID: 15325795 DOI: 10.1016/j.imlet.2004.05.015]
48 **Wilson AG**, di Giovine FS, Blakemore AI, Duff GW. Single base polymorphism in the human tumour necrosis factor alpha (TNF alpha) gene detectable by NcoI restriction of PCR product. *Hum Mol Genet* 1992; **1**: 353 [PMID: 1363876]
49 **Hirv K**, Seyfarth M, Uibo R, Kull K, Salupere R, Latza U, Rink L. Polymorphisms in tumour necrosis factor and adhesion molecule genes in patients with inflammatory bowel disease: associations with HLA-DR and -DQ alleles and subclinical markers. *Scand J Gastroenterol* 1999; **34**: 1025-1032 [PMID: 10563674]
50 **Yap LM**, Ahmad T, Jewell DP. The contribution of HLA genes to IBD susceptibility and phenotype. *Best Pract Res Clin Gastroenterol* 2004; **18**: 577-596 [PMID: 15157829 DOI: 10.1016/j.bpg.2004.01.003]
51 **Moffatt MF**, Cookson WO. Tumour necrosis factor haplotypes and asthma. *Hum Mol Genet* 1997; **6**: 551-554 [PMID: 9097957]
52 **Witte JS**, Palmer LJ, O'Connor RD, Hopkins PJ, Hall JM. Relation between tumour necrosis factor polymorphism TNFalpha-308 and risk of asthma. *Eur J Hum Genet* 2002; **10**: 82-85 [PMID: 11896460 DOI: 10.1038/sj.ejhg.5200746]
53 **Balding J**, Kane D, Livingstone W, Mynett-Johnson L, Bresnihan B, Smith O, FitzGerald O. Cytokine gene polymorphisms: association with psoriatic arthritis susceptibility and severity. *Arthritis Rheum* 2003; **48**: 1408-1413 [PMID: 12746914 DOI: 10.1002/art.10935]
54 **Mulcahy B**, Waldron-Lynch F, McDermott MF, Adams C, Amos CI, Zhu DK, Ward RH, Clegg DO, Shanahan F, Molloy MG, O'Gara F. Genetic variability in the tumor necrosis factor-lymphotoxin region influences susceptibility to rheumatoid arthritis. *Am J Hum Genet* 1996; **59**: 676-683 [PMID: 8751869]
55 **Ferguson LR**, Huebner C, Petermann I, Gearry RB, Barclay ML, Demmers P, McCulloch A, Han DY. Single nucleotide polymorphism in the tumor necrosis factor-alpha gene affects inflammatory bowel diseases risk. *World J Gastroenterol* 2008; **14**: 4652-4661 [PMID: 18698679]
56 **Fidder HH**, Heijmans R, Chowers Y, Bar-Meir S, Avidan B, Pena AS, Crusius JB. TNF-857 polymorphism in Israeli Jewish patients with inflammatory bowel disease. *Int J Immunogenet* 2006; **33**: 81-85 [PMID: 16611251 DOI: 10.1111/j.1744-313X.2006.00572.x]
57 **Cao Q**, Zhu Q, Wu ML, Hu WL, Gao M, Si JM. Genetic susceptibility to ulcerative colitis in the Chinese Han ethnic

population: association with TNF polymorphisms. *Chin Med J (Engl)* 2006; **119**: 1198-1203 [PMID: 16863613]
58 **Lu Z**, Chen L, Li H, Zhao Y, Lin L. Effect of the polymorphism of tumor necrosis factor-alpha-308 G/A gene promoter on the susceptibility to ulcerative colitis: a meta-analysis. *Digestion* 2008; **78**: 44-51 [PMID: 18827481 DOI: 10.1159/000158605]
59 **Castro-Santos P**, Suarez A, López-Rivas L, Mozo L, Gutierrez C. TNFalpha and IL-10 gene polymorphisms in inflammatory bowel disease. Association of -1082 AA low producer IL-10 genotype with steroid dependency. *Am J Gastroenterol* 2006; **101**: 1039-1047 [PMID: 16573780 DOI: 10.1111/j.1572-0241.2006.00501.x]
60 **Cucchiara S,** Latiano A, Palmieri O, Canani RB, D'Inca R, Guariso G, Vieni G, De Venuto D, Riegler G, De'Angelis GL, Guagnozzi D, Bascietto C, Miele E, Valvano MR, Bossa F, Annese V. Polymorphisms of tumor necrosis factor-alpha but not MDR1 influence response to medical therapy in pediatric-onset inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 2007; **44**: 171-179 [DOI: 10.1097/MPG.0b013e31802c41f3]
61 **Sýkora J**, Subrt I, Dìdek P, Siala K, Schwarz J, Machalová V, Varvarovská J, Pazdiora P, Pozler O, Stozický F. Cytokine tumor necrosis factor-alpha A promoter gene polymorphism at position -308 G--& gt; A and pediatric inflammatory bowel disease: implications in ulcerative colitis and Crohn's disease. *J Pediatr Gastroenterol Nutr* 2006; **42**: 479-487 [PMID: 16707968 DOI: 10.1097/01.mpg.0000221917.80887.9e]
62 **Levine A**, Karban A, Eliakim R, Shaoul R, Reif S, Pacht A, Wardi J, Yakir B, Silver EL. A polymorphism in the TNF-alpha promoter gene is associated with pediatric onset and colonic location of Crohn's disease. *Am J Gastroenterol* 2005; **100**: 407-413 [PMID: 15667501 DOI: 10.1111/j.1572-0241.2005.41126.x]
63 **Lamhonwah AM**, Skaug J, Scherer SW, Tein I. A third human carnitine/organic cation transporter (OCTN3) as a candidate for the 5q31 Crohn's disease locus (IBD5). *Biochem Biophys Res Commun* 2003; **301**: 98-101 [PMID: 12535646]
64 **Wu X**, George RL, Huang W, Wang H, Conway SJ, Leibach FH, Ganapathy V. Structural and functional characteristics and tissue distribution pattern of rat OCTN1, an organic cation transporter, cloned from placenta. *Biochim Biophys Acta* 2000; **1466**: 315-327 [PMID: 10825452]
65 **Wu X**, Huang W, Prasad PD, Seth P, Rajan DP, Leibach FH, Chen J, Conway SJ, Ganapathy V. Functional characteristics and tissue distribution pattern of organic cation transporter 2 (OCTN2), an organic cation/carnitine transporter. *J Pharmacol Exp Ther* 1999; **290**: 1482-1492 [PMID: 10454528]
66 **Elimrani I**, Lahjouji K, Seidman E, Roy MJ, Mitchell GA, Qureshi I. Expression and localization of organic cation/carnitine transporter OCTN2 in Caco-2 cells. *Am J Physiol Gastrointest Liver Physiol* 2003; **284**: G863-G871 [PMID: 12684216 DOI: 10.1152/ajpgi.00220.2002]
67 **Burckhardt G**, Wolff NA. Structure of renal organic anion and cation transporters. *Am J Physiol Renal Physiol* 2000; **278**: F853-F866 [PMID: 10836973]
68 **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]
69 **García-Miranda P**, Durán JM, Peral MJ, Ilundáin AA. Developmental maturation and segmental distribution of rat small intestinal L-carnitine uptake. *J Membr Biol* 2005; **206**: 9-16 [PMID: 16440177 DOI: 10.1007/s00232-005-0769-0]
70 **Kato Y**, Sugiura M, Sugiura T, Wakayama T, Kubo Y, Kobayashi D, Sai Y, Tamai I, Iseki S, Tsuji A. Organic cation/carnitine transporter OCTN2 (Slc22a5) is responsible for carnitine transport across apical membranes of small intestinal epithelial cells in mouse. *Mol Pharmacol* 2006; **70**: 829-837 [PMID: 16754783 DOI: 10.1124/mol.106.024158]
71 **Peltekova VD**, Wintle RF, Rubin LA, Amos CI, Huang Q, Gu X, Newman B, Van Oene M, Cescon D, Greenberg G, Griffiths AM, St George-Hyslop PH, Siminovitch KA. Functional variants of OCTN cation transporter genes are associated with Crohn disease. *Nat Genet* 2004; **36**: 471-475 [PMID: 15107849 DOI: 10.1038/ng1339]
72 **Martini M**, Ferrara AM, Giachelia M, Panieri E, Siminovitch K, Galeotti T, Larocca LM, Pani G. Association of the OCTN1/1672T variant with increased risk for colorectal cancer in young individuals and ulcerative colitis patients. *Inflamm Bowel Dis* 2012; **18**: 439-448 [PMID: 21793125 DOI: 10.1002/ibd.21814]
73 **Seth P**, Wu X, Huang W, Leibach FH, Ganapathy V. Mutations in novel organic cation transporter (OCTN2), an organic cation/carnitine transporter, with differential effects on the organic cation transport function and the carnitine transport function. *J Biol Chem* 1999; **274**: 33388-33392 [PMID: 10559218]
74 **Durán JM**, Peral MJ, Calonge ML, Ilundáin AA. Functional characterization of intestinal L-carnitine transport. *J Membr Biol* 2002; **185**: 65-74 [PMID: 11891565]
75 **Lin Z**, Nelson L, Franke A, Poritz L, Li TY, Wu R, Wang Y, MacNeill C, Thomas NJ, Schreiber S, Koltun WA. OCTN1 variant L503F is associated with familial and sporadic inflammatory bowel disease. *J Crohns Colitis* 2010; **4**: 132-138 [PMID: 21122496 DOI: 10.1016/j.crohns.2009.09.003]
76 **Schinkel AH**. The physiological function of drug-transporting P-glycoproteins. *Semin Cancer Biol* 1997; **8**: 161-170 [PMID: 9441946 DOI: 10.1006/scbi.1997.0068]
77 **Ho GT**, Moodie FM, Satsangi J. Multidrug resistance 1 gene (P-glycoprotein 170): an important determinant in gastrointestinal disease? *Gut* 2003; **52**: 759-766 [PMID: 12692067]
78 **Juliano RL**, Ling V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. *Biochim Biophys Acta* 1976; **455**: 152-162 [PMID: 990323]
79 **Thiebaut F**, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc Natl Acad Sci USA* 1987; **84**: 7735-7738 [PMID: 2444983]
80 **Cordon-Cardo C**, O'Brien JP, Casals D, Rittman-Grauer L, Biedler JL, Melamed MR, Bertino JR. Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites. *Proc Natl Acad Sci USA* 1989; **86**: 695-698 [PMID: 2563168]
81 **Schwab M**, Schaeffeler E, Marx C, Fromm MF, Kaskas B, Metzler J, Stange E, Herfarth H, Schoelmerich J, Gregor M, Walker S, Cascorbi I, Roots I, Brinkmann U, Zanger UM, Eichelbaum M. Association between the C3435T MDR1 gene polymorphism and susceptibility for ulcerative colitis. *Gastroenterology* 2003; **124**: 26-33 [PMID: 12512026 DOI: 10.1053/gast.2003.50010]
82 **Glas J**, Török HP, Schiemann U, Folwaczny C. MDR1 gene polymorphism in ulcerative colitis. *Gastroenterology* 2004; **126**: 367 [PMID: 14724799]
83 **Annese V**, Valvano MR, Palmieri O, Latiano A, Bossa F, Andriulli A. Multidrug resistance 1 gene in inflammatory bowel disease: a meta-analysis. *World J Gastroenterol* 2006; **12**: 3636-3644 [PMID: 16773678]
84 **Hoffmeyer S**, Burk O, von Richter O, Arnold HP, Brockmöller J, Johne A, Cascorbi I, Gerloff T, Roots I, Eichelbaum M, Brinkmann U. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc Natl Acad Sci USA* 2000; **97**: 3473-3478 [PMID: 10716719 DOI: 10.1073/pnas.050585397]
85 **Tanabe M**, Ieiri I, Nagata N, Inoue K, Ito S, Kanamori Y, Takahashi M, Kurata Y, Kigawa J, Higuchi S, Terakawa N, Otsubo K. Expression of P-glycoprotein in human placenta: relation to genetic polymorphism of the multidrug

resistance (MDR)-1 gene. *J Pharmacol Exp Ther* 2001; **297**: 1137-1143 [PMID: 11356939]
86 **Nakamura T**, Sakaeda T, Horinouchi M, Tamura T, Aoyama N, Shirakawa T, Matsuo M, Kasuga M, Okumura K. Effect of the mutation (C3435T) at exon 26 of the MDR1 gene on expression level of MDR1 messenger ribonucleic acid in duodenal enterocytes of healthy Japanese subjects. *Clin Pharmacol Ther* 2002; **71**: 297-303 [PMID: 11956513 DOI: 10.1067/mcp.2002.122055]
87 **Kim RB**, Leake BF, Choo EF, Dresser GK, Kubba SV, Schwarz UI, Taylor A, Xie HG, McKinsey J, Zhou S, Lan LB, Schuetz JD, Schuetz EG, Wilkinson GR. Identification of functionally variant MDR1 alleles among European Americans and African Americans. *Clin Pharmacol Ther* 2001; **70**: 189-199 [PMID: 11503014 DOI: 10.1067/mcp.2001.117412]
88 **Brinar M**, Cukovic-Cavka S, Bozina N, Ravic KG, Markos P, Ladic A, Cota M, Krznaric Z, Vucelic B. MDR1 polymorphisms are associated with inflammatory bowel disease in a cohort of Croatian IBD patients. *BMC Gastroenterol* 2013; **13**: 57 [PMID: 23537364 DOI: 10.1186/1471-230X-13-57]
89 **Osuga T**, Sakaeda T, Nakamura T, Yamada T, Koyama T, Tamura T, Aoyama N, Okamura N, Kasuga M, Okumura K. MDR1 C3435T polymorphism is predictive of later onset of ulcerative colitis in Japanese. *Biol Pharm Bull* 2006; **29**: 324-329 [PMID: 16462040]
90 **Ho GT**, Nimmo ER, Tenesa A, Fennell J, Drummond H, Mowat C, Arnott ID, Satsangi J. Allelic variations of the multidrug resistance gene determine susceptibility and disease behavior in ulcerative colitis. *Gastroenterology* 2005; **128**: 288-296 [PMID: 15685540]
91 **Potocnik U**, Ferkolj I, Glavac D, Dean M. Polymorphisms in multidrug resistance 1 (MDR1) gene are associated with refractory Crohn disease and ulcerative colitis. *Genes Immun* 2004; **5**: 530-539 [PMID: 15505619 DOI: 10.1038/sj.gene.6364123]
92 **Palmieri O**, Latiano A, Valvano R, D'Incà R, Vecchi M, Sturniolo GC, Saibeni S, Bossa F, Latiano T, Devoto M, Andriulli A, Annese V. Multidrug resistance 1 gene polymorphisms are not associated with inflammatory bowel disease and response to therapy in Italian patients. *Aliment Pharmacol Ther* 2005; **22**: 1129-1138 [PMID: 16305727 DOI: 10.1111/j.1365-2036.2005.02701.x]
93 **Dudarewicz M**, Barańska M, Rychlik-Sych M, Trzciński R, Dziki A, Skrętkowicz J. C3435T polymorphism of the ABCB1/MDR1 gene encoding P-glycoprotein in patients with inflammatory bowel disease in a Polish population. *Pharmacol Rep* 2012; **64**: 343-350 [PMID: 22661185]
94 **Huebner C**, Browning BL, Petermann I, Han DY, Philpott M, Barclay M, Gearry R, McCulloch A, Demmers P, Ferguson LR. Genetic analysis of MDR1 and inflammatory bowel disease reveals protective effect of heterozygous variants for ulcerative colitis. *Inflamm Bowel Dis* 2009; **15**: 1784-1793 [PMID: 19685447 DOI: 10.1002/ibd.21019]
95 **Fiedler T**, Büning C, Reuter W, Pitre G, Gentz E, Schmidt HH, Büttner J, Ockenga J, Gerloff T, Meisel C, Lochs H, Roots I, Köpke K, Johne A. Possible role of MDR1 two-locus genotypes for young-age onset ulcerative colitis but not Crohn's disease. *Eur J Clin Pharmacol* 2007; **63**: 917-925 [PMID: 17665184 DOI: 10.1007/s00228-007-0334-0]
96 **Onnie CM**, Fisher SA, Pattni R, Sanderson J, Forbes A, Lewis CM, Mathew CG. Associations of allelic variants of the multidrug resistance gene (ABCB1 or MDR1) and inflammatory bowel disease and their effects on disease behavior: a case-control and meta-analysis study. *Inflamm Bowel Dis* 2006; **12**: 263-271 [PMID: 16633048 DOI: 10.1097/01.MIB.0000209791.98866.ba]
97 **Urcelay E**, Mendoza JL, Martín MC, Mas A, Martínez A, Taxonera C, Fernandez-Arquero M, Díaz-Rubio M, de la Concha EG. MDR1 gene: susceptibility in Spanish Crohn's disease and ulcerative colitis patients. *Inflamm Bowel Dis* 2006; **12**: 33-37 [PMID: 16374256]
98 **Fritz JH**, Ferrero RL, Philpott DJ, Girardin SE. Nod-like proteins in immunity, inflammation and disease. *Nat Immunol* 2006; **7**: 1250-1257 [PMID: 17110941 DOI: 10.1038/ni1412]
99 **Hysi P**, Kabesch M, Moffatt MF, Schedel M, Carr D, Zhang Y, Boardman B, von Mutius E, Weiland SK, Leupold W, Fritzsch C, Klopp N, Musk AW, James A, Nunez G, Inohara N, Cookson WO. NOD1 variation, immunoglobulin E and asthma. *Hum Mol Genet* 2005; **14**: 935-941 [PMID: 15718249 DOI: 10.1093/hmg/ddi087]
100 **Ting JP**, Kastner DL, Hoffman HM. CATERPILLERs, pyrin and hereditary immunological disorders. *Nat Rev Immunol* 2006; **6**: 183-195 [PMID: 16498449 DOI: 10.1038/nri1788]
101 **Verma R**, Ahuja V, Paul J. Detection of single-nucleotide polymorphisms in the intron 9 region of the nucleotide oligomerization domain-1 gene in ulcerative colitis patients of North India. *J Gastroenterol Hepatol* 2012; **27**: 96-103 [PMID: 21722177 DOI: 10.1111/j.1440-1746.2011.06832.x]
102 **McGovern DP**, Hysi P, Ahmad T, van Heel DA, Moffatt MF, Carey A, Cookson WO, Jewell DP. Association between a complex insertion/deletion polymorphism in NOD1 (CARD4) and susceptibility to inflammatory bowel disease. *Hum Mol Genet* 2005; **14**: 1245-1250 [PMID: 15790594 DOI: 10.1093/hmg/ddi135]
103 **Van Limbergen J**, Russell RK, Nimmo ER, Törkvist L, Lees CW, Drummond HE, Smith L, Anderson NH, Gillett PM, McGrogan P, Hassan K, Weaver LT, Bisset WM, Mahdi G, Arnott ID, Sjöqvist U, Lördal M, Farrington SM, Dunlop MG, Wilson DC, Satsangi J. Contribution of the NOD1/CARD4 insertion/deletion polymorphism +32656 to inflammatory bowel disease in Northern Europe. *Inflamm Bowel Dis* 2007; **13**: 882-889 [PMID: 17285593 DOI: 10.1002/ibd.20124]
104 **Tremelling M**, Hancock L, Bredin F, Sharpstone D, Bingham SA, Parkes M. Complex insertion/deletion polymorphism in NOD1 (CARD4) is not associated with inflammatory bowel disease susceptibility in East Anglia panel. *Inflamm Bowel Dis* 2006; **12**: 967-971 [PMID: 17012967 DOI: 10.1097/01.mib.0000234131.89971.e5]
105 **Aderem A**, Ulevitch RJ. Toll-like receptors in the induction of the innate immune response. *Nature* 2000; **406**: 782-787 [PMID: 10963608 DOI: 10.1038/35021228]
106 **Beutler B**. Inferences, questions and possibilities in Toll-like receptor signalling. *Nature* 2004; **430**: 257-263 [PMID: 15241424 DOI: 10.1038/nature02761]
107 **Kawai T**, Akira S. Innate immune recognition of viral infection. *Nat Immunol* 2006; **7**: 131-137 [PMID: 16424890 DOI: 10.1038/ni1303]
108 **Cario E**, Podolsky DK. Differential alteration in intestinal epithelial cell expression of toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. *Infect Immun* 2000; **68**: 7010-7017 [PMID: 11083826]
109 **Cario E**, Rosenberg IM, Brandwein SL, Beck PL, Reinecker HC, Podolsky DK. Lipopolysaccharide activates distinct signaling pathways in intestinal epithelial cell lines expressing Toll-like receptors. *J Immunol* 2000; **164**: 966-972 [PMID: 10623846]
110 **Rachmilewitz D**, Katakura K, Karmeli F, Hayashi T, Reinus C, Rudensky B, Akira S, Takeda K, Lee J, Takabayashi K, Raz E. Toll-like receptor 9 signaling mediates the anti-inflammatory effects of probiotics in murine experimental colitis. *Gastroenterology* 2004; **126**: 520-528 [PMID: 14762789]
111 **Rakoff-Nahoum S**, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell* 2004; **118**: 229-241 [PMID: 15260992 DOI: 10.1016/j.cell.2004.07.002]
112 **Lee J**, Mo JH, Katakura K, Alkalay I, Rucker AN, Liu YT, Lee HK, Shen C, Cojocaru G, Shenouda S, Kagnoff M, Eckmann L, Ben-Neriah Y, Raz E. Maintenance of colonic

homeostasis by distinctive apical TLR9 signalling in intestinal epithelial cells. *Nat Cell Biol* 2006; **8**: 1327-1336 [PMID: 17128265 DOI: 10.1038/ncb1500]

113 **Zhang D**, Zhang G, Hayden MS, Greenblatt MB, Bussey C, Flavell RA, Ghosh S. A toll-like receptor that prevents infection by uropathogenic bacteria. *Science* 2004; **303**: 1522-1526 [PMID: 15001781 DOI: 10.1126/science.1094351]

114 **Vermeire S,** Rutgeerts P, Van Steen K, Joossens S, Claessens G, Pierik M, Peeters M, Vlietinck R. Genome wide scan in a Flemish inflammatory bowel disease population: support for the IBD4 locus, population heterogeneity, and epistasis. *Gut* 2004; **53**: 980-986

115 **Medzhitov R**. Toll-like receptors and innate immunity. *Nat Rev Immunol* 2001; **1**: 135-145 [PMID: 11905821 DOI: 10.1038/35100529]

116 **Barton GM**, Medzhitov R. Toll-like receptor signaling pathways. *Science* 2003; **300**: 1524-1525 [PMID: 12791976 DOI: 10.1126/science.1085536]

117 **Hemmi H**, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, Matsumoto M, Hoshino K, Wagner H, Takeda K, Akira S. A Toll-like receptor recognizes bacterial DNA. *Nature* 2000; **408**: 740-745 [PMID: 11130078 DOI: 10.1038/35047123]

118 **Lund J**, Sato A, Akira S, Medzhitov R, Iwasaki A. Toll-like receptor 9-mediated recognition of Herpes simplex virus-2 by plasmacytoid dendritic cells. *J Exp Med* 2003; **198**: 513-520 [PMID: 12900525 DOI: 10.1084/jem.20030162]

119 **Török HP**, Glas J, Tonenchi L, Mussack T, Folwaczny C. Polymorphisms of the lipopolysaccharide-signaling complex in inflammatory bowel disease: association of a mutation in the Toll-like receptor 4 gene with ulcerative colitis. *Clin Immunol* 2004; **112**: 85-91 [PMID: 15207785 DOI: 10.1016/j.clim.2004.03.002]

120 **Franchimont D**, Vermeire S, El Housni H, Pierik M, Van Steen K, Gustot T, Quertinmont E, Abramowicz M, Van Gossum A, Devière J, Rutgeerts P. Deficient host-bacteria interactions in inflammatory bowel disease? The toll-like receptor (TLR)-4 Asp299gly polymorphism is associated with Crohn's disease and ulcerative colitis. *Gut* 2004; **53**: 987-992 [PMID: 15194649]

121 **Manolakis AC**, Kapsoritakis AN, Kapsoritaki A, Tiaka EK, Oikonomou KA, Lotis V, Vamvakopoulou D, Davidi I, Vamvakopoulos N, Potamianos SP. Readressing the role of Toll-like receptor-4 alleles in inflammatory bowel disease: colitis, smoking, and seroreactivity. *Dig Dis Sci* 2013; **58**: 371-380 [PMID: 22918682 DOI: 10.1007/s10620-012-2348-4]

122 **Meena NK**, Verma R, Verma N, Ahuja V, Paul J. TLR4 D299G polymorphism modulates cytokine expression in ulcerative colitis. *J Clin Gastroenterol* 2013; **47**: 773-780 [PMID: 23470644 DOI: 10.1097/MCG.0b013e31828a6e93]

123 **Oostenbrug LE**, Drenth JP, de Jong DJ, Nolte IM, Oosterom E, van Dullemen HM, van der Linde K, te Meerman GJ, van der Steege G, Kleibeuker JH, Jansen PL. Association between Toll-like receptor 4 and inflammatory bowel disease. *Inflamm Bowel Dis* 2005; **11**: 567-575 [PMID: 15905704]

124 **Rigoli L**, Romano C, Caruso RA, Lo Presti MA, Di Bella C, Procopio V, Lo Giudice G, Amorini M, Costantino G, Sergi MD, Cuppari C, Calabro GE, Gallizzi R, Salpietro CD, Fries W. Clinical significance of NOD2/CARD15 and Toll-like receptor 4 gene single nucleotide polymorphisms in inflammatory bowel disease. *World J Gastroenterol* 2008; **14**: 4454-4461 [PMID: 18680223]

125 **Kim EJ**, Chung WC, Lee KM, Paik CN, Jung SH, Lee BI, Chae HS, Choi KY. Association between toll-like receptors/CD14 gene polymorphisms and inflammatory bowel disease in Korean population. *J Korean Med Sci* 2012; **27**: 72-77 [PMID: 22219617 DOI: 10.3346/jkms.2012.27.1.72]

126 **Okayama N**, Fujimura K, Suehiro Y, Hamanaka Y, Fujiwara M, Matsubara T, Maekawa T, Hazama S, Oka M, Nohara H, Kayano K, Okita K, Hinoda Y. Simple genotype analysis of the Asp299Gly polymorphism of the Toll-like receptor-4 gene that is associated with lipopolysaccharide hyporesponsiveness. *J Clin Lab Anal* 2002; **16**: 56-58 [PMID: 11835533 DOI: 10.1002/jcla.2075]

127 **Shen XY**, Shi RH, Wang Y, Zhang HJ, Zhou XQ, Shen FC, Li KB. [Toll-like receptor gene polymorphisms and susceptibility to inflammatory bowel disease in Chinese Han and Caucasian populations]. *Zhonghua Yi Xue Za Zhi* 2010; **90**: 1416-1420 [PMID: 20646633]

128 **Guo QS**, Xia B, Jiang Y, Morré SA, Cheng L, Li J, Crusius JB, Peña AS. Polymorphisms of CD14 gene and TLR4 gene are not associated with ulcerative colitis in Chinese patients. *Postgrad Med J* 2005; **81**: 526-529 [PMID: 16085746 DOI: 10.1136/pgmj.2004.030825]

129 **Fuse K**, Katakura K, Sakamoto N, Ohira H. Toll-like receptor 9 gene mutations and polymorphisms in Japanese ulcerative colitis patients. *World J Gastroenterol* 2010; **16**: 5815-5821 [PMID: 21155002]

130 **Török HP**, Glas J, Tonenchi L, Bruennler G, Folwaczny M, Folwaczny C. Crohn's disease is associated with a toll-like receptor-9 polymorphism. *Gastroenterology* 2004; **127**: 365-366 [PMID: 15236225]

131 **Salmi M**, Granfors K, MacDermott R, Jalkanen S. Aberrant binding of lamina propria lymphocytes to vascular endothelium in inflammatory bowel diseases. *Gastroenterology* 1994; **106**: 596-605 [PMID: 8119529]

132 **Nakamura S**, Ohtani H, Watanabe Y, Fukushima K, Matsumoto T, Kitano A, Kobayashi K, Nagura H. In situ expression of the cell adhesion molecules in inflammatory bowel disease. Evidence of immunologic activation of vascular endothelial cells. *Lab Invest* 1993; **69**: 77-85 [PMID: 7687311]

133 **Ohto H**, Maeda H, Shibata Y, Chen RF, Ozaki Y, Higashihara M, Takeuchi A, Tohyama H. A novel leukocyte differentiation antigen: two monoclonal antibodies TM2 and TM3 define a 120-kd molecule present on neutrophils, monocytes, platelets, and activated lymphoblasts. *Blood* 1985; **66**: 873-881 [PMID: 2412619]

134 **Newman PJ**, Albelda SM. Cellular and molecular aspects of PECAM-1. *Nouv Rev Fr Hematol* 1992; **34 Suppl**: S9-13 [PMID: 1340533]

135 **Newman PJ**, Berndt MC, Gorski J, White GC, Lyman S, Paddock C, Muller WA. PECAM-1 (CD31) cloning and relation to adhesion molecules of the immunoglobulin gene superfamily. *Science* 1990; **247**: 1219-1222 [PMID: 1690453]

136 **Vaporciyan AA**, DeLisser HM, Yan HC, Mendiguren II, Thom SR, Jones ML, Ward PA, Albelda SM. Involvement of platelet-endothelial cell adhesion molecule-1 in neutrophil recruitment in vivo. *Science* 1993; **262**: 1580-1582 [PMID: 8248808]

137 **Gao C**, Sun W, Christofidou-Solomidou M, Sawada M, Newman DK, Bergom C, Albelda SM, Matsuyama S, Newman PJ. PECAM-1 functions as a specific and potent inhibitor of mitochondrial-dependent apoptosis. *Blood* 2003; **102**: 169-179 [PMID: 12649141 DOI: 10.1182/blood-2003-01-0003]

138 **Piali L**, Hammel P, Uherek C, Bachmann F, Gisler RH, Dunon D, Imhof BA. CD31/PECAM-1 is a ligand for alpha v beta 3 integrin involved in adhesion of leukocytes to endothelium. *J Cell Biol* 1995; **130**: 451-460 [PMID: 7542249]

139 **Pooley N**, Ghosh L, Sharon P. Up-regulation of E-selectin and intercellular adhesion molecule-1 differs between Crohn's disease and ulcerative colitis. *Dig Dis Sci* 1995; **40**: 219-225 [PMID: 7529672]

140 **Steeber DA**, Green NE, Sato S, Tedder TF. Humoral immune responses in L-selectin-deficient mice. *J Immunol* 1996; **157**: 4899-4907 [PMID: 8943394]

141 **Khazen D**, Jendoubi-Ayed S, Aleya WB, Sfar I, Mouelhi L, Matri S, Najjar T, Filali A, Gorgi Y, Abdallah TB, Ayed K. Polymorphism in ICAM-1, PECAM-1, E-selectin, and L-selectin genes in Tunisian patients with inflammatory bowel disease. *Eur J Gastroenterol Hepatol* 2009; **21**: 167-175 [PMID: 19212205 DOI: 10.1097/MEG.0b013e32830e6fc8]

142 **Akdis M**, Burgler S, Crameri R, Eiwegger T, Fujita H, Gomez E, Klunker S, Meyer N, O'Mahony L, Palomares O, Rhyner C, Ouaked N, Schaffartzik A, Van De Veen W, Zeller S, Zimmermann M, Akdis CA. Interleukins, from 1 to 37, and interferon-γ: receptors, functions, and roles in diseases. *J Allergy Clin Immunol* 2011; **127**: 701-21.e1-70 [PMID: 21377040 DOI: 10.1016/j.jaci.2010.11.050]

143 **Dinarello CA**. Immunological and inflammatory functions of the interleukin-1 family. *Annu Rev Immunol* 2009; **27**: 519-550 [PMID: 19302047 DOI: 10.1146/annurev.immunol.021908.132612]

144 **Dinarello CA**. Interleukin-1, interleukin-1 receptors and interleukin-1 receptor antagonist. *Int Rev Immunol* 1998; **16**: 457-499 [PMID: 9646173 DOI: 10.3109/08830189809043005]

145 **Yamamoto-Furusho JK**, Santiago-Hernández JJ, Pérez-Hernández N, Ramírez-Fuentes S, Fragoso JM, Vargas-Alarcón G. Interleukin 1 β (IL-1B) and IL-1 antagonist receptor (IL-1RN) gene polymorphisms are associated with the genetic susceptibility and steroid dependence in patients with ulcerative colitis. *J Clin Gastroenterol* 2011; **45**: 531-535 [PMID: 20975573 DOI: 10.1097/MCG.0b013e3181faec51]

146 **Malek TR**. The biology of interleukin-2. *Annu Rev Immunol* 2008; **26**: 453-479 [PMID: 18062768 DOI: 10.1146/annurev.immunol.26.021607.090357]

147 **Festen EA**, Goyette P, Scott R, Annese V, Zhernakova A, Lian J, Lefèbvre C, Brant SR, Cho JH, Silverberg MS, Taylor KD, de Jong DJ, Stokkers PC, Mcgovern D, Palmieri O, Achkar JP, Xavier RJ, Daly MJ, Duerr RH, Wijmenga C, Weersma RK, Rioux JD. Genetic variants in the region harbouring IL2/IL21 associated with ulcerative colitis. *Gut* 2009; **58**: 799-804 [PMID: 19201773]

148 **Boulanger MJ**, Chow DC, Brevnova EE, Garcia KC. Hexameric structure and assembly of the interleukin-6/IL-6 alpha-receptor/gp130 complex. *Science* 2003; **300**: 2101-2104 [PMID: 12829785 DOI: 10.1126/science.1083901]

149 **Balding J**, Livingstone WJ, Conroy J, Mynett-Johnson L, Weir DG, Mahmud N, Smith OP. Inflammatory bowel disease: the role of inflammatory cytokine gene polymorphisms. *Mediators Inflamm* 2004; **13**: 181-187 [PMID: 15223609 DOI: 10.1080/09511920410001713529]

150 **Cantor MJ**, Nickerson P, Bernstein CN. The role of cytokine gene polymorphisms in determining disease susceptibility and phenotype in inflammatory bowel disease. *Am J Gastroenterol* 2005; **100**: 1134-1142 [PMID: 15842590 DOI: 10.1111/j.1572-0241.2005.40979.x]

151 **Yoshimura T**, Matsushima K, Tanaka S, Robinson EA, Appella E, Oppenheim JJ, Leonard EJ. Purification of a human monocyte-derived neutrophil chemotactic factor that has peptide sequence similarity to other host defense cytokines. *Proc Natl Acad Sci USA* 1987; **84**: 9233-9237 [PMID: 3480540]

152 **Holmes WE**, Lee J, Kuang WJ, Rice GC, Wood WI. Structure and functional expression of a human interleukin-8 receptor. *Science* 1991; **253**: 1278-1280 [PMID: 1840701]

153 **Miller MD**, Krangel MS. Biology and biochemistry of the chemokines: a family of chemotactic and inflammatory cytokines. *Crit Rev Immunol* 1992; **12**: 17-46 [PMID: 1418604]

154 **Walczak A**, Przybylowska K, Dziki L, Sygut A, Chojnacki C, Chojnacki J, Dziki A, Majsterek I. The lL-8 and IL-13 gene polymorphisms in inflammatory bowel disease and colorectal cancer. *DNA Cell Biol* 2012; **31**: 1431-1438 [PMID: 22741617]

155 **Li K**, Yao S, Liu S, Wang B, Mao D. Genetic polymorphisms of interleukin 8 and risk of ulcerative colitis in the Chinese population. *Clin Chim Acta* 2009; **405**: 30-34 [PMID: 19348790 DOI: 10.1016/j.cca.2009.03.053]

156 **Deniz G**, Erten G, Kücüksezer UC, Kocacik D, Karagiannidis C, Aktas E, Akdis CA, Akdis M. Regulatory NK cells suppress antigen-specific T cell responses. *J Immunol* 2008; **180**: 850-857 [PMID: 18178824]

157 **Franke A**, Balschun T, Karlsen TH, Sventoraityte J, Nikolaus S, Mayr G, Domingues FS, Albrecht M, Nothnagel M, Ellinghaus D, Sina C, Onnie CM, Weersma RK, Stokkers PC, Wijmenga C, Gazouli M, Strachan D, McArdle WL, Vermeire S, Rutgeerts P, Rosenstiel P, Krawczak M, Vatn MH, Mathew CG, Schreiber S. Sequence variants in IL10, ARPC2 and multiple other loci contribute to ulcerative colitis susceptibility. *Nat Genet* 2008; **40**: 1319-1323 [PMID: 18836448 DOI: 10.1038/ng.221]

158 **Doecke JD**, Simms LA, Zhao ZZ, Huang N, Hanigan K, Krishnaprasad K, Roberts RL, Andrews JM, Mahy G, Bampton P, Lewindon P, Florin T, Lawrance IC, Gearry RB, Montgomery GW, Radford-Smith GL. Genetic susceptibility in IBD: overlap between ulcerative colitis and Crohn's disease. *Inflamm Bowel Dis* 2013; **19**: 240-245 [PMID: 23348120 DOI: 10.1097/MIB.0b013e3182810041]

159 **Andersen V**, Ernst A, Christensen J, Østergaard M, Jacobsen BA, Tjønneland A, Krarup HB, Vogel U. The polymorphism rs3024505 proximal to IL-10 is associated with risk of ulcerative colitis and Crohns disease in a Danish case-control study. *BMC Med Genet* 2010; **11**: 82 [PMID: 20509889 DOI: 10.1186/1471-2350-11-82]

160 **Tedde A**, Laura Putignano A, Bagnoli S, Congregati C, Milla M, Sorbi S, Genuardi M, Papi L. Interleukin-10 promoter polymorphisms influence susceptibility to ulcerative colitis in a gender-specific manner. *Scand J Gastroenterol* 2008; **43**: 712-718 [PMID: 18569989 DOI: 10.1080/00365520701885507]

161 **Garza-González E**, Pérez-Pérez GI, Mendoza-Ibarra SI, Flores-Gutiérrez JP, Bosques-Padilla FJ. Genetic risk factors for inflammatory bowel disease in a North-eastern Mexican population. *Int J Immunogenet* 2010; **37**: 355-359 [PMID: 20518842 DOI: 10.1111/j.1744-313X.2010.00932.x]

162 **Marrakchi R**, Moussa A, Ouerhani S, Bougatef K, Bouhaha R, Messai Y, Rouissi K, Khadimallah I, Khodjet-el-Khil H, Najar T, Benammar-Elgaaeid A. Interleukin 10 promoter region polymorphisms in inflammatory bowel disease in Tunisian population. *Inflamm Res* 2009; **58**: 155-160 [PMID: 19184348 DOI: 10.1007/s00011-008-8265-5]

163 **Ahirwar DK**, Kesarwani P, Singh R, Ghoshal UC, Mittal RD. Role of tumor necrosis factor-alpha (C-863A) polymorphism in pathogenesis of inflammatory bowel disease in Northern India. *J Gastrointest Cancer* 2012; **43**: 196-204 [PMID: 21249467 DOI: 10.1007/s12029-010-9238-9]

164 **Hsieh CS**, Macatonia SE, Tripp CS, Wolf SF, O'Garra A, Murphy KM. Development of TH1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. *Science* 1993; **260**: 547-549 [PMID: 8097338]

165 **Wolf SF**, Sieburth D, Sypek J. Interleukin 12: a key modulator of immune function. *Stem Cells* 1994; **12**: 154-168 [PMID: 7911046 DOI: 10.1002/stem.5530120203]

166 **Parham C**, Chirica M, Timans J, Vaisberg E, Travis M, Cheung J, Pflanz S, Zhang R, Singh KP, Vega F, To W, Wagner J, O'Farrell AM, McClanahan T, Zurawski S, Hannum C, Gorman D, Rennick DM, Kastelein RA, de Waal Malefyt R, Moore KW. A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rbeta1 and a novel cytokine receptor subunit, IL-23R. *J Immunol* 2002; **168**: 5699-5708 [PMID: 12023369]

167 **Glas J**, Seiderer J, Wagner J, Olszak T, Fries C, Tillack C, Friedrich M, Beigel F, Stallhofer J, Steib C, Wetzke M, Göke B, Ochsenkühn T, Diegelmann J, Czamara D, Brand S. Analysis of IL12B gene variants in inflammatory bowel disease. *PLoS One* 2012; **7**: e34349 [PMID: 22479607 DOI: 10.1371/journal.pone.0034349]

168 **Yamazaki K**, Takahashi A, Takazoe M, Kubo M, Onouchi Y, Fujino A, Kamatani N, Nakamura Y, Hata A. Positive association of genetic variants in the upstream region of NKX2-3 with Crohn's disease in Japanese patients. *Gut* 2009; **58**: 228-232 [PMID: 18936107 DOI: 10.1136/gut.2007.140764]

169 **Kobori A**, Yagi Y, Imaeda H, Ban H, Bamba S, Tsujikawa T, Saito Y, Fujiyama Y, Andoh A. Interleukin-33 expression is

specifically enhanced in inflamed mucosa of ulcerative colitis. *J Gastroenterol* 2010; **45**: 999-1007 [PMID: 20405148 DOI: 10.1007/s00535-010-0245-1]

170 **Seiderer J**, Elben I, Diegelmann J, Glas J, Stallhofer J, Tillack C, Pfennig S, Jürgens M, Schmechel S, Konrad A, Göke B, Ochsenkühn T, Müller-Myhsok B, Lohse P, Brand S. Role of the novel Th17 cytokine IL-17F in inflammatory bowel disease (IBD): upregulated colonic IL-17F expression in active Crohn's disease and analysis of the IL17F p.His161Arg polymorphism in IBD. *Inflamm Bowel Dis* 2008; **14**: 437-445 [PMID: 18088064 DOI: 10.1002/ibd.20339]

171 **Schwartz S**, Beaulieu JF, Ruemmele FM. Interleukin-17 is a potent immuno-modulator and regulator of normal human intestinal epithelial cell growth. *Biochem Biophys Res Commun* 2005; **337**: 505-509 [PMID: 16198312 DOI: 10.1016/j.bbrc.2005.09.075]

172 **Arisawa T**, Tahara T, Shibata T, Nagasaka M, Nakamura M, Kamiya Y, Fujita H, Nakamura M, Yoshioka D, Arima Y, Okubo M, Hirata I, Nakano H. The influence of polymorphisms of interleukin-17A and interleukin-17F genes on the susceptibility to ulcerative colitis. *J Clin Immunol* 2008; **28**: 44-49 [PMID: 17828618 DOI: 10.1007/s10875-007-9125-8]

173 **Franke A**, McGovern DP, Barrett JC, Wang K, Radford-Smith GL, Ahmad T, Lees CW, Balschun T, Lee J, Roberts R, Anderson CA, Bis JC, Bumpstead S, Ellinghaus D, Festen EM, Georges M, Green T, Haritunians T, Jostins L, Latiano A, Mathew CG, Montgomery GW, Prescott NJ, Raychaudhuri S, Rotter JI, Schumm P, Sharma Y, Simms LA, Taylor KD, Whiteman D, Wijmenga C, Baldassano RN, Barclay M, Bayless TM, Brand S, Büning C, Cohen A, Colombel JF, Cottone M, Stronati L, Denson T, De Vos M, D'Inca R, Dubinsky M, Edwards C, Florin T, Franchimont D, Gearry R, Glas J, Van Gossum A, Guthery SL, Halfvarson J, Verspaget HW, Hugot JP, Karban A, Laukens D, Lawrance I, Lemann M, Levine A, Libioulle C, Louis E, Mowat C, Newman W, Panés J, Phillips A, Proctor DD, Regueiro M, Russell R, Rutgeerts P, Sanderson J, Sans M, Seibold F, Steinhart AH, Stokkers PC, Torkvist L, Kullak-Ublick G, Wilson D, Walters T, Targan SR, Brant SR, Rioux JD, D'Amato M, Weersma RK, Kugathasan S, Griffiths AM, Mansfield JC, Vermeire S, Duerr RH, Silverberg MS, Satsangi J, Schreiber S, Cho JH, Annese V, Hakonarson H, Daly MJ, Parkes M. Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. *Nat Genet* 2010; **42**: 1118-1125 [PMID: 21102463 DOI: 10.1038/ng.717]

174 **Dinarello CA**. Interleukin-18. *Methods* 1999; **19**: 121-132 [PMID: 10525448 DOI: 10.1006/meth.1999.0837]

175 **Aizawa Y**, Sutoh S, Matsuoka M, Negishi M, Torii A, Miyakawa Y, Sugisaka H, Nakamura M, Toda G. Association of interleukin-18 gene single-nucleotide polymorphisms with susceptibility to inflammatory bowel disease. *Tissue Antigens* 2005; **65**: 88-92 [PMID: 15663745 DOI: 10.1111/j.1399-0039.2005.00336.x]

176 **Takagawa T**, Tamura K, Takeda N, Tomita T, Ohda Y, Fukunaga K, Hida N, Ohnishi K, Hori K, Kosaka T, Fukuda Y, Ikeuchi H, Yamamura T, Miwa H, Matsumoto T. Association between IL-18 gene promoter polymorphisms and inflammatory bowel disease in a Japanese population. *Inflamm Bowel Dis* 2005; **11**: 1038-1043 [PMID: 16306765]

177 **Ben Aleya W**, Sfar I, Habibi I, Mouelhi L, Aouadi H, Makhlouf M, Ayed-Jendoubi S, Najjar T, Ben Abdallah T, Ayed K, Gorgi Y. Interleukin-18 gene polymorphisms in tunisian patients with inflammatory bowel disease. *Digestion* 2011; **83**: 269-274 [PMID: 21273776]

178 **Bin C**, Zhirong Z, Xiaoqin W, Minhu C, Mei L, Xiang G, Baili C, Pinjin H. Contribution of rs11465788 in IL23R gene to Crohn's disease susceptibility and phenotype in Chinese population. *J Genet* 2009; **88**: 191-196 [PMID: 19700857]

179 **Cargill M**, Schrodi SJ, Chang M, Garcia VE, Brandon R, Callis KP, Matsunami N, Ardlie KG, Civello D, Catanese JJ, Leong DU, Panko JM, McAllister LB, Hansen CB, Papenfuss J, Prescott SM, White TJ, Leppert MF, Krueger GG, Begovich AB. A large-scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes. *Am J Hum Genet* 2007; **80**: 273-290 [PMID: 17236132 DOI: 10.1086/511051]

180 **Sáfrány E**, Pazár B, Csöngei V, Járomi L, Polgár N, Sipeky C, Horváth IF, Zeher M, Poór G, Melegh B. Variants of the IL23R gene are associated with ankylosing spondylitis but not with Sjögren syndrome in Hungarian population samples. *Scand J Immunol* 2009; **70**: 68-74 [PMID: 19522770 DOI: 10.1111/j.1365-3083.2009.02265.x]

181 **Safrany E**, Szell M, Csongei V, Jaromi L, Sipeky C, Szabo T, Kemeny L, Nagy J, Melegh B. Polymorphisms of the IL23R gene are associated with psoriasis but not with immunoglobulin A nephropathy in a Hungarian population. *Inflammation* 2011; **34**: 603-608 [PMID: 20978829]

182 **Safrany E**, Melegh B. Functional variants of the interleukin-23 receptor gene in non-gastrointestinal autoimmune diseases. *Curr Med Chem* 2009; **16**: 3766-3774 [PMID: 19747142]

183 **Duerr RH**, Taylor KD, Brant SR, Rioux JD, Silverberg MS, Daly MJ, Steinhart AH, Abraham C, Regueiro M, Griffiths A, Dassopoulos T, Bitton A, Yang H, Targan S, Datta LW, Kistner EO, Schumm LP, Lee AT, Gregersen PK, Barmada MM, Rotter JI, Nicolae DL, Cho JH. A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. *Science* 2006; **314**: 1461-1463 [PMID: 17068223 DOI: 10.1126/science.1135245]

184 **Yu P**, Shen F, Zhang X, Cao R, Zhao X, Liu P, Tu H, Yang X, Shi R, Zhang H. Association of single nucleotide polymorphisms of IL23R and IL17 with ulcerative colitis risk in a Chinese Han population. *PLoS One* 2012; **7**: e44380 [PMID: 22984500 DOI: 10.1371/journal.pone.0044380]

185 **Zhao XD**, Shen FC, Zhang HJ, Shen XY, Wang YM, Yang XZ, Tu HM, Tai YH, Shi RH. [Association of interleukin-23 receptor gene polymorphisms with susceptibility and phenotypes of inflammatory bowel diseases in Jiangsu Han population]. *Zhonghua Nei Ke Za Zhi* 2011; **50**: 935-941 [PMID: 22333126]

186 **Einarsdottir E**, Koskinen LL, Dukes E, Kainu K, Suomela S, Lappalainen M, Ziberna F, Korponay-Szabo IR, Kurppa K, Kaukinen K, Adány R, Pocsai Z, Széles G, Färkkilä M, Turunen U, Halme L, Paavola-Sakki P, Not T, Vatta S, Ventura A, Löfberg R, Torkvist L, Bresso F, Halfvarson J, Mäki M, Kontula K, Saarialho-Kere U, Kere J, D'Amato M, Saavalainen P. IL23R in the Swedish, Finnish, Hungarian and Italian populations: association with IBD and psoriasis, and linkage to celiac disease. *BMC Med Genet* 2009; **10**: 8 [PMID: 19175939 DOI: 10.1186/1471-2350-10-8]

187 **Sarlos P**, Varszegi D, Csongei V, Magyari L, Jaromi L, Nagy L, Melegh B. Susceptibility to ulcerative colitis in Hungarian patients determined by gene-gene interactions. *World J Gastroenterol* 2014; **20**: 219-227 [PMID: 24415875]

188 **Pène J**, Chevalier S, Preisser L, Vénéreau E, Guilleux MH, Ghannam S, Molès JP, Danger Y, Ravon E, Lesaux S, Yssel H, Gascan H. Chronically inflamed human tissues are infiltrated by highly differentiated Th17 lymphocytes. *J Immunol* 2008; **180**: 7423-7430 [PMID: 18490742]

189 **Silverberg MS**, Cho JH, Rioux JD, McGovern DP, Wu J, Annese V, Achkar JP, Goyette P, Scott R, Xu W, Barmada MM, Klei L, Daly MJ, Abraham C, Bayless TM, Bossa F, Griffiths AM, Ippoliti AF, Lahaie RG, Latiano A, Paré P, Proctor DD, Regueiro MD, Steinhart AH, Targan SR, Schumm LP, Kistner EO, Lee AT, Gregersen PK, Rotter JI, Brant SR, Taylor KD, Roeder K, Duerr RH. Ulcerative colitis-risk loci on chromosomes 1p36 and 12q15 found by genome-wide association study. *Nat Genet* 2009; **41**: 216-220 [PMID: 19122664 DOI: 10.1038/ng.275]

190 **McGovern DP**, Gardet A, Törkvist L, Goyette P, Essers J,

Taylor KD, Neale BM, Ong RT, Lagacé C, Li C, Green T, Stevens CR, Beauchamp C, Fleshner PR, Carlson M, D' Amato M, Halfvarson J, Hibberd ML, Lördal M, Padyukov L, Andriulli A, Colombo E, Latiano A, Palmieri O, Bernard EJ, Deslandres C, Hommes DW, de Jong DJ, Stokkers PC, Weersma RK, Sharma Y, Silverberg MS, Cho JH, Wu J, Roeder K, Brant SR, Schumm LP, Duerr RH, Dubinsky MC, Glazer NL, Haritunians T, Ippoliti A, Melmed GY, Siscovick DS, Vasiliauskas EA, Targan SR, Annese V, Wijmenga C, Pettersson S, Rotter JI, Xavier RJ, Daly MJ, Rioux JD, Seielstad M. Genome-wide association identifies multiple ulcerative colitis susceptibility loci. *Nat Genet* 2010; **42**: 332-337 [PMID: 20228799 DOI: 10.1038/ng.549]

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

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

193 **Reinisch W**, Sandborn WJ, Hommes DW, D'Haens G, Hanauer S, Schreiber S, Panaccione R, Fedorak RN, Tighe MB, Huang B, Kampman W, Lazar A, Thakkar R. Adalimumab for induction of clinical remission in moderately to severely active ulcerative colitis: results of a randomised controlled trial. *Gut* 2011; **60**: 780-787 [PMID: 21209123]

194 **Sandborn WJ**, van Assche G, Reinisch W, Colombel JF, D' Haens G, Wolf DC, Kron M, Tighe MB, Lazar A, Thakkar RB. Adalimumab induces and maintains clinical remission in patients with moderate-to-severe ulcerative colitis. *Gastroenterology* 2012; **142**: 257-65.e1-3 [PMID: 22062358 DOI: 10.1053/j.gastro.2011.10.032]

195 **Sandborn WJ**, Feagan BG, Marano C, Zhang H, Strauss R, Johanns J, Adedokun OJ, Guzzo C, Colombel JF, Reinisch W, Gibson PR, Collins J, Järnerot G, Rutgeerts P. Subcutaneous golimumab maintains clinical response in patients with moderate-to-severe ulcerative colitis. *Gastroenterology* 2014; **146**: 96-109.e1 [PMID: 23770005 DOI: 10.1053/j.gastro.2013.06.010]

196 **Sandborn WJ**, Hanauer SB, Katz S, Safdi M, Wolf DG, Baerg RD, Tremaine WJ, Johnson T, Diehl NN, Zinsmeister AR. Etanercept for active Crohn's disease: a randomized, double-blind, placebo-controlled trial. *Gastroenterology* 2001; **121**: 1088-1094 [PMID: 11677200]

197 **Tang C**, Chen S, Qian H, Huang W. Interleukin-23: as a drug target for autoimmune inflammatory diseases. *Immunology* 2012; **135**: 112-124 [PMID: 22044352 DOI: 10.1111/j.1365-2567.2011.03522.x]

198 **Sandborn WJ**, Gasink C, Gao LL, Blank MA, Johanns J, Guzzo C, Sands BE, Hanauer SB, Targan S, Rutgeerts P, Ghosh S, de Villiers WJ, Panaccione R, Greenberg G, Schreiber S, Lichtiger S, Feagan BG. Ustekinumab induction and maintenance therapy in refractory Crohn's disease. *N Engl J Med* 2012; **367**: 1519-1528 [PMID: 23075178]

199 **Hueber W**, Sands BE, Lewitzky S, Vandemeulebroecke M, Reinisch W, Higgins PD, Wehkamp J, Feagan BG, Yao MD, Karczewski M, Karczewski J, Pezous N, Bek S, Bruin G, Mellgard B, Berger C, Londei M, Bertolino AP, Tougas G, Travis SP. Secukinumab, a human anti-IL-17A monoclonal antibody, for moderate to severe Crohn's disease: unexpected results of a randomised, double-blind placebo-controlled trial. *Gut* 2012; **61**: 1693-1700 [PMID: 22595313]

200 **Sandborn WJ**, Ghosh S, Panes J, Vranic I, Su C, Rousell S, Niezychowski W. Tofacitinib, an oral Janus kinase inhibitor, in active ulcerative colitis. *N Engl J Med* 2012; **367**: 616-624 [PMID: 22894574 DOI: 10.1056/NEJMoa1112168]

201 **Targan SR**, Feagan BG, Fedorak RN, Lashner BA, Panaccione R, Present DH, Spehlmann ME, Rutgeerts PJ, Tulassay Z, Volfova M, Wolf DC, Hernandez C, Bornstein J, Sandborn WJ. Natalizumab for the treatment of active Crohn's disease: results of the ENCORE Trial. *Gastroenterology* 2007; **132**: 1672-1683 [PMID: 17484865]

202 **Feagan BG**, Greenberg GR, Wild G, Fedorak RN, Paré P, McDonald JW, Dubé R, Cohen A, Steinhart AH, Landau S, Aguzzi RA, Fox IH, Vandervoort MK. Treatment of ulcerative colitis with a humanized antibody to the alpha4beta7 integrin. *N Engl J Med* 2005; **352**: 2499-2507 [PMID: 15958805 DOI: 10.1056/NEJMoa042982]

203 **Feagan BG**, Rutgeerts P, Sands BE, Hanauer S, Colombel JF, Sandborn WJ, Van Assche G, Axler J, Kim HJ, Danese S, Fox I, Milch C, Sankoh S, Wyant T, Xu J, Parikh A. Vedolizumab as induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2013; **369**: 699-710 [PMID: 23964932 DOI: 10.1056/NEJMoa1215734]

204 **Zuk O**, Hechter E, Sunyaev SR, Lander ES. The mystery of missing heritability: Genetic interactions create phantom heritability. *Proc Natl Acad Sci USA* 2012; **109**: 1193-1198 [PMID: 22223662 DOI: 10.1073/pnas.1119675109]

P- Reviewer: Akarsu M, Gazouli M, Yamakawa M
S- Editor: Wen LL **L- Editor**: A **E- Editor:** Lu YJ

World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4291/wjgp.v5.i3.322

World J Gastrointest Pathophysiol 2014 August 15; 5(3): 322-334
ISSN 2150-5330 (online)

REVIEW

Current status of predictive biomarkers for neoadjuvant therapy in esophageal cancer

Norihisa Uemura, Tadashi Kondo

Norihisa Uemura, Department of Gastroenterological Surgery, Aichi Cancer Center Hospital, Nagoya 464-8681, Aichi, Japan
Tadashi Kondo, Division of Pharmacoproteomics, National Cancer Center Research Institute, Tokyo 104-0045, Japan
Author contributions: Uemura N and Kondo T equally contributed to this study.
Correspondence to: Tadashi Kondo, MD, PhD, Division of Pharmacoproteomics, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. takondo@ncc.go.jp
Telephone: +81-3-35422511 Fax: +81-3-35475298
Received: December 25, 2013 Revised: January 27, 2014
Accepted: April 25, 2014
Published online: August 15, 2014

Abstract

Neoadjuvant therapy has been proven to be extremely valuable and is widely used for advanced esophageal cancer. However, a significant proportion of treated patients (60%-70%) does not respond well to neoadjuvant treatments and develop severe adverse effects. Therefore, predictive markers for individualization of multimodality treatments are urgently needed in esophageal cancer. Recently, molecular biomarkers that predict the response to neoadjuvant therapy have been explored in multimodal approaches in esophageal cancer and successful examples of biomarker identification have been reported. In this review, promising candidates for predictive molecular biomarkers developed by using multiple molecular approaches are reviewed. Moreover, treatment strategies based on the status of predicted biomarkers are discussed, while considering the international differences in the clinical background. However, in the absence of adequate treatment options related to the results of the biomarker test, the usefulness of these diagnostic tools is limited and new effective therapies for biomarker-identified nonresponders to cancer treatment should be concurrent with the progress of predictive technologies. Further improvement in the prognosis of esophageal cancer patients can be achieved through the introduction of novel therapeutic approaches in clinical practice.

Key words: Esophageal cancer; Neoadjuvant therapy; Response prediction; Molecular biomarker; Chemoradiation

Core tip: To achieve individualization of neoadjuvant therapy for locally advanced esophageal cancers, predictive biomarkers are urgently needed. Biomarker development using multimodal approaches, including gene expression profiling, single nucleotide polymorphisms, microRNAs, proteomics, immunohistochemistry, serum biomarkers and conventional blood tests, seem promising. Independent validation studies will establish novel prognostic modalities based on molecular biomarkers. Progress of predictive modalities and further studies on the molecular background of patients with a poor prognosis will facilitate the development of new effective therapies for patients resistant to the present neoadjuvant therapy. Prognostic stratification of patients will promote efforts toward novel therapeutic strategies.

Uemura N, Kondo T. Current status of predictive biomarkers for neoadjuvant therapy in esophageal cancer. *World J Gastrointest Pathophysiol* 2014; 5(3): 322-334 Available from: URL: http://www.wjgnet.com/2150-5330/full/v5/i3/322.htm DOI: http://dx.doi.org/10.4291/wjgp.v5.i3.322

INTRODUCTION

Esophageal cancer is the fifth most common cause of cancer-related death for men and the eighth for women worldwide[1]. Despite the use of modern surgical tech-

niques in combination with radio- and chemotherapy, early recurrence is common and the overall 5-year survival rate remains below 40%[2]. Consequently, there is a great interest in multimodal approaches to the treatment of esophageal cancer and neoadjuvant chemotherapy, alone or in combination with chemoradiotherapy (CRT), is becoming the standard approach of care in locally advanced esophageal cancers. Randomized trials of different neoadjuvant therapy protocols have been conducted in patients with locally advanced cancers. Meta-analyses of those randomized trials have revealed only modest survival advantages, except in the case of patients who achieved a complete histopathological response and seemed to highly benefit from a neoadjuvant regimen[3-9]. However, a significant proportion (60%-70%) of treated patients did not respond well to these treatments and experienced severe adverse effects[8,10]. In addition, nonresponsive patients may lose the option of surgical resection after ineffective chemotherapy[11] and the prognosis of nonresponders has been found to be inferior to that for patients treated by surgery alone[12]. While there is an obvious correlation between the response and prognosis, the response to chemotherapy or radiotherapy is variable, even when patients are at the same clinical stage. Thus, an accurate risk stratification of cancer patients for therapy is of paramount importance for avoiding potential morbidity due to ineffective treatment and prevention of further disease progression. With this background, identification of predictive markers would allow accurate risk stratification and individualization of multimodality treatment for patients with locally advanced esophageal cancer[13].

In recent years, molecular biomarkers that can predict the response to neoadjuvant therapy in esophageal cancer have been investigated by using multidimensional approaches. Global expression transcriptomics and proteomics studies allow for simultaneous screening of several thousand molecules and knowledge-based methodologies such as immunohistochemistry are focused on a specific molecule or pathway. These approaches are based on their own unique principles and the performance of predictive molecular biomarkers developed by using each approach seems to be equally promising. Here, we have reviewed the current status of molecular biomarkers predictive for response to neoadjuvant therapy in esophageal cancer. We have focused on predictive markers that can be used to analyze pretreatment samples such as diagnostic biopsies or serum specimens obtained before neoadjuvant treatment. These biomarkers will help avoid unnecessarily invasive treatments. We have summarized promising candidates for predictive molecular biomarkers in esophageal cancer according to the type of development modality.

MOLECULAR BIOMARKERS FOR RESPONSE PREDICTION

Gene expression profiling

High throughput technology such as gene expression microarray has been considered as one of the most powerful tools for understanding the biological characteristics of malignancies. Microarray-based gene expression profiling generates quantitative expression data for thousands of genes, which can be further analyzed by various bioinformatics approaches to identify the most informative genes relevant to cancer prognosis. In particular, the gene expression signatures determined by microarrays have been used to predict the response to neoadjuvant treatment among cancer patients[14].

Maher *et al*[15] investigated gene expression profiles in a cohort comprising 13 patients who were the most responsive or resistant to a standard combination of chemotherapy and radiation therapy. The authors identified five genes (*EPB41L3*, *RNPC1*, *RTKN*, *STAT5B* and *NMES1*) as predictive biomarkers by using DNA microarrays and validated the results by qRT-PCR, confirming that the expression level of five genes could be used to predict the response to neoadjuvant CRT in esophageal cancer with 95% accuracy. Luthra *et al*[16] profiled pretreatment endoscopic cancer biopsies from 19 patients using an AffymetrixU133A Chip (Santa Clara, CA) and noted correlation of the molecular profiles with pathological response to neoadjuvant treatments. The authors reported that the expression levels of three genes (*PERP*, *S100A2 and SPRR3*) helped discriminate between patients with complete histopathological response and those resistant to treatment, with high sensitivity (86%) and specificity (85%). Schauer *et al*[17] performed microarray analysis in 47 patients who had a locally advanced esophageal adenocarcinoma (AC) and had undergone neoadjuvant chemotherapy with cisplatin, leucovorin and 5-fluorouracil, followed by resection. The authors found that the gene encoding the ephrin B3 receptor showed the most prominent differential expression between responders and nonresponders and validated these results by immunohistochemistry. Motoori *et al*[18] performed comprehensive gene expression profiling of pretreatment biopsy samples from 25 patients with esophageal squamous cell carcinoma (SCC) to identify expression patterns predictive for cisplatin-based neoadjuvant chemotherapy. Their system consisted of 199 most informative genes and had the prediction accuracy of 82%. Duong *et al*[19] performed microarray analysis for 46 esophageal cancer patients, that is, 21 SCC and 25 AC patients for whom neoadjuvant CRT had been recommended. Their study was based on two-color competitive hybridization to a cDNA array printed at the Peter MacCallum Cancer Centre Microarray Core Facility[19] and identified a 32-gene classifier that could be used to predict a response to neoadjuvant CRT in SCCs, whereas a negative predictive profile was observed for AC patients.

These examples suggest that gene expression profiling is a powerful tool to identify gene sets for selection of optimal and personalized therapy for patients with esophageal cancer. In breast cancer, mRNA expression signatures strongly predictive of metastasis have been identified and a novel prognostic test for assessing the

risk of metastasis and benefits of chemotherapy has been introduced in clinical settings. This test, named MammaPrint, effectively identifies breast cancer patients with a high risk of recurrence after local treatment alone[20]. The Oncotype DX assay (Genomic Health, Redwood, CA) is another test aimed at better discerning breast cancer patients who would benefit from chemotherapy and those who can safely avoid it. By using the Oncotype DX, we measured the status of 21 genes and could predict the benefits of chemotherapy and the rate of cancer recurrence in 10 years[21]. Similar diagnostic predictive tests are desired for esophageal cancer; however, in this case, different prognostic biomarkers have been identified by using similar technical platforms. The results of these studies need further validation in order to forward their clinical application.

Single nucleotide polymorphisms

In the process of generating a draft sequence of the human genome, it has become clear that the extent of genetic variation is much larger than previously estimated[22,23]. The most common sequence variation in the human genome is the stable substitution of a single base called single-nucleotide polymorphism (SNP). By definition, SNP has a minor allele frequency of greater than 1% in at least one population[24]. Most SNPs are silent and do not alter gene expression or function. The cancer genomics research on SNP variation provides an opportunity for the detection of molecular biomarkers predictive of the response to cancer therapy[25].

Wu *et al*[26] investigated the association between SNPs in multigenic cascades involved in radiation and chemotherapy-dependent responses and clinical outcomes for esophageal cancer patients. The authors applied the pathway-based approach to examine the impact of a comprehensive SNP panel on clinical outcomes in 210 esophageal cancer patients and found that among the genes involved in DNA base excision repair, the variant alleles R399Q in the *XRCC1* gene were significantly associated with the absence of complete pathological response and poor survival. Warnecke-Eberz *et al*[27] investigated a panel of selected gene SNPs to predict responses to neoadjuvant radiochemotherapy in 52 esophageal cancer patients. The authors showed that SNP of C118T in the *ERCC1* gene and the rarely occurring AA genotype of the *XRCC1* gene were predictive of therapy response. Both *ERCC1* and *XRCC1* genes are components of the nucleotide excision repair pathway that protects the integrity of the genome by removing a wide variety of DNA lesions including inter- and intra-strand crosslinks caused by platinum agents or radiation[28]. These SNPs in *ERCC1* appeared to have functional significance because a low intra-tumoral expression of the ERCC1 protein was found to be strongly associated with a major pathological response[29,30]. Moreover, Brabender *et al*[31] reported that *ERCC1* RNA expression in peripheral blood could be a predictor of the response to neoadjuvant therapy. Functional contribution of SNPs in other genes involved in nucleotide excision repair should be investigated for further understanding of the pathogenesis of esophageal cancer.

Clinical applications of SNP testing in cancer are quite realistic. In other types of cancer, the cancer genomics research on SNP variation has provided clinical applications. For example, genetic polymorphisms of the *UGT1A1* gene would affect inter-individual variations in the toxic response to irinotecan by altering the bioavailability of the irinotecan active metabolite SN-38[32,33]. Genetic testing for the presence of the UGT1A1*28 allele has been approved by the FDA and has become available in hospitals. Similar tests for genetic polymorphisms in esophageal cancer would be extremely useful and validation studies for the predictive potential of SNPs would promote their introduction in clinics.

MicroRNAs

MicroRNAs (miRNAs) are short (19-24 nucleotides) noncoding RNA sequences involved in the regulation of gene expression *via* the inhibition of mRNA translation[33,34]. Many lines of evidence suggest that miRNAs exist stably in tissues and body fluids and play a key role in various biological processes, including carcinogenesis. Aberrant miRNA expression has been shown to correlate with the inhibition of tumor suppressor genes or inappropriate activation of oncogenes. Recent studies have shown that the abnormal miRNA expression patterns frequently detected in esophageal cancers have strong prognostic values[35-38]. The predictive utility of miRNAs has also been demonstrated by global expression studies.

Odenthal *et al*[39] assessed miRNA profiles of responders and nonresponders to neoadjuvant therapy for esophageal cancer in order to identify possible predictive markers. The authors found that the pre-therapeutic intra-tumor expression of miR-192 and miR-194 was significantly associated with the histopathological response of esophageal SCCs to multimodal therapy. Using pretreatment biopsy specimens, Ko *et al*[40] showed that the miRNA expression profile was significantly different between groups with and without complete pathological response. Among the 71 differentially regulated miRNAs, five showed the difference of more than two-fold; these included miR-296[41], which has recently been shown to be of prognostic significance in esophageal cancer. The inhibition of miR-296 also resulted in the increased chemosensitivity of esophageal cancer cells to standard chemotherapeutic agents such as 5-fluorouracil and cisplatin[41]. Tanaka *et al*[42] investigated the serum levels of miR-21, miR-145, miR-200c and let-7c by qRT-PCR in 64 esophageal cancer patients treated with neoadjuvant chemotherapy. The authors revealed a significant correlation of miR-200c high expression with poor response to chemotherapy. The possible prognostic utility of miR-200c was also reported by Hamano *et al*[43], who in a study of 98 patients found that miR-200c was involved in resistance to chemotherapy. Lynam-Lennon *et al*[44] demonstrated that resistance to radiation was sig-

nificantly associated with the downregulation of miR-31 and that the ectopic re-expression of miR-31 considerably restored radiosensitivity of the resistant cells. The authors also showed that miR-31 expression was markedly reduced in patients with poor pathological response to neoadjuvant CRT, whereas the expression of the miR-31-regulated DNA repair genes significantly increased[44].

Clinical application of miRNAs as predictive biomarkers is quite feasible because miRNAs are relatively stable and their expression levels can be quantitatively assessed by qRT-PCR. Currently, several clinical trials have already been approved by the FDA to evaluate the value of serum miRNAs in therapeutic response prediction (http://clinicaltrials.gov). Clinical trials evaluating serum miRNAs include the search for predictors of therapeutic response in ovarian carcinoma and miRNA profiling of breast cancer in patients undergoing neoadjuvant or adjuvant treatment[45]. Further functional studies would hopefully validate the functional relevance of miRNAs in esophageal cancer and result in diagnostic and novel therapeutic approaches.

Proteomics

The proteome is a functional translation of the genome. The genomic aberrations in cancer cells are translated to the proteome determining cancer phenotypes and regulating tumor behavior. Because proteins are the main executioner biomolecules, which influence the molecular pathways in normal and tumor cells, proteomic markers are closer and more relevant to cancer initiation and progression than other biomarkers. Proteomic studies can therefore generate unique data related to cancer phenotypes. Many lines of evidence have demonstrated the discordance between mRNA and protein expression[46-48]. In addition, DNA sequence and mRNA expression cannot accurately predict post-translational modifications such as phosphorylation and glycosylation, which play a key role in regulating the malignant behavior of cancer cells. Taken together, proteomic studies can provide valuable information for biomarker identification in various cancers[49-51].

Aichler *et al*[52] analyzed proteomic changes associated with response to chemotherapy by MALDI imaging mass spectrometry using pre-therapeutic biopsy samples of 23 esophageal ACs. Proteins related to clinical response were identified by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The authors discovered that clinical response to cisplatin was associated with the defects in the mitochondrial respiratory chain of cancer cells caused by the loss of specific cytochrome c oxidase subunits. Maher *et al*[53] examined the proteomic profiles of serum samples by using surface-enhanced laser desorption/ionization time-of-flight (SELDL-TOF) mass spectrometry and validated the results with an enzyme-linked immunosorbent assay. By comparing pre-treatment serum samples from 16 poor responders and 15 good responders, the authors found that higher serum levels of complement factors C4a and C3a were significantly associated with favorable response to treatments. The leave-one-out cross-validation analysis revealed that these serum proteins could predict the response to neoadjuvant CRT with a sensitivity and specificity of 78.6% and 83.3%, respectively.

Although there are various reports about biomarker candidates identified by proteomics studies, only a few of them have been proven to be clinically useful[54] because of the lack of independent validation studies. However, the prognostic utility of protein biomarkers has been successfully validated for gastrointestinal stromal tumors in extensive multi-institutional studies[55]. Further validation studies will promote the clinical application of promising protein biomarkers for esophageal cancer.

Immunohistochemistry

By focusing on functionally important molecules or pathways, discovery of biomarker candidates can be performed effectively. Global expression studies based on statistical data may not be able to identify functionally important genes and proteins because expression levels do not always reflect functional activity. In this sense, a knowledge-dependent approach such as immunohistochemistry has unique advantages over the other methods for expression assessment because it allows for the analysis of a large number of formalin-fixed and paraffin-embedded tissue sample archives and provides detailed spacious information not available by other methods. Immunohistochemistry has been successfully used for hypothesis-driven biomarker discovery[56].

Solid tumors are driven and managed by a small population of cancer stem cells (CSCs), tumor-initiating cells or cancer stem-like cells[57-60]. Among these cells, CSCs are found to be more resistant to treatment[61,62]; therefore, CSC markers have been considered promising candidates for predictive biomarkers. Previous reports have demonstrated the importance of CSC markers including growth factor receptors, tumor suppressor genes and DNA-repair pathway factors in malignant features of esophageal cancer cells. Smit *et al*[63] investigated the expression of CSC markers, *in vitro* growth of spheroids, sensitivity to radiation and *in vivo* growth of several esophageal cancer-derived cell sub-populations. The authors found that the CD44+/CD24- subpopulation of esophageal cancer cells exhibited a higher proliferation rate and sphere forming potential and was more radioresistant *in vitro* than unselected or CD44+/CD24+ cells. In a study of the archival pre-neoadjuvant CRT biopsy material from esophageal AC patients (N = 27), CD44+/CD24- cells could only be identified in 50% (9/18) of poor responders to neoadjuvant CRT, but never (0/9) in complete responders. These results warrant further investigation into the possible clinical utility of CD44+/CD24- phenotype as a predictive biomarker for the response to CRT in patients with esophageal cancer.

Human epidermal growth factor receptors 1 and 2

(EGFR and HER2/neu) are known to be involved in malignant transformation and tumor growth. Yamamoto *et al*[64] assessed the expression of EGFR, HER2/neu, HER3, Ki-67 and p53 by immunohistochemistry in 37 esophageal SCC patients treated with neoadjuvant chemotherapy and found that EGFR expression correlated with pathological response to neoadjuvant chemotherapy. Akamatsu *et al*[65] reported similar findings in 34 patients who had esophageal SCC and were receiving neoadjuvant CRT, *i.e.*, positive staining for HER2/neu was found to be associated with CRT resistance. In contrast, Arsenijevic *et al*[66] and Schena *et al*[67] found no statistically significant difference between EGFR and HER2/neu expression and the clinical response to neoadjuvant CRT. Further verification studies are necessary to clarify the role of EGFR and HER2 expression in the response of esophageal cancer patients to CRT.

The tumor suppressor gene p53, which is involved in cell cycle regulation, apoptosis and DNA repair, has been identified as an important molecular factor in the response to neoadjuvant therapy in patients with esophageal cancer[68]. However, the predictive value of p53 status for chemotherapy response in esophageal cancer patients has not been established. Kitamura *et al*[69] performed a study involving 95 patients with esophageal SCC and showed that p53 protein expression was significantly associated with increased sensitivity to neoadjuvant CRT. In contrast to these findings, Shimada *et al*[70] demonstrated that p53 protein expression was negatively associated with histopathological response to chemotherapy, whereas other similar studies did not find any predictive value for p53 in multimodality therapy for esophageal cancer[66,71]. Zhang *et al*[72] conducted a meta-analysis of 28 studies comprising 1497 cases to elucidate the correlation of p53 status with the response to chemotherapy-based treatment. The authors concluded that patients with low expression of wild-type p53 had higher rates of complete pathological response to neoadjuvant CRT. The clinical significance of p53 as a predictive biomarker for the treatment of esophageal cancer should be further evaluated.

DNA repair pathways are essential for the cell responses to DNA damage induced by CRT. Aberrant regulation of DNA repair proteins is frequently reported in cancers and the reduced expression of these proteins correlated with poor prognosis in esophageal cancers[73-75]. Alexander *et al*[76] assessed major DNA repair proteins such as XPF, FANCD2, PAR, MLH1, PARP1 and phosphorylated MAPKAP kinase 2 in 79 patients with esophageal cancer by tissue microarray. The authors showed that higher scores for MLH1 and lower scores for FANCD2 were significantly associated with pathological response to neoadjuvant CRT on multivariable analysis.

Expression of heat-shock proteins (HSPs) and glucose-regulated proteins (GRPs) can be induced in cells following exposure to different insults, allowing cells to survive stress conditions. The regulation and expression of these proteins have an important impact on the biology of esophageal cancer with respect to prognosis[77] and response to chemotherapy[78]. Slotta-Huspenina *et al*[79] assessed HSPs and GRPs by reverse phase protein arrays (RPPAs), immunohistochemistry and quantitative RT–PCR in pretherapeutic biopsies of 90 patients with esophageal AC. The authors showed that low expression of HSP90, HSP27 and p-HSP27$^{(Ser15, Ser78, Ser82)}$ and high expression of GRP78, GRP94, HSP70 and HSP60 were significantly associated with pathological response to neoadjuvant chemotherapy.

Even with the advances in modern technologies, the emergence of new biomarkers for esophageal cancer has been relatively slow because biomarker discovery has been generally hypothesis-driven and depended on investigation of individual genes or proteins. Data-driven approaches such as global expression studies provide a considerable number of biomarker candidates and once their functional and clinical significance is established, they are worth validating by immunohistochemistry. Immunohistochemistry is an established clinical examination method and further validation studies on biomarker candidates confirmed by immunohistochemistry should be relatively easily performed. A possible utility of these candidate proteins as predictive biomarkers for neoadjuvant CRT should be further validated.

Serum biomarkers with response to treatments

The hypothesis-driven approach is used to examine serum proteins, which have been previously established as biomarkers but have not been considered as predictive biomarker candidates. Serum samples can be obtained by a minimally invasive procedure at a relatively low cost and thus can be repeatedly examined. There are several reports that conventional serum biomarkers could be predictive in esophageal cancer.

Makuuchi *et al*[80] examined the expression levels of 84 cytokines in serum samples obtained from 37 esophageal SCC patients treated with neoadjuvant CRT. They found that the level of serum soluble IL-6 receptor was significantly higher in 30 patients who failed to achieve a complete histological response, thereby revealing a correlation between serum IL-6 receptor levels and the histological response to neoadjuvant CRT. These observations suggest that persistent systemic inflammation can be a possible mechanism of resistance to CRT therapy in esophageal cancers.

Brabender *et al*[81] assessed thymidylate synthetase and dihydropyrimidine dehydrogenase RNA expression in the peripheral blood of 29 patients who had esophageal cancer and had been treated with neoadjuvant CRT. The authors showed that high thymidylate synthetase expression was associated with a minor response to neoadjuvant treatment, while there was no significant association between dihydropyrimidine dehydrogenase and treatment response. They also reported that the specificity of response prediction reached 100% when the levels of thymidylate synthetase and dihydropyrimidine dehydro-

genase were assessed simultaneously.

Only a few serum biomarkers have been examined for predictive utility in cancers and it is challenging to investigate the rest of them. Such an examination does not require significant sample volumes and it is quite feasible to examine multiple serum biomarkers in identical cohorts. Serum biomarkers can be routinely examined in the clinical setting and their application to the prediction of treatment responses seems to be quite promising.

Common blood tests

Data obtained by common blood tests can be an indicator of response to neoadjuvant therapy. It is noteworthy that, although serum examination may lack specificity and sensitivity, its combination with common blood tests can provide predictive stratification of esophageal cancer patients for chemotherapy.

Sato *et al*[82] investigated the correlation between the pre-therapeutic neutrophil to lymphocyte ratio (NLR) and pathological response to neoadjuvant chemotherapy in patients with advanced esophageal cancer. The authors showed that the pretreatment NLR (< 2.2/ ⩾ 2.2) was significantly correlated with pathological response: the pathological response rates were 56% and 21% in patients with the NLR < 2.2 and NLR > 2.2, respectively. Similar results were reported by Noble *et al*[83], who examined the correlation of blood-borne inflammatory and nutritional markers with response to neoadjuvant chemotherapy in radically treated esophagogastric cancer patients. The authors demonstrated that only serum albumin ($P = 0.037$) had a predictive value for the pathological response to chemotherapy and that a higher NLR was associated with poor overall survival. In contrast, Hsu *et al*[84] reported that none of the clinical parameters, including blood profiles, images and baseline tumor characteristics, predicted the response to CRT.

Cancer always unfolds on a background of chronic inflammation and it is an interesting idea that inflammatory markers can also serve as prognostic biomarkers for cancer therapy. On the other hand, parameters of systemic inflammation can be confounding factors in a cancer biomarker study. Stricter sample stratification for biomarker studies and extensive independent validation by independent researchers may distinguish true biomarkers from the confounding factors. The results obtained by current studies seem to be promising and further validation will confirm the prognostic utility of candidate biomarkers for clinical applications (Table 1).

TREATMENT STRATEGY BASED ON THE STATUS OF PREDICTIVE BIOMARKERS

As described above, a number of molecules have emerged as predictive candidate biomarkers for the treatment of esophageal cancers and will hopefully result in establishment of biomarkers for routine clinical use. By combining several promising markers in a cross-modality manner, we may be able to develop versatile predictive tools that are more effective than single markers. This approach should be achieved by linking the biomarker components to stratified patient information. The diagnostic kit may be developed such that it gets a local makeover to adjust for variations in clinical therapeutic approaches. The effectiveness of response prediction depends on therapeutic strategies, including the surgical procedure and neoadjuvant therapy, and the clinical background of patients with esophageal cancer. For example, neoadjuvant chemotherapy with cisplatin plus 5-fluorouracil is the current standard treatment for locally advanced esophageal cancer in Japan[85], while neoadjuvant CRT with cisplatin plus 5-fluorouracil is the standard in Western countries[86]. In Japan, a three-arm Phase Ⅲ trial started in November 2012 to confirm the superiority of docetaxel and cisplatin plus 5-fluorouracil over cisplatin plus 5-fluorouracil and the superiority of cisplatin plus 5-fluorouracil with CRT over cisplatin plus 5-fluorouracil as neoadjuvant therapy for esophageal SCC[87]. If neoadjuvant chemotherapy is combined with radiation therapy, the prediction kit should include the biomarkers associated with sensitivity to radiation, such as RNA-binding protein RNPC1[88]. On the other hand, if the combination chemotherapy regimen includes docetaxel, a docetaxel-specific biomarker, such as RPN2[89], should be present. In addition, a predominant histological type of esophageal cancer has been found to exhibit region-dependent differences. Thus, SCC is the predominant histological type of esophageal carcinoma worldwide; however, in Australia, the United Kingdom, the United States, and some Western European countries (*e.g.*, Finland, France, and the Netherlands), the incidence of esophageal AC now exceeds that of SCC[90,91]. In a study on 8562 patients who underwent surgical resection, Merkow *et al*[92] found that the only factor predictive of pathological complete response was SCC histology. The response pattern to neoadjuvant therapy is different in each histological type[93]. Thus, to increase the specificity of response prediction, different molecules can serve as biomarkers depending on histological type. Any article clubbing two diseases together is not appropriate. Surgical procedures are also different in each country. Surgical options for the resection of esophageal carcinoma include the following: trans-hiatal esophagectomy and trans-thoracic approaches, such as Ivor Lewis esophagectomy (abdominal and right thoracic approach also called the Lewis-Tanner approach), the three-incision modified McKeown esophagectomy (involving laparotomy, right thoracotomy, neck anastomosis, and left thoracotomy) and the left thoraco-abdominal approach[94-100]. In Japan and several other countries, extended lymphadenectomy is a common procedure, but this is not the case elsewhere[101-103]. In conclusion, because the sensitivity and specificity of response prediction vary according to regional differences in therapeutic strategies and clinical background, it may be necessary to customize a prediction kit for each country rather than to adopt a universal prediction strategy.

Table 1 Molecular biomarkers for predicting the response to neoadjuvant therapy in esophageal cancer

Modality/biomarker	*N*	Histology	Neoadjuvant therapy	Sensitivity	Specificity	PPV	NPV	Accuracy	Ref.
Gene expression profiling									
5 genes (EPB41L3, RNPC1, RTKN, STAT5B, and NMES1)	13	Squamous-23% Adeno-77%	CRT; 5-FU and cisplatin, 40.05-44 Gy	100%	91%	NA	NA	95%	[15]
3 genes (PERP, S100A2, and SPRR3)	19	Squamous-11% Adeno-84%	CRT; 5-FU, docetaxel and irinotecan, 50.4 Gy	86%	85%	75%	92%	85%	[16]
Ephrin B3 receptor	47	Adeno-100%	CT; 5-FU, cisplatin and leucovorin	89%	84%	89%	84%	87%	[17]
199 genes	25	Squamous-100%	CT; 5-FU, cisplatin and adriamycin	68%	93%	88%	79%	82%	[18]
32 genes	46	Squamous-46% Adeno-54%	CRT; 5-FU and cisplatin, 35-50 Gy	100%	67%	55%	100%	76%	[19]
Single nucleotide polymorphisms									
XRCC1 R399Q	210	Squamous-17% Adeno-83%	CRT; 5-FU, cisplatin and paclitaxel, RT (NA)	NA	NA	NA	NA	NA	[26]
ERCC1 C118T/XRCC1 A194G	52	Squamous-60% Adeno-40%	CRT; 5-FU and cisplatin, 36 Gy	54/5%	67/100%	80/100%	37/59%	58/60%	[27]
MicroRNAs									
miR-192, miR-194	8	Squamous-25% Adeno-75%	CRT; 5-FU and cisplatin, 40 Gy	NA	NA	NA	NA	NA	[39]
HS-240, has-miR-296, has-miR-141, has-miR-31, HS-217	25	Squamous-20% Adeno-80%	CRT; cisplatin and irinotecan, 50.4 Gy	NA	NA	NA	NA	NA	[40]
Serum miR-200c	64	Squamous-100%	CT; 5-FU, cisplatin and adriamycin or docetaxel	68%	62%	53%	75%	64%	[42]
miR-200c	98	Squamous-91%	CT; 5-FU, cisplatin and adriamycin	NA	NA	NA	NA	NA	[43]
miR-31	19	Squamous-5% Adeno-95%	CRT; 5-FU and cisplatin, 40.05 Gy	NA	NA	NA	NA	NA	[44]
Proteomics									
Mitochondrial respiratory chain complexes	69	Adeno-100%	CT; 5-FU and cisplatin	50%	93%	82%	74%	71%	[52]
C4a, C3a	31	Squamous and adeno; NA	CRT; 5-FU and cisplatin, 40-44 Gy	79%	83%	NA	NA	81%	[53]
Immunohistochemistry									
CD44+/CD24-	27	Adeno-100%	CRT; NA	50%	100%	100%	50%	67%	[63]
EGFR	37	Squamous-100%	CT; 5-FU, cisplatin and docetaxel	93%	55%	58%	92%	70%	[64]
HER2/neu	34	Squamous-100%	CRT; 5-FU and cisplatin or leucovorin, 39.6-40 Gy	69%	71%	60%	79%	71%	[65]
p53 (wild-type)	1497	Squamous-91% Adeno-9%	CRT or CT (meta-analysis)	NA	NA	NA	NA	NA	[72]
MLH1, FANCD2	79	Squamous-27% Adeno-71%	CRT; 5-FU, cisplatin and/or paclitaxel, 45-64.8 Gy	20%	100%	100%	22%	35%	[76]
Heat-shock proteins and glucose-regulated proteins	90	Adeno-100%	CT; 5-FU, cisplatin or oxaliplatin	61%	63%	53%	70%	62%	[79]
Serum biomarker									
Serum soluble interleukin-6 receptor	37	Squamous-100%	CRT; 5-FU and cisplatin, 40 Gy	NA	NA	NA	NA	NA	[80]
Thymidylate synthetase and dihydropyrimidine dehydrogenase	29	Squamous-34% Adeno-66%	CRT; 5-FU and cisplatin, 36 Gy	20%	100%	100%	36%	45%	[81]
Common blood tests									
Neutrophil-to-lymphocyte ratio	83	Squamous-84%	CT; 5-FU and cisplatin	71%	66%	56%	79%	68%	[82]
Albumin	246	Squamous-13% Adeno-86%	CT; cisplatin, epirubicin and 5-FU or capecitabine, or epirubicin and oxaliplatin	NA	NA	NA	NA	NA	[83]

PPV: Positive predict value; NPV: Negative predict value; Squamous: Squamous cell carcinoma; Adeno: Adenocarcinoma; CRT: Chemoradiotherapy; CT: Chemotherapy; 5-FU: 5-fluorouracil; NA: Not available.

Pathological nonresponders to neoadjuvant therapy for esophageal cancer demonstrate no survival benefits compared to patients treated with primary esophagectomy[12]. Factors predicting the response to neoadjuvant therapy may help to reduce the number of unnecessarily treated patients and lead to the investigation of new and more effective therapeutic strategies for the unresponsive group. However, if there are no effective therapies for nonresponders, predicting the response to neoadjuvant therapy is tantamount to abandoning nonresponders to their fate. Further improvement in outcomes for the patient with esophageal cancer cannot be

achieved without improvement of the prognosis of nonresponders. Therefore, the development of new effective therapies for nonresponders concurrently with progress in predictive methodology is necessary. Recently, novel therapeutic approaches, such as new targeted strategies, epigenetic therapeutics, monoclonal antibody therapy and carbon-ion radiotherapy, are being developed[104-105]. Although initially many of these studies involved patients with metastatic disease, these therapies are now being increasingly investigated in the preoperative setting as components of multimodality therapy[104]. The efficacy of targeted agents for neoadjuvant therapy of patients with esophageal cancer has yet to be established in previous and ongoing clinical trials[105]. Additional trials to examine new targeted agents have been performed. Further improvement of the prognosis of esophageal cancer patients can be achieved through the introduction of these novel therapeutic approaches in practice, which provides prognostic improvement for nonresponders identified by predictive biomarkers.

CLINICAL APPLICATION OF BIOMARKERS

Advances in modern omics technologies and the integration of the results into clinical practice provide valuable opportunities for biomarker discovery research. As discussed in this review, considerable numbers of promising biomarkers in esophageal cancer have been established and more biomarker candidates are likely to be identified by the application of novel technologies. These biomarkers have been discovered through a hypothesis-driven approach by medical doctors for specific clinical applications and they seem to have great potential in providing benefits to patients. However, only a few of the biomarkers discovered in the last decade have been introduced into clinical practice and skepticism about the clinical utility of biomarkers in the diagnosis and treatment of cancer has been expressed[106]. As discussed here, treatments based on the results of biomarker studies should be further developed to benefit all patient subgroups. To establish the reliability of biomarkers before clinical trials, the reproducibility of the results should be assessed by independent investigators. However, we found that none of the biomarkers reviewed in this article had been validated by other researchers. Small sample sizes may be the most serious obstacle for validation of predictive biomarkers. Although it is generally accepted that multi-institutional and inter-disciplinary collaboration is required for biomarker validation, until now no serious validation studies have been performed for any predictive biomarkers in esophageal cancer and this issue requires further analysis.

CONCLUSION

We have reviewed the current status of biomarkers in esophageal cancer, especially focusing on the utility for predicting responses to neoadjuvant therapy. The reported biomarkers seem to be promising because they have been developed based on clinical research and their predictive performance has been examined by using clinical samples. Further validation and functional evaluation will increase the reliability of these biomarkers. Combined use of the reported biomarkers may increase prognostic performance and this concept is worth further research. Prognostic modalities should be tailored to specific clinical therapeutic approaches that differ according to individual cases. The development of new effective therapies for nonresponders can be hoped for with the progress in predictive techniques. Further understanding of the molecular mechanisms underlying the resistance to CRT in cancers can be achieved by investigating the functional effects of biomarkers on the malignant properties of tumor cells and such efforts will pave the way to novel therapeutic strategies.

REFERENCES

1 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]

2 **Rice TW**, Rusch VW, Apperson-Hansen C, Allen MS, Chen LQ, Hunter JG, Kesler KA, Law S, Lerut TE, Reed CE, Salo JA, Scott WJ, Swisher SG, Watson TJ, Blackstone EH. Worldwide esophageal cancer collaboration. *Dis Esophagus* 2009; **22**: 1-8 [PMID: 19196264 DOI: 10.1111/j.1442-2050.2008.00901.x]

3 **Urschel JD**, Vasan H. A meta-analysis of randomized controlled trials that compared neoadjuvant chemoradiation and surgery to surgery alone for resectable esophageal cancer. *Am J Surg* 2003; **185**: 538-543 [PMID: 12781882 DOI: 10.1016/S0002-9610(03)00066-7]

4 **Kaklamanos IG**, Walker GR, Ferry K, Franceschi D, Livingstone AS. Neoadjuvant treatment for resectable cancer of the esophagus and the gastroesophageal junction: a meta-analysis of randomized clinical trials. *Ann Surg Oncol* 2003; **10**: 754-761 [PMID: 12900366]

5 **Malthaner RA**, Wong RK, Rumble RB, Zuraw L. Neoadjuvant or adjuvant therapy for resectable esophageal cancer: a systematic review and meta-analysis. *BMC Med* 2004; **2**: 35 [PMID: 15447788 DOI: 10.1186/1741-7015-2-35]

6 **Fiorica F**, Di Bona D, Schepis F, Licata A, Shahied L, Venturi A, Falchi AM, Craxì A, Cammà C. Preoperative chemoradiotherapy for oesophageal cancer: a systematic review and meta-analysis. *Gut* 2004; **53**: 925-930 [PMID: 15194636]

7 **Greer SE**, Goodney PP, Sutton JE, Birkmeyer JD. Neoadjuvant chemoradiotherapy for esophageal carcinoma: a meta-analysis. *Surgery* 2005; **137**: 172-177 [PMID: 15674197 DOI: 10.1016/j.surg.2004.06.033]

8 **Gebski V**, Burmeister B, Smithers BM, Foo K, Zalcberg J, Simes J. Survival benefits from neoadjuvant chemoradiotherapy or chemotherapy in oesophageal carcinoma: a meta-analysis. *Lancet Oncol* 2007; **8**: 226-234 [PMID: 17329193 DOI: 10.1016/S1470-2045(07)70039-6]

9 **Xu XH**, Peng XH, Yu P, Xu XY, Cai EH, Guo P, Li K. Neoadjuvant chemotherapy for resectable esophageal carcinoma: a meta-analysis of randomized clinical trials. *Asian Pac J Cancer Prev* 2012; **13**: 103-110 [PMID: 22502650]

10 **Nguyen NP**, Krafft SP, Vinh-Hung V, Vos P, Almeida F, Jang S, Ceizyk M, Desai A, Davis R, Hamilton R, Modarresifar H, Abraham D, Smith-Raymond L. Feasibility of tomotherapy to reduce normal lung and cardiac toxicity for distal esophageal cancer compared to three-dimensional

radiotherapy. *Radiother Oncol* 2011; **101**: 438-442 [PMID: 21908064 DOI: 10.1016/j.radonc.2011.07.015]
11 **Blencowe NS**, McNair AG, Davis CR, Brookes ST, Blazeby JM. Standards of outcome reporting in surgical oncology: a case study in esophageal cancer. *Ann Surg Oncol* 2012; **19**: 4012-4018 [PMID: 22820935 DOI: 10.1245/s10434-012-2497-x]
12 **Dittrick GW**, Weber JM, Shridhar R, Hoffe S, Melis M, Almhanna K, Barthel J, McLoughlin J, Karl RC, Meredith KL. Pathologic nonresponders after neoadjuvant chemoradiation for esophageal cancer demonstrate no survival benefit compared with patients treated with primary esophagectomy. *Ann Surg Oncol* 2012; **19**: 1678-1684 [PMID: 22045465 DOI: 10.1245/s10434-011-2078-4]
13 **Vallböhmer D**, Hölscher AH, DeMeester S, DeMeester T, Salo J, Peters J, Lerut T, Swisher SG, Schröder W, Bollschweiler E, Hofstetter W. A multicenter study of survival after neoadjuvant radiotherapy/chemotherapy and esophagectomy for ypT0N0M0R0 esophageal cancer. *Ann Surg* 2010; **252**: 744-749 [PMID: 21037429 DOI: 10.1097/SLA.0b013e3181fb8dde]
14 **Quackenbush J**. Microarray analysis and tumor classification. *N Engl J Med* 2006; **354**: 2463-2472 [PMID: 16760446 DOI: 10.1056/NEJMra042342]
15 **Maher SG**, Gillham CM, Duggan SP, Smyth PC, Miller N, Muldoon C, O'Byrne KJ, Sheils OM, Hollywood D, Reynolds JV. Gene expression analysis of diagnostic biopsies predicts pathological response to neoadjuvant chemoradiotherapy of esophageal cancer. *Ann Surg* 2009; **250**: 729-737 [PMID: 19801928 DOI: 10.1097/SLA.0b013e3181bce7e1]
16 **Luthra R**, Wu TT, Luthra MG, Izzo J, Lopez-Alvarez E, Zhang L, Bailey J, Lee JH, Bresalier R, Rashid A, Swisher SG, Ajani JA. Gene expression profiling of localized esophageal carcinomas: association with pathologic response to preoperative chemoradiation. *J Clin Oncol* 2006; **24**: 259-267 [PMID: 16344314 DOI: 10.1200/JCO.2005.03.3688]
17 **Schauer M**, Janssen KP, Rimkus C, Raggi M, Feith M, Friess H, Theisen J. Microarray-based response prediction in esophageal adenocarcinoma. *Clin Cancer Res* 2010; **16**: 330-337 [PMID: 20028767 DOI: 10.1158/1078-0432.CCR-09-1673]
18 **Motoori M**, Takemasa I, Yamasaki M, Komori T, Takeno A, Miyata H, Takiguchi S, Fujiwara Y, Yasuda T, Yano M, Matsuura N, Matsubara K, Monden M, Mori M, Doki Y. Prediction of the response to chemotherapy in advanced esophageal cancer by gene expression profiling of biopsy samples. *Int J Oncol* 2010; **37**: 1113-1120 [PMID: 20878059 DOI: 10.3892/ijo_00000763]
19 **Duong C**, Greenawalt DM, Kowalczyk A, Ciavarella ML, Raskutti G, Murray WK, Phillips WA, Thomas RJ. Pretreatment gene expression profiles can be used to predict response to neoadjuvant chemoradiotherapy in esophageal cancer. *Ann Surg Oncol* 2007; **14**: 3602-3609 [PMID: 17896157 DOI: 10.1245/s10434-007-9550-1]
20 **van 't Veer LJ**, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, Peterse HL, van der Kooy K, Marton MJ, Witteveen AT, Schreiber GJ, Kerkhoven RM, Roberts C, Linsley PS, Bernards R, Friend SH. Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 2002; **415**: 530-536 [PMID: 11823860 DOI: 10.1038/415530a]
21 **Paik S**, Shak S, Tang G, Kim C, Baker J, Cronin M, Baehner FL, Walker MG, Watson D, Park T, Hiller W, Fisher ER, Wickerham DL, Bryant J, Wolmark N. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med* 2004; **351**: 2817-2826 [PMID: 15591335 DOI: 10.1056/NEJMoa041588]
22 **Lander ES,** Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W, Funke R, Gage D, Harris K, Heaford A, Howland J, Kann L, Lehoczky J, LeVine R, McEwan P, McKernan K, Meldrim J, Mesirov JP, Miranda C, Morris W, Naylor J, Raymond C, Rosetti M, Santos R, Sheridan A, Sougnez C, Stange-Thomann N, Stojanovic N, Subramanian A, Wyman D, Rogers J, Sulston J, Ainscough R, Beck S, Bentley D, Burton J, Clee C, Carter N, Coulson A, Deadman R, Deloukas P, Dunham A, Dunham I, Durbin R, French L, Grafham D, Gregory S, Hubbard T, Humphray S, Hunt A, Jones M, Lloyd C, McMurray A, Matthews L, Mercer S, Milne S, Mullikin JC, Mungall A, Plumb R, Ross M, Shownkeen R, Sims S, Waterston RH, Wilson RK, Hillier LW, McPherson JD, Marra MA, Mardis ER, Fulton LA, Chinwalla AT, Pepin KH, Gish WR, Chissoe SL, Wendl MC, Delehaunty KD, Miner TL, Delehaunty A, Kramer JB, Cook LL, Fulton RS, Johnson DL, Minx PJ, Clifton SW, Hawkins T, Branscomb E, Predki P, Richardson P, Wenning S, Slezak T, Doggett N, Cheng JF, Olsen A, Lucas S, Elkin C, Uberbacher E, Frazier M, Gibbs RA, Muzny DM, Scherer SE, Bouck JB, Sodergren EJ, Worley KC, Rives CM, Gorrell JH, Metzker ML, Naylor SL, Kucherlapati RS, Nelson DL, Weinstock GM, Sakaki Y, Fujiyama A, Hattori M, Yada T, Toyoda A, Itoh T, Kawagoe C, Watanabe H, Totoki Y, Taylor T, Weissenbach J, Heilig R, Saurin W, Artiguenave F, Brottier P, Bruls T, Pelletier E, Robert C, Wincker P, Smith DR, Doucette-Stamm L, Rubenfield M, Weinstock K, Lee HM, Dubois J, Rosenthal A, Platzer M, Nyakatura G, Taudien S, Rump A, Yang H, Yu J, Wang J, Huang G, Gu J, Hood L, Rowen L, Madan A, Qin S, Davis RW, Federspiel NA, Abola AP, Proctor MJ, Myers RM, Schmutz J, Dickson M, Grimwood J, Cox DR, Olson MV, Kaul R, Raymond C, Shimizu N, Kawasaki K, Minoshima S, Evans GA, Athanasiou M, Schultz R, Roe BA, Chen F, Pan H, Ramser J, Lehrach H, Reinhardt R, McCombie WR, de la Bastide M, Dedhia N, Blocker H, Hornischer K, Nordsiek G, Agarwala R, Aravind L, Bailey JA, Bateman A, Batzoglou S, Birney E, Bork P, Brown DG, Burge CB, Cerutti L, Chen HC, Church D, Clamp M, Copley RR, Doerks T, Eddy SR, Eichler EE, Furey TS, Galagan J, Gilbert JG, Harmon C, Hayashizaki Y, Haussler D, Hermjakob H, Hokamp K, Jang W, Johnson LS, Jones TA, Kasif S, Kaspryzk A, Kennedy S, Kent WJ, Kitts P, Koonin EV, Korf I, Kulp D, Lancet D, Lowe TM, McLysaght A, Mikkelsen T, Moran JV, Mulder N, Pollara VJ, Ponting CP, Schuler G, Schultz J, Slater G, Smit AF, Stupka E, Szustakowski J, Thierry-Mieg D, Thierry-Mieg J, Wagner L, Wallis J, Wheeler R, Williams A, Wolf YI, Wolfe KH, Yang SP, Yeh RF, Collins F, Guyer MS, Peterson J, Felsenfeld A, Wetterstrand KA, Patrinos A, Morgan MJ, de Jong P, Catanese JJ, Osoegawa K, Shizuya H, Choi S, Chen YJ; International Human Genome Sequencing C. Initial sequencing and analysis of the human genome. *Nature* 2001; **409:** 860-921 [PMID: 11237011 DOI: 10.1038/35057062]
23 **Venter JC**, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA, Gocayne JD, Amanatides P, Ballew RM, Huson DH, Wortman JR, Zhang Q, Kodira CD, Zheng XH, Chen L, Skupski M, Subramanian G, Thomas PD, Zhang J, Gabor Miklos GL, Nelson C, Broder S, Clark AG, Nadeau J, McKusick VA, Zinder N, Levine AJ, Roberts RJ, Simon M, Slayman C, Hunkapiller M, Bolanos R, Delcher A, Dew I, Fasulo D, Flanigan M, Florea L, Halpern A, Hannenhalli S, Kravitz S, Levy S, Mobarry C, Reinert K, Remington K, Abu-Threideh J, Beasley E, Biddick K, Bonazzi V, Brandon R, Cargill M, Chandramouliswaran I, Charlab R, Chaturvedi K, Deng Z, Di Francesco V, Dunn P, Eilbeck K, Evangelista C, Gabrielian AE, Gan W, Ge W, Gong F, Gu Z, Guan P, Heiman TJ, Higgins ME, Ji RR, Ke Z, Ketchum KA, Lai Z, Lei Y, Li Z, Li J, Liang Y, Lin X, Lu F, Merkulov GV, Milshina N, Moore HM, Naik AK, Narayan VA, Neelam B, Nusskern D, Rusch DB, Salzberg S, Shao W, Shue B, Sun J, Wang Z, Wang A, Wang X, Wang J, Wei M, Wides R, Xiao C, Yan C, Yao A, Ye J, Zhan M, Zhang W, Zhang H, Zhao Q, Zheng L, Zhong F, Zhong W, Zhu S, Zhao S, Gilbert D, Baumhueter S, Spier G, Carter C, Cravchik A, Woodage T,

Ali F, An H, Awe A, Baldwin D, Baden H, Barnstead M, Barrow I, Beeson K, Busam D, Carver A, Center A, Cheng ML, Curry L, Danaher S, Davenport L, Desilets R, Dietz S, Dodson K, Doup L, Ferriera S, Garg N, Gluecksmann A, Hart B, Haynes J, Haynes C, Heiner C, Hladun S, Hostin D, Houck J, Howland T, Ibegwam C, Johnson J, Kalush F, Kline L, Koduru S, Love A, Mann F, May D, McCawley S, McIntosh T, McMullen I, Moy M, Moy L, Murphy B, Nelson K, Pfannkoch C, Pratts E, Puri V, Qureshi H, Reardon M, Rodriguez R, Rogers YH, Romblad D, Ruhfel B, Scott R, Sitter C, Smallwood M, Stewart E, Strong R, Suh E, Thomas R, Tint NN, Tse S, Vech C, Wang G, Wetter J, Williams S, Williams M, Windsor S, Winn-Deen E, Wolfe K, Zaveri J, Zaveri K, Abril JF, Guigo R, Campbell MJ, Sjolander KV, Karlak B, Kejariwal A, Mi H, Lazareva B, Hatton T, Narechania A, Diemer K, Muruganujan A, Guo N, Sato S, Bafna V, Istrail S, Lippert R, Schwartz R, Walenz B, Yooseph S, Allen D, Basu A, Baxendale J, Blick L, Caminha M, Carnes-Stine J, Caulk P, Chiang YH, Coyne M, Dahlke C, Mays A, Dombroski M, Donnelly M, Ely D, Esparham S, Fosler C, Gire H, Glanowski S, Glasser K, Glodek A, Gorokhov M, Graham K, Gropman B, Harris M, Heil J, Henderson S, Hoover J, Jennings D, Jordan C, Jordan J, Kasha J, Kagan L, Kraft C, Levitsky A, Lewis M, Liu X, Lopez J, Ma D, Majoros W, McDaniel J, Murphy S, Newman M, Nguyen T, Nguyen N, Nodell M, Pan S, Peck J, Peterson M, Rowe W, Sanders R, Scott J, Simpson M, Smith T, Sprague A, Stockwell T, Turner R, Venter E, Wang M, Wen M, Wu D, Wu M, Xia A, Zandieh A, Zhu X. The sequence of the human genome. *Science* 2001; **291**: 1304-1351 [PMID: 11181995 DOI: 10.1126/science.1058040]

24 **Risch NJ**. Searching for genetic determinants in the new millennium. *Nature* 2000; **405**: 847-856 [PMID: 10866211 DOI: 10.1038/35015718]

25 **Glinsky GV**. Integration of HapMap-based SNP pattern analysis and gene expression profiling reveals common SNP profiles for cancer therapy outcome predictor genes. *Cell Cycle* 2006; **5**: 2613-2625 [PMID: 17172834]

26 **Wu X**, Gu J, Wu TT, Swisher SG, Liao Z, Correa AM, Liu J, Etzel CJ, Amos CI, Huang M, Chiang SS, Milas L, Hittelman WN, Ajani JA. Genetic variations in radiation and chemotherapy drug action pathways predict clinical outcomes in esophageal cancer. *J Clin Oncol* 2006; **24**: 3789-3798 [PMID: 16785472 DOI: 10.1200/JCO.2005.03.6640]

27 **Warnecke-Eberz U**, Vallböhmer D, Alakus H, Kütting F, Lurje G, Bollschweiler E, Wienand-Dorweiler A, Drebber U, Hölscher AH, Metzger R. ERCC1 and XRCC1 gene polymorphisms predict response to neoadjuvant radiochemotherapy in esophageal cancer. *J Gastrointest Surg* 2009; **13**: 1411-1421 [PMID: 19421825 DOI: 10.1007/s11605-009-0881-z]

28 **Houtsmuller AB**, Rademakers S, Nigg AL, Hoogstraten D, Hoeijmakers JH, Vermeulen W. Action of DNA repair endonuclease ERCC1/XPF in living cells. *Science* 1999; **284**: 958-961 [PMID: 10320375]

29 **Kim MK**, Cho KJ, Kwon GY, Park SI, Kim YH, Kim JH, Song HY, Shin JH, Jung HY, Lee GH, Choi KD, Kim SB. ERCC1 predicting chemoradiation resistance and poor outcome in oesophageal cancer. *Eur J Cancer* 2008; **44**: 54-60 [PMID: 17976974 DOI: 10.1016/j.ejca.2007.09.006]

30 **Schneider S**, Uchida K, Brabender J, Baldus SE, Yochim J, Danenberg KD, Salonga D, Chen P, Tsao-Wei D, Groshen S, Hoelscher AH, Schneider PM, Danenberg PV. Downregulation of TS, DPD, ERCC1, GST-Pi, EGFR, and HER2 gene expression after neoadjuvant three-modality treatment in patients with esophageal cancer. *J Am Coll Surg* 2005; **200**: 336-344 [PMID: 15737843 DOI: 10.1016/j.jamcollsurg.2004.10.035]

31 **Brabender J**, Vallböhmer D, Grimminger P, Hoffmann AC, Ling F, Lurje G, Bollschweiler E, Schneider PM, Hölscher AH, Metzger R. ERCC1 RNA expression in peripheral blood predicts minor histopathological response to neoadjuvant radio-chemotherapy in patients with locally advanced cancer of the esophagus. *J Gastrointest Surg* 2008; **12**: 1815-1821 [PMID: 18769985 DOI: 10.1007/s11605-008-0668-7]

32 **Ando Y**, Saka H, Ando M, Sawa T, Muro K, Ueoka H, Yokoyama A, Saitoh S, Shimokata K, Hasegawa Y. Polymorphisms of UDP-glucuronosyltransferase gene and irinotecan toxicity: a pharmacogenetic analysis. *Cancer Res* 2000; **60**: 6921-6926 [PMID: 11156391]

33 **Minami H**, Sai K, Saeki M, Saito Y, Ozawa S, Suzuki K, Kaniwa N, Sawada J, Hamaguchi T, Yamamoto N, Shirao K, Yamada Y, Ohmatsu H, Kubota K, Yoshida T, Ohtsu A, Saijo N. Irinotecan pharmacokinetics/pharmacodynamics and UGT1A genetic polymorphisms in Japanese: roles of UGT1A1*6 and *28. *Pharmacogenet Genomics* 2007; **17**: 497-504 [PMID: 17558305]

34 **Bhatti I**, Lee A, Lund J, Larvin M. Small RNA: a large contributor to carcinogenesis? *J Gastrointest Surg* 2009; **13**: 1379-1388 [PMID: 19373515 DOI: 10.1007/s11605-009-0887-6]

35 **Guo Y**, Chen Z, Zhang L, Zhou F, Shi S, Feng X, Li B, Meng X, Ma X, Luo M, Shao K, Li N, Qiu B, Mitchelson K, Cheng J, He J. Distinctive microRNA profiles relating to patient survival in esophageal squamous cell carcinoma. *Cancer Res* 2008; **68**: 26-33 [PMID: 18172293 DOI: 10.1158/0008-5472.CAN-06-4418]

36 **Hu Y**, Correa AM, Hoque A, Guan B, Ye F, Huang J, Swisher SG, Wu TT, Ajani JA, Xu XC. Prognostic significance of differentially expressed miRNAs in esophageal cancer. *Int J Cancer* 2011; **128**: 132-143 [PMID: 20309880 DOI: 10.1002/ijc.25330]

37 **Mathé EA**, Nguyen GH, Bowman ED, Zhao Y, Budhu A, Schetter AJ, Braun R, Reimers M, Kumamoto K, Hughes D, Altorki NK, Casson AG, Liu CG, Wang XW, Yanaihara N, Hagiwara N, Dannenberg AJ, Miyashita M, Croce CM, Harris CC. MicroRNA expression in squamous cell carcinoma and adenocarcinoma of the esophagus: associations with survival. *Clin Cancer Res* 2009; **15**: 6192-6200 [PMID: 19789312 DOI: 10.1158/1078-0432.CCR-09-1467]

38 **Akagi I**, Miyashita M, Ishibashi O, Mishima T, Kikuchi K, Makino H, Nomura T, Hagiwara N, Uchida E, Takizawa T. Relationship between altered expression levels of MIR21, MIR143, MIR145, and MIR205 and clinicopathologic features of esophageal squamous cell carcinoma. *Dis Esophagus* 2011; **24**: 523-530 [PMID: 21453382 DOI: 10.1111/j.1442-2050.2011.01177.x]

39 **Odenthal M**, Bollschweiler E, Grimminger PP, Schröder W, Brabender J, Drebber U, Hölscher AH, Metzger R, Vallböhmer D. MicroRNA profiling in locally advanced esophageal cancer indicates a high potential of miR-192 in prediction of multimodality therapy response. *Int J Cancer* 2013; **133**: 2454-2463 [PMID: 23649428 DOI: 10.1002/ijc.28253]

40 **Ko MA**, Zehong G, Virtanen C, Guindi M, Waddell TK, Keshavjee S, Darling GE. MicroRNA expression profiling of esophageal cancer before and after induction chemoradiotherapy. *Ann Thorac Surg* 2012; **94**: 1094-1102; discussion 1102-1103 [PMID: 22939244 DOI: 10.1016/j.athoracsur.2012.04.145]

41 **Hong L**, Han Y, Zhang H, Li M, Gong T, Sun L, Wu K, Zhao Q, Fan D. The prognostic and chemotherapeutic value of miR-296 in esophageal squamous cell carcinoma. *Ann Surg* 2010; **251**: 1056-1063 [PMID: 20485139 DOI: 10.1097/SLA.0b013e3181dd4ea9]

42 **Tanaka K**, Miyata H, Yamasaki M, Sugimura K, Takahashi T, Kurokawa Y, Nakajima K, Takiguchi S, Mori M, Doki Y. Circulating miR-200c levels significantly predict response to chemotherapy and prognosis of patients undergoing neoadjuvant chemotherapy for esophageal cancer. *Ann Surg Oncol* 2013; **20** Suppl 3: S607-S615 [PMID: 23838916 DOI: 10.1245/s10434-013-3093-4]

43 **Hamano R**, Miyata H, Yamasaki M, Kurokawa Y, Hara J,

Moon JH, Nakajima K, Takiguchi S, Fujiwara Y, Mori M, Doki Y. Overexpression of miR-200c induces chemoresistance in esophageal cancers mediated through activation of the Akt signaling pathway. *Clin Cancer Res* 2011; **17**: 3029-3038 [PMID: 21248297 DOI: 10.1158/1078-0432.CCR-10-2532]

44 **Lynam-Lennon N**, Reynolds JV, Marignol L, Sheils OM, Pidgeon GP, Maher SG. MicroRNA-31 modulates tumour sensitivity to radiation in oesophageal adenocarcinoma. *J Mol Med* ***(Berl)*** 2012; **90**: 1449-1458 [PMID: 22706599 DOI: 10.1007/s00109-012-0924-x]

45 **Zhang J**, Zhao H, Gao Y, Zhang W. Secretory miRNAs as novel cancer biomarkers. *Biochim Biophys Acta* 2012; **1826**: 32-43 [PMID: 22440944 DOI: 10.1016/j.bbcan.2012.03.001]

46 **Chen G**, Gharib TG, Huang CC, Taylor JM, Misek DE, Kardia SL, Giordano TJ, Iannettoni MD, Orringer MB, Hanash SM, Beer DG. Discordant protein and mRNA expression in lung adenocarcinomas. *Mol Cell Proteomics* 2002; **1**: 304-313 [PMID: 12096112]

47 **Varambally S**, Yu J, Laxman B, Rhodes DR, Mehra R, Tomlins SA, Shah RB, Chandran U, Monzon FA, Becich MJ, Wei JT, Pienta KJ, Ghosh D, Rubin MA, Chinnaiyan AM. Integrative genomic and proteomic analysis of prostate cancer reveals signatures of metastatic progression. *Cancer Cell* 2005; **8**: 393-406 [PMID: 16286247 DOI: 10.1016/j.ccr.2005.10.001]

48 **Gygi SP**, Rochon Y, Franza BR, Aebersold R. Correlation between protein and mRNA abundance in yeast. *Mol Cell Biol* 1999; **19**: 1720-1730 [PMID: 10022859]

49 **Uemura N**, Nakanishi Y, Kato H, Saito S, Nagino M, Hirohashi S, Kondo T. Transglutaminase 3 as a prognostic biomarker in esophageal cancer revealed by proteomics. *Int J Cancer* 2009; **124**: 2106-2115 [PMID: 19142970 DOI: 10.1002/ijc.24194]

50 **Hanash SM**, Pitteri SJ, Faca VM. Mining the plasma proteome for cancer biomarkers. *Nature* 2008; **452**: 571-579 [PMID: 18385731 DOI: 10.1038/nature06916]

51 **Cox J**, Mann M. Is proteomics the new genomics? *Cell* 2007; **130**: 395-398 [PMID: 17693247 DOI: 10.1016/j.cell.2007.07.032]

52 **Aichler M**, Elsner M, Ludyga N, Feuchtinger A, Zangen V, Maier SK, Balluff B, Schöne C, Hierber L, Braselmann H, Meding S, Rauser S, Zischka H, Aubele M, Schmitt M, Feith M, Hauck SM, Ueffing M, Langer R, Kuster B, Zitzelsberger H, Höfler H, Walch AK. Clinical response to chemotherapy in oesophageal adenocarcinoma patients is linked to defects in mitochondria. *J Pathol* 2013; **230**: 410-419 [PMID: 23592244 DOI: 10.1002/path.4199]

53 **Maher SG**, McDowell DT, Collins BC, Muldoon C, Gallagher WM, Reynolds JV. Serum proteomic profiling reveals that pretreatment complement protein levels are predictive of esophageal cancer patient response to neoadjuvant chemoradiation. *Ann Surg* 2011; **254**: 809-816; discussion 816-817 [PMID: 22005152 DOI: 10.1097/SLA.0b013e31823699f2]

54 **Fung ET**. A recipe for proteomics diagnostic test development: the OVA1 test, from biomarker discovery to FDA clearance. *Clin Chem* 2010; **56**: 327-329 [PMID: 20110452 DOI: 10.1373/clinchem.2009.140855]

55 **Kondo T**, Suehara Y, Kikuta K, Kubota D, Tajima T, Mukaihara K, Ichikawa H, Kawai A. Proteomic approach toward personalized sarcoma treatment: lessons from prognostic biomarker discovery in gastrointestinal stromal tumor. *Proteomics Clin Appl* 2013; **7**: 70-78 [PMID: 23281253 DOI: 10.1002/prca.201200085]

56 **Taylor CR**, Levenson RM. Quantification of immunohistochemistry--issues concerning methods, utility and semiquantitative assessment II. *Histopathology* 2006; **49**: 411-424 [PMID: 16978205 DOI: 10.1111/j.1365-2559.2006.02513.x]

57 **Al-Hajj M**, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA* 2003; **100**: 3983-3988 [PMID: 12629218 DOI: 10.1073/pnas.0530291100]

58 **Zhu L**, Gibson P, Currle DS, Tong Y, Richardson RJ, Bayazitov IT, Poppleton H, Zakharenko S, Ellison DW, Gilbertson RJ. Prominin 1 marks intestinal stem cells that are susceptible to neoplastic transformation. *Nature* 2009; **457**: 603-607 [PMID: 19092805 DOI: 10.1038/nature07589]

59 **O'Brien CA**, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature* 2007; **445**: 106-110 [PMID: 17122772 DOI: 10.1038/nature05372]

60 **Vermeulen L**, De Sousa E Melo F, van der Heijden M, Cameron K, de Jong JH, Borovski T, Tuynman JB, Todaro M, Merz C, Rodermond H, Sprick MR, Kemper K, Richel DJ, Stassi G, Medema JP. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. *Nat Cell Biol* 2010; **12**: 468-476 [PMID: 20418870 DOI: 10.1038/ncb2048]

61 **Phillips TM**, McBride WH, Pajonk F. The response of CD24(-/low)/CD44+ breast cancer-initiating cells to radiation. *J Natl Cancer Inst* 2006; **98**: 1777-1785 [PMID: 17179479 DOI: 10.1093/jnci/djj495]

62 **Todaro M**, Alea MP, Di Stefano AB, Cammareri P, Vermeulen L, Iovino F, Tripodo C, Russo A, Gulotta G, Medema JP, Stassi G. Colon cancer stem cells dictate tumor growth and resist cell death by production of interleukin-4. *Cell Stem Cell* 2007; **1**: 389-402 [PMID: 18371377 DOI: 10.1016/j.stem.2007.08.001]

63 **Smit JK**, Faber H, Niemantsverdriet M, Baanstra M, Bussink J, Hollema H, van Os RP, Plukker JT, Coppes RP. Prediction of response to radiotherapy in the treatment of esophageal cancer using stem cell markers. *Radiother Oncol* 2013; **107**: 434-441 [PMID: 23684587 DOI: 10.1016/j.radonc.2013.03.027]

64 **Yamamoto Y**, Yamai H, Seike J, Yoshida T, Takechi H, Furukita Y, Kajiura K, Minato T, Bando Y, Tangoku A. Prognosis of esophageal squamous cell carcinoma in patients positive for human epidermal growth factor receptor family can be improved by initial chemotherapy with docetaxel, fluorouracil, and cisplatin. *Ann Surg Oncol* 2012; **19**: 757-765 [PMID: 21947696 DOI: 10.1245/s10434-011-2071-y]

65 **Akamatsu M**, Matsumoto T, Oka K, Yamasaki S, Sonoue H, Kajiyama Y, Tsurumaru M, Sasai K. c-erbB-2 oncoprotein expression related to chemoradioresistance in esophageal squamous cell carcinoma. *Int J Radiat Oncol Biol Phys* 2003; **57**: 1323-1327 [PMID: 14630269 DOI: 10.1016/S0360-3016(03)00782-X]

66 **Arsenijevic T**, Micev M, Nikolic V, Gavrilovic D, Radulovic S, Pesko P. Is there a correlation between molecular markers and response to neoadjuvant chemoradiotherapy in locally advanced squamous cell esophageal cancer? *J BUON* 2012; **17**: 706-711 [PMID: 23335529]

67 **Schena M**, La Rovere E, Solerio D, Bustreo S, Barone C, Daniele L, Buffoni L, Bironzo P, Sapino A, Gasparri G, Ciuffreda L, Ricardi U. Neoadjuvant chemo-radiotherapy for locally advanced esophageal cancer: a monocentric study. *Tumori* 2012; **98**: 451-457 [PMID: 23052161 DOI: 10.1700/1146.12639]

68 **Vallböhmer D**, Lenz HJ. Predictive and prognostic molecular markers in outcome of esophageal cancer. *Dis Esophagus* 2006; **19**: 425-432 [PMID: 17069584 DOI: 10.1111/j.1442-2050.2006.00622.x]

69 **Kitamura K**, Saeki H, Kawaguchi H, Araki K, Ohno S, Kuwano H, Maehara Y, Sugimachi K. Immunohistochemical status of the p53 protein and Ki-67 antigen using biopsied specimens can predict a sensitivity to neoadjuvant therapy in patients with esophageal cancer. *Hepatogastroenterology* 2000; **47**: 419-423 [PMID: 10791203]

70 **Shimada Y**, Watanabe G, Yamasaki S, Maeda M, Kawabe A, Kaganoi JI, Itami A, Fukumoto M, Kanda Y, Imamura M. Histological response of cisplatin predicts patients' survival in oesophageal cancer and p53 protein accumulation in pretreatment biopsy is associated with cisplatin sensitivity. *Eur J Cancer* 2000; **36**: 987-993 [PMID: 10885602]

71 **Sarbia M**, Ott N, Pühringer-Oppermann F, Brücher BL. The

predictive value of molecular markers (p53, EGFR, ATM, CHK2) in multimodally treated squamous cell carcinoma of the oesophagus. *Br J Cancer* 2007; **97**: 1404-1408 [PMID: 17940507 DOI: 10.1038/sj.bjc.6604037]

72 **Zhang SS**, Huang QY, Yang H, Xie X, Luo KJ, Wen J, Cai XL, Yang F, Hu Y, Fu JH. Correlation of p53 status with the response to chemotherapy-based treatment in esophageal cancer: a meta-analysis. *Ann Surg Oncol* 2013; **20**: 2419-2427 [PMID: 23515910 DOI: 10.1245/s10434-012-2859-4]

73 **Kishi K**, Doki Y, Yano M, Yasuda T, Fujiwara Y, Takiguchi S, Kim S, Higuchi I, Monden M. Reduced MLH1 expression after chemotherapy is an indicator for poor prognosis in esophageal cancers. *Clin Cancer Res* 2003; **9**: 4368-4375 [PMID: 14555508]

74 **Nam TK**, Lee JH, Cho SH, Chung IJ, Ahn SJ, Song JY, Yoon MS, Chung WK, Nah BS. Low hMLH1 expression prior to definitive chemoradiotherapy predicts poor prognosis in esophageal squamous cell carcinoma. *Cancer Lett* 2008; **260**: 109-117 [PMID: 18053639 DOI: 10.1016/j.canlet.2007.10.026]

75 **Uehara H**, Miyamoto M, Kato K, Cho Y, Kurokawa T, Murakami S, Fukunaga A, Ebihara Y, Kaneko H, Hashimoto H, Murakami Y, Shichinohe T, Kawarada Y, Itoh T, Okushiba S, Kondo S, Katoh H. Deficiency of hMLH1 and hMSH2 expression is a poor prognostic factor in esophageal squamous cell carcinoma. *J Surg Oncol* 2005; **92**: 109-115 [PMID: 16231369 DOI: 10.1002/jso.20332]

76 **Alexander BM**, Wang XZ, Niemierko A, Weaver DT, Mak RH, Roof KS, Fidias P, Wain J, Choi NC. DNA repair biomarkers predict response to neoadjuvant chemoradiotherapy in esophageal cancer. *Int J Radiat Oncol Biol Phys* 2012; **83**: 164-171 [PMID: 22000749 DOI: 10.1016/j.ijrobp.2011.05.033]

77 **Langer R**, Feith M, Siewert JR, Wester HJ, Hoefler H. Expression and clinical significance of glucose regulated proteins GRP78 (BiP) and GRP94 (GP96) in human adenocarcinomas of the esophagus. *BMC Cancer* 2008; **8**: 70 [PMID: 18331622 DOI: 10.1186/1471-2407-8-70]

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

79 **Slotta-Huspenina J**, Wolff C, Drecoll E, Feith M, Bettstetter M, Malinowsky K, Bauer L, Becker K, Ott K, Höfler H, Becker KF, Langer R. A specific expression profile of heat-shock proteins and glucose-regulated proteins is associated with response to neoadjuvant chemotherapy in oesophageal adenocarcinomas. *Br J Cancer* 2013; **109**: 370-378 [PMID: 23839491 DOI: 10.1038/bjc.2013.319]

80 **Makuuchi Y**, Honda K, Osaka Y, Kato K, Kojima T, Daiko H, Igaki H, Ito Y, Hoshino S, Tachibana S, Watanabe T, Furuta K, Sekine S, Umaki T, Watabe Y, Miura N, Ono M, Tsuchida A, Yamada T. Soluble interleukin-6 receptor is a serum biomarker for the response of esophageal carcinoma to neoadjuvant chemoradiotherapy. *Cancer Sci* 2013; **104**: 1045-1051 [PMID: 23648090 DOI: 10.1111/cas.12187]

81 **Brabender J**, Metzger R, Vallböhmer D, Ling F, Neiss S, Bollschweiler E, Schneider PM, Hölscher AH, Grimminger PP. Roles of thymidylate synthase and dihydropyrimidine dehydrogenase expression in blood as predictors of response to multimodal therapy in esophageal cancer. *Surgery* 2012; **151**: 306-312 [PMID: 21982526 DOI: 10.1016/j.surg.2011.07.018]

82 **Sato H**, Tsubosa Y, Kawano T. Correlation between the pretherapeutic neutrophil to lymphocyte ratio and the pathologic response to neoadjuvant chemotherapy in patients with advanced esophageal cancer. *World J Surg* 2012; **36**: 617-622 [PMID: 22223293 DOI: 10.1007/s00268-011-1411-1]

83 **Noble F**, Hopkins J, Curtis N, Kelly JJ, Bailey IS, Byrne JP, Bateman AC, Bateman AR, Underwood TJ. The role of systemic inflammatory and nutritional blood-borne markers in predicting response to neoadjuvant chemotherapy and survival in oesophagogastric cancer. *Med Oncol* 2013; **30**: 596 [PMID: 23690267 DOI: 10.1007/s12032-013-0596-6]

84 **Hsu PK**, Chien LI, Huang CS, Hsieh CC, Wu YC, Hsu WH, Chou TY. Comparison of survival among neoadjuvant chemoradiation responders, non-responders and patients receiving primary resection for locally advanced oesophageal squamous cell carcinoma: does neoadjuvant chemoradiation benefit all? *Interact Cardiovasc Thorac Surg* 2013; **17**: 460-466 [PMID: 23728085 DOI: 10.1093/icvts/ivt216]

85 **Ando N**, Kato H, Igaki H, Shinoda M, Ozawa S, Shimizu H, Nakamura T, Yabusaki H, Aoyama N, Kurita A, Ikeda K, Kanda T, Tsujinaka T, Nakamura K, Fukuda H. A randomized trial comparing postoperative adjuvant chemotherapy with cisplatin and 5-fluorouracil versus preoperative chemotherapy for localized advanced squamous cell carcinoma of the thoracic esophagus (JCOG9907). *Ann Surg Oncol* 2012; **19**: 68-74 [PMID: 21879261 DOI: 10.1245/s10434-011-2049-9]

86 **Almhanna K**, Shridhar R, Meredith KL. Neoadjuvant or adjuvant therapy for resectable esophageal cancer: is there a standard of care? *Cancer Control* 2013; **20**: 89-96 [PMID: 23571699]

87 **Nakamura K**, Kato K, Igaki H, Ito Y, Mizusawa J, Ando N, Udagawa H, Tsubosa Y, Daiko H, Hironaka S, Fukuda H, Kitagawa Y. Three-arm phase III trial comparing cisplatin plus 5-FU (CF) versus docetaxel, cisplatin plus 5-FU (DCF) versus radiotherapy with CF (CF-RT) as preoperative therapy for locally advanced esophageal cancer (JCOG1109, NExT study). *Jpn J Clin Oncol* 2013; **43**: 752-755 [PMID: 23625063 DOI: 10.1093/jjco/hyt061]

88 **Hötte GJ**, Linam-Lennon N, Reynolds JV, Maher SG. Radiation sensitivity of esophageal adenocarcinoma: the contribution of the RNA-binding protein RNPC1 and p21-mediated cell cycle arrest to radioresistance. *Radiat Res* 2012; **177**: 272-279 [PMID: 22214381]

89 **Kurashige J**, Watanabe M, Iwatsuki M, Kinoshita K, Saito S, Nagai Y, Ishimoto T, Baba Y, Mimori K, Baba H. RPN2 expression predicts response to docetaxel in oesophageal squamous cell carcinoma. *Br J Cancer* 2012; **107**: 1233-1238 [PMID: 22955852 DOI: 10.1038/bjc.2012.396]

90 **Lepage C**, Rachet B, Jooste V, Faivre J, Coleman MP. Continuing rapid increase in esophageal adenocarcinoma in England and Wales. *Am J Gastroenterol* 2008; **103**: 2694-2699 [PMID: 18853967 DOI: 10.1111/j.1572-0241.2008.02191.x]

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

92 **Merkow RP**, Bilimoria KY, McCarter MD, Chow WB, Ko CY, Bentrem DJ. Use of multimodality neoadjuvant therapy for esophageal cancer in the United States: assessment of 987 hospitals. *Ann Surg Oncol* 2012; **19**: 357-364 [PMID: 21769460 DOI: 10.1245/s10434-011-1945-3]

93 **Bollschweiler E**, Metzger R, Drebber U, Baldus S, Vallböhmer D, Kocher M, Hölscher AH. Histological type of esophageal cancer might affect response to neo-adjuvant radiochemotherapy and subsequent prognosis. *Ann Oncol* 2009; **20**: 231-238 [PMID: 18836090 DOI: 10.1093/annonc/mdn622]

94 **Pennathur A**, Luketich JD. Resection for esophageal cancer: strategies for optimal management. *Ann Thorac Surg* 2008; **85**: S751-S756 [PMID: 18222210 DOI: 10.1016/j.athoracsur.2007.11.078]

95 **Pennathur A**, Zhang J, Chen H, Luketich JD. The "best operation" for esophageal cancer? *Ann Thorac Surg* 2010; **89**: S2163-S2167 [PMID: 20494003 DOI: 10.1016/j.athoracsur.2010.03.068]

96 **Hagen JA**, DeMeester SR, Peters JH, Chandrasoma P, De-

Meester TR. Curative resection for esophageal adenocarcinoma: analysis of 100 en bloc esophagectomies. *Ann Surg* 2001; **234**: 520-530; discussion 530-531 [PMID: 11573045]
97 **Orringer MB**, Marshall B, Iannettoni MD. Transhiatal esophagectomy: clinical experience and refinements. *Ann Surg* 1999; **230**: 392-400; discussion 400-403 [PMID: 10493486]
98 **Altorki N**, Kent M, Ferrara C, Port J. Three-field lymph node dissection for squamous cell and adenocarcinoma of the esophagus. *Ann Surg* 2002; **236**: 177-183 [PMID: 12170022 DOI: 10.1097/01.SLA.0000021583.51164.F4]
99 **Swanson SJ**, Batirel HF, Bueno R, Jaklitsch MT, Lukanich JM, Allred E, Mentzer SJ, Sugarbaker DJ. Transthoracic esophagectomy with radical mediastinal and abdominal lymph node dissection and cervical esophagogastrostomy for esophageal carcinoma. *Ann Thorac Surg* 2001; **72**: 1918-1924; discussion 1918-1924 [PMID: 11789772]
100 **Visbal AL**, Allen MS, Miller DL, Deschamps C, Trastek VF, Pairolero PC. Ivor Lewis esophagogastrectomy for esophageal cancer. *Ann Thorac Surg* 2001; **71**: 1803-1808 [PMID: 11426751]
101 **Hiranyatheb P,** Osugi H. Radical lymphadenectomy in esophageal cancer: from the past to the present. *Dis Esophagus* 2013 Jun 24; Epub ahead of print [PMID: 23796327 DOI: 10.1111/dote.12091]
102 **Wong J**, Weber J, Almhanna K, Hoffe S, Shridhar R, Karl R, Meredith KL. Extent of lymphadenectomy does not predict survival in patients treated with primary esophagectomy. *J Gastrointest Surg* 2013; **17**: 1562-1568; discussion 1569 [PMID: 23818125 DOI: 10.1007/s11605-013-2259-5]
103 **Stiles BM**, Nasar A, Mirza FA, Lee PC, Paul S, Port JL, Altorki NK. Worldwide Oesophageal Cancer Collaboration guidelines for lymphadenectomy predict survival following neoadjuvant therapy. *Eur J Cardiothorac Surg* 2012; **42**: 659-664 [PMID: 22491667 DOI: 10.1093/ejcts/ezs105]
104 **Akutsu Y**, Yasuda S, Nagata M, Izumi Y, Okazumi S, Shimada H, Nakatani Y, Tsujii H, Kamada T, Yamada S, Matsubara H. A phase I/II clinical trial of preoperative short-course carbon-ion radiotherapy for patients with squamous cell carcinoma of the esophagus. *J Surg Oncol* 2012; **105**: 750-755 [PMID: 22012645 DOI: 10.1002/jso.22127]
105 **Toomey PG**, Vohra NA, Ghansah T, Sarnaik AA, Pilon-Thomas SA. Immunotherapy for gastrointestinal malignancies. *Cancer Control* 2013; **20**: 32-42 [PMID: 23302905]
106 **Saijo N**. Critical comments for roles of biomarkers in the diagnosis and treatment of cancer. *Cancer Treat Rev* 2012; **38**: 63-67 [PMID: 21652149 DOI: 10.1016/j.ctrv.2011.02.004]

P- Reviewer: Goenka MK, Kim GH, Scherer A **S- Editor**: Ji FF
L- Editor: Roemmele A **E- Editor:** Lu YJ

World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4291/wjgp.v5.i3.335

World J Gastrointest Pathophysiol 2014 August 15; 5(3): 335-343
ISSN 2150-5330 (online)

REVIEW

Epidemiological studies of esophageal cancer in the era of genome-wide association studies

An-Hui Wang, Yuan Liu, Bo Wang, Yi-Xuan He, Ye-Xian Fang, Yong-Ping Yan

An-Hui Wang, Bo Wang, Yong-Ping Yan, Department of Epidemiology, School of Public Health, Fourth Military Medical University, Xi'an 710032, Shaanxi Province, China
Yuan Liu, Clinic of Xi'an Communication College, Xi'an 710032, Shaanxi Province, China
Yi-Xuan He, Ye-Xian Fang, Medical Student of Fourth Military Medical University, Xi'an 710032, Shaanxi Province, China
Author contributions: Wang AH contributed to the conception, design, editing and revision of the manuscript; Liu Y, He YX and Fang YX contributed to drafting the article; Wang B and Yan YP contributed to manuscript review and revision.
Correspondence to: An-Hui Wang, Associate Professor, Department of Epidemiology, School of Public Health, Fourth Military Medical University, No. 169 Changle West Road, Xi'an 710032, Shaanxi Province, China. wanganhui@hotmail.com
Telephone: +86-29-84774871 Fax: +86-29-84774876
Received: January 27, 2014 Revised: April 17, 2014
Accepted: May 31, 2014
Published online: August 15, 2014

Abstract

Esophageal cancer (EC) caused about 395000 deaths in 2010. China has the most cases of EC and EC is the fourth leading cause of cancer death in China. Esophageal squamous cell carcinoma (ESCC) is the predominant histologic type (90%-95%), while the incidence of esophageal adenocarcinoma (EAC) remains extremely low in China. Traditional epidemiological studies have revealed that environmental carcinogens are risk factors for EC. Molecular epidemiological studies revealed that susceptibility to EC is influenced by both environmental and genetic risk factors. Of all the risk factors for EC, some are associated with the risk of ESCC and others with the risk of EAC. However, the details and mechanisms of risk factors involved in the process for EC are unclear. The advanced methods and techniques used in human genome studies bring a great opportunity for researchers to explore and identify the details of those risk factors or susceptibility genes involved in the process of EC. Human genome epidemiology is a new branch of epidemiology, which leads the epidemiology study from the molecular epidemiology era to the era of genome wide association studies (GWAS). Here we review the epidemiological studies of EC (especially ESCC) in the era of GWAS, and provide an overview of the general risk factors and those genomic variants (genes, SNPs, miRNAs, proteins) involved in the process of ESCC.

Key words: Esophageal cancer; Epidemiology; Genome wide association study; Single nucleotide polymorphism; MicroRNA

Core tip: Epidemiological study methods advance as the science and technique progress. In the era of genome wide association studies (GWAS), human genome epidemiology (HuGE) provide a great chance for epidemiologists and clinical scientists to explore the cause of disease and evaluate genomic biomarkers for diagnosis or prognosis. More and more epidemiological studies use GWAS methods to analyze genomic variants and the association with esophageal cancer. Here we review epidemiological studies of esophageal cancer in the era of GWAS, and briefly introduce the case-control study and cohort study methods in HuGE studies.

Wang AH, Liu Y, Wang B, He YX, Fang YX, Yan YP. Epidemiological studies of esophageal cancer in the era of genome-wide association studies. *World J Gastrointest Pathophysiol* 2014; 5(3): 335-343 Available from: URL: http://www.wjgnet.com/2150-5330/full/v5/i3/335.htm DOI: http://dx.doi.org/10.4291/wjgp.v5.i3.335

INTRODUCTION

Esophageal cancer (EC) caused about 395000 deaths

Table 1 Major risk factors for esophageal cancer

Risk factor	Ref.
Cigarette smoking (tobacco use)	Fan *et al*[12], Oze *et al*[7]
Alcohol drinking (alcohol consumption)[1]	Oze *et al*[13], Fan *et al*[12], Islami *et al*[5]
Drinking hot tea or soup at high temperature	Wu *et al*[14]
Food mutagens	Yokokawa *et al*[15], Zhang *et al*[16]
Family history	Turati *et al*[9], Gao *et al*[17]
Nutritional deficiency	Tran *et al*[18]
Poor oral hygiene/ESCC	Dar *et al*[8]
Coffee consumption[2]	Naganuma *et al*[11]
HPV infection	Li *et al*[19], Cui *et al*[20]
Obesity	Chen *et al*[21]

[1]Alcohol consumption depends on the quantity of alcohol intake; [2]Coffee consumption: reverse relation. ESCC: Esophageal squamous cell carcinoma.

in 2010[1]. The incidence rate and mortality rate varied among different geographic and ethnic populations. China has the most cases of esophageal cancer. EC is the fourth leading cause of cancer associated death in China. Esophageal squamous cell carcinoma (ESCC) is the predominant histologic type (90%-95%), while the incidence of esophageal adenocarcinoma (EAC) remains extremely low in China.

Traditional epidemiological studies have identified that environmental carcinogens play a critical role in the process of EC. Molecular epidemiological studies revealed that susceptibility to EC is associated with both genomic and non-genomic factors and the interaction between genomic and non-genomic factors. Of all the factors, some are associated with ESCC and others with EAC. Human genome epidemiology (HuGE) is denoted as "an emerging field of inquiry that uses systematic applications of epidemiologic methods and approaches in population-based studies of the impact of human genetic variation on health and disease"[2]. HuGE emerged after the sequencing of human genome was accomplished[3,4]. The characteristic of HuGE is the techniques applied in studies, especially the technique of DNA microarray chips used in genome-wide association studies (GWAS). These techniques can compare millions of SNPs between genome DNA from cases and controls. In this review we focus on the epidemiological studies of EC in the era of GWAS.

GENERAL RISK FACTORS FOR EC

The incidence of EC is associated with age. More than 85% of EC patients were diagnosed at an age more than 55 years old. The incidence of EC in males is higher than that in females. Esophageal reflux disease (GERD) is a risk factor of EAC. GERD is also a risk factor for Barrett's esophagus (BE), and BE is associated with an increased risk for EC. Asian, especially Chinese, are more like to have an onset of EC than other populations.

Tobacco use (tobacco smoking, tobacco chewing, *etc.*) is a predominant risk factor for EC, especially ESCC. Alcohol drinking can also increase the risk of EC. Alcohol drinking is more likely to increase the risk of ESCC. People exposed to both tobacco use and alcohol had the risk of EC much more than those exposed to smoking or drinking alone. The risk of ESCC increased as the quantity of alcohol intake increased. The association between alcohol drinking and an increased risk of EC was more likely observed in Asian populations than in others[5]. Alcohol consumption and cigarette smoking are risk factors for ESCC in China and Japan[6,7].

Overweight or obese is associated with a higher risk of EAC. A diet with more fruits or/and vegetables is reported to reduce the risk of EC. On the contrary some diet habit may raise the risk for EC. Drinking very hot liquids frequently may increase the risk of ESCC. Overeating is the risk factor for EAC.

Infection with human papillomavirus (HPV) is associated with a number of cancers. HPV infection has been observed in about one-third of EC patients in Asia and South Africa.

Risk factors for EC varied among different countries, which may explain in part by the social-economic difference. The risk factors for EC are different between high- and low-incidence areas[6]. A study in Kashmir[8] recruited 703 cases and 1664 controls and found an inverse association between tooth cleaning and ESCC risk. A study based on a network of Italian and Swiss case-control studies found that a family history of oral and pharyngeal cancer was associated with an increased risk for EC[9]. In China individuals with a family history of EC were found to have an increased risk for EC[10]. The Miyagi Cohort Study found that people who drink one or more cups of coffee per day compared with those who did not drink have a lower risk of EC and oral pharyngeal cancer[11]. The major risk factors for EC are summarized in Table 1.

GENERAL VIEW OF EPIDEMIOLOGY IN THR EAR OF GWAS

Epidemiology studies in the era of GWAS are characterized by large sample size and the use of the technique of microarray. HuGE has advanced to the stage of GWAS[22-26]. Table 2 shows the genomic variants identified to be associated with ESCC. Some of those genetic variants was confirmed in other populations and some others were not identified in other populations. GWAS in China showed that variants in several chromosome regions conferred an increased risk of EC, but only genetic variants in alcohol-metabolizing genes were risk factors for ESCC in Japanese[6,22-26]. A 2-step GWAS including 1070 cases and 2836 controls identified that single nucleotide polymorphisms (SNPs) rs671, rs1229984, alcohol consumption, and tobacco use were risk factors for ESCC[23].

Genetic polymorphisms can affect the susceptibility

Table 2 Genomic variants identified to be associated with esophageal squamous cell carcinoma

Loci associated with ESCC	Method/design	Case sample size	Control sample size	Ref.
PLCE1 (10q23 rs227422) and C20orf54 (20p13)	GWAS	1077	1733	Wang *et al*[22]
ALDH2 (4q21-23, rs671) and ADH1B (12q24, rs1229984)	GWAS	1070	2836	Cui *et al*[23]
PLCE1 (10q23 rs2274223)	GWAS	2115	3302	Abnet *et al*[24]
ALDH2 (4q23, rs671) and ADH1B (12q24.11–13, rs1229984)	GWAS	1071	2762	Tanaka *et al*[25]
5q11 (rs10052657) 21q22 (rs2014300), 6p21 (rs10484761), 10q23 (rs2274223), and 12q24 (rs2074356, rs11066280)	GWAS	2031	2044	Wu *et al*[26]
CYP1A1 A2455G polymorphism(Ile/Val, rs1048943)	Meta-analysis (13 case-control studies)	1881	3786	Shen *et al*[27]
CYP1A1/CYP2E1	Case-control study	565 /482	468/466	Wang *et al*[28]
(MTHFR) C677T and A1298C polymorphisms with ESCC	Meta-analysis (15 case-control studies)	3213	4354	Fang *et al*[29]
rs1014867 polymorphisms in FAT4 gene	Case-control study	2139	2273	Du *et al*[30]
Interleukin 1B rs16944	Case-control study	380	380	Zheng *et al*[31]
CHRNA5-A3-B4 rs667282 TT/TG	Case-control study	866	952	Wang *et al*[32]
rs1494961, rs1229984 and rs1789924, and rs671	Case-control study	2139	2273	Gao *et al*[33]
Genetic variants in DNA repair pathway genes/(EGFR) signaling pathway	Case-control study	1942	2111	Li *et al*[34], Li *et al*[35]
Sex hormone metabolic genes	Case-control study	1026	1452	Hyland *et al*[36]
Chromosome 1 open reading frame 10 (C1orf10)	Case-control study	991	984	Zhang *et al*[37]

ESCC: Esophageal squamous cell carcinoma; GWAS: Genome wide association studies; PLCE1: Phospholipase C epsilon 1; C20orf54: Chromosome 20 open reading frame 54; ADH1B: Alcohol dehydrogenase; ALDH2: Acetaldehyde dehydrogenase.

to EC. Cytochrome P450 1A1 (CYP1A1) enzyme is a member of the CYP superfamily and prone to mutation, and an association between CYP1A1 enzyme activity and the risk of developing EC was revealed[38]. A meta-analysis uncovered that the A2455G polymorphism (Ile/Val, rs1048943) was a risk factor for EC[27]. By combining the technique of DNA microarray and epidemiology data of EC patients living in North or South China, the polymorphisms of CYP1A1 and CYP2E1 were studied[28]. In South area there was a significant association between CYP1A1 m2 polymorphism and EC. In North area there were significant associations between CYP2E1 Pst I and CYP2E Rsa I polymorphisms and EC. A significantly increased risk of ESCC was identified for smokers with the methylenetetrahydrofolate reductase (MTHFR) 677T allele[29]. MTHFR 677T and MTHFR 1298C conferred an increased risk for ESCC in Chinese population than in other populations. Four SNPs (rs1014867, rs12508222, rs1039808 and rs1567047) in FAT4 as potential risk factors for EC were studied[30]. The T allele of rs1014867 (Pro4972Ser) was associated with a reduced risk for EC[30]. The functional IL1B rs16944G > A polymorphism might be associated with the risk of ESCC and IL3 rs2073506 G > A polymorphism was a risk factor for ESCC with higher TNM stages[31]. CHRNA5-A3-B4 rs667282 TT/TG genotypes were risk factors of ESCC in Chinese[32]. In China, a case-control study including 2139 cases and 2,273 controls was carried out to evaluate the associations of six reported SNPs (rs1494961, rs1229984, rs1789924, rs971074, rs671 and rs4767364) with risk of ESCC. Results indicate that rs1494961, rs1229984, rs1789924, and rs671 may be used as biomarkers for ESCC[33]. Based on the SNPs identified in GWAS, 25 SNPs, 4 non-genomic factors (sex, age, tobacco use and alcohol drinking) and their associations with ESCC risk were studied[39]. Results indicate that genomic factors, none-genomic factors and their interactions can predict who are at high risk for ESCC. In contrast to association with a risk of ESCC in Asians, the PLCE1 rs2274223 and RFT2 13042395 SNPs were not associated with a risk of EC in Dutch Caucasians[40]. GWAS also identified three SNPs (rs10419226 in CRTC1, rs11789015 in BARX1 and rs2687201 near FOXP1) that were associated with a risk of EAC and BE[41].

GENOMIC VARIANTS IN PATHWAY GENES AND THEIR ASSOCIATIONS WITH EC

A GWAS aimed to explore the DNA repair pathway genes as risk factors for ESCC and GC was carried out[34]. One thousand six hundred and seventy-five SNPs were genotyped in cases (ESCC, GC) and controls from Shanxi and Linxian[34]. The DNA repair pathway genes were found to be risk factors for ESCC. CHEK2 was significantly associated with ESCC. Li *et al*[35] explored 3443 SNPs in genes involved in the EGFR signaling pathway in a study including 1942 ESCC cases, 1758 GC cases, and 2111 controls. Gene-level analyses found that GNAI3, CHRNE, PAK4, WASL, and ITCH were associated with a risk of ESCC[35]. A study analyzed 797 SNPs in 51 sex hormone metabolic genes in 1026 cases and 1452 controls[36]. Six genes including SULT2B1, CYP1B1, CYP3A7, CYP3A5, SHBG and CYP11A1 were identified as risk factors for ESCC[36]. Chromosome 1 open reading frame 10 (C1orf10), which is involved in heat shock and ethanol response, is either absent or down-regulated in ESCC tissues. Six strongly linked SNPs in a region of 7 kb were observed in a case-control study[37]. Compared

Table 3 MicroRNA expression and their associations with esophageal squamous cell carcinoma[45]

MiRNA	Compared to normal esophageal tissue	Proved targets
miR-10a	Decreased	HOXA3, HOXB1, HOXB3 HOXD4, HOXD10
miR-21	Increased	PCDCD4, NFIB, PTEN, TPM1
miR-93	Increased	FUSA, E2F1, TP53, INP1
miR-129	Increased	LATS2
miR-203	Increased/ decreased	ABL1, TP53INP1, SOCS3
miR-205	Decreased/increased	ZEB1, ZEB2, E2F5, HER3, ERBB3, PRKCE, LRP1
miR-375	Decreased	PDK1

with -1139GG, -1139CC genotype was a risk factor for ESCC[37].

The HuGE progressed form the discovery of novel genes or SNPs to the functional or mechanistic study of those genes or SNPs. Moreover, HuGE studies try to screen some of those genes, SNPs or miRNAs that are clinical treatment targets or biomarkers for diagnosis or prognosis. A low mtDNA copy number variant (CNV) was a risk factor for EAC[42]. A case-control study was carried out to analyze the relationship between SNPs (rs17417407, rs2274223 and rs22744224) in PLCE1 and susceptibility to ESCC[43]. Rs2274223G was identified to be a risk factor for ESCC, and rs2274224G was observed as a favorable factor for ESCC[43]. Phenotypes for rs17417407T, rs2274223G and rs2274224G were observed as risk factors for ESCC. Genomic polymorphisms in PLCE1 can affect the risk of ESCC in Chinese population exposed to tobacco smoking[43].

Zhang *et al*[37] found that there was an interaction between the -1139G/C genotype in C1orf10 and smoking, which increases the risk of ESCC. An HPV gene chip was used to detect HPV genotypes in 183 EC cases and 89 controls[20]. The frequency of seven HPV genotypes (16, 18, 35, 52, 6, 11, 43) in EC tissues was higher (31.7%) than that in controls (9.0%, $P < 0.001$), indicating that HPV infection was a risk factor for EC in Kazakh population. Moreover, heterozygote rs2274223 in PLCE1 was associated with an increased risk of HPV infection[20].

MICRORNAS AND THEIR ASSOCIATIONS WITH EC

MicroRNAs (miRNAs) are non-coding RNAs that modulate the translation of RNAs. MiRNAs have been involved in cancer initiation and development. Different miRNAs show differential expression levels in EC tissue or EC cell lines. The levels of miR-145 and miR-143 were decreased in ESCC tissues. An inverse association between miR-143 expression levels and cancer invasion or metastasis was identified[44]. Results showed that miR-143 may act as a suppressor in the process of ESCC. MiRNA microarray technique can be used to explore the profiles of miRNAs in ESCC cell lines. MiR-10a and MiR-205 were observed as potential specific biomarkers for ESCC (Table 3)[45].

Kan and Meltzer[46] reviewed miRNAs in BE and EAC. They surmised the following: (1) miRNA profiles were different between BE and EAC; (2) miR-196a is overexpressed in EAC tissues and is favorable to EAC cell survival; miR-196a might be a biomarker during the carcinogenesis from BE to EAC; and (3) the miR-106b-25 polycistron is involved in EC progression via suppression of p21 and Bim. The potential role of miRNAs in GC and EC and the mechanisms of action have been reviewed previously[47].

MiRNAs participate in the process of carcinogenesis by affecting the expression of genes to regulate cell apoptosis, proliferation and invasion. Some miRNAs have been proved to be associated with the characteristics of cancer or the survival time of patients, and those miRNAs might be valuable as biomarkers for diagnosis or prognosis prediction. A greater understanding of functions of miRNAs in EC could provide more details about the mechanisms of carcinogenesis (Table 4)[44,47,48].

A study explored the expression of miRNAs in ESCC and found that 15 miRNAs were down-regulated[48]. Four miRNAs (miR-145, miR-30a-3p, miR-133a and miR-133b) were decreased in ESCC and might act as tumor suppressors. Three miRNAs (miR-133b, miR-133a and miR-145) can directly inhibit FSCN1 expression, which might decrease the risk for ESCC[48]. A hospital based case-control study including 380 cases and 380 controls was carried out to observe the association of SNPs in miRNAs with genetic susceptibility to ESCC[49]. Female individuals or people who never smoke or drink have a lower risk for ESCC if they carry MiR-196a2 rs11614913 T > C[49]. Zhang *et al*[50] reported that up-regulation of miR-203 in EC cells can significantly increase apoptosis and decrease miR-21 expression. MiR-203 overexpression can also inhibit cell invasion, migration and proliferation, and may act as a tumor suppressor in EC.

CLINICAL RESEARCH OF GENOMIC BIOMARKERS FOR EC

EC is a disease with a poor prognosis[51]. It is urgent to identify valuable biomarkers involved in the diagnosis, progress or therapy targets for ESCC. Qi[52] reviewed the proteins, identified by proteomics, which were associated with the process of ESCC. The mechanisms of action of the proteins identified by proteomics and involved in the progress of ESCC were also discussed[53].

Loss of chromosome 19p is one of the most frequent allelic imbalances in ESCC. Down-regulation of DIRAS1 was associated with a poor survival rate. About 50% of ESCC cases had down-regulation of DIRAS1, and this down-regulation was associated with unfavorable clinical characteristics such as lymph node metastasis and low survival rate[53]. A GWAS observed the relationship between SNPs and the survival of ESCC

Table 4 Common miRNA expression profiles in esophageal cancer[47]

ESCC		EAC	
Up-regulated	Down-regulated	Up-regulated	Down-regulated
miR-21	Let-7c	miR-21	Let-7c
miR-155	miR-1	miR-28	miR-203
miR-93	MiR-99a	miR-3a-5p	miR-205
miR-129	miR-100	miR-143-145 cluster	miR-23a
	miR-133a	miR-192	miR-27a
	miR-143-145 cluster	miR-194	miR-27b
	miR-203	miR-215	miR-31
	miR-375		miR-99a
			miR-100

ESCC: Esophageal squamous cell carcinoma; EAC: Esophageal adenocarcinoma.

patients[54]. Results showed that SLC39A6 overexpression was associated with a shorter period of survival, which indicated that SLC39A6 might be a target for ESCC therapy[54]. HOTAIR, a well-known long non-coding RNA, has been reported to associate with ESCC. It was found that HOTAIR was overexpressed in ESCC compared to normal esophageal tissues[55,56]. Overexpression of HOTAIR was associated with poorer prognosis. The HOTAIR /WIF-1 axis was identified to play an important role in cell metastasis and might be a target for ESCC therapy. PIK3CA mutations in ESCC are associated with longer survival, suggesting its role as a prognostic biomarker[57]. Proteomic methods were used to evaluate proteins as potential biomarkers for ESCC[58], and 33 proteins overexpressed and 14 proteins down-regulated in ESCC were identified[58]. The expression of fos related antigen 1 (Fra-1) was identified as an unfavorable factor for prognosis[59]. The effect of SNPs of long intergenic non-coding RNAs on ESCC was studied by Wu *et al*[60]. 52 SNPs were studied in 1493 ESCC cases and 1553 controls in China[60]. Compared with the AA genotype of rs11752942, AG and GG reduced the risk of ESCC. Rs11752942G allele could significantly down-regulate the expression level of lincRNA-uc003opf.1[60]. These results indicated that rs11752942 in lincRNAuc003opf. 1 exon was a biomarker for susceptibility to ESCC. Sakai *et al*[61] reviewed the most recent studies on miRNAs in EC and/or BE. Four miRNAs were identified as diagnostic biomarkers and five miRNAs were supposed to be valuable biomarkers for diagnosis and prognosis. The progress in miRNAs identified in EC is exciting, but there is still a lot of work to be done before those miRNAs can be used as biomarkers for diagnosis, efficacy evaluation or prognosis prediction.

EPIDEMIOLOGICAL STUDY DESIGN IN THE ERA OF GWAS

The advantages and disadvantages of case-control and cohort studies in the era of GWAS have been previously discussed in detail[62]. The great majority of GWAS conducted to date have used the case-control design, in which genome or SNPs were compared between tissues from esophageal cancer patients or esophageal cancer free controls[22,26]. Other risk factors for EC were also investigated and analyzed to search for the genetic and environmental factors influencing EC. Case-control design not only allows to study multiple factors that might associate with disease, but also permits a more detailed evaluation of risk factor exposure, such as tobacco use, alcohol drinking, occupational, HPV infection, family history of EC or dietary history. However, there are several biases that are related with the selection of cases and controls. If cases can be representative of all persons who develop EC, the bias from case selection in a case-control study is limited. However, cases in most of the case-control studies are often hospital based, typically through review of medical records, and those with early death have great chance not to be included, leading to survival bias. Theoretically, controls should be representative of all persons at risk for EC. In fact, selecting controls in a case-control study is the most difficult aspect. The evaluation of risk factor exposure should avoid bias, which is related to measuring exposures. Case-control studies are often easier and cheaper to conduct than cohort studies.

The major merit of the cohort study is that recall bias is controlled by collecting exposure prior to disease outcome. Cases identified in cohort are incident and free of survival bias. Results of cohort studies can be used to explain the cause of disease. The disadvantages of cohort studies include the requirement of large sample size if the incidence of disease is low, expensive cost for genomic test, and long term follow-up[63]. Due to reasons of cost and efficiency fewer GWAS use cohort study design. More and more case-control studies were carried out with large sample sizes, to explore the genomic and environmental risk factors for EC[23,25].

GWAS use high-throughput microarray technologies to analyze genetic SNPs, miRNAs or proteins and evaluate their association with disease or with clinical utilities (biomarkers for diagnosis or prognosis). Since 2005, more than 100 loci for more than 40 diseases have been discovered and confirmed. Many SNPs were first observed to be associated with disease risk. GWAS have some advantages in identifying genetic variants associated with disease. GWAS also have some limitations, including type I and type II errors and biases due to poor representative of participants. Two step or multi-step GWAS are recommended in epidemiological case-control studies.

CONCLUSION

The flood of GWAS findings from case-control studies has led to the increasing need for subsequent confirmation and functional studies in experimental systems to identify the biological mechanisms of the association be-

tween genomic variants and EC. Epidemiological studies of EC in the era of GWAS have explored the genomic variants affecting signaling, epigenetic regulation, RNAs, proteins and pathways involved in cell proliferation or invasion. However, much work remains to be done including identifying the biomarkers for screening, efficacy evaluation and prognosis prediction. In the future, more and more epidemiological studies will take the advantages of population-based, very large sample-sized GWAS.

REFERENCES

1 **Lozano R**, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Barker-Collo S, Bartels DH, Bell ML, Benjamin EJ, Bennett D, Bhalla K, Bikbov B, Bin Abdulhak A, Birbeck G, Blyth F, Bolliger I, Boufous S, Bucello C, Burch M, Burney P, Carapetis J, Chen H, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahodwala N, De Leo D, Degenhardt L, Delossantos A, Denenberg J, Des Jarlais DC, Dharmaratne SD, Dorsey ER, Driscoll T, Duber H, Ebel B, Erwin PJ, Espindola P, Ezzati M, Feigin V, Flaxman AD, Forouzanfar MH, Fowkes FG, Franklin R, Fransen M, Freeman MK, Gabriel SE, Gakidou E, Gaspari F, Gillum RF, Gonzalez-Medina D, Halasa YA, Haring D, Harrison JE, Havmoeller R, Hay RJ, Hoen B, Hotez PJ, Hoy D, Jacobsen KH, James SL, Jasrasaria R, Jayaraman S, Johns N, Karthikeyan G, Kassebaum N, Keren A, Khoo JP, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Lipnick M, Lipshultz SE, Ohno SL, Mabweijano J, MacIntyre MF, Mallinger L, March L, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM, McAnulty JH, McDermott MM, McGrath J, Mensah GA, Merriman TR, Michaud C, Miller M, Miller TR, Mock C, Mocumbi AO, Mokdad AA, Moran A, Mulholland K, Nair MN, Naldi L, Narayan KM, Nasseri K, Norman P, O'Donnell M, Omer SB, Ortblad K, Osborne R, Ozgediz D, Pahari B, Pandian JD, Rivero AP, Padilla RP, Perez-Ruiz F, Perico N, Phillips D, Pierce K, Pope CA 3rd, Porrini E, Pourmalek F, Raju M, Ranganathan D, Rehm JT, Rein DB, Remuzzi G, Rivara FP, Roberts T, De León FR, Rosenfeld LC, Rushton L, Sacco RL, Salomon JA, Sampson U, Sanman E, Schwebel DC, Segui-Gomez M, Shepard DS, Singh D, Singleton J, Sliwa K, Smith E, Steer A, Taylor JA, Thomas B, Tleyjeh IM, Towbin JA, Truelsen T, Undurraga EA, Venketasubramanian N, Vijayakumar L, Vos T, Wagner GR, Wang M, Wang W, Watt K, Weinstock MA, Weintraub R, Wilkinson JD, Woolf AD, Wulf S, Yeh PH, Yip P, Zabetian A, Zheng ZJ, Lopez AD, Murray CJ, AlMazroa MA, Memish ZA. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 2012; **380**:2095–2128 [PMID: 23245604 DOI: 10.1016/S0140-6736(12)61728-0]

2 **Khoury MJ**, Dorman JS. The Human Genome Epidemiology Network. *Am J Epidemiol* 1998; **148**: 1-3 [PMID: 9663396]

3 **Lander ES**, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W, Funke R, Gage D, Harris K, Heaford A, Howland J, Kann L, Lehoczky J, LeVine R, McEwan P, McKernan K, Meldrim J, Mesirov JP, Miranda C, Morris W, Naylor J, Raymond C, Rosetti M, Santos R, Sheridan A, Sougnez C, Stange-Thomann N, Stojanovic N, Subramanian A, Wyman D, Rogers J, Sulston J, Ainscough R, Beck S, Bentley D, Burton J, Clee C, Carter N, Coulson A, Deadman R, Deloukas P, Dunham A, Dunham I, Durbin R, French L, Grafham D, Gregory S, Hubbard T, Humphray S, Hunt A, Jones M, Lloyd C, McMurray A, Matthews L, Mercer S, Milne S, Mullikin JC, Mungall A, Plumb R, Ross M, Shownkeen R, Sims S, Waterston RH, Wilson RK, Hillier LW, McPherson JD, Marra MA, Mardis ER, Fulton LA, Chinwalla AT, Pepin KH, Gish WR, Chissoe SL, Wendl MC, Delehaunty KD, Miner TL, Delehaunty A, Kramer JB, Cook LL, Fulton RS, Johnson DL, Minx PJ, Clifton SW, Hawkins T, Branscomb E, Predki P, Richardson P, Wenning S, Slezak T, Doggett N, Cheng JF, Olsen A, Lucas S, Elkin C, Uberbacher E, Frazier M, Gibbs RA, Muzny DM, Scherer SE, Bouck JB, Sodergren EJ, Worley KC, Rives CM, Gorrell JH, Metzker ML, Naylor SL, Kucherlapati RS, Nelson DL, Weinstock GM, Sakaki Y, Fujiyama A, Hattori M, Yada T, Toyoda A, Itoh T, Kawagoe C, Watanabe H, Totoki Y, Taylor T, Weissenbach J, Heilig R, Saurin W, Artiguenave F, Brottier P, Bruls T, Pelletier E, Robert C, Wincker P, Smith DR, Doucette-Stamm L, Rubenfield M, Weinstock K, Lee HM, Dubois J, Rosenthal A, Platzer M, Nyakatura G, Taudien S, Rump A, Yang H, Yu J, Wang J, Huang G, Gu J, Hood L, Rowen L, Madan A, Qin S, Davis RW, Federspiel NA, Abola AP, Proctor MJ, Myers RM, Schmutz J, Dickson M, Grimwood J, Cox DR, Olson MV, Kaul R, Raymond C, Shimizu N, Kawasaki K, Minoshima S, Evans GA, Athanasiou M, Schultz R, Roe BA, Chen F, Pan H, Ramser J, Lehrach H, Reinhardt R, McCombie WR, de la Bastide M, Dedhia N, Blöcker H, Hornischer K, Nordsiek G, Agarwala R, Aravind L, Bailey JA, Bateman A, Batzoglou S, Birney E, Bork P, Brown DG, Burge CB, Cerutti L, Chen HC, Church D, Clamp M, Copley RR, Doerks T, Eddy SR, Eichler EE, Furey TS, Galagan J, Gilbert JG, Harmon C, Hayashizaki Y, Haussler D, Hermjakob H, Hokamp K, Jang W, Johnson LS, Jones TA, Kasif S, Kaspryzk A, Kennedy S, Kent WJ, Kitts P, Koonin EV, Korf I, Kulp D, Lancet D, Lowe TM, McLysaght A, Mikkelsen T, Moran JV, Mulder N, Pollara VJ, Ponting CP, Schuler G, Schultz J, Slater G, Smit AF, Stupka E, Szustakowski J, Thierry-Mieg D, Thierry-Mieg J, Wagner L, Wallis J, Wheeler R, Williams A, Wolf YI, Wolfe KH, Yang SP, Yeh RF, Collins F, Guyer MS, Peterson J, Felsenfeld A, Wetterstrand KA, Patrinos A, Morgan MJ, de Jong P, Catanese JJ, Osoegawa K, Shizuya H, Choi S, Chen YJ; International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. *Nature* 2001; **409**: 860–921 [PMID: 11237011]

4 **Venter JC**, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA, Gocayne JD, Amanatides P, Ballew RM, Huson DH, Wortman JR, Zhang Q, Kodira CD, Zheng XH, Chen L, Skupski M, Subramanian G, Thomas PD, Zhang J, Gabor Miklos GL, Nelson C, Broder S, Clark AG, Nadeau J, McKusick VA, Zinder N, Levine AJ, Roberts RJ, Simon M, Slayman C, Hunkapiller M, Bolanos R, Delcher A, Dew I, Fasulo D, Flanigan M, Florea L, Halpern A, Hannenhalli S, Kravitz S, Levy S, Mobarry C, Reinert K, Remington K, Abu-Threideh J, Beasley E, Biddick K, Bonazzi V, Brandon R, Cargill M, Chandramouliswaran I, Charlab R, Chaturvedi K, Deng Z, Di Francesco V, Dunn P, Eilbeck K, Evangelista C, Gabrielian AE, Gan W, Ge W, Gong F, Gu Z, Guan P, Heiman TJ, Higgins ME, Ji RR, Ke Z, Ketchum KA, Lai Z, Lei Y, Li Z, Li J, Liang Y, Lin X, Lu F, Merkulov GV, Milshina N, Moore HM, Naik AK, Narayan VA, Neelam B, Nusskern D, Rusch DB, Salzberg S, Shao W, Shue B, Sun J, Wang Z, Wang A, Wang X, Wang J, Wei M, Wides R, Xiao C, Yan C, Yao A, Ye J, Zhan M, Zhang W, Zhang H, Zhao Q, Zheng L, Zhong F, Zhong W, Zhu S, Zhao S, Gilbert D, Baumhueter S, Spier G, Carter C, Cravchik A, Woodage T, Ali F, An H, Awe A, Baldwin D, Baden H, Barnstead M, Barrow I, Beeson K, Busam D, Carver A, Center A, Cheng ML, Curry L, Danaher S, Davenport L, Desilets R, Dietz S, Dodson K, Doup L, Ferriera S, Garg N, Gluecksmann A, Hart B, Haynes J, Haynes C, Heiner C, Hladun S, Hostin D, Houck J, Howland T, Ibegwam C,

Johnson J, Kalush F, Kline L, Koduru S, Love A, Mann F, May D, McCawley S, McIntosh T, McMullen I, Moy M, Moy L, Murphy B, Nelson K, Pfannkoch C, Pratts E, Puri V, Qureshi H, Reardon M, Rodriguez R, Rogers YH, Romblad D, Ruhfel B, Scott R, Sitter C, Smallwood M, Stewart E, Strong R, Suh E, Thomas R, Tint NN, Tse S, Vech C, Wang G, Wetter J, Williams S, Williams M, Windsor S, Winn-Deen E, Wolfe K, Zaveri J, Zaveri K, Abril JF, Guigó R, Campbell MJ, Sjolander KV, Karlak B, Kejariwal A, Mi H, Lazareva B, Hatton T, Narechania A, Diemer K, Muruganujan A, Guo N, Sato S, Bafna V, Istrail S, Lippert R, Schwartz R, Walenz B, Yooseph S, Allen D, Basu A, Baxendale J, Blick L, Caminha M, Carnes-Stine J, Caulk P, Chiang YH, Coyne M, Dahlke C, Mays A, Dombroski M, Donnelly M, Ely D, Esparham S, Fosler C, Gire H, Glanowski S, Glasser K, Glodek A, Gorokhov M, Graham K, Gropman B, Harris M, Heil J, Henderson S, Hoover J, Jennings D, Jordan C, Jordan J, Kasha J, Kagan L, Kraft C, Levitsky A, Lewis M, Liu X, Lopez J, Ma D, Majoros W, McDaniel J, Murphy S, Newman M, Nguyen T, Nguyen N, Nodell M, Pan S, Peck J, Peterson M, Rowe W, Sanders R, Scott J, Simpson M, Smith T, Sprague A, Stockwell T, Turner R, Venter E, Wang M, Wen M, Wu D, Wu M, Xia A, Zandieh A, Zhu X. The sequence of the human genome. *Science* 2001; **291**: 1304-1351 [PMID: 11181995]

5 **Islami F**, Fedirko V, Tramacere I, Bagnardi V, Jenab M, Scotti L, Rota M, Corrao G, Garavello W, Schüz J, Straif K, Negri E, Boffetta P, La Vecchia C. Alcohol drinking and esophageal squamous cell carcinoma with focus on light-drinkers and never-smokers: a systematic review and meta-analysis. *Int J Cancer* 2011; **129**: 2473-2484 [PMID: 21190191 DOI: 10.1002/ijc.25885]

6 **Lin Y**, Totsuka Y, He Y, Kikuchi S, Qiao Y, Ueda J, Wei W, Inoue M, Tanaka H. Epidemiology of esophageal cancer in Japan and China. *J Epidemiol* 2013; **23**: 233-242 [PMID: 23629646 DOI: 10.2188/jea.JE20120162]

7 **Oze I**, Matsuo K, Ito H, Wakai K, Nagata C, Mizoue T, Tanaka K, Tsuji I, Tamakoshi A, Sasazuki S, Inoue M, Tsugane S. Cigarette smoking and esophageal cancer risk: an evaluation based on a systematic review of epidemiologic evidence among the Japanese population. *Jpn J Clin Oncol* 2012; **42**: 63-73 [PMID: 22131340 DOI: 10.1093/jjco/hyr170]

8 **Dar NA**, Islami F, Bhat GA, Shah IA, Makhdoomi MA, Iqbal B, Rafiq R, Lone MM, Abnet CC, Boffetta P. Poor oral hygiene and risk of esophageal squamous cell carcinoma in Kashmir. *Br J Cancer* 2013; **109**: 1367-1372 [PMID: 23900216 DOI: 10.1038/bjc.2013.437]

9 **Turati F**, Edefonti V, Bosetti C, Ferraroni M, Malvezzi M, Franceschi S, Talamini R, Montella M, Levi F, Dal Maso L, Serraino D, Polesel J, Negri E, Decarli A, La Vecchia C. Family history of cancer and the risk of cancer: a network of case-control studies. *Ann Oncol* 2013; **24**: 2651-2656 [PMID: 23884440 DOI: 10.1093/annonc/mdt280]

10 **Wang AH**, Sun CS, Li LS, Huang JY, Chen QS, Xu DZ. Genetic susceptibility and environmental factors of esophageal cancer in Xi'an. *World J Gastroenterol* 2004; **10**: 940-944 [PMID: 15052670]

11 **Naganuma T**, Kuriyama S, Kakizaki M, Sone T, Nakaya N, Ohmori-Matsuda K, Nishino Y, Fukao A, Tsuji I. Coffee consumption and the risk of oral, pharyngeal, and esophageal cancers in Japan: the Miyagi Cohort Study. *Am J Epidemiol* 2008; **168**: 1425-1432 [PMID: 18974083 DOI: 10.1093/aje/kwn282]

12 **Fan Y**, Yuan JM, Wang R, Gao YT, Yu MC. Alcohol, tobacco, and diet in relation to esophageal cancer: the Shanghai Cohort Study. *Nutr Cancer* 2008; **60**: 354-363 [PMID: 18444169 DOI: 10.1080/01635580701883011]

13 **Oze I**, Matsuo K, Wakai K, Nagata C, Mizoue T, Tanaka K, Tsuji I, Sasazuki S, Inoue M, Tsugane S. Alcohol drinking and esophageal cancer risk: an evaluation based on a systematic review of epidemiologic evidence among the Japanese population. *Jpn J Clin Oncol* 2011; **41**: 677-692 [PMID: 21430021 DOI: 10.1093/jjco/hyr026]

14 **Wu M**, Zhao JK, Zhang ZF, Han RQ, Yang J, Zhou JY, Wang XS, Zhang XF, Liu AM, van' t Veer P, Kok FJ, Kampman E. Smoking and alcohol drinking increased the risk of esophageal cancer among Chinese men but not women in a high-risk population. *Cancer Causes Control* 2011; **22**: 649-657 [PMID: 21321789 DOI: 10.1007/s10552-011-9737-4]

15 **Yokokawa Y**, Ohta S, Hou J, Zhang XL, Li SS, Ping YM, Nakajima T. Ecological study on the risks of esophageal cancer in Ci-Xian, China: the importance of nutritional status and the use of well water. *Int J Cancer* 1999; **83**: 620-624 [PMID: 10521797]

16 **Zhang N**, Yu C, Wen D, Chen J, Ling Y, Terajima K, Akazawa K, Shan B, Wang S. Association of nitrogen compounds in drinking water with incidence of esophageal squamous cell carcinoma in Shexian, China. *Tohoku J Exp Med* 2012; **226**: 11-17 [PMID: 22146401]

17 **Gao Y**, Hu N, Han X, Giffen C, Ding T, Goldstein A, Taylor P. Family history of cancer and risk for esophageal and gastric cancer in Shanxi, China. *BMC Cancer* 2009; **9**: 269 [PMID: 19656375 DOI: 10.1186/1471-2407-9-269]

18 **Tran GD**, Sun XD, Abnet CC, Fan JH, Dawsey SM, Dong ZW, Mark SD, Qiao YL, Taylor PR. Prospective study of risk factors for esophageal and gastric cancers in the Linxian general population trial cohort in China. *Int J Cancer* 2005; **113**: 456-463 [PMID: 15455378]

19 **Li X**, Gao C, Yang Y, Zhou F, Li M, Jin Q, Gao L. Systematic review with meta-analysis: the association between human papillomavirus infection and oesophageal cancer. *Aliment Pharmacol Ther* 2014; **39**: 270-281 [PMID: 24308856 DOI: 10.1111/apt.12574]

20 **Cui X**, Chen Y, Liu L, Li L, Hu J, Yang L, Liang W, Li F. Heterozygote of PLCE1 rs2274223 increases susceptibility to human papillomavirus infection in patients with esophageal carcinoma among the Kazakh populations. *J Med Virol* 2014; **86**: 608-617 [PMID: 24127316 DOI: 10.1002/jmv.23775]

21 **Chen Q**, Zhuang H, Liu Y. The association between obesity factor and esophageal caner. *J Gastrointest Oncol* 2012; **3**: 226-231 [PMID: 22943013 DOI: 10.3978/j.issn.2078-6891.2012.026]

22 **Wang LD**, Zhou FY, Li XM, Sun LD, Song X, Jin Y, Li JM, Kong GQ, Qi H, Cui J, Zhang LQ, Yang JZ, Li JL, Li XC, Ren JL, Liu ZC, Gao WJ, Yuan L, Wei W, Zhang YR, Wang WP, Sheyhidin I, Li F, Chen BP, Ren SW, Liu B, Li D, Ku JW, Fan ZM, Zhou SL, Guo ZG, Zhao XK, Liu N, Ai YH, Shen FF, Cui WY, Song S, Guo T, Huang J, Yuan C, Huang J, Wu Y, Yue WB, Feng CW, Li HL, Wang Y, Tian JY, Lu Y, Yuan Y, Zhu WL, Liu M, Fu WJ, Yang X, Wang HJ, Han SL, Chen J, Han M, Wang HY, Zhang P, Li XM, Dong JC, Xing GL, Wang R, Guo M, Chang ZW, Liu HL, Guo L, Yuan ZQ, Liu H, Lu Q, Yang LQ, Zhu FG, Yang XF, Feng XS, Wang Z, Li Y, Gao SG, Qige Q, Bai LT, Yang WJ, Lei GY, Shen ZY, Chen LQ, Li EM, Xu LY, Wu ZY, Cao WK, Wang JP, Bao ZQ, Chen JL, Ding GC, Zhuang X, Zhou YF, Zheng HF, Zhang Z, Zuo XB, Dong ZM, Fan DM, He X, Wang J, Zhou Q, Zhang QX, Jiao XY, Lian SY, Ji AF, Lu XM, Wang JS, Chang FB, Lu CD, Chen ZG, Miao JJ, Fan ZL, Lin RB, Liu TJ, Wei JC, Kong QP, Lan Y, Fan YJ, Gao FS, Wang TY, Xie D, Chen SQ, Yang WC, Hong JY, Wang L, Qiu SL, Cai ZM, Zhang XJ. Genome-wide association study of esophageal squamous cellcarcinoma in Chinese subjects identifies susceptibility loci at PLCE1 and C20orf54. *Nat Genet* 2010; **42**: 759-763 [PMID: 20729853 DOI: 10.1038/ng.648]

23 **Cui R**, Kamatani Y, Takahashi A, Usami M, Hosono N, Kawaguchi T, Tsunoda T, Kamatani N, Kubo M, Nakamura Y, Matsuda K. Functional variants in ADH1B and ALDH2 coupled with alcohol and smoking synergistically enhance esophageal cancer risk. *Gastroenterology* 2009; **137**: 1768-1775 [PMID: 19698717 DOI: 10.1053/j.gastro.2009.07.070]

24 **Abnet CC**, Freedman ND, Hu N, Wang Z, Yu K, Shu XO, Yuan JM, Zheng W, Dawsey SM, Dong LM, Lee MP, Ding T, Qiao YL, Gao YT, Koh WP, Xiang YB, Tang ZZ, Fan JH, Wang C, Wheeler W, Gail MH, Yeager M, Yuenger J, Hutchinson A, Jacobs KB, Giffen CA, Burdett L, Fraumeni JF, Tucker MA, Chow WH, Goldstein AM, Chanock SJ, Taylor PR. A shared susceptibility locus in PLCE1 at 10q23 for gastric adenocarcinoma and esophageal squamous cell carcinoma. *Nat Genet* 2010; **42**: 764-767 [PMID: 20729852 DOI: 10.1038/ng.649]

25 **Tanaka F**, Yamamoto K, Suzuki S, Inoue H, Tsurumaru M, Kajiyama Y, Kato H, Igaki H, Furuta K, Fujita H, Tanaka T, Tanaka Y, Kawashima Y, Natsugoe S, Setoyama T, Tokudome S, Mimori K, Haraguchi N, Ishii H, Mori M. Strong interaction between the effects of alcohol consumption and smoking on oesophageal squamous cell carcinoma among individuals with ADH1B and/or ALDH2 risk alleles. *Gut* 2010; **59**: 1457-1464 [PMID: 20833657 DOI: 10.1136/gut.2009.205724]

26 **Wu C**, Hu Z, He Z, Jia W, Wang F, Zhou Y, Liu Z, Zhan Q, Liu Y, Yu D, Zhai K, Chang J, Qiao Y, Jin G, Liu Z, Shen Y, Guo C, Fu J, Miao X, Tan W, Shen H, Ke Y, Zeng Y, Wu T, Lin D. Genome-wide association study identifies three new susceptibility loci for esophageal squamous-cell carcinoma in Chinese populations. *Nat Genet* 2011; **43**: 679-684 [PMID: 21642993 DOI: 10.1038/ng.849]

27 **Shen FF**, Zhou FY, Xue QS, Pan Y, Zheng L, Zhang H, Wang LD, Zheng HF. Association between CYP1A1 polymorphisms and esophageal cancer: a meta-analysis. *Mol Biol Rep* 2013; **40**: 6035-6042 [PMID: 24065535 DOI: 10.1007/s11033-013-2713-1]

28 **Wang D**, Su M, Tian D, Liang S, Zhang J. Associations between CYP1A1 and CYP2E1 polymorphisms and susceptibility to esophageal cancer in Chaoshan and Taihang areas of China. *Cancer Epidemiol* 2012; **36**: 276-282 [PMID: 22088806 DOI: 10.1016/j.canep.2011.10.008]

29 **Fang Y**, Xiao F, An Z, Hao L. Systematic review on the relationship between genetic polymorphisms of methylenetetrahydrofolate reductase and esophageal squamous cell carcinoma. *Asian Pac J Cancer Prev* 2011; **12**: 1861-1866 [PMID: 22126580]

30 **Du J**, Ji J, Gao Y, Xu L, Xu J, Zhu C, Gu H, Jiang J, Li H, Ma H, Hu Z, Jin G, Guo W, Chen X, Shen H. Nonsynonymous polymorphisms in FAT4 gene are associated with the risk of esophageal cancer in an Eastern Chinese population. *Int J Cancer* 2013; **133**: 357-361 [PMID: 23319386 DOI: 10.1002/ijc.28033]

31 **Zheng L**, Yin J, Wang L, Wang X, Shi Y, Shao A, Tang W, Ding G, Liu C, Chen S, Gu H. Interleukin 1B rs16944 G> A polymorphism was associated with a decreased risk of esophageal cancer in a Chinese population. *Clin Biochem* 2013; **46**: 1469-1473 [PMID: 23726808 DOI: 10.1016/j.clinbiochem.2013.05.050]

32 **Wang Y**, Wu H, Liu Q, Wang C, Fu L, Wang H, Zhu W, Fu W, Lv Y, Wang S, Hu L. Association of CHRNA5-A3-B4 variation with esophageal squamous cell carcinoma risk and smoking behaviors in a Chinese population. *PLoS One* 2013; **8**: e67664 [PMID: 23844051 DOI: 10.1371/journal.pone.0067664]

33 **Gao Y**, He Y, Xu J, Xu L, Du J, Zhu C, Gu H, Ma H, Hu Z, Jin G, Chen X, Shen H. Genetic variants at 4q21, 4q23 and 12q24 are associated with esophageal squamous cell carcinoma risk in a Chinese population. *Hum Genet* 2013; **132**: 649-656 [PMID: 23430454 DOI: 10.1007/s00439-013-1276-5]

34 **Li WQ**, Hu N, Hyland PL, Gao Y, Wang ZM, Yu K, Su H, Wang CY, Wang LM, Chanock SJ, Burdett L, Ding T, Qiao YL, Fan JH, Wang Y, Xu Y, Shi JX, Gu F, Wheeler W, Xiong XQ, Giffen C, Tucker MA, Dawsey SM, Freedman ND, Abnet CC, Goldstein AM, Taylor PR. Genetic variants in DNA repair pathway genes and risk of esophageal squamous cell carcinoma and gastric adenocarcinoma in a Chinese population. *Carcinogenesis* 2013; **34**: 1536-1542 [PMID: 23504502 DOI: 10.1093/carcin/bgt094]

35 **Li WQ**, Hu N, Wang Z, Yu K, Su H, Wang L, Wang C, Chanock SJ, Burdett L, Ding T, Qiao YL, Fan JH, Wang Y, Xu Y, Giffen C, Xiong X, Murphy G, Tucker MA, Dawsey SM, Freedman ND, Abnet CC, Goldstein AM, Taylor PR. Genetic variants in epidermal growth factor receptor pathway genes and risk of esophageal squamous cell carcinoma and gastric cancer in a Chinese population. *PLoS One* 2013; **8**: e68999 [PMID: 23874846 DOI: 10.1371/journal.pone.0068999]

36 **Hyland PL**, Freedman ND, Hu N, Tang ZZ, Wang L, Wang C, Ding T, Fan JH, Qiao YL, Golozar A, Wheeler W, Yu K, Yuenger J, Burdett L, Chanock SJ, Dawsey SM, Tucker MA, Goldstein AM, Abnet CC, Taylor PR. Genetic variants in sex hormone metabolic pathway genes and risk of esophageal squamous cell carcinoma. *Carcinogenesis* 2013; **34**: 1062-1068 [PMID: 23358850 DOI: 10.1093/carcin/bgt030]

37 **Zhang W**, Chen X, Luo A, Lin D, Tan W, Liu Z. Genetic variants of C1orf10 and risk of esophageal squamous cell carcinoma in a Chinese population. *Cancer Sci* 2009; **100**: 1695-1700 [PMID: 19558548 DOI: 10.1111/j.1349-7006.2009.01240.x]

38 **Agundez JA**. Cytochrome P450 gene polymorphism and cancer. *Curr Drug Metab* 2004; **5**: 211-224 [PMID: 15180491]

39 **Chang J**, Huang Y, Wei L, Ma B, Miao X, Li Y, Hu Z, Yu D, Jia W, Liu Y, Tan W, He Z, Ke Y, Wu T, Shen H, Zeng Y, Wu C, Lin D. Risk prediction of esophageal squamous-cell carcinoma with common genetic variants and lifestyle factors in Chinese population. *Carcinogenesis* 2013; **34**: 1782-1786 [PMID: 23536576 DOI: 10.1093/carcin/bgt106]

40 **Dura P**, Bregitha CV, te Morsche RH, Roelofs HM, Kristinsson JO, Wobbes T, Witteman BJ, Tan AC, Drenth JP, Peters WH. GWAS-uncovered SNPs in PLCE1 and RFT2 genes are not implicated in Dutch esophageal adenocarcinoma and squamous cell carcinoma etiology. *Eur J Cancer Prev* 2013; **22**: 417-419 [PMID: 23222411 DOI: 10.1097/CEJ.0b013e32835c7f53]

41 **Levine DM**, Ek WE, Zhang R, Liu X, Onstad L, Sather C, Lao-Sirieix P, Gammon MD, Corley DA, Shaheen NJ, Bird NC, Hardie LJ, Murray LJ, Reid BJ, Chow WH, Risch HA, Nyrén O, Ye W, Liu G, Romero Y, Bernstein L, Wu AH, Casson AG, Chanock SJ, Harrington P, Caldas I, Debiram-Beecham I, Caldas C, Hayward NK, Pharoah PD, Fitzgerald RC, Macgregor S, Whiteman DC, Vaughan TL. A genome-wide association study identifies new susceptibility loci for esophageal adenocarcinoma and Barrett's esophagus. *Nat Genet* 2013; **45**: 1487-1493 [PMID: 24121790 DOI: 10.1038/ng.2796]

42 **Xu E**, Sun W, Gu J, Chow WH, Ajani JA, Wu X. Association of mitochondrial DNA copy number in peripheral blood leukocytes with risk of esophageal adenocarcinoma. *Carcinogenesis* 2013; **34**: 2521-2524 [PMID: 23803692 DOI: 10.1093/carcin/bgt230]

43 **Duan F**, Xie W, Cui L, Wang P, Song C, Qu H, Wang K, Zhang J, Dai L. Novel functional variants locus in PLCE1 and susceptibility to esophageal squamous cell carcinoma: based on published genome-wide association studies in a central Chinese population. *Cancer Epidemiol* 2013; **37**: 647-652 [PMID: 23688607 DOI: 10.1016/j.canep.2013.04.009]

44 **Ni Y**, Meng L, Wang L, Dong W, Shen H, Wang G, Liu Q, Du J. MicroRNA-143 functions as a tumor suppressor in human esophageal squamous cell carcinoma. *Gene* 2013; **517**: 197-204 [PMID: 23276710 DOI: 10.1016/j.gene.2012.12.031]

45 **Matsushima K**, Isomoto H, Kohno S, Nakao K. MicroRNAs and esophageal squamous cell carcinoma. *Digestion* 2010; **82**: 138-144 [PMID: 20588024 DOI: 10.1159/000310918]

46 **Kan T**, Meltzer SJ. MicroRNAs in Barrett's esophagus and esophageal adenocarcinoma. *Curr Opin Pharmacol* 2009; **9**: 727-732 [PMID: 19773200 DOI: 10.1016/j.coph.2009.08.009]

47 **Song JH**, Meltzer SJ. MicroRNAs in pathogenesis, diagno-

sis, and treatment of gastroesophageal cancers. *Gastroenterology* 2012; **143**: 35-47.e2 [PMID: 22580099]
48 **Kano M**, Seki N, Kikkawa N, Fujimura L, Hoshino I, Akutsu Y, Chiyomaru T, Enokida H, Nakagawa M, Matsubara H. miR-145, miR-133a and miR-133b: Tumor-suppressive miRNAs target FSCN1 in esophageal squamous cell carcinoma. *Int J Cancer* 2010; **127**: 2804-2814 [PMID: 21351259 DOI: 10.1002/ijc.25284]
49 **Wei J**, Zheng L, Liu S, Yin J, Wang L, Wang X, Shi Y, Shao A, Tang W, Ding G, Liu C, Chen S, Gu H. MiR-196a2 rs11614913 T & gt; C polymorphism and risk of esophageal cancer in a Chinese population. *Hum Immunol* 2013; **74**: 1199-1205 [PMID: 23792053 DOI: 10.1016/j.humimm.2013.06.012]
50 **Zhang F**, Yang Z, Cao M, Xu Y, Li J, Chen X, Gao Z, Xin J, Zhou S, Zhou Z, Yang Y, Sheng W, Zeng Y. MiR-203 suppresses tumor growth and invasion and down-regulates MiR-21 expression through repressing Ran in esophageal cancer. *Cancer Lett* 2014; **342**: 121-129 [PMID: 24001611 DOI: 10.1016/j.canlet.2013.08.037]
51 **Kim T**, Grobmyer SR, Smith R, Ben-David K, Ang D, Vogel SB, Hochwald SN. Esophageal cancer--the five year survivors. *J Surg Oncol* 2011; **103**: 179-183 [PMID: 21259254 DOI: 10.1002/jso.21784]
52 **Qi YJ**, Chao WX, Chiu JF. An overview of esophageal squamous cell carcinoma proteomics. *J Proteomics* 2012; **75**: 3129-3137 [PMID: 22564818 DOI: 10.1016/j.jprot.2012.04.025]
53 **Zhu YH**, Fu L, Chen L, Qin YR, Liu H, Xie F, Zeng T, Dong SS, Li J, Li Y, Dai Y, Xie D, Guan XY. Downregulation of the novel tumor suppressor DIRAS1 predicts poor prognosis in esophageal squamous cell carcinoma. *Cancer Res* 2013; **73**: 2298-2309 [PMID: 23436800 DOI: 10.1158/0008-5472.CAN-12-2663]
54 **Wu C**, Li D, Jia W, Hu Z, Zhou Y, Yu D, Tong T, Wang M, Lin D, Qiao Y, Zhou Y, Chang J, Zhai K, Wang M, Wei L, Tan W, Shen H, Zeng Y, Lin D. Genome-wide association study identifies common variants in SLC39A6 associated with length of survival in esophageal squamous-cell carcinoma. *Nat Genet* 2013; **45**: 632-638 [PMID: 23644492 DOI: 10.1038/ng.2638]
55 **Ge XS**, Ma HJ, Zheng XH, Ruan HL, Liao XY, Xue WQ, Chen YB, Zhang Y, Jia WH. HOTAIR, a prognostic factor in esophageal squamous cell carcinoma, inhibits WIF-1 expression and activates Wnt pathway. *Cancer Sci* 2013; **104**: 1675-1682 [PMID: 24118380 DOI: 10.1111/cas.12296]
56 **Li X**, Wu Z, Mei Q, Li X, Guo M, Fu X, Han W. Long non-coding RNA HOTAIR, a driver of malignancy, predicts negative prognosis and exhibits oncogenic activity in oesophageal squamous cell carcinoma. *Br J Cancer* 2013; **109**: 2266-2278 [PMID: 24022190 DOI: 10.1038/bjc.2013.548]
57 **Shigaki H**, Baba Y, Watanabe M, Murata A, Ishimoto T, Iwatsuki M, Iwagami S, Nosho K, Baba H. PIK3CA mutation is associated with a favorable prognosis among patients with curatively resected esophageal squamous cell carcinoma. *Clin Cancer Res* 2013; **19**: 2451-2459 [PMID: 23532889 DOI: 10.1158/1078-0432.CCR-12-3559]
58 **Zhang J**, Wang K, Zhang J, Liu SS, Dai L, Zhang JY. Using proteomic approach to identify tumor-associated proteins as biomarkers in human esophageal squamous cell carcinoma. *J Proteome Res* 2011; **10**: 2863-2872 [PMID: 21517111 DOI: 10.1021/pr200141c]
59 **Usui A**, Hoshino I, Akutsu Y, Sakata H, Nishimori T, Murakami K, Kano M, Shuto K, Matsubara H. The molecular role of Fra-1 and its prognostic significance in human esophageal squamous cell carcinoma. *Cancer* 2012; **118**: 3387-3396 [PMID: 22028113 DOI: 10.1002/cncr.26652]
60 **Wu H**, Zheng J, Deng J, Hu M, You Y, Li N, Li W, Lu J, Zhou Y. A genetic polymorphism in lincRNA-uc003opf.1 is associated with susceptibility to esophageal squamous cell carcinoma in Chinese populations. *Carcinogenesis* 2013; **34**: 2908-2917 [PMID: 23872665 DOI: 10.1093/carcin/bgt252]
61 **Sakai NS**, Samia-Aly E, Barbera M, Fitzgerald RC. A review of the current understanding and clinical utility of miRNAs in esophageal cancer. *Semin Cancer Biol* 2013; **23**: 512-521 [PMID: 24013023 DOI: 10.1016/j.semcancer.2013.08.005]
62 **Manolio T**. Case-control and cohort studies in the age of genome-wide associations. In: Khoury MJ, Bedrosian SR, Gwinn M, Higgins JPT, Ioannidis JPA, Little J, editors. Human genome epidemiology. Oxford: Oxford University Press, 2010: 100-119
63 **Pearson TA**, Manolio TA. How to interpret a genome-wide association study. *JAMA* 2008; **299**: 1335-1344 [PMID: 18349094 DOI: 10.1001/jama.299]

P- Reviewer: Bustamante-Balen M, Ding XW, Lisotti A
S- Editor: Ji FF **L- Editor**: Wang TQ **E- Editor:** Lu YJ

World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4291/wjgp.v5.i3.344

World J Gastrointest Pathophysiol 2014 August 15; 5(3): 344-354
ISSN 2150-5330 (online)

REVIEW

Perihilar cholangiocarcinoma: Current therapy

Wei Zhang, Lu-Nan Yan

Wei Zhang, Lu-Nan Yan, Department of Liver Surgery, Liver Transplantation Division, West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China
Author contributions: Zhang W and Yan LN contributed equally to this work; Zhang W and Yan LN designed and performed the research; ZW wrote the paper.
Correspondence to: Lu-Nan Yan, MD, PhD, Department of Liver Surgery, Liver Transplantation Division, West China Hospital, Sichuan University, Wuhou District, Chengdu 610041, Sichuan Province, China. yanlunan688@163.com
Telephone: +86-28-81812453 Fax: +86-28-85423724
Received: January 16, 20104 Revised: April 11, 2014
Accepted: June 10, 2014
Published online: August 15, 2014

Abstract

Perihilar cholangiocarcinoma, which is a rare primary malignancy, originates from the epithelial cells of the bile duct. Usually invading the periductal tissues and the lymph nodes, perihilar cholangiocarcinoma is commonly diagnosed in the advanced stage of the disease and has a dismal prognosis. Currently, complete hepatectomy is the primary therapy for curing this disease. Perioperative assessment and available surgical procedures can be considered for achieving a negative margin resection, which is associated with long-term survival and better quality of life. For patients with unresectable cholangiocarcinoma, several palliative treatments have been demonstrated to produce a better outcome; and liver transplantation for selected patients with perihilar cholangiocarcinoma is promising and desirable. However, the role of palliative treatments and liver transplantation was controversial and requires more evidence and substantial validity from multiple institutions. In this article, we summarize the data from multiple institutions and discuss the resectability, mortality, morbidity and outcome with different approaches.

Key words: Cholangiocarcinoma; Klatskin tumor; Surgery; Liver transplantation; Therapy

Core tip: Perihilar cholangiocarcinoma is a type of malignant tumor with vague and insidious symptoms, and is often diagnosed at an advanced stage. Currently, negative margin resection (R0) is the only way to cure patients with perihilar cholangiocarcinoma. In this article, we describe the surgical procedure and the criteria for operation and illustrate the palliative therapy and liver transplantation options for unresectable perihilar cholangiocarcinoma.

Zhang W, Yan LN. Perihilar cholangiocarcinoma: Current therapy. *World J Gastrointest Pathophysiol* 2014; 5(3): 344-354 Available from: URL: http://www.wjgnet.com/2150-5330/full/v5/i3/344.htm DOI: http://dx.doi.org/10.4291/wjgp.v5.i3.344

INTRODUCTION

Cholangiocarcinoma, which is a rare malignant tumor, constitutes less than 1% of all human malignancies[1]. The spectrum of cholangiocarcinoma is divided into three types, according to the anatomical location. Perihilar cholangiocarcinoma (PHC) is the most common type of the malignant tumor accounting for 50%-67% of all cases, followed by distal cholangiocarcinoma (DCCA) and intrahepatic cholangiocarcinoma (ICCA), which account for 27%-42% and 6%-8%, respectively[2,3]. When first described by Klatskin, PHC was commonly called Klatskin tumor[4]. Ben-Menachem summarized that the most common risk factors of PHC were liver flukes, primary sclerosing cholangitis, choledochal cysts, hepatolithiasis and cirrhosis, which account for 10% of the cases[5]. Patients with PHC are usually admitted to the hospital with severe painless jaundice and are diagnosed at an advanced stage, which means a poor prognosis and a shortened life span.

Complete resection is recognized as an effective therapy for many carcinomas. Similarly, resection has long

been demonstrated to be the best option for patients with PHC, and is associated with long-term survival and better quality of life[6]. PHC surgery was previously considered to be a challenge for hepatobiliary surgeons, because of the complex, intimate and variable anatomical relationship of the bile duct and vascular structures[7]. Because of the anatomical characters and the slow progression of the tumor, palliative procedures have been used to treat cancers involving the hepatic hilus, whereas definitive surgery can only be applied to a minority of patients with well-localized lesions[8]. From 1955 to 1973, Longmire collected 63 patients with extrahepatic cholangiocarcinoma (ECCA), and 34 of those patients had lesions that originated near the confluence of the hepatic duct. However, only six patients (18%) were likely candidates for hepatic resection. Guthrie *et al*[9] gathered 107 patients with ECCA divided into two periods, 1980-1985 and 1986-1991. They found that the overall resectablity rate (17%) was similar to that reported in other studies, while the use of percutaneous transhepatic cholangiography decreased and the use of endoscopic retrograde cholangiography increased in the second period. However, palliative treatments had unsatisfactory results and were associated with a high incidence of recurrent cholangitis and jaundice. Furthermore, the palliative approaches did not provide a method for curing the tumors; the techniques only served to relieve the symptoms of biliary obstruction.

With the development of radiology, oncology, liver transplantation and a better understanding of the pathways of tumor spread, surgical methods have recently improved significantly. Radical resection with a microscopically negative margin is believed to be the only way to cure patients with PHC. During recent decades, various surgical innovations and strategies have been introduced to achieve this goal. Currently, left or right hepatic resection, routine caudate lobe resection, lymphadenectomy, vascular resection and portal vein arterialization were promoted to improve outcome in patients with PHC. Nevertheless, for those patients who were not candidates for curative resection, several palliative treatments, such as chemotherapy, radiotherapy and photodynamic therapy, could be used to improve the quality of their life.

SURGERY

Staging and assessment of resectability

For various types of cancers, the American Joint Committee on Cancer (AJCC) TNM staging system is the most useful classification. The latest AJCC edition (7th edition) separates the ECCA into PHC and DCCA, which shows that the two subtypes have their own characteristics in pathology, treatment and prognosis. Based on the primary tumor (T), regional lymph nodes (N) and metastasis (M), the stage group is divided into 0-Ⅳ. Except for the "basic stage", the TNM classification has additional descriptions for residual tumor and histological grade. This classification is usually associated with the histological classification, also known as pathological staging, which is mostly used to stage tumors after surgical resection[10]. However, the majority of experts thought that the classification failed to indicate local respectability of the tumor and to distinguish between various surgical options, which limited the use of the staging system in the preoperative setting[11].

Proposed in the 1970s, the Bismuth-Corlette classification is the most useful stage system for predicting the resectability and for assessing the longitudinal intraductal extension of resection. Four types are classified according to the location and the longitudinal extension of the tumor in the biliary tree. Type Ⅰ lesions involve the common hepatic duct immediately below the confluence; Type Ⅱ lesions involve the hepatic bile duct confluence, that is beyond the confluence; Type Ⅲa and Ⅲb lesions occlude the common hepatic duct and either the right or the left hepatic duct, respectively; and Type Ⅳ lesions involve the confluence and both right and left hepatic ducts[12,13]. In Bismuth's opinion, Types Ⅰ and Ⅱ lesions would require only a local resection of the bile duct with a hepaticojejunostomy reconstruction, whereas the right or left hepatectomy for Type Ⅲa or Ⅲb lesions and hepatectomy plus liver transplantation for Type Ⅳ lesions, could be a contraindication for resection[13]. However, the Bismuth classification fails to describe the radical extension of the cancerous lesion and cannot provide complete information concerning vascular involvement and lymph node involvement, distant metastasis and liver atrophy. Thus, the staging system is primarily used as a convenient guideline for a surgical approach.

Combining the radial and longitudinal extensions of PHC, a preoperative clinical staging system was introduced by Jarnagin and Blumgart at Memorial Sloan-Kettering Cancer Center (MSKCC). This system, which was formally summarized and published in 2001, is also known as the T-staging system , and consists of local tumor extent, biliary duct, portal vein and hepatic lobar atrophy (Table 1)[14,15]. This system could be used to stratify patients preoperatively for the likelihood of respectability and to counsel patients on the potential for an R0 resection. In 2007, Chen *et al*[16] used this staging system to assess 85 patients with PHC. The 1-year survival rates of T1, T2 and T3 patients were 71.8%, 50.8% and 12.9%, respectively; whereas the 3-year survival rates were 34.4%, 18.2% and 0%, respectively[16]. The patients with PHC in the T1 and T2 stages were likely candidates for curative resection, whereas those in the T3 stage could not achieve R0 resection even if they had undergone resection[16]. Another retrospective test in 380 patients showed that the R0 resection rates for T1, T2 and T3 patients were 44.1%, 36.1% and 1.3%, respectively; whereas the median survival was 22.8, 23 and 10.8 mo, respectively[17]. Both surveys demonstrated that the T stage was associated with resectability and long-term survival. Moreover, the MSKCC provided the criteria for unresectable PHC, which included the following: locally

Table 1 Memorial sloan-kettering cancer center classification

Stage	Criteria
T1	Tumor involving biliary confluence ± unilateral extension to second-order biliary radicles.
T2	Tumor involving biliary confluence ± unilateral extension to second-order biliary radicles and ipsilateral portal vein involvement ± ipsilateral hepatic lobar atrophy
T3	Tumor involving biliary confluence + bilateral extension to second-order biliary radicles; or unilateral extension to second-order biliary radicles with contralateral portal vein involvement; or unilateral extension to second-order biliary radicles with contralateral hepatic lobar atrophy; or main or bilateral portal venous involvement

Table 2 Criteria for unresectability[15]

Patient factors
Medically unfit or otherwise unable to tolerate a major operation
Hepatic cirrhosis
Local tumor-related factors
Tumor extension to secondary biliary radicles bilaterally
Encasement or occlusion of the main portal vein proximal to its bifurcation
Atrophy of one hepatic lobe with contralateral portal vein branch encasement or occlusion
Atrophy of one hepatic lobe with contralateral tumor extension to secondary biliary radicles
Unilateral tumor extension to secondary biliary radicles with contralateral portal vein branch
encasement or occlusion
Metastatic disease
Histologically proven metastases to N2 lymph nodes[1]
Lung, liver, or peritoneal metastases

[1]Metastatic disease to peripancreatic, periduodenal, celiac, superior mesenteric, or posterior pancreaticoduodenal lymph nodes was considered to represent disease not amenable to a potentially curative resection. By contrast, metastatic disease to cystic duct, pericholedochal, hilar, or portal lymph nodes (*i.e.*, within the hepatoduodenal ligament) did not necessarily constitute unresectability.

advanced tumor extending bilaterally to the secondary biliary radicles, unilateral sectional bile ducts with contralateral portal vein branch involvement, encasement or occlusion of the primary portal vein proximal to its bifurcation, and atrophy of one hepatic lobe with contralateral tumor extension to sectional bile ducts (Table 2)[15].

A recent report indicated that a new system was designed by the international cholangiocarcinoma group, which incorporated the size of the tumor, the extent of the disease in the biliary system, the involvement of the hepatic artery and portal vein, the involvement of lymph nodes, distant metastases, and the volume of the putative remnant liver after resection[10]. Despite its comprehensiveness, this new classification must be validated and accepted.

We searched the key words "hilar cholangiocarcinoma", "Klatskin tumor" and "resection" using Pubmed and Medline, and we summarized the respectability and the outcomes from different institutions in different periods. The results of the surgical treatment are shown in Table 3[2,3,8,15,17-51]. Although the data were not fully calculated and were derived from tertiary referral centers, the number of patients with PHC who had undergone the resection was small, and only few large institutions contained more than 300 cases[42,50,51]. These findings attested to the rarity of this disease; additionally, these results indicated that the majority of patients lost the opportunity to undergo a curative operation when diagnosed, and therefore, these patients were not counted in the total number of study participants. Table 3 shows that the resectability rate was significantly variable, ranging from 28% to 95%, and that the curative resection rate ranged from 14% to 95%. This wide variability may be attributed to the differences in the sample content, the broad range of dates for inclusion, the characteristics of patients in different geographical areas, the methods of patient selection and the preoperative techniques in these studies.

Surgical procedures and strategies

In several reports, the surgical procedures were as follows: (1) preoperative biliary drainage was conducted to reduce the serum bilirubin concentration below 2 mg/dL; (2) preoperative percutaneous transhepatic portal embolization was performed when the volume of the liver remnant was estimated to be less than 40%; (3) the operative procedures for hilar resection were determined and planned using multidetector row computed tomography (MRCT); (4) the skeletonization of the portal vein and hepatic artery was performed using nodal clearance around the head of the pancreas; (5) portal vein resection and reconstruction were conducted before hepatic dissection if necessary; (6) frozen sections of the resected margins of the bile duct were investigated; and (7) lymph nodes in the hepatoduodenal ligament, around the head of pancreas and around the common hepatic artery were completely removed, whereas lymph nodes in the para-aortic region were removed, if possible, with a curative resection[44]. In other institutions, the surgical procedure included the hepatic artery resection, reconstruction and arterioportal shunt.

Obstructive jaundice, which is the most common symptom in patients with PHC, may increase the in-hospital mortality by 10% and is associated with many complications, such as bacterial translocation, malnutrition, renal insufficiency and postoperative liver dysfunction[52,53]. To avoid the risk of hepatic resection, preoperative biliary drainage (PBD) is recommended by many surgical teams. Percutaneous transhepatic biliary drainage (PTBD) had previously been widely used; however, several prospective randomized studies showed

Table 3 Results of surgical resection for perihilar cholangiocarcinoma

Ref.	Published year	Resections	Resectablity (%)	Negative margin (%)	Liver resection (%)	Morbidity	Mortality	5-yr survival rate (%)
Hadjis *et al*[18]	1990	27	NA	56	60	NA	7	22
Nakeeb *et al*[2]	1996	109	56	26	14	47	4	11
Su *et al*[19]	1996	49	28	49	57	47	10	15
Klempnauer *et al*[20]	1997	151	45	77	77	NA	10	28
Miyazaki *et al*[21]	1998	76	NA	71	86	34	13	26
Neuhaus *et al*[22]	1999	80	NA	61	85	55	8	22
Kosuge *et al*[8]	1999	65	73	52	80	37	9	33
Gerhards *et al*[23]	2000	112	NA	14	29	65	18	NA
Nimura *et al*[24]	2000	142	80	61	90	49	9	26
Todoroki *et al*[25]	2000	101	89	14	58	14	4	28
Jarnagin *et al*[15]	2001	80	50	78	78	64	10	26
Kawarada *et al*[27]	2002	65	89	64	75	28	2.3	26
Capussotti *et al*[26]	2002	36	NA	89	83	47	3	27
Kawasaki *et al*[28]	2003	79	75	68	87	14	1.3	22
Seyama *et al*[29]	2003	87	94	64	67	43	0	40
Rea *et al*[32]	2004	46	NA	80	100	52	9	26
Kondo *et al*[31]	2004	40	95	95	65	48	0	NA
I.Jitsma *et al*[30]	2004	42	NA	65	100	76	12	19
Hemming *et al*[33]	2005	53	50	80	98	40	9	35
Jarnagin *et al*[34]	2005	106	70	77	82	62	8	NA
Dinant *et al*[35]	2006	99	NA	31	38	66	15	27
DeOliveira *et al*[3]	2007	173	62	19	20	61	5	10
Ito *et al*[36]	2008	38	55	63	53	32	0	33
Konstadoulakis *et al*[37]	2008	59	81	68.6	86.4	25.5	6.8	34.9
Igami *et al*[41]	2010	298	70	74	98	43	2	42
Hirano *et al*[40]	2010	146	NA	87	87	44	3.4	35.5
Lee *et al*[42]	2010	302	86	70.9	89	43	1.7	32.5
Unno *et al*[44]	2010	125	NA	63.2	100	48.7	8	34.7
Ercolani *et al*[38]	2010	51	49.6	72.5	98	51	10	34.1
Shimizu *et al*[43]	2010	224	NA	69.1	78	47.6	10.7	30.3
Giuliante *et al*[39]	2010	43	29	77	93	52.5	6.9	36.1
Regimbeau *et al*[45]	2011	56	NA	76.9	100	72	8	NA
Young *et al*[48]	2012	83	92	42.2	93	62.7	7	20
Saxena *et al*[47]	2012	54	64	64.3	42	45.2	2.4	24
Ribero *et al*[46]	2012	82	NA	81.7	91.5	64.6	9.7	28
De Jong *et al*[50]	2012	305	NA	64.2	73	NA	10.6	20.2
Matsuo *et al*[17]	2012	157	78	76	90	59.2	7.6	37.5
Cheng *et al*[49]	2012	176	34	78.4	97	26.3	2.9	13.5
Nagino *et al*[51]	2013	574	76.1	76.5	96.7	57.3	4.7	32.5

NA: Not applicable.

that PTBD had no benefit in postoperative morbidity and mortality but increased potential risks, such as vascular injury, infectious complications and tumor seeding metastasis[54-56]. Currently, endoscopic nasobiliary drainage (ENBD) is performed instead of PTBD because of fewer complications and better outcomes. More recently, the Nagoya Institute demonstrated that unilateral ENBD of the future remnant lobe(s) exhibited a high success rate as an effective and suitable PBD method even in BC type Ⅲ to Ⅳ lesions[57]. To avoid the postoperative liver dysfunction resulting from extended hepatic resection, many institutions have promoted portal vein embolization (PVE) to increase volume of the future liver remnant (FLR). In several cautious surgical centers, when the FLR was 40% or less of the total liver volume, PVE was performed because the serum bilirubin level had decreased to less than 10 mg/dL[41,46]. Subsequently, surgery was performed after 2-4 wk of liver hypertrophy due to clonal expansion and cellular response[58].

When determining the surgical approach, the local excision, hepatectomy, and extended hepatectomy with or without caudate resection should be considered. In the Bismuth's opinion, Bismuth Type Ⅰ and Ⅱ would require only a local resection. Recently, bile duct resection alone without hepatectomy has been largely abandoned in favor of a more aggressive approach. Capussotti *et al*[59] conducted a systematic review of the effect of local resection compared with hepatectomy. In the pathologic aspect, the isolated bile duct cannot be adequately resected, because of the following: the necessity for wide surgical margins; neoplastic extension along the perineural sheaths and segment 1 neoplastic invasion. From another perspective, the R0 resection rate was higher after combined liver resection, although, in the earlier years of its application, local resection could be associated with fewer complications and shorter lengths of hospital stay[15,21,35]. In conclusion, according to this systematic review, local resection should only be scheduled for small papillary Klatskin tumors without bile duct confluence involvement confined to the bile duct wall[59]. Because

of the rarity and the advanced stage of the disease at the time of diagnosis, a local resection was rarely performed.

Despite the incomplete accuracy, the Bismuth classification initiated the idea of wider resection for PHC[13]. Table 3 shows that liver resection rates increased from 14% to 100% with an increased R0 resection rate. The common liver resection strategies are as follows: right or left hepatectomy (resection of hepatic segments 5, 6, 7, 8 or 2, 3, 4 ± 1), right or left hepatic trisectionectomy, also called extended right or left hepatectomy (resection of hepatic segments 4, 5, 6, 7, 8 or 2, 3, 4, 5, 8 ± 1), and central hepatectomy. Bisectionectomy or more was defined as a major hepatectomy; sectionectomy or less was defined as a minor hepatectomy[38]. Currently, for those patients with Bismuth type Ⅰ and Ⅱ, the right hepatectomy with caudate lobectomy was recommended, which has been demonstrated to decrease the rate of recurrence[29]. However, for those patients with Bismuth types Ⅲ and Ⅳ lesions, the approaches varied in different institutions. Recently, Cheng *et al*[49] reported 171 patients with PHC of Bismuth types Ⅲ and Ⅳ lesions. For Bismuth Type Ⅲ lesions, right, left or central hepatectomy with caudate lobectomy was performed. For Bismuth Ⅳ lesions, the right or left hepatectomy or extended right or left hepatectomy with caudate lobectomy was conducted to increase the negative margin rates. The choice of surgical side may depend on the predominance of the tumor; however, the right trisectionectomy is indicated for centrally located tumors because of the length of each hepatic duct, the location of the hilar common bile duct in the hepatoduodenal ligament, the ease of complete caudate lobectomy and portal vein reconstruction, and the frequent involvement of the right hepatic artery[7,28]. The left hepatectomy is considered to be a more complicated procedure than the right hepatectomy and requires greater skill, especially in cases involving portal vein resection and reconstruction. Moreover, preserving the right hepatic artery and the right portal vein could be an oncological problem with left or extended left resection, which could increase the tumor cell dissemination. Therefore, the rate of left hepatectomies is approximately 25%-30% of all resections[60]. In the study by Shimizu *et al*[43], the R0 resection was achieved in all 7 patients who underwent right trisectionectomy, but in only 8 (61.5%) of 13 patients who underwent left trisectionectomy. This finding suggests that a more extended resection from the right side, but not from the left side, may provide greater potential for curability. However, several authors believed that the left extended hepatectomy could achieve the same result. Nagino *et al*[51] analyzed the patients with PHC who underwent surgery and compared the surgical strategies in different periods (Table 4). From their experience, the incidence of left hepatic trisectionectomies gradually increased while the incidence of central hepatectomies decreased. Totally, the left or extended left hepatectomy represented nearly 55% of all of the resections performed on patients with PHC.

Nimura *et al*[61] introduced the concept of routine caudate lobectomy (CL). Bilateral biliary branches of the caudate lobe are confluent with the right hepatic duct, the left hepatic duct, the confluence of these and the right posterior hepatic duct. Therefore, the caudate lobe is usually involved in PHC in 40% to 98% of patients, which indicates a need for CL[61-63]. Moreover, routine CL combined with resection had high curative resectablity rates and increased the likelihood of long-term survival for patients with advanced stage PHC[49]. Similarly, Kow *et al*[64] showed that the patients with CL had a significantly better overall survival rate of 64.0 mo compared to the survival rate of 34.6 mo in type Ⅲ PHC patients in the group without CL. Although mechanisms for CL have not been established, the outcome remains optimistic while undertaking CL in PHC.

A major hepatectomy combined with pancreatoduodenectomy, for example, hepatopancreatoduodenectomy (HPD), was routinely used in the PHC surgery in several institutions. This procedure occupied 12.9% of the total surgery cases, and was indicated in the following cases: (1) diffusely infiltrating tumors of the entire extrahepatic bile duct; and (2) downward superficial spreading, or bulky nodal metastases of the pancreatoduodenal region (Table 4)[65]. Therefore, HPB provides an important method for treating spreading unresectable cholangiocarcinoma; thus, it is now the fourth standard procedure following hepatectomy, bile duct resection, and pancreatoduodenectomy[66].

In several high-volume samples, PHC was frequently reported to metastasize via the lymphatics in 24% to 75% of the patients[42,51]. Moreover, many authors had demonstrated that lymph node metastasis had a negative impact on survival in PHC[3,28,29,31,33,42,51]. Thereafter, lymphadenectomy played a crucial role in the outcome of patients with PHC. However, the 5-year survival rate is related to the location of the metastasis of the lymph node. Therefore, lymph node metastasis that is confined to the hepatic pedicle or the hepatoduodenal ligament is not a reason for abandoning resection. The tumor positive lymph nodes along the common hepatic artery or celiac axis are usually considered a contraindication for resection[7]. Kitagawa *et al*[67] showed that, in 110 patients after resection of PHC, there was a 5-year survival rate of 31%, if the lymph nodes were negative. However, in patients suffering from a local or a para-aortic lymph node infiltration, the 5-year survival rates were 15% and 12%, respectively. Interestingly, in the same report, 12% of the patients with positive para-aortic lymph nodes who lived more than 5 years were found to have macroscopically negative nodes in surgery[67]. Although the routine lymph node dissection beyond the hepatoduodenal ligament is not generally recommended, several authors still believe that lymph node dissection is beneficial.

Due to the intimate relationship between the bile duct and vessels, PHC could usually infiltrate the portal vein and hepatic artery. The indication for portal vein

Table 4 Surgery performed according to the time period[51] *n* (%)

	Total	Time period				*P*
		Earlier period		Later period		
		1997-1990	1991-2000	2001-2005	2006-2010	
Number of patients resected	574	72	116	168	218	
Resectability	574/754 (76.1)	72/93 (77.4)	116/148 (78.4)	168/216 (77.8)	218/297 (73.4)	0.406
Type of hepatectomy[1]						< 0.001
S1,4,5,6,7,8	43 (7.5)	5 (6.9)	11 (9.5)	4 (2.4)	23 (10.6)	
S1,5,6,7,8	177 (30.8)	17 (23.6)	40 (34.5)	53 (31.5)	67 (30.7)	
S1,2,3,4,5,8,	110 (19.2)	4 (5.6)	12 (10.3)	29 (17.3)	65 (29.8)	
S1,2,3,4	187 (32.6)	27 (37.5)	35 (30.2)	68 (40.5)	57 (26.1)	
S1,4,5,8/S1,5,8/S1,4/S1	38 (6.6)	13 (18.1)	10 (8.6)	11 (6.5)	4 (1.8)	
Without hepatectomy	19 (3.3)	6 (8.3)	8 (6.9)	3 (1.8)	2 (0.9)	
Combined resection						
Pancreatoduodenectomy	74 (12.9)	9 (12.5)	13 (11.2)	20 (11.9)	32 (14.7)	0.553
Portal vein resection	206 (35.9)	23 (31.9)	36 (31.0)	58 (34.5)	89 (40.8)	0.116
Wedge resection	36	15	6	10	5	
Segmental resection	170	8	30	48	84	
Hepatic artery resection	76 (13.2)	0	5 (4.3)	25 (14.9)	46 (21.1)	< 0.001
Operative time, min[2]	668 ± 134	664 ± 162	787 ± 170	675 ± 145	605 ± 134	< 0.001
Blood loss, mL[2]	2491 ± 2156	4414 ± 2791	3773 ± 3024	1898 ± 1268	1768 ± 1130	< 0.001
Homologous blood transfusion	271 (47.2)	68 (94.4)	93 (80.2)	46 (27.4)	64 (29.4)	< 0.001

Homologous blood includes packed red blood cell and fresh-frozen plasma. Note that P indicates the statistical difference between the earlier period (1977-2000) and the later period (2001-2010). [1]Expressed as Couinaud's hepatic segments resected; [2]Excluding 19 patients who did not undergo hepatectomy.

resection (PVR) and reconstruction for PHC is controversial. Previously, tumors involving the portal vein were considered unresectable. However, more recently, several surgeons have advocated this approach and its clinical benefit has been validated in many studies[22,28,29,31,33,42,50]. de Jong *et al*[50] reported the results of the analysis of an international, multicenter database from seven major hepatobiliary centers. They found that the PVR for PHC was associated with a greater risk for 30-d and 90-d perioperative mortality. Nevertheless, they thought that PVR should be undertaken, when necessary, to extirpate all of the disease because of its association with long-term survival in several patients with PHC[50]. Similarly, Nanigo recommended that PVR should be performed only when the vessel adhered to and could not be freed from the tumor during the skeletonization resection of the hepatoduodenal ligament and that PVR should not be performed as a routine procedure because it lacked scientific validation[68]. Because of the short distance between the tumor and the portal vein, Neuhaus *et al*[22] proposed a "no-touch" concept in 1999 and recommended routine PVR to achieve a wider distal radicality. Additionally, Neuhaus *et al*[69] proposed a survey to compare the effect of the "no-touch" resection with the traditional curative resection. The 5-year survival rate was significantly higher in the "no-touch" group at 58% compared to 29% in the traditional curative resection group ($P = 0.021$). However, this new technique has not been accepted by many institutions because it lacks scientific validation and more random studies are warranted for additional investigation.

In earlier reports, few institutions proposed the surgical strategy of hepatic resection combined with hepatic artery resection in patients with advanced PHC. In small samples, the outcome and survival rates were disappointing. Therefore, many authors did not recommend this surgical strategy[43,62,70]. Shimizu *et al*[43] showed that all of the nine patients undergoing left-sided hepatectomy combined with hepatic artery resection lived less than 3 years, and they considered that the hepatic artery resection was a primary prognostic factor (RR = 3.063; 95%CI: 1.289-7.282). However, in 2010, the Nagoya Institute reported their experiences with major hepatectomies with simultaneous resections and reconstructions of the portal vein and hepatic artery; the investigators showed that the challenging surgery could be performed with an acceptable mortality rate of 2% and offered a better likelihood of long-term survival with a 5-year survival rate of 30%[71]. Currently, the number of patients undergoing hepatic artery resection has been increasing (Table 4). In the institute's published data of 107 patients, the majority of patients (95%) underwent left-sided hepatectomies, of which 59% were left trisectionectomies and 36% were left hepatectomies. The overall mortality rate was 2.8% and the 5-year survival rate was 34.1%. The resected hepatic arteries were reconstructed primarily by end-to-end anastomosis, with an arterioportal shunt or an interposition graft using the radial artery or great saphenous vein[68]. For those patients who are unable to undergo hepatic artery reconstruction after resection, portal vein arterialization (PVA) could be a new approach. Using this method, adequate oxygen delivery to hepatocytes and biliary ducts can be assured. Moreover, several animal experiments showed that PVA could promote hepatic cell proliferation and enhance liver regeneration after extended hepatic resection[72]. The clini-

cal significance of hepatic artery resection is debatable, yet also promising and encouraging.

Morbidity and mortality

In Table 3, we summarize the morbidity and mortality which show significant variations, ranging from 14% to 76% and from 0% to 18%, respectively. Sano *et al*[73] defined the complications as major when they resulted in organ failure or required another surgery or interventional radiology, such as liver failure, lung failure and renal failure[51,73]. Complications that were classified as minor include pleural effusion necessitating thoracocentesis, wound infection, intra-abdominal infection with positive culture of the drainage fluid, delayed gastric emptying, anastomotic leakage, clinically silent pancreatic fistula with amylase-rich serous fluid or contaminated fluid with positive culture, and bile leakage from the raw surface of the liver healing spontaneously or responding to conservative management[73]. The most common complications observed in most institutions were infective complications, especially during earlier years of the use of these procedures, representing 50% or more of the observed complications[3,15,36]. Nagiono *et al*[51] compared the complications between the earlier years and the more recent years, and they demonstrated that the incidence of grade C liver failure, which is clinically serious, decreased markedly from 18.2% from 1977 to 1990 to 3.2% from 2006 to 2010. Wound sepsis was the second most common complication, followed by intra-abdominal abscess and bile leakage[51].

The operative mortality included all in-hospital deaths as defined by Sano. All postoperative complications that affected the outcome or lengthened the hospital stay were considered. Death may be associated with acute liver failure after extended right hepatectomy and combined portal vein resection, and sepsis with multiorgan failure[45]. Overall, these extended liver as well as vascular resections were found to be significant predictors of increased mortality[23]. In addition to liver function, operative time and blood loss may be associated with mortality[51]. Several reports have demonstrated that preoperative portal vein embolization may decrease mortality even with extended hepatectomy[73].

Outcomes and recurrence

The average 5-year survival rates after resection for PHC range from 11% to 42% (Table 3). Factors associated with favorable outcome include the following: R0 resection, no lymph node metastasis, absence of perineural and perivascular invasion, and well-differentiated histological grade. Complete resection with negative histologic margins is the only modifiable factor and, for that reason, the primary aim of surgical therapy. Recently, several reports demonstrated that patients undergoing R1 resection (microscopically positive margin) had a longer overall survival rate than patients with unresectable PHC[36,74]. Moreover, patients undergoing R0 resections with a margin less than 5 mm had the same survival rate as those patients undergoing R1 resections[29]. The surgeons were encouraged to perform more aggressive surgery to achieve a better outcome.

Few studies have analyzed recurrence patterns and time to recurrence in patients with PHC. In several reports, tumor recurrence rates can be as high as 50% to 76%, and the median time to recurrence rates has been reported to be 12 to 43 mo[36,47,75,76]. The most common site of recurrence is a local site, followed by the liver, lymph node, peritoneum and other organs. Only histologic grade was associated with recurrence-free survival[47]. Generally, the patients with recurrent disease are not candidates for curative therapy and can only receive adjuvant therapy to improve long-term outcome.

ORTHOTOPIC LIVER TRANSPLANTATION

Theoretically, orthotopic liver transplantation (OLT) offers the advantage of the resection of all of the structures that may be affected by tumor, for example, the portal vein, bilateral hepatic ducts and atrophic liver lobes. Compared to surgical resection, OLT has several advantages: (1) patients with Bismuth Ⅳ type lesions and peripheral vascular lesions cannot undergo resection; (2) patients with PHC arising from primary sclerosing cholangitis (PSC) will tolerate resection poorly because of the underlying liver impairment; (3) dissection in the hepatic hilum has the potential for causing spillage, which is an adverse prognostic factor; and (4) a clear circumferential margin is usually not achievable, which might increase the recurrence rates of PHC[77]. However, in the early years of the application of this procedure, the results were disappointing. The Cincinnati Transplant Tumor Registry collected global data between 1968 and 1997. The 1-, 2-, and 5-year survival rates were 72%, 48%, and 23%, respectively. Eighty four percent of the patients had a recurrence within 2 years of transplantation[78]. This undesirable result may have been associated with the unselected patients who had distant metastasis. Despite this finding, PHC was considered to be a relative contraindication to OLT due to the lack of organs. Interestingly, several investigations found that those patients with negative margins in transplantation and the absence of regional lymph node metastases had a better survival rate. Moreover, 22% of the patients receiving radiotherapy and chemotherapy alone had a 5-year survival, which inspired several surgeons to explore a new OLT approach for PHC.

From 1987 to 2000, Miyazaki *et al*[70] collected 17 patients who were treated with systemic chemotherapy and intraluminal bile duct irradiation as they awaited liver transplantation. Eleven patients underwent liver transplantation, and until 2000, five patients were alive without evidence of tumor recurrence with a median follow-up of 7.5 years (range, 2.8-14.5 years). In 1994, the Mayo Clinic developed a protocol employing preoperative chemoradiation therapy followed by liver transplantation, which showed encouraging results. Currently,

Table 5 Criteria for neoadjuvant therapy and liver transplantation[82]

Diagnosis of cholangiocarcinoma
Transcatheter biopsy or brush cytology
CA-19.9 > 100 mg/mL and/or a mass on cross-sectional imaging with a malignant appearing stricture on cholangiography
Biliary ploidy by FISH with a malignant appearing stricture on cholangiography
Unresectable tumor above cystic duct
Pancreatoduodenectomy for microscopic involvement of the common bile duct
Resectable cholangiocarcinoma arising in PSC
Radial tumor diameter ≤ 3 cm
Absence of intra- and extrahepatic metastases
Candidate for liver transplantation

PSC: Primary sclerosing cholangitis.

according to the Mayo Clinic protocol, patients receive EBRT (a target dose of 4500 cGy) with protracted venous infusion of 5-FU (225 mg/m^2 per day). Following this treatment, transcatheter iridium-192 brachytherapy (a target dose of 2000 cGy) is administered. Subsequently, the patients receive oral capecitabine (1000 mg/m^2 per day in two divided doses) until the time of OLT. Importantly, a staging laparotomy is performed on all of the patients before OLT to rule out metastatic disease. Only the patients with negative staging operations are eligible for transplantation[79]. Although there is a high dropout rate as patients await liver transplantation, the 5-year survival rate could achieve approximately 65% to 70%. However, the majority of patients undertaking OLT were diagnosed with PSC, and only 58% patients had histologically proven cancer which limited the use of OLT[80].

In 1996, Pichlmayr *et al*[81] proposed the indications for OLT in patients with PHC as follows: (1) unresectablity in presumed UICC stage Ⅱ confirmed by laparotomy; (2) status postresection with the intention for R0 with R or R2 positive resection margins due to advanced central tumor infiltration; and (3) local intrahepatic recurrence. After additional exploration and analysis of PHC, the Mayo Clinic proposed their criteria for neoadjuvant therapy and liver transplantation[82] (Table 5). These types of patients would be excluded if they had the following: (1) intrahepatic cholangiocarcinoma; (2) uncontrolled infection; (3) prior radiation or chemotherapy; (4) prior biliary resection or attempted resection; (5) intrahepatic metastases; (6) evidence of extrahepatic disease; (7) history of other malignancy within 5 years; and (8) transperitoneal biopsy[82]. Although the Mayo Clinic protocol has been accepted in the majority of institutions, the role of OLT requires additional substantial evidence and data confirmation from multiple institutions.

REFERENCES

1 **Siegel R**, Naishadham D, Jemal A. Cancer statistics, 2013. *CA Cancer J Clin* 2013; **63**: 11-30 [PMID: 23335087]
2 **Nakeeb A**, Pitt HA, Sohn TA, Coleman J, Abrams RA, Piantadosi S, Hruban RH, Lillemoe KD, Yeo CJ, Cameron JL. Cholangiocarcinoma. A spectrum of intrahepatic, perihilar, and distal tumors. *Ann Surg* 1996; **224**: 463-473; discussion 473-475 [PMID: 8857851]
3 **DeOliveira ML**, Cunningham SC, Cameron JL, Kamangar F, Winter JM, Lillemoe KD, Choti MA, Yeo CJ, Schulick RD. Cholangiocarcinoma: thirty-one-year experience with 564 patients at a single institution. *Ann Surg* 2007; **245**: 755-762 [PMID: 17457168 DOI: 10.1097/01.sla.0000251366.62632.d3]
4 **Klatskin G**. Adenocarcinoma of the hepatic duct at its bifurcation within the porta hepatis. an unusual tumor with distinctive clinical and pathological features. *Am J Med* 1965; **38**: 241-256 [PMID: 14256720]
5 **Ben-Menachem T**. Risk factors for cholangiocarcinoma. *Eur J Gastroenterol Hepatol* 2007; **19**: 615-617 [PMID: 17625428 DOI: 10.1097/MEG.0b013e328224b935]
6 **Launois B**, Campion JP, Brissot P, Gosselin M. Carcinoma of the hepatic hilus. Surgical management and the case for resection. *Ann Surg* 1979; **190**: 151-157 [PMID: 223508]
7 **Ramos E**. Principles of surgical resection in hilar cholangiocarcinoma. *World J Gastrointest Oncol* 2013; **5**: 139-146 [PMID: 23919108 DOI: 10.4251/wjgo.v5.i7.139]
8 **Kosuge T**, Yamamoto J, Shimada K, Yamasaki S, Makuuchi M. Improved surgical results for hilar cholangiocarcinoma with procedures including major hepatic resection. *Ann Surg* 1999; **230**: 663-671 [PMID: 10561090]
9 **Guthrie CM**, Haddock G, De Beaux AC, Garden OJ, Carter DC. Changing trends in the management of extrahepatic cholangiocarcinoma. *Br J Surg* 1993; **80**: 1434-1439 [PMID: 7504567]
10 **Deoliveira ML**, Schulick RD, Nimura Y, Rosen C, Gores G, Neuhaus P, Clavien PA. New staging system and a registry for perihilar cholangiocarcinoma. *Hepatology* 2011; **53**: 1363-1371 [PMID: 21480336 DOI: 10.1002/hep.24227]
11 **de Jong MC**, Hong SM, Augustine MM, Goggins MG, Wolfgang CL, Hirose K, Schulick RD, Choti MA, Anders RA, Pawlik TM. Hilar cholangiocarcinoma: tumor depth as a predictor of outcome. *Arch Surg* 2011; **146**: 697-703 [PMID: 21690446 DOI: 10.1001/archsurg.2011.122]
12 **Bismuth H**, Corlette MB. Intrahepatic cholangioenteric anastomosis in carcinoma of the hilus of the liver. *Surg Gynecol Obstet* 1975; **140**: 170-178 [PMID: 1079096]
13 **Bismuth H**, Nakache R, Diamond T. Management strategies in resection for hilar cholangiocarcinoma. *Ann Surg* 1992; **215**: 31-38 [PMID: 1309988]
14 **Burke EC**, Jarnagin WR, Hochwald SN, Pisters PW, Fong Y, Blumgart LH. Hilar Cholangiocarcinoma: patterns of spread, the importance of hepatic resection for curative operation, and a presurgical clinical staging system. *Ann Surg* 1998; **228**: 385-394 [PMID: 9742921]
15 **Jarnagin WR**, Fong Y, DeMatteo RP, Gonen M, Burke EC, Bodniewicz BS J, Youssef BA M, Klimstra D, Blumgart LH. Staging, resectability, and outcome in 225 patients with hilar cholangiocarcinoma. *Ann Surg* 2001; **234**: 507-517; discussion 517-519 [PMID: 11573044]

16 **Chen RF**, Li ZH, Zhou JJ, Wang J, Chen JS, Lin Q, Tang QB, Peng NF, Jiang ZP, Zhou QB. Preoperative evaluation with T-staging system for hilar cholangiocarcinoma. *World J Gastroenterol* 2007; **13**: 5754-5759 [PMID: 17963304]

17 **Matsuo K**, Rocha FG, Ito K, D'Angelica MI, Allen PJ, Fong Y, Dematteo RP, Gonen M, Endo I, Jarnagin WR. The Blumgart preoperative staging system for hilar cholangiocarcinoma: analysis of resectability and outcomes in 380 patients. *J Am Coll Surg* 2012; **215**: 343-355 [PMID: 22749003 DOI: 10.1016/j.jamcollsurg.2012.05.025]

18 **Hadjis NS**, Blenkharn JI, Alexander N, Benjamin IS, Blumgart LH. Outcome of radical surgery in hilar cholangiocarcinoma. *Surgery* 1990; **107**: 597-604 [PMID: 2162082]

19 **Su CH**, Tsay SH, Wu CC, Shyr YM, King KL, Lee CH, Lui WY, Liu TJ, P'eng FK. Factors influencing postoperative morbidity, mortality, and survival after resection for hilar cholangiocarcinoma. *Ann Surg* 1996; **223**: 384-394 [PMID: 8633917]

20 **Klempnauer J**, Ridder GJ, von Wasielewski R, Werner M, Weimann A, Pichlmayr R. Resectional surgery of hilar cholangiocarcinoma: a multivariate analysis of prognostic factors. *J Clin Oncol* 1997; **15**: 947-954 [PMID: 9060532]

21 **Miyazaki M**, Ito H, Nakagawa K, Ambiru S, Shimizu H, Shimizu Y, Kato A, Nakamura S, Omoto H, Nakajima N, Kimura F, Suwa T. Aggressive surgical approaches to hilar cholangiocarcinoma: hepatic or local resection? *Surgery* 1998; **123**: 131-136 [PMID: 9481397]

22 **Neuhaus P**, Jonas S, Bechstein WO, Lohmann R, Radke C, Kling N, Wex C, Lobeck H, Hintze R. Extended resections for hilar cholangiocarcinoma. *Ann Surg* 1999; **230**: 808-818; discussion 819 [PMID: 10615936]

23 **Gerhards MF**, van Gulik TM, de Wit LT, Obertop H, Gouma DJ. Evaluation of morbidity and mortality after resection for hilar cholangiocarcinoma--a single center experience. *Surgery* 2000; **127**: 395-404 [PMID: 10776430]

24 **Nimura Y**, Kamiya J, Kondo S, Nagino M, Uesaka K, Oda K, Sano T, Yamamoto H, Hayakawa N. Aggressive preoperative management and extended surgery for hilar cholangiocarcinoma: Nagoya experience. *J Hepatobiliary Pancreat Surg* 2000; **7**: 155-162 [PMID: 10982608 DOI: 10.1007/s005340000070155.534]

25 **Todoroki T**, Kawamoto T, Koike N, Takahashi H, Yoshida S, Kashiwagi H, Takada Y, Otsuka M, Fukao K. Radical resection of hilar bile duct carcinoma and predictors of survival. *Br J Surg* 2000; **87**: 306-313 [PMID: 10718799 DOI: 10.1046/j.1365-2168.2000.01343.x]

26 **Capussotti L**, Muratore A, Polastri R, Ferrero A, Massucco P. Liver resection for hilar cholangiocarcinoma: in-hospital mortality and longterm survival. *J Am Coll Surg* 2002; **195**: 641-647 [PMID: 12437251]

27 **Kawarada Y**, Das BC, Naganuma T, Tabata M, Taoka H. Surgical treatment of hilar bile duct carcinoma: experience with 25 consecutive hepatectomies. *J Gastrointest Surg* 2002; **6**: 617-624 [PMID: 12127130]

28 **Kawasaki S**, Imamura H, Kobayashi A, Noike T, Miwa S, Miyagawa S. Results of surgical resection for patients with hilar bile duct cancer: application of extended hepatectomy after biliary drainage and hemihepatic portal vein embolization. *Ann Surg* 2003; **238**: 84-92 [PMID: 12832969 DOI: 10.1097/01.sla.0000074984.83031.02]

29 **Seyama Y**, Kubota K, Sano K, Noie T, Takayama T, Kosuge T, Makuuchi M. Long-term outcome of extended hemihepatectomy for hilar bile duct cancer with no mortality and high survival rate. *Ann Surg* 2003; **238**: 73-83 [PMID: 12832968 DOI: 10.1097/01.sla.0000074960.55004.72]

30 **IJitsma AJ**, Appeltans BM, de Jong KP, Porte RJ, Peeters PM, Slooff MJ. Extrahepatic bile duct resection in combination with liver resection for hilar cholangiocarcinoma: a report of 42 cases. *J Gastrointest Surg* 2004; **8**: 686-694 [PMID: 15358329 DOI: 10.1016/j.gassur.2004.04.006]

31 **Kondo S**, Hirano S, Ambo Y, Tanaka E, Okushiba S, Morikawa T, Katoh H. Forty consecutive resections of hilar cholangiocarcinoma with no postoperative mortality and no positive ductal margins: results of a prospective study. *Ann Surg* 2004; **240**: 95-101 [PMID: 15213624]

32 **Rea DJ**, Munoz-Juarez M, Farnell MB, Donohue JH, Que FG, Crownhart B, Larson D, Nagorney DM. Major hepatic resection for hilar cholangiocarcinoma: analysis of 46 patients. *Arch Surg* 2004; **139**: 514-523; discussion 523-525 [PMID: 15136352 DOI: 10.1001/archsurg.139.5.514]

33 **Hemming AW**, Reed AI, Fujita S, Foley DP, Howard RJ. Surgical management of hilar cholangiocarcinoma. *Ann Surg* 2005; **241**: 693-699; discussion 693-699 [PMID: 15849505]

34 **Jarnagin WR**, Bowne W, Klimstra DS, Ben-Porat L, Roggin K, Cymes K, Fong Y, DeMatteo RP, D'Angelica M, Koea J, Blumgart LH. Papillary phenotype confers improved survival after resection of hilar cholangiocarcinoma. *Ann Surg* 2005; **241**: 703-712; discussion 712-714 [PMID: 15849506]

35 **Dinant S**, Gerhards MF, Rauws EA, Busch OR, Gouma DJ, van Gulik TM. Improved outcome of resection of hilar cholangiocarcinoma (Klatskin tumor). *Ann Surg Oncol* 2006; **13**: 872-880 [PMID: 16614876 DOI: 10.1245/aso.2006.05.053]

36 **Ito F**, Agni R, Rettammel RJ, Been MJ, Cho CS, Mahvi DM, Rikkers LF, Weber SM. Resection of hilar cholangiocarcinoma: concomitant liver resection decreases hepatic recurrence. *Ann Surg* 2008; **248**: 273-279 [PMID: 18650638 DOI: 10.1097/SLA.0b013e31817f2bfd]

37 **Konstadoulakis MM**, Roayaie S, Gomatos IP, Labow D, Fiel MI, Miller CM, Schwartz ME. Aggressive surgical resection for hilar cholangiocarcinoma: is it justified? Audit of a single center's experience. *Am J Surg* 2008; **196**: 160-169 [PMID: 18466862 DOI: 10.1016/j.amjsurg.2007.07.033]

38 **Ercolani G**, Zanello M, Grazi GL, Cescon M, Ravaioli M, Del Gaudio M, Vetrone G, Cucchetti A, Brandi G, Ramacciato G, Pinna AD. Changes in the surgical approach to hilar cholangiocarcinoma during an 18-year period in a Western single center. *J Hepatobiliary Pancreat Sci* 2010; **17**: 329-337 [PMID: 20464563 DOI: 10.1007/s00534-009-0249-5]

39 **Giuliante F**, Ardito F, Vellone M, Nuzzo G. Liver resections for hilar cholangiocarcinoma. *Eur Rev Med Pharmacol Sci* 2010; **14**: 368-370 [PMID: 20496550]

40 **Hirano S**, Kondo S, Tanaka E, Shichinohe T, Tsuchikawa T, Kato K, Matsumoto J, Kawasaki R. Outcome of surgical treatment of hilar cholangiocarcinoma: a special reference to postoperative morbidity and mortality. *J Hepatobiliary Pancreat Sci* 2010; **17**: 455-462 [PMID: 19820891 DOI: 10.1007/s00534-009-0208-1]

41 **Igami T**, Nishio H, Ebata T, Yokoyama Y, Sugawara G, Nimura Y, Nagino M. Surgical treatment of hilar cholangiocarcinoma in the "new era": the Nagoya University experience. *J Hepatobiliary Pancreat Sci* 2010; **17**: 449-454 [PMID: 19806294 DOI: 10.1007/s00534-009-0209-0]

42 **Lee SG**, Song GW, Hwang S, Ha TY, Moon DB, Jung DH, Kim KH, Ahn CS, Kim MH, Lee SK, Sung KB, Ko GY. Surgical treatment of hilar cholangiocarcinoma in the new era: the Asan experience. *J Hepatobiliary Pancreat Sci* 2010; **17**: 476-489 [PMID: 19851704 DOI: 10.1007/s00534-009-0204-5]

43 **Shimizu H**, Kimura F, Yoshidome H, Ohtsuka M, Kato A, Yoshitomi H, Furukawa K, Miyazaki M. Aggressive surgical resection for hilar cholangiocarcinoma of the left-side predominance: radicality and safety of left-sided hepatectomy. *Ann Surg* 2010; **251**: 281-286 [PMID: 20054275 DOI: 10.1097/SLA.0b013e3181be0085]

44 **Unno M**, Katayose Y, Rikiyama T, Yoshida H, Yamamoto K, Morikawa T, Hayashi H, Motoi F, Egawa S. Major hepatectomy for perihilar cholangiocarcinoma. *J Hepatobiliary Pancreat Sci* 2010; **17**: 463-469 [PMID: 19941010 DOI: 10.1007/s00534-009-0206-3]

45 **Regimbeau JM**, Fuks D, Le Treut YP, Bachellier P, Belghiti J, Boudjema K, Baulieux J, Pruvot FR, Cherqui D, Farges O.

Surgery for hilar cholangiocarcinoma: a multi-institutional update on practice and outcome by the AFC-HC study group. *J Gastrointest Surg* 2011; **15**: 480-488 [PMID: 21249527 DOI: 10.1007/s11605-011-1414-0]

46 **Ribero D**, Amisano M, Lo Tesoriere R, Rosso S, Ferrero A, Capussotti L. Additional resection of an intraoperative margin-positive proximal bile duct improves survival in patients with hilar cholangiocarcinoma. *Ann Surg* 2011; **254**: 776-781; discussion 781-783 [PMID: 22042470 DOI: 10.1097/SLA.0b013e3182368f85]

47 **Saxena A**, Chua TC, Chu FC, Morris DL. Improved outcomes after aggressive surgical resection of hilar cholangiocarcinoma: a critical analysis of recurrence and survival. *Am J Surg* 2011; **202**: 310-320 [PMID: 21871986 DOI: 10.1016/j.amjsurg.2010.08.041]

48 **Young AL**, Igami T, Senda Y, Adair R, Farid S, Toogood GJ, Prasad KR, Lodge JP. Evolution of the surgical management of perihilar cholangiocarcinoma in a Western centre demonstrates improved survival with endoscopic biliary drainage and reduced use of blood transfusion. *HPB* (Oxford) 2011; **13**: 483-493 [PMID: 21689232 DOI: 10.1111/j.1477-2574.2011.00328.x]

49 **Cheng QB**, Yi B, Wang JH, Jiang XQ, Luo XJ, Liu C, Ran RZ, Yan PN, Zhang BH. Resection with total caudate lobectomy confers survival benefit in hilar cholangiocarcinoma of Bismuth type III and IV. *Eur J Surg Oncol* 2012; **38**: 1197-1203 [PMID: 22992326 DOI: 10.1016/j.ejso.2012.08.009]

50 **de Jong MC**, Marques H, Clary BM, Bauer TW, Marsh JW, Ribero D, Majno P, Hatzaras I, Walters DM, Barbas AS, Mega R, Schulick RD, Choti MA, Geller DA, Barroso E, Mentha G, Capussotti L, Pawlik TM. The impact of portal vein resection on outcomes for hilar cholangiocarcinoma: a multi-institutional analysis of 305 cases. *Cancer* 2012; **118**: 4737-4747 [PMID: 22415526 DOI: 10.1002/cncr.27492]

51 **Nagino M**, Ebata T, Yokoyama Y, Igami T, Sugawara G, Takahashi Y, Nimura Y. Evolution of surgical treatment for perihilar cholangiocarcinoma: a single-center 34-year review of 574 consecutive resections. *Ann Surg* 2013; **258**: 129-140 [PMID: 23059502 DOI: 10.1097/SLA.0b013e3182708b57]

52 **Iacono C**, Ruzzenente A, Campagnaro T, Bortolasi L, Valdegamberi A, Guglielmi A. Role of preoperative biliary drainage in jaundiced patients who are candidates for pancreatoduodenectomy or hepatic resection: highlights and drawbacks. *Ann Surg* 2013; **257**: 191-204 [PMID: 23013805 DOI: 10.1097/SLA.0b013e31826f4b0e]

53 **Guiu B**, Bize P, Demartines N, Lesurtel M, Denys A. Simultaneous biliary drainage and portal vein embolization before extended hepatectomy for hilar cholangiocarcinoma: preliminary experience. *Cardiovasc Intervent Radiol* 2014; **37**: 698-704 [PMID: 23842686 DOI: 10.1007/s00270-013-0699-7]

54 **Hatfield AR**, Tobias R, Terblanche J, Girdwood AH, Fataar S, Harries-Jones R, Kernoff L, Marks IN. Preoperative external biliary drainage in obstructive jaundice. A prospective controlled clinical trial. *Lancet* 1982; **2**: 896-899 [PMID: 6126752]

55 **McPherson GA**, Benjamin IS, Hodgson HJ, Bowley NB, Allison DJ, Blumgart LH. Pre-operative percutaneous transhepatic biliary drainage: the results of a controlled trial. *Br J Surg* 1984; **71**: 371-375 [PMID: 6372935]

56 **Pitt HA**, Gomes AS, Lois JF, Mann LL, Deutsch LS, Longmire WP. Does preoperative percutaneous biliary drainage reduce operative risk or increase hospital cost? *Ann Surg* 1985; **201**: 545-553 [PMID: 2986562]

57 **Kawashima H**, Itoh A, Ohno E, Itoh Y, Ebata T, Nagino M, Goto H, Hirooka Y. Preoperative endoscopic nasobiliary drainage in 164 consecutive patients with suspected perihilar cholangiocarcinoma: a retrospective study of efficacy and risk factors related to complications. *Ann Surg* 2013; **257**: 121-127 [PMID: 22895398 DOI: 10.1097/SLA.0b013e318262b2e9]

58 **Abdalla EK**, Hicks ME, Vauthey JN. Portal vein embolization: rationale, technique and future prospects. *Br J Surg* 2001; **88**: 165-175 [PMID: 11167863 DOI: 10.1046/j.1365-2168.2001.01658.x]

59 **Capussotti L**, Vigano L, Ferrero A, Muratore A. Local surgical resection of hilar cholangiocarcinoma: is there still a place? *HPB* (Oxford) 2008; **10**: 174-178 [PMID: 18773049 DOI: 10.1080/13651820801992534]

60 **Otto G**, Romaneehsen B, Hoppe-Lotichius M, Bittinger F. Hilar cholangiocarcinoma: resectability and radicality after routine diagnostic imaging. *J Hepatobiliary Pancreat Surg* 2004; **11**: 310-318 [PMID: 15549429 DOI: 10.1007/s00534-004-0912-9]

61 **Nimura Y**, Hayakawa N, Kamiya J, Kondo S, Shionoya S. Hepatic segmentectomy with caudate lobe resection for bile duct carcinoma of the hepatic hilus. *World J Surg* 1990; **14**: 535-443; discussion 544 [PMID: 2166381]

62 **Ogura Y**, Mizumoto R, Tabata M, Matsuda S, Kusuda T. Surgical treatment of carcinoma of the hepatic duct confluence: analysis of 55 resected carcinomas. *World J Surg* 1993; **17**: 85-92; discussion 92-93 [PMID: 8447147]

63 **Sugiura Y**, Nakamura S, Iida S, Hosoda Y, Ikeuchi S, Mori S, Sugioka A, Tsuzuki T. Extensive resection of the bile ducts combined with liver resection for cancer of the main hepatic duct junction: a cooperative study of the Keio Bile Duct Cancer Study Group. *Surgery* 1994; **115**: 445-451 [PMID: 8165536]

64 **Kow AW**, Wook CD, Song SC, Kim WS, Kim MJ, Park HJ, Heo JS, Choi SH. Role of caudate lobectomy in type III A and III B hilar cholangiocarcinoma: a 15-year experience in a tertiary institution. *World J Surg* 2012; **36**: 1112-1121 [PMID: 22374541 DOI: 10.1007/s00268-012-1497-0]

65 **Nagino M**. Perihilar cholangiocarcinoma: a surgeon's viewpoint on current topics. *J Gastroenterol* 2012; **47**: 1165-1176 [PMID: 22847554 DOI: 10.1007/s00535-012-0628-6]

66 **Ebata T**, Yokoyama Y, Igami T, Sugawara G, Takahashi Y, Nimura Y, Nagino M. Hepatopancreatoduodenectomy for cholangiocarcinoma: a single-center review of 85 consecutive patients. *Ann Surg* 2012; **256**: 297-305 [PMID: 22750757 DOI: 10.1097/SLA.0b013e31826029ca]

67 **Kitagawa Y**, Nagino M, Kamiya J, Uesaka K, Sano T, Yamamoto H, Hayakawa N, Nimura Y. Lymph node metastasis from hilar cholangiocarcinoma: audit of 110 patients who underwent regional and paraaortic node dissection. *Ann Surg* 2001; **233**: 385-392 [PMID: 11224627]

68 **Nagino M**. Cutting edge of an aggressive surgical approach for perihilar cholangiocarcinoma. *Updates Surg* 2013; **65**: 81-83 [PMID: 23460256 DOI: 10.1007/s13304-013-0204-5]

69 **Neuhaus P**, Thelen A, Jonas S, Puhl G, Denecke T, Veltzke-Schlieker W, Seehofer D. Oncological superiority of hilar en bloc resection for the treatment of hilar cholangiocarcinoma. *Ann Surg Oncol* 2012; **19**: 1602-1608 [PMID: 21964888 DOI: 10.1245/s10434-011-2077-5]

70 **Miyazaki M**, Kato A, Ito H, Kimura F, Shimizu H, Ohtsuka M, Yoshidome H, Yoshitomi H, Furukawa K, Nozawa S. Combined vascular resection in operative resection for hilar cholangiocarcinoma: does it work or not? *Surgery* 2007; **141**: 581-588 [PMID: 17462457 DOI: 10.1016/j.surg.2006.09.016]

71 **Nagino M**, Nimura Y, Nishio H, Ebata T, Igami T, Matsushita M, Nishikimi N, Kamei Y. Hepatectomy with simultaneous resection of the portal vein and hepatic artery for advanced perihilar cholangiocarcinoma: an audit of 50 consecutive cases. *Ann Surg* 2010; **252**: 115-123 [PMID: 20531001 DOI: 10.1097/SLA.0b013e3181e463a7]

72 **Chen YL**, Chen WB, Wan YY, Li WG, Huang ZQ, Wu XT, Yang J, Yang L. Effects of partial portal vein arterialization on liver regeneration after hepatectomy in minipigs with obstructive jaundice. *Chin Med J* (Engl) 2012; **125**: 2302-2305 [PMID: 22882852]

73 **Sano T**, Shimada K, Sakamoto Y, Yamamoto J, Yamasaki S, Kosuge T. One hundred two consecutive hepatobiliary resections for perihilar cholangiocarcinoma with zero mor-

tality. *Ann Surg* 2006; **244**: 240-247 [PMID: 16858186 DOI: 10.1097/01.sla.0000217605.66519.38]
74 **Schiffman SC**, Reuter NP, McMasters KM, Scoggins CR, Martin RC. Overall survival peri-hilar cholangiocarcinoma: R1 resection with curative intent compared to primary endoscopic therapy. *J Surg Oncol* 2012; **105**: 91-96 [PMID: 21815152 DOI: 10.1002/jso.22054]
75 **Hasegawa S**, Ikai I, Fujii H, Hatano E, Shimahara Y. Surgical resection of hilar cholangiocarcinoma: analysis of survival and postoperative complications. *World J Surg* 2007; **31**: 1256-1263 [PMID: 17453285 DOI: 10.1007/s00268-007-9001-y]
76 **Cannon RM**, Brock G, Buell JF. Surgical resection for hilar cholangiocarcinoma: experience improves resectability. *HPB* (Oxford) 2012; **14**: 142-149 [PMID: 22221577 DOI: 10.1111/j.1477-2574.2011.00419.x]
77 **Pandey D**, Lee KH, Tan KC. The role of liver transplantation for hilar cholangiocarcinoma. *Hepatobiliary Pancreat Dis Int* 2007; **6**: 248-253 [PMID: 17548246]
78 **Meyer CG**, Penn I, James L. Liver transplantation for cholangiocarcinoma: results in 207 patients. *Transplantation* 2000; **69**: 1633-1637 [PMID: 10836374]
79 **Gores GJ**, Darwish Murad S, Heimbach JK, Rosen CB. Liver transplantation for perihilar cholangiocarcinoma. *Dig Dis* 2013; **31**: 126-129 [PMID: 23797134 DOI: 10.1159/000347207]
80 **Ito F**, Cho CS, Rikkers LF, Weber SM. Hilar cholangiocarcinoma: current management. *Ann Surg* 2009; **250**: 210-218 [PMID: 19638920 DOI: 10.1097/SLA.0b013e3181afe0ab]
81 **Pichlmayr R**, Weimann A, Klempnauer J, Oldhafer KJ, Maschek H, Tusch G, Ringe B. Surgical treatment in proximal bile duct cancer. A single-center experience. *Ann Surg* 1996; **224**: 628-638 [PMID: 8916878]
82 **Rosen CB**, Heimbach JK, Gores GJ. Liver transplantation for cholangiocarcinoma. *Transpl Int* 2010; **23**: 692-697 [PMID: 20497401]

P- Reviewer: Chetty R, Plentz RR **S- Editor**: Wen LL
L- Editor: Wang TQ **E- Editor:** Lu YJ

World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4291/wjgp.v5.i3.355

World J Gastrointest Pathophysiol 2014 August 15; 5(3): 355-358
ISSN 2150-5330 (online)

Helicobacter pylori as a risk factor for central serous chorioretinopathy: Literature review

Aránzazu Mateo-Montoya, Martine Mauget-Faÿse

Aránzazu Mateo-Montoya, Martine Mauget-Faÿse, Ophthalmology Service, Fondation Ophtalmologique Adolphe de Rothschild, 75019 Paris, France
Author contributions: Mauget-Faÿse M designed the study; Mateo-Montoya A performed the research and wrote the paper; Mauget-Faÿse M and Mateo-Montoya A reviewed the paper and approved it.
Correspondence to: Aránzazu Mateo-Montoya, MD, Ophthalmology Service, Fondation Ophtalmologique Adolphe de Rothschild, 25 rue Manin, 75019 Paris, France. arancha.mateo@gmail.com
Telephone: +33-148-036671 Fax: +33-148-036523
Received: January 26, 2014 Revised: May 4, 2014
Accepted: May 28, 2014
Published online: August 15, 2014

Abstract

Helicobacter pylori (*H. pylori*), a Gram-negative bacterium, is one of the most frequent causes of gastrointestinal infections worldwide. It has been associated as a pathogen for the human body with many systemic diseases, including different eye diseases. We will focus on a specific eye disease called idiopathic central serous chorioretinopathy (ICSCR). This disease is characterized by a serous detachment of the neurosensory retina in the macular region, which affects the vision to different degrees. Currently, the pathophysiology of ICSCR is not clear and there is no effective treatment. However, several potential risk factors have been elucidated. One of the factors that has more frequently been associated with ICSCR is stress. As *H. pylori* was identified as a possible etiological factor for occlusive arterial diseases in young people who were particularly stressed, it was thought that *H. pylori* might also be present in ICSCR. Therefore, some physicians started to test its presence in patents with ICSCR. If *H. pylori* happened to be associated with ICSCR, the treatment of gastrointestinal infection could also improve visual symptoms and help to remediate this eye disease. Although *H. pylori* is highly prevalent in the general population, a true correlation seems to exist. We present a review on the relationship between ICSCR and *H. pylori*.

Key words: *Helicobacter pylori*; Idiopathic central serous chorioretinopathy; Retina; Eye disease; Occlusive arterial disease

Core tip: *Helicobacter pylori* (*H. pylori*) has been associated with many systemic diseases. We focus on a specific eye disease called idiopathic central serous chorioretinopathy (ICSCR), which is characterized by a serous detachment of the neurosensory retina in the macular region and affects vision to different degrees. One factor frequently associated with ICSCR is stress. As *H. pylori* was identified as a possible etiological factor for occlusive arterial diseases in young people who were particularly stressed, it was thought that *H. pylori* might also be present in ICSCR. We present a review on the relationship between ICSCR and *H. pylori*.

Mateo-Montoya A, Mauget-Faÿse M. *Helicobacter pylori* as a risk factor for central serous chorioretinopathy: Literature review. *World J Gastrointest Pathophysiol* 2014; 5(3): 355-358 Available from: URL: http://www.wjgnet.com/2150-5330/full/v5/i3/355.htm DOI: http://dx.doi.org/10.4291/wjgp.v5.i3.355

INTRODUCTION

Helicobacter pylori (*H. pylori*), a Gram-negative bacterium, is one of the most frequent causes of gastrointestinal infections worldwide. It has been associated as a pathogen for the human body with many systemic diseases, including vascular (atherosclerosis and cardiovascular diseases, Raynaud's syndrome, primary headache), autoimmune (Sjögren syndrome, autoimmune thyroiditis, idiopathic arrythmias, Parkinson's disease, nonarterial anterior

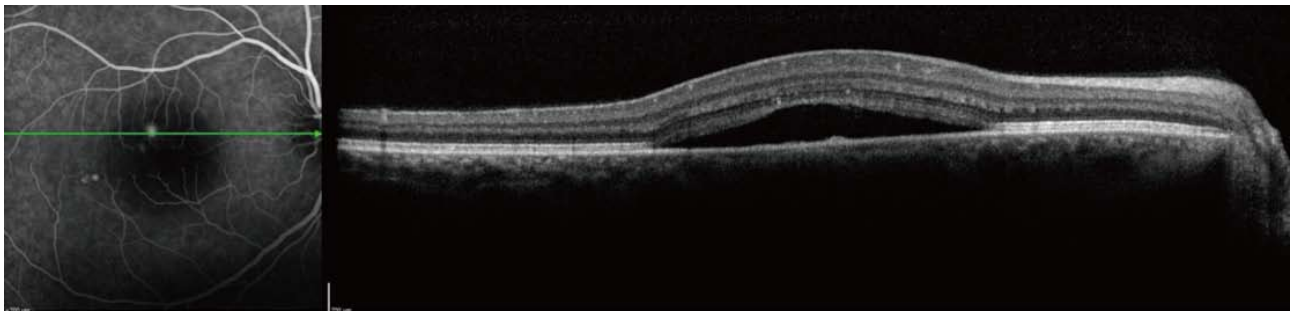

Figure 1 Optical coherence tomography image showing separation of the sensory retina from the retinal pigment epithelium.

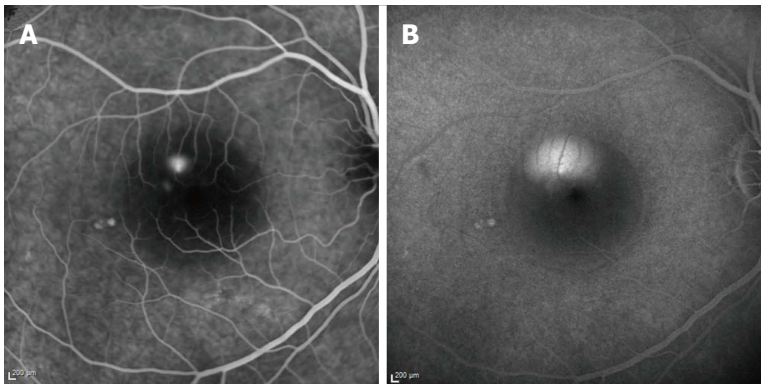

Figure 2 Fluorescein angiography at 2 (A) and 20 (B) min. A: The early phase shows a hyperfluorescent spot due to leakage of dye through the RPE; B: During the late venous phase, fluorescein passes into the subretinal space and spreads until the entire area is filled with dye.

optic ischemic neuropathy) and skin diseases (urticaria, rosacea), iron deficiency anemia, growth retardation, late menarche, extra-gastric MALT lymphoma, duodenal ulcer, gastric cancer, gastro-oesophageal reflux disease, diabetes mellitus, hepatic encephalopathy, sudden infant death syndrome, and anorexia of aging[1-4].

H. pylori has also been associated with eye diseases such as Sjögren syndrome, blepharitis, glaucoma, uveitis and idiopathic central serous chorioretinopathy (ICSCR)[5-9].

Our review focuses on the relation between ICSCR and *H. pylori*. ICSCR was first described by von Graefe in 1886[10]. ICSCR affects middle-aged adults (between 25-45 years old), predominantly men, and is characterized by a serous detachment of the neurosensory retina in the macular region. It is usually unilateral (90% of the patients). Patients may develop metamorphopsia, central positive scotoma, micropsia, and impaired color vision. Additional retinal findings include retinal pigment epithelium (RPE) detachment, RPE atrophic tracks, capillary telangiectasia, retinal or choroidal neovascularisation, and intraretinal deposits[11-13], which may be visualized with fluorescein and indocyanine green angiography, and optical coherence tomography (OCT). (Figures 1 and 2).

Most of the cases spontaneously resolve with recovery of good visual function. However, recurrences have been observed in 50% or more of the cases[14]. A small percentage of subjects experience chronic decompensation of the RPE and develop severe vision loss.

The pathophysiology of ICSCR is poorly understood. It is thought that damage to the RPE active fluid transport mechanisms that usually dehydrate the subretinal space may play a role[14]. Cigarette smoking, systemic hypertension, pregnancy, allergic respiratory disease, antibiotic or alcohol ingestion[15], sildenafil citrate[16] or systemic corticosteroids[17], sympathomimetic agents[18], antiphospholipid antibodies[19], retinitis pigmentosa[20], psoriasis[21], and endogenous mineralcorticoid dysfunction[17] have been cited as potential risk factors for this disease. ICSCR has also been reported in patients with a benign tumor of the adrenal gland[22], cryoglobulinemia[23], systemic lupus erythematosus[24], or after bone marrow transplantation[25]; and has been strongly associated with individuals with type A personality[26].

Currently, there is no effective treatment for ICSCR. Photodynamic therapy with verteporfin has been used in the last few years. Although it decreases serous detachment and improves visual acuity, it results in scotomas in some patients. A new treatment has recently been proposed based on oral eplerenone. Experimental data has shown that central chorioretinopathy could result from an overactivity of the mineralcorticoid receptor pathway in choroid vessels. Eplerenone is a mineralcorticoid receptor antagonist and has therefore been considered as a potential treatment for ICSCR. Randomized controlled trials are needed to confirm if this therapy could help in the treatment of ICSCR[17].

DISCUSSION

HP was first associated with ICSCR in 2000. A French team (Mauget-Faÿse *et al*[7]) presented their first results on a poster at the Association for Research in Vision and Ophthalmology (ARVO) congress. Knowing that

H. pylori was identified as a possible etiological factor for occlusive arterial diseases in young people who were particularly stressed[27], the authors of this study decided to test the presence of HP infection in ICSCR patients. As occlusive arterial disease shared some characteristics with ICSCR (*i.e.*, associated with type A personality and ischemia), it was believed that HP infection might be a common factor.

This prospective pilot study of 16 patients affected by active ICSCR or by its variant, diffuse retinal epitheliopathy, found that the prevalence of *H. pylori* infection; determined by one of more of the following methods: histology of gastric biopsy specimens, C-urea breath test, or serology test (Boehringer Mannhein test); was significantly higher in subjects with ICSCR[7]. A complementary study including more patients and confirming the results was published in 2004[28]. A few months earlier, a case report of a 43-year-old man suggested that ICSCR recurrences were associated with the presence of *H. pylori*. Resolution of ICSCR was correlated with the eradication of the bacterium using the conventional triple-therapy regimen (amoxicillin, clarithromycin, omeprazole)[29].

Some further studies confirmed this relationship. A Spanish team observed that 68.75% of ICSCR patients were infected with *H. pylori*, compared with 30% of the control population[30]. Recently, Casella *et al*[18] suggested that chronic ICSCR patients could be infected with *H. pylori* and that the treatment of the infection could have a positive impact on the outcome of chronic ICSCR regarding the improvement of final best-corrected visual acuity and resolution of the serous detachment. Lastly, Dang *et al*[17] reported that, although *H. pylori* eradication does not increase visual acuity and does not diminish subretinal fluid, it could benefit central retinal sensitivity in ICSCR patients. A statistical difference was observed in central retinal sensitivity at 3 mo after HP eradication therapy. Macular sensitivity was measured using microperimeter-1 (Nidek, Vigonza, Italy) after pupil dilatation and not with contrast sensitivity charts. Thirty-three stimulus points located in the area of the central 15° diameter around the macula were examined. The average sensitivity of the 33 points was defined as the central retinal sensitivity[17].

It is difficult to determine the potential role of *H. pylori* in the pathogenesis of ICSCR. Giusti elucidated several hypotheses regarding pathogenesis[31]. A possible explanation might be the link between *H. pylori* infection and atherosclerosis. A cross reactivity of anti-Cag A antibodies, whose presence is more frequently associated in atherosclerosis, and the presence of immunoglobulin-G (Ig-G) antibody have been considered as risk factors for endothelial dysfunction[32]. Another mechanism is the role of heat shock proteins expressed by several pathogens, *e.g.*, *H. pylori*. It has been hypothesized that an immune response against antigens located on pathogenic organisms would cross-react with homologous host proteins, *e.g.*, with the endothelial vascular wall[33].

Further implications of *H. pylori* infections have lately been proposed: increase of lipids and fibrinogen levels[32], upregulation of endothelial adhesion molecules and increase of polymorphonuclear leucocyte adhesion[34], and increase of platelet activation and aggregation[35].

CONCLUSION

Several studies indicate that many ICSCR patients could be infected with *H. pylori* and that the treatment of the infection could have a positive impact on the outcome of the disease. Due to the high prevalence of *H. pylori* infection in the general population, it is difficult to establish a true correlation. Prospective and masked clinical trials are necessary to confirm the relationship between ICSCR and *H. pylori*, as well as the benefits to ICSCR patients from receiving *H. pylori* treatment.

REFERENCES

1 **De Koster E**, De Bruyne I, Langlet P, Deltenre M. Evidence based medicine and extradigestive manifestations of Helicobacter pylori. *Acta Gastroenterol Belg* 2000; **63**: 388-392 [PMID: 11233523]

2 **Carloni E**, Cremonini F, Di Caro S, Padalino C, Gerardino L, Santoliquido A, Colasanti S, Pola P, Gasbarrini A. Helicobacter pylori-related extradigestive diseases and effects of eradication therapy. *Dig Liver Dis* 2000; **32** Suppl 3: S214-S216 [PMID: 11245299 DOI: 10.1016/S1590-8658(00)80282-0]

3 **Zbinden R**. Expanding the spectrum of Helicobacter pylori-associated diseases. *Infection* 2005; **33**: 49 [PMID: 15827869 DOI: 10.1007/s15010-005-7205-3]

4 **Roussos A**, Philippou N, Gourgoulianis KI. Helicobacter pylori infection and respiratory diseases: a review. *World J Gastroenterol* 2003; **9**: 5-8 [PMID: 12508341]

5 **El Miedany YM**, Baddour M, Ahmed I, Fahmy H. Sjogren's syndrome: concomitant H. pylori infection and possible correlation with clinical parameters. *Joint Bone Spine* 2005; **72**: 135-141 [PMID: 15797493 DOI: 10.1016/j.jbspin.2004.04.005]

6 **Saccà SC**, Pascotto A, Venturino GM, Prigione G, Mastromarino A, Baldi F, Bilardi C, Savarino V, Brusati C, Rebora A. Prevalence and treatment of Helicobacter pylori in patients with blepharitis. *Invest Ophthalmol Vis Sci* 2006; **47**: 501-508 [PMID: 16431942 DOI: 10.1167/iovs.05-0323]

7 **Mauget-Faÿsse M**, Kodjikian L, Quaranta M, Ben Ezra D, Trepsat C, Mion F, Mégraud F. [Helicobacter pylori in central serous chorioretinopathy and diffuse retinal epitheliopathy. Results of the first prospective pilot study]. *J Fr Ophtalmol* 2002; **25**: 1021-1025 [PMID: 12527825]

8 **Otasevic L**, Zlatanovic G, Stanojevic-Paovic A, Miljkovic-Selimovic B, Dinic M, Djordjevic-Jocic J, Stankovic A. Helicobacter pylori: an underestimated factor in acute anterior uveitis and spondyloarthropathies? *Ophthalmologica* 2007; **221**: 6-13 [PMID: 17183194 DOI: 10.1159/000096515]

9 **Izzotti A**, Saccà SC, Bagnis A, Recupero SM. Glaucoma and Helicobacter pylori infection: correlations and controversies. *Br J Ophthalmol* 2009; **93**: 1420-1427 [PMID: 19854738 DOI: 10.1136/bjo.2008.150409]

10 **Von Graefe A.** Ueber centrale rezidivierende keratitis. *Albrecht von Graefes Arch Klin Ophthalmol* 1886; **12**: 211

11 **Iida T**, Yannuzzi LA, Spaide RF, Borodoker N, Carvalho CA, Negrao S. Cystoid macular degeneration in chronic central serous chorioretinopathy. *Retina* 2003; **23**: 1-7; quiz 137-138 [PMID: 12652224 DOI: 10.1097/00006982-200302000-00001]

12 **Teschner S**, Noack J, Birngruber R, Schmidt-Erfurth U.

Characterization of leakage activity in exudative chorioretinal disease with three-dimensional confocal angiography. *Ophthalmology* 2003; **110**: 687-697 [PMID: 12689887]
13 **Spaide RF**. Deposition of yellow submacular material in central serous chorioretinopathy resembling adult-onset foveomacular vitelliform dystrophy. *Retina* 2004; **24**: 301-304 [PMID: 15097895 DOI: 10.1097/00006982-200404000-00019]
14 **Albert DM,** Jakobiec FA. Principles and practice of ophthalmology. Philadelpia: Saunders, 1994
15 **Haimovici R**, Koh S, Gagnon DR, Lehrfeld T, Wellik S. Risk factors for central serous chorioretinopathy: a case-control study. *Ophthalmology* 2004; **111**: 244-249 [PMID: 15019370 DOI: 10.1016/j.ophtha.2003.09.024]
16 **Allibhai ZA**, Gale JS, Sheidow TS. Central serous chorioretinopathy in a patient taking sildenafil citrate. *Ophthalmic Surg Lasers Imaging* 2004; **35**: 165-167 [PMID: 15088831]
17 **Dang Y**, Mu Y, Zhao M, Li L, Guo Y, Zhu Y. The effect of eradicating Helicobacter pylori on idiopathic central serous chorioretinopathy patients. *Ther Clin Risk Manag* 2013; **9**: 355-360 [PMID: 24043941 DOI: 10.2147/TCRM.S50407]
18 **Casella AM**, Berbel RF, Bressanim GL, Malaguido MR, Cardillo JA. Helicobacter pylori as a potential target for the treatment of central serous chorioretinopathy. *Clinics* (Sao Paulo) 2012; **67**: 1047-1052 [PMID: 23018302 DOI: 10.6061/clinics/2012(09)11]
19 **Costen MT**, Parkin BT, Davison CR, Crick MP. Central serous chorioretinopathy and antiphospholipid antibodies--results of a pilot study. *Eye* (Lond) 2004; **18**: 938 [PMID: 15002021 DOI: 10.1038/sj.eye.6701348]
20 **Dorenboim Y**, Rehany U, Rumelt S. Central serous chorioretinopathy associated with retinitis pigmentosa. *Graefes Arch Clin Exp Ophthalmol* 2004; **242**: 346-349 [PMID: 14997320 DOI: 10.1007/s00417-003-0819-1]
21 **Nucci C**, Corsi A, Mancino R, Macri G. Central serous chorioretinopathy in patients with psoriasis. *Acta Ophthalmol Scand* 2004; **82**: 105-107 [PMID: 14738494 DOI: 10.1111/j.1600-0420.2004.00189c.x]
22 **Katsimpris JM**, Vandoros M, Petropoulos IK, Andrikopoulos P. Central serous chorioretinopathy associated with adrenal myelolipoma. *Klin Monbl Augenheilkd* 2003; **220**: 199-203 [PMID: 12664380 DOI: 10.1055/s-2003-38187]
23 **Zamir E**, Chowers I. Central serous chorioretinopathy in a patient with cryoglobulinaemia. *Eye* (Lond) 1999; **13** (Pt 2): 265-266 [PMID: 10450398 DOI: 10.1038/eye.1999.67]
24 **Cunningham ET**, Alfred PR, Irvine AR. Central serous chorioretinopathy in patients with systemic lupus erythematosus. *Ophthalmology* 1996; **103**: 2081-2090 [PMID: 9003342 DOI: 10.1016/S0161-6420(96)30385-0]
25 **Karashima K**, Fujioka S, Harino S. Two cases of central serous chorioretinopathy treated with photocoagulation after bone marrow transplantation. *Retina* 2002; **22**: 651-653 [PMID: 12441737 DOI: 10.1097/00006982-200210000-00022]
26 **Spahn C**, Wiek J, Burger T, Hansen L. Psychosomatic aspects in patients with central serous chorioretinopathy. *Br J Ophthalmol* 2003; **87**: 704-708 [PMID: 12770965 DOI: 10.1136/bjo.87.6.704]
27 **Libby P**, Egan D, Skarlatos S. Roles of infectious agents in atherosclerosis and restenosis: an assessment of the evidence and need for future research. *Circulation* 1997; **96**: 4095-4103 [PMID: 9403635 DOI: 10.1161/01.CIR.96.11.4095]
28 **Ahnoux-Zabsonre A**, Quaranta M, Mauget-Faÿsse M. [Prevalence of Helicobacter pylori in central serous chorioretinopathy and diffuse retinal epitheliopathy: a complementary study]. *J Fr Ophtalmol* 2004; **27**: 1129-1133 [PMID: 15687922 DOI: 10.1016/S0181-5512(04)96281-X]
29 **Giusti C**. [Central serous chorioretinopathy: a new extragastric manifestation of Helicobacter pylori?: Analysis of a clinical case]. *Clin Ter* 2001; **152**: 393-397 [PMID: 11865536]
30 **Asensio-Sánchez VM**, Rodríguez-Delgado B, García-Herrero E, Cabo-Vaquera V, García-Loygorri C. [Central serous chorioretinopathy as an extradigestive manifestation of Helicobacter pylori gastric infection]. *Arch Soc Esp Oftalmol* 2008; **83**: 177-182 [PMID: 18311677 DOI: 10.4321/S0365-66912008000300009]
31 **Giusti C**. Association of Helicobacter pylori with central serous chorioretinopathy: hypotheses regarding pathogenesis. *Med Hypotheses* 2004; **63**: 524-527 [PMID: 15288381 DOI: 10.1016/j.mehy.2004.02.020]
32 **Franceschi F**, Sepulveda AR, Gasbarrini A, Pola P, Silveri NG, Gasbarrini G, Graham DY, Genta RM. Cross-reactivity of anti-CagA antibodies with vascular wall antigens: possible pathogenic link between Helicobacter pylori infection and atherosclerosis. *Circulation* 2002; **106**: 430-434 [PMID: 12135941 DOI: 10.1161/01.CIR.0000024100.90140.19]
33 **Lamb DJ**, El-Sankary W, Ferns GA. Molecular mimicry in atherosclerosis: a role for heat shock proteins in immunisation. *Atherosclerosis* 2003; **167**: 177-185 [PMID: 12818399]
34 **Byrne MF**, Corcoran PA, Atherton JC, Sheehan KM, Murray FE, Fitzgerald DJ, Murphy JF. Stimulation of adhesion molecule expression by Helicobacter pylori and increased neutrophil adhesion to human umbilical vein endothelial cells. *FEBS Lett* 2002; **532**: 411-414 [PMID: 12482602 DOI: 10.1016/S0014-5793(02)03728-6]
35 **Byrne MF**, Kerrigan SW, Corcoran PA, Atherton JC, Murray FE, Fitzgerald DJ, Cox DM. Helicobacter pylori binds von Willebrand factor and interacts with GPIb to induce platelet aggregation. *Gastroenterology* 2003; **124**: 1846-1854 [PMID: 12806618 DOI: 10.1016/S0016-5085(03)00397-4]

P- Reviewer: Velez-Montoya R **S- Editor**: Wen LL
L- Editor: A **E- Editor:** Lu YJ

World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4291/wjgp.v5.i3.359

World J Gastrointest Pathophysiol 2014 August 15; 5(3): 359-365
ISSN 2150-5330 (online)

MINIREVIEWS

Risk of cardiovascular disease in inflammatory bowel disease

Nynne Nyboe Andersen, Tine Jess

Nynne Nyboe Andersen, Tine Jess, Department of Epidemiology Research, Statens Serum Institut, DK-2300 Copenhagen, Denmark
Author contributions: Andersen NN collected the material and drafted the manuscript; Jess T discussed the topic and revised the manuscript.
Correspondence to: Nynne Nyboe Andersen, MD, Department of Epidemiology Research, Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen S, Denmark. nyna@ssi.dk
Telephone: +45-32-683139 Fax: +45-32-683165
Received: December 11, 2013 Revised: April 22, 2014
Accepted: May 28, 2014
Published online: August 15, 2014

Abstract

Abundant scientific evidence supporting an association between inflammatory bowel disease (IBD) and venous thromboembolic events, caused by an IBD related hypercoagulability, is acknowledged and thromboprophylactic treatment strategies are now implemented in the management of IBD patients. In contrary, the risk of arterial thromboembolic disease, as ischemic heart disease, cerebrovascular events, and mesenteric ischemia in patients with IBD remains uncertain and the magnitude of a potentially increased risk is continuously debated, with ambiguous risk estimates among studies. The evident role of inflammation in the pathogenesis of atherosclerosis forms the basis of a biological plausible link; the chronic systemic inflammation in IBD patients increases the risk of atherosclerosis and thereby the risk of thrombotic events. Further, studies have shown that the burden of traditional risk factors for atherosclerosis, such as obesity, diabetes mellitus, and dyslipidemia is lower in IBD populations, thus further strengthen the role of non-traditional risk factors, as chronic inflammation in the linking of the two disease entities. Likewise, mortality from cardiovascular disease in IBD remains questioned. The aim of the current review is to give an up-date on the existing evidence of the possible association between IBD and cardiovascular disease and to discuss traditional and non-traditional risk factors.

Key words: Inflammatory bowel disease; cardiovascular disease; Risk; Ulcerative colitis; Crohn's disease

Core tip: The increased risk of venous thromboembolic events in inflammatory bowel disease (IBD) patients is well-established and prophylactic strategies are implemented in current guidelines. The risk of arterial thromboembolic complications in IBD remains uncertain. Together, the systemic inflammation in patients with IBD and the inflammation-driven development of atherosclerosis form the basis of a potential association between the two disease entities. The present review will provide a summary of the existing literature on the association between IBD and thromboembolic diseases and discuss potential risk and preventive factors.

Andersen NN, Jess T. Risk of cardiovascular disease in inflammatory bowel disease. *World J Gastrointest Pathophysiol* 2014; 5(3): 359-365 Available from: URL: http://www.wjgnet.com/2150-5330/full/v5/i3/359.htm DOI: http://dx.doi.org/10.4291/wjgp.v5.i3.359

INTRODUCTION

Inflammatory bowel disease (IBD), comprising ulcerative colitis (UC) and Crohn's disease (CD) are systemic, chronic inflammatory conditions that predominately affect the gastrointestinal tract but are also characterized by numerous extraintestinal manifestations, assumedly caused by concomitant systemic inflammation. It is well-established that the risk of venous thromboembolic event is increased in IBD patients[1], primarily during flares[2], potentially due to an inflammation induced state

of hypercoagulability. However, the true magnitude of this risk and the associated mortality rate remains debated.

In the last decade, it has become increasingly evident that chronic systemic inflammation plays a pivotal role in the pathogenesis of atherosclerosis[3]. Further, the observation of increased thickness of the carotid intimal-media (a measure of atherosclerotic burden), endothelia dysfunction, and atherogenic alterations in the lipid profile of patients with IBD has further fuelled the hypothesis of a potential increased risk of atherosclerosis-driven vascular diseases in IBD[4-6]. Likewise, an increased risk of cardiovascular diseases (CVD) in other inflammatory conditions as rheumatoid arthritis[7], psoriasis[8] and systemic lupus erythematous[9] is now established, independent of traditional cardiovascular risk factors. Currently, reported results on risk of CVD in IBD have been ambiguous with studies revealing an increased risk of both ischemic heart disease (IHD) and cerebrovascular accidents (CVA) while others have shown no association[10-13]. Additionally, a few studies have suggested that IBD patients have a lower burden of some of the traditional risk factors for CVD, such as hypertension, diabetes mellitus, dyslipidemia, and obesity, and that non-traditional risk factors could play an important role for IBD patients[13,14]. Overall, this has led to an ongoing debate of whether the risk of arterial thrombotic disease is increased in IBD patients, what the underlying mechanisms are, and whether a strategy for disease specific risk assessment should be implemented in the management of IBD patients.

The aim of the current review is to give an update on the existing evidence on risk of atherosclerosis-related vascular disease, including ischemic heart disease, cerebrovascular accidents, mesenteric thrombosis, and venous thromboembolic events and associated risk factors and mortality rates in patients with IBD and further to evaluate on future prospects and preventive factors.

VENOUS THROMBOEMBOLIC EVENTS

The association between venous thromboembolic events (VTEs), comprising deep venous thrombosis (DVT) and pulmonary embolism (PE), and IBD was indicated as early as in 1936 by Bargen *et al*[15]. In 1986, fifty years after the suggested association, Talbot *et al*[16] was the first to report valid results on the incidence of VTE's in 7199 IBD patients from the Mayo Clinic, US and revealed a potentially increased risk.

VTEs are a serious concern with a significant morbidity and mortality. The risk of VTEs is associated with the hypercoagulability related to IBD. The specific clotting mechanism have been attributed to a range of factors including thrombocytosis[17], increased levels of clotting factors V/Ⅷ/fibrinogen[18], acquired antithrombin Ⅲ deficiency[19,20] and decreased levels of protein C and S[21-23]. The exact mechanism, the interplay between the variable factors and whether the hypercoagulability is a secondary phenomenon to IBD or represents an underlying pathological mechanism for IBD remain uncertain.

In 2001, the first large population-based study on risk of VTE's in IBD was reported from the Canadian Manitoba database. In a cohort of 5,529 IBD patients matched 1:10 with healthy controls from the general population, the risk of DVT and PE was significantly increased in IBD patients compared to controls (incidence rate ratio, IRR = 3.54, 95%CI: 2.9-4.3; and IRR = 3.3, 95%CI: 2.5-4.3 for DVT and PE respectively). IBD patients < 40 years of age were at particular high risk of VTEs with a six-fold increased risk (IRR = 6.02; 95%CI: 3.92-9.12). No sex or IBD subtype differences were observed[24]. This study led to the introduction of thromboprophylaxis as the standard care for IBD patients with active inflammation admitted to hospital. A later population-based study from the United Kingdom by Grainge *et al*[2] sought to elucidate the risk of VTE's during different stages of disease activity as they hypothesized that the more severe inflammation the greater risk of VTEs. In 13756 IBD patients, matched with 71627 non-IBD controls, the risk of developing VTE's was similar to the results from Canada with a hazard ratio (HR) of 3.4 (95%CI: 2.7-4.3). Further the study found that the risk of VTEs during a flare (defined as the period 120 d after a new corticosteroid prescription) was much more prominent with a HR of 8.4 (95%CI: 5.5-12.8). The highest relative risk of VTEs was found for IBD patients non-hospitalized during a flare with an almost 16-fold increased risk (HR = 15.8; 95%CI: 9.8-25.5). A recent meta-analysis identified 10 studies assessing the risk of VTEs in 72205 IBD patients and 891840 controls and found that the overall risk of VTEs in IBD was increased by 96% compared to the general population (RR = 1.96; 95%CI: 1.67-2.30)[25]. No difference in risk was found between UC and CD. The meta-analysis further confirmed that the risk of VTEs was greater in studies including IBD patients in general (RR = 2.48; 95%CI: 2.04-3.00) compared to studies evaluating on hospitalized IBD patients (RR 1.47; 95%CI: 1.17-1.86). This observation is potentially due to an effect of thromboprophylactic treatment strategies for hospitalized IBD patients.

Only few studies have evaluated on mortality rates in VTE complicated IBD patients. From the Mayo Clinic, Solem *et al*[26] reported a 22% mortality rate after a median follow-up of 1.8 years among 98 IBD patients diagnosed with VTE however, no comparison was made with post-VTE mortality rates in the general population. A large nation-wide population-based study from the United States by Nguyen and Sam[27], including more than a hundred thousand IBD patients, revealed that the in-hospital mortality was significantly higher for IBD patients with VTE compared with non-VTE IBD patients and this was valid for both CD (17.0 *vs* 4.2 per 1000 hospitalizations, $P < 0.0001$) and UC (37.4 *vs* 9.9 per 1000 hospitalizations, $P < 0.0001$). The excess mortality associated with VTE was 2.1 fold higher for

IBD patients than non-IBD individuals with VTEs ($P < 0.0001$) thereby indicating that VTEs have a more severe prognosis in IBD patients than in non-IBD individuals.

To summarize, it appears evident that IBD is a moderate independent risk factor for the development of VTEs and that the risk is highest among IBD patients with a flare in disease, not admitted to hospital. Further, there is a significant mortality associated with VTEs in IBD patients that is even greater than in non-IBD patient with VTEs. This calls for the importance of preventative and treatment strategies of VTEs in the IBD population, especially in the light of results from a recent survey involving 591 United States physicians; only 35% would give pharmacologic VTE prophylaxis to a hospitalized patient with severe UC[28].

ARTERIAL THROMBOEMBOLISM

In contrast to the well-established association between IBD and VTE, the risk of arterial thromboembolic events (ATE) in IBD is less elucidated in the literature. In the following, for simplicity, ATE will comprehend ischemic heart disease (IHD), cerebrovascular disease (CVD) and mesenteric ischemia.

Several circumstances could suggest that IBD patients are at increased risk of ATE. First of all IBD patients, particular CD patients are more likely to be current or past smokers. Further, some IBD-related drugs, *e.g.*, corticosteroids which increases the blood pressure and change the glucose homeostasis, and in contrary, the avoidance of aspirin-containing medications (due to potential fear of exacerbating IBD) could potentially increase the risk of ATE in IBD. Additionally, the presence of a chronic systemic inflammation in IBD, a well-known independent risk factor for atherosclerosis, assumedly augments the risk.

ISCHEMIC HEART DISEASE

Ischemic heart disease is caused by atherosclerotic plaque formation in coronary arteries and it is the most common type of heart disease and the leading cause of death in the world. Several inflammatory mediators as high C-reactive protein, and further up-stream inflammation markers such as tumor necrosis factor-α, interleukin-6 and 18 and the CD40 ligand are involved in the pathogenesis of both chronic inflammatory conditions including IBD and atherosclerosis[17,29]. Further, studies have revealed that IBD patients, compared to non-IBD individuals, have an increased carotid intima-media thickness, a surrogate marker for IHD and have a higher risk of early onset of atherosclerosis[6,30]. Thus, it appears biologically plausible that IBD patients carry an augmented risk of IHD compared to the general population.

In 2008, the first large study on risk of IHD in IBD patients, a population-based study from the Manitoba Database, Canada conducted by Bernstein *et al*[31], reported a 26% increased risk (IRR = 1.26; 95%CI: 1.11-1.44) of IHD in 8060 IBD patients compared to non-IBD individuals. No difference in risk was observed between sex and subtype of IBD.

In contrary, a retrospective matched cohort study from United States by Ha *et al*[10] including 17487 IBD patients did not reveal any overall increased risk of IHD in either CD or UC, but in sub-analyses the risk of myocardial infarction was significantly increased in IBD women aged above 40 years (HR = 1.16; $P = 0.003$).

In a matched cohort study by Yarur *et al*[13] from 2011, the risk of IHD was assessed among 356 IBD patients and 712 matched controls and the authors reported a nearly 3-fold increased risk of IHD in IBD (HR = 2.85; 95%CI: 1.82-4.46). A nationwide Danish population-based cohort study of 4570820 individuals by Rungoe *et al*[12] reported a lower, although significant increased risk of IHD (IRR = 1.59; 95%CI: 1.50-1.69) in IBD patients compared to non-IBD individuals[12]. Analyzing risk of IHD solely in the first three months and during the first year after IBD diagnosis revealed particularly high risk estimates (IRR = 4.57; 95%CI: 3.89-5.36 and IRR = 2.13; 95%CI: 1.91-2.38 respectively), hence also reflecting the potential role of ascertainment bias when assessing two chronic diseases (*i.e.* that hospitalization for one of the diseases increases the potential for discovery and recording of the other disease). However, analyses disregarding the first year after diagnosis and fully adjusted for co-morbidity related medications revealed a persistent 22% increased risk of IHD over time (IRR = 1.22; 95%CI: 1.14-1.30). A following population-based Danish study by Kristensen *et al*[11] reported risk of myocardial infarction (MI) in more than 20.000 IBD patients according to disease activity. Analyses revealed an increased risk of MI in IBD patients during flare (RR = 1.49; 95%CI: 1.16-1.93) and during persistent activity (RR = 2.05; 95%CI: 1.58-2.65), whereas the risk was not increased during periods of remission (RR = 1.01; 95%CI: 0.89-1.15). In accordance with the Danish findings, a meta-analysis on risk of IHD in IBD by Singh *et al*[32] reported a 19% increased risk of IHD in IBD patients (OR = 1.19; 95%CI: 1.08-1.31) with the risk being higher in female gender (OR = 1.26; 95%CI: 1.18-1-35). Interestingly, another meta-analysis by Fumery *et al*[25], solely including observational studies on risk of IHD in IBD did not (potentially due to lack of power) reveal a statistically increased risk, although the magnitude of risk was similar (RR = 1.23; 95%CI: 0.94-1.62). The main difference between the two meta-analyses was the inclusion of a cross-sectional study by Sridhar *et al*[33] only in the latter meta-analysis; a study that contrary to expected found an inverse association between IHD and hospitalized IBD patients with a significant protective effect of IBD on risk of IHD (OR = 0.60; 95%CI: 0.56-0.65). With results paradoxical to the hypothesis authors explained this protective association could be caused by a direct result of Berkson's fallacy[34], a form of selection bias that causes hospital cases and non-hospital controls in a case control study to be systemati-

cally different from one another, leading to a systematically higher exposure rate among hospital patients, and thereby distorting the risk estimate. This explanation is strengthened by the fact that the risk of IHD is increased in the meta-analysis by Fumery *et al*[25], when studies including hospitalized IBD patients were omitted (RR = 1.35; 95%CI: 1.19-1.52).

CEREBROVASCULAR DISEASE

Several case reports of ischemic stroke in remarkably young patients with CD has additionally led to the hypothesis of a potential association between IBD and CVE[35-38].

Bernstein and colleagues reported a slightly increased risk of cerebrovascular disease in patients with CD (but not UC) in a population based setting (IRR = 1.32; 95%CI: 1.05-1.66), but adjustments were insufficient, lacking several important cerebrovascular risk factors, such as smoking, obesity and hypertension[31]. A population-based case-control study from the United States evaluated on risk of ischemic stroke among 8054 CD patients matched with 161078 non-CD patients and results revealed an insignificant overall increased risk of ischemic stroke (OR = 1.10; 95%CI: 0.85-1.43)[39]. A significant almost 3-fold increased risk of ischemic stroke was estimated in younger CD patients below 50 years of age (OR = 2.93; 95%CI: 1.44-5.89). A large United States conducted population-based matched cohort study found no overall increased risk of cerebrovascular disease in IBD patients, but stratified analyses revealed a significantly increased risk of stroke among women with IBD below the age of 40 compared to non-IBD controls (HR = 2.1, $P < 0.05$)[10]. Only in a Danish setting an overall slightly increased risk of stroke in IBD patients has been estimated (RR = 1.15; 95%CI: 1.04-1.27)[11] and during flares this risk was further increased (RR = 1.53; 95%CI: 1.22-1.92).

The meta-analysis by Singh *et al*[32] reported pooled OR from five studies on cerebrovascular events in IBD and the meta-analysis revealed an adjusted 18% increased risk of CVE in IBD (OR = 1.18; 95%CI: 1.09-1.27), with a higher magnitude of risk estimates in women and patients at younger age.

INTESTINAL ISCHEMIA

The association between intestinal ischemia (including acute/chronic mesenteric ischemia and ischemic colitis) and IBD is vaguely elucidated. A population-based case-control study from the United Kingdom from 2011 studied risk factors for intestinal ischemia from the General Practice Research Database (GPRD)[40]. Of the 71 cases of intestinal ischemia derived from the database only one patient had intestinal ischemia and IBD corresponding to an insignificant 4-fold increased risk (OR = 4.19; 95%CI: 0.46-38.43). From the Nationwide Inpatient Sample (NIS), the largest inpatient database in the United States, the risk of mesenteric ischemia was assessed among nearly 150000 discharges with a diagnosis of IBD and revealed a significant association between IBD and mesenteric ischemia (adjusted OR = 3.4; 95%CI: 2.90-4.00) with a higher risk among UC patients (OR = 5.3; 95%CI: 4.24-6.74) than CD patients (2.58; 95%CI: 2.09-3.17). Young females with UC in the age group from 18 -39 years had the highest risk (OR = 15.48; 95%CI: 8.98-26.67). Likewise, a large cohort study[10] reported increased risk of mesenteric ischemia in IBD patients with a HR of 11.2 compared with controls ($P < 0.0001$) and found the risk to be highest in UC patients (HR = 12.5; $P < 0.0001$) and females aged between 18-39 years (HR = 22.3; $P < 0.0001$). Although the absolute risk may be limited, mesenteric ischemia remains a very serious condition and IBD practitioners should be aware of the importance of recognizing these events.

CARDIOVASCULAR MORTALITY

Several studies have assessed the mortality rate from CVD in IBD and reports on both increased and decreased mortality rates exist[41,42]. In a recent meta-analysis by Bewtra *et al*[43] of cause-specific standardized mortality ratios in both population-based and inception cohort studies of IBD patients, no increased mortality from cardiovascular disease in neither UC nor CD was found (SMR_{UC} = 0.90; 95%CI: 0.80-1.02 and SMR_{CD} = 1.00; 95%CI: 0.88-1.13). Similar insignificant risk estimates of cardiovascular mortality in IBD patients was reported in the meta-analysis by Fumery and colleges (pooled SMR = 1.03; 95%CI: 0.93-1.14)[25]. Nevertheless, it is important to keep in mind that although cardiovascular mortality is a hard end-point and less prone to ascertainment bias it does not capture the entire spectrum of cardiovascular disease and with improving therapeutic options the mortality rate is decreasing and observational studies on the association between IBD and cardiovascular mortality often does not reach statistical significance due to the low mortality rates. In the large-scale population-based study by Kristensen *et al*[11] with non-increased overall CV mortality among patients in remission (RR = 0.98; 95%CI: 0.89-1.09), authors were able to show increased CV mortality during flares (RR = 2.32; 95%CI: 2.01-2.68) and in patients with persistent disease activity (RR = 2.50; 95%CI: 2.14-2.92).

RISK FACTORS

The traditional risk factors for CVD are hypertension, diabetes mellitus, obesity, smoking, dyslipidemia, and physical inactivity.

A small Indian study by Sappati Biyyani *et al*[14] aimed at evaluating the presence of traditional atherosclerotic risk factors in patients with IBD and coronary artery disease (CAD) compared to a control group (only CAD) by using the Framingham risk score. The Framingham

risk score is a 10-year risk of CAD score based on the following risk factors: age, hypertension, diabetes mellitus, tobacco use and dyslipidemia. Among 42 cases and 137 controls the Framingham risk score was significantly lower in patients with both IBD and CAD compared to controls (8.1 *vs* 10.0; $P = 0.002$). Yarur *et al*[13] further assessed traditional and nontraditional risk factors in IBD related CAD and found that several traditional risk factors usually linked with patients' anthropometric status were less common in IBD. Kristensen *et al*[11] made subgroup analyses stratifying IBD patients according to presence of traditional risk factors and showed a strong association between the number of risk factors and the risk of cardiovascular events. Additionally it is interesting that this study found an association between disease activity and risk of CV events, thereby supporting the hypothesis that the chronic inflammation acts as a risk factor for CVD in IBD patients. This is in accordance with another Danish study by Rungoe and colleagues stratifying risk according to use of oral corticosteroids, used as a proxy for both current and later disease activity, and the study revealed a higher risk of IHD in IBD patients with a history of oral corticosteroids compared to never users (IRR = 1.37 *vs* 1.23 respectively; $P < 0.01$)[12].

POTENTIAL PREVENTIVE TREATMENTS

Considering chronic systemic inflammation as a potential nontraditional risk factor for CVD in IBD, it is interesting to evaluate the effect of treatments lowering the inflammatory burden on risk of CVD; despite the fact that anti-inflammatory therapy as treatment for atherosclerosis has received little attention. However, only few studies have addressed the impact of inflammation lowering drugs use in the management of IBD on risk of CVD.

In the study by Bewtra *et al*[44] sub-analyses stratifying between users and non-users of 5-aminosalicylic (5-ASA), a drug potentially possessing aspirin like properties, revealed a significant decreased risk of IHD in IBD patients receiving 5-ASA compared to never users (IRR = 1.16 *vs* 1.36 respectively; $P = 0.02$)[12]. Restricting analyses to long-term use of 5-ASA (defined as three or more redeemed prescriptions) further strengthened the finding of a preventive effect of 5-ASA on IHD (further decrease in IRR = of IHD to 1.08; 95%CI: 0.98-1.19). Interestingly, this observation of a preventive effect of 5-ASA on IHD was only present in IBD patients receiving oral corticosteroids which in this case was used as a proxy for disease severity. These results could indicate that only IBD patients with more severe disease or increased disease activity, are at increased risk of IHD and in this case the aspirin-like moiety of 5-ASA may have preventive properties.

As stated previously, the pro-inflammatory cytokine TNF-α plays an important role in the inflammatory process in both the intestine and in development of atherosclerosis. Accordingly, biological drugs impairing this cytokine, *e.g.*, infliximab and adalimumab, have been outlined not only as potential preventive treatments lowering the risk of CVD in IBD but also as a potential treatment for atherosclerotic disease as IHD in the general population. The direct and indirect effects of the TNF-α cytokine on the cardiovascular system is very complex and to some extend paradoxical. It is beyond the scope of the present review to give a detailed description of the pathological effects of TNF-α, but overall TNF-α tends to have both beneficial and harmful effects on the cardiovascular system, both in *in vitro* and *in vivo* studies; suggestively caused by a TNF-α concentration-related difference in effect and activation of different receptors[45-49]. This might also be the reason for conflicting results in studies evaluating the effect of TNF-α antagonist as a potential treatment option for atherosclerosis and IHD[48,50].

A study by Greenberg *et al*[51] evaluated on CV events associated with TNF-α antagonist treatment among more than 10000 patients with reumathoid arthritis (RA) and found that TNF-α antagonists treatment was associated with a reduced risk of cardiovascular events compared to RA patients treated with traditional disease-modifying antirheumatic drugs (HR = 0.39; 95%CI: 0.19-0.82). The risk of CVD, including both IHD and CVE, in IBD patients treated with TNF-α antagonists was elucidated in a Danish population-based study including more than 50000 IBD patients. Thirty-one TNF-α antagonist-exposed patients and 2641 unexposed patients developed IHD, yielding an adjusted RR of 0.85 (95%CI: 0.59-1.24) whereas the risk of CVE associated with TNF-α antagonists was 1.42 (95%CI: 0.82-2.45)[52]. Thus, point estimates indicate a protective effect of TNF-α antagonist on IHD but at the same time suggest TNF-α antagonists to be a risk factor for CVE, though noteworthy none of the estimates reached statistical significance. The complexity of TNF-α and the therapies targeting the cytokine demands for forthcoming intensive and thorough research in the field before any clear evaluation can be fulfilled.

A recent interest has been raised to the HMG-CoA-reductase inhibitors (statins), drugs mainly used for hyperlipidemia but comprise pleiotropic properties as pro-apoptotic, anti-angiogenic, and anti-inflammatory effects. The anti-inflammatory capacity of statins has been evaluated in IBD patients in a large retrospective study by Crockett *et al*[53] revealing a 18% reduction in initiation of oral steroids in IBD patients (HR = 0.82; 95%CI: 0.71-0.94) and an even greater reduction for UC patients (HR = 0.75; 95%CI: 0.62, 0.91). Future studies are needed to clarify the beneficial effect of statins in IBD and whether a potential synergetic effect may develop due to the potential of both lowering the risk of atherosclerosis and the inflammation in IBD.

CONCLUSION

The association between venous thromboembolic events and IBD is well-established and may cause significant

morbidity and mortality. Although antithrombotic prophylactic treatment is recommended for hospitalized IBD patients, surveys have shown that these recommendations are by far not followed in practice and greater attention to this issue is warranted.

Regarding arterial thromboembolic diseases, it seems plausible and it is further supported by recent literature, that the risk of CVD is increased in IBD patients, particularly during flares. The elevated risk is most likely due to an increased atherosclerotic burden triggered by inflammatory mediators, such as CRP, interleukin 6, and TNF-α.

Future large, prospective longitudinal studies are needed to determine the true risk of CVD in IBD and to further characterize preventive and risk factors. It is of particular interest whether tight control of the IBD-related inflammation could lower the progression and early development of atherosclerosis in these patients.

REFERENCES

1 **Murthy SK**, Nguyen GC. Venous thromboembolism in inflammatory bowel disease: an epidemiological review. *Am J Gastroenterol* 2011; **106**: 713-718 [PMID: 21407182 DOI: 10.1038/ajg.2011.53]

2 **Grainge MJ**, West J, Card TR. Venous thromboembolism during active disease and remission in inflammatory bowel disease: a cohort study. *Lancet* 2010; **375**: 657-663 [PMID: 20149425 DOI: 10.1016/S0140-6736(09)61963-2]

3 **Hansson GK**. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* 2005; **352**: 1685-1695 [PMID: 15843671 DOI: 10.1056/NEJMra043430]

4 **Theocharidou E**, Gossios TD, Griva T, Giouleme O, Douma S, Athyros VG, Karagiannis A. Is There an Association Between Inflammatory Bowel Diseases and Carotid Intima-media Thickness? Preliminary Data. *Angiology* 2013 Jun 6; Epub ahead of print [PMID: 23748978 DOI: 10.1177/0003319713489876]

5 **Roifman I**, Sun YC, Fedwick JP, Panaccione R, Buret AG, Liu H, Rostom A, Anderson TJ, Beck PL. Evidence of endothelial dysfunction in patients with inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2009; **7**: 175-182 [PMID: 19121648 DOI: 10.1016/j.cgh.2008.10.021]

6 **van Leuven SI**, Hezemans R, Levels JH, Snoek S, Stokkers PC, Hovingh GK, Kastelein JJ, Stroes ES, de Groot E, Hommes DW. Enhanced atherogenesis and altered high density lipoprotein in patients with Crohn's disease. *J Lipid Res* 2007; **48**: 2640-2646 [PMID: 17890779 DOI: 10.1194/jlr.M700176-JLR200]

7 **Avina-Zubieta JA**, Thomas J, Sadatsafavi M, Lehman AJ, Lacaille D. Risk of incident cardiovascular events in patients with rheumatoid arthritis: a meta-analysis of observational studies. *Ann Rheum Dis* 2012; **71**: 1524-1529 [PMID: 22425941 DOI: 10.1136/annrheumdis-2011-200726]

8 **Patel RV**, Shelling ML, Prodanovich S, Federman DG, Kirsner RS. Psoriasis and vascular disease-risk factors and outcomes: a systematic review of the literature. *J Gen Intern Med* 2011; **26**: 1036-1049 [PMID: 21472501 DOI: 10.1007/s11606-011-1698-5]

9 **Cervera R**, Khamashta MA, Font J, Sebastiani GD, Gil A, Lavilla P, Mejía JC, Aydintug AO, Chwalinska-Sadowska H, de Ramón E, Fernández-Nebro A, Galeazzi M, Valen M, Mathieu A, Houssiau F, Caro N, Alba P, Ramos-Casals M, Ingelmo M, Hughes GR. Morbidity and mortality in systemic lupus erythematosus during a 10-year period: a comparison of early and late manifestations in a cohort of 1,000 patients. *Medicine* (Baltimore) 2003; **82**: 299-308 [PMID: 14530779 DOI: 10.1097/01.md.0000091181.93122.55]

10 **Ha C**, Magowan S, Accortt NA, Chen J, Stone CD. Risk of arterial thrombotic events in inflammatory bowel disease. *Am J Gastroenterol* 2009; **104**: 1445-1451 [PMID: 19491858 DOI: 10.1038/ajg.2009.81]

11 **Kristensen SL**, Ahlehoff O, Lindhardsen J, Erichsen R, Jensen GV, Torp-Pedersen C, Nielsen OH, Gislason GH, Hansen PR. Disease activity in inflammatory bowel disease is associated with increased risk of myocardial infarction, stroke and cardiovascular death--a Danish nationwide cohort study. *PLoS One* 2013; **8**: e56944 [PMID: 23457642 DOI: 10.1371/journal.pone.0056944]

12 **Rungoe C**, Basit S, Ranthe MF, Wohlfahrt J, Langholz E, Jess T. Risk of ischaemic heart disease in patients with inflammatory bowel disease: a nationwide Danish cohort study. *Gut* 2013; **62**: 689-694 [PMID: 22961677 DOI: 10.1136/gutjnl-2012-303285]

13 **Yarur AJ**, Deshpande AR, Pechman DM, Tamariz L, Abreu MT, Sussman DA. Inflammatory bowel disease is associated with an increased incidence of cardiovascular events. *Am J Gastroenterol* 2011; **106**: 741-747 [PMID: 21386828 DOI: 10.1038/ajg.2011.63]

14 **Sappati Biyyani RS**, Fahmy NM, Baum E, Nelson KM, King JF. Inflammatory bowel disease and coronary artery disease. *Indian J Gastroenterol* 2009; **28**: 28-30 [PMID: 19529899 DOI: 10.1007/s12664-009-0006-3]

15 **Bargen JA**, Barker NW. Extensive arterial and venous thrombosis complicating chronic ulcerative colitis. *Arch Intern Med* 1938; **31**: 17-31

16 **Talbot RW**, Heppell J, Dozois RR, Beart RW. Vascular complications of inflammatory bowel disease. *Mayo Clin Proc* 1986; **61**: 140-145 [PMID: 3080643]

17 **Hatoum OA**, Binion DG. The vasculature and inflammatory bowel disease: contribution to pathogenesis and clinical pathology. *Inflamm Bowel Dis* 2005; **11**: 304-313 [PMID: 15735437 DOI: 10.1097/01.MIB.0000160772.78951.61]

18 **Lam A**, Borda IT, Inwood MJ, Thomson S. Coagulation studies in ulcerative colitis and Crohn's disease. *Gastroenterology* 1975; **68**: 245-251 [PMID: 1116673]

19 **Chamouard P**, Grunebaum L, Wiesel ML, Frey PL, Wittersheim C, Sapin R, Baumann R, Cazenave JP. Prothrombin fragment 1 + 2 and thrombin-antithrombin III complex as markers of activation of blood coagulation in inflammatory bowel diseases. *Eur J Gastroenterol Hepatol* 1995; **7**: 1183-1188 [PMID: 8789309]

20 **Souto JC**, Martínez E, Roca M, Mateo J, Pujol J, González D, Fontcuberta J. Prothrombotic state and signs of endothelial lesion in plasma of patients with inflammatory bowel disease. *Dig Dis Sci* 1995; **40**: 1883-1889 [PMID: 7555437]

21 **Aadland E**, Odegaard OR, Røseth A, Try K. Free protein S deficiency in patients with chronic inflammatory bowel disease. *Scand J Gastroenterol* 1992; **27**: 957-960 [PMID: 1455194]

22 **Aadland E**, Odegaard OR, Røseth A, Try K. Free protein S deficiency in patients with Crohn's disease. *Scand J Gastroenterol* 1994; **29**: 333-335 [PMID: 8047807]

23 **Jorens PG**, Hermans CR, Haber I, Kockx MM, Vermylen J, Parizel GA. Acquired protein C and S deficiency, inflammatory bowel disease and cerebral arterial thrombosis. *Blut* 1990; **61**: 307-310 [PMID: 2148695]

24 **Bernstein CN**, Blanchard JF, Houston DS, Wajda A. The incidence of deep venous thrombosis and pulmonary embolism among patients with inflammatory bowel disease: a population-based cohort study. *Thromb Haemost* 2001; **85**: 430-434 [PMID: 11307809]

25 **Fumery M**, Xiaocang C, Dauchet L, Gower-Rousseau C, Peyrin-Biroulet L, Colombel JF. Thromboembolic events and cardiovascular mortality in inflammatory bowel diseases: A meta-analysis of observational studies. *J Crohns Colitis* 2013 Jun 1; Epub ahead of print [PMID: 24183231 DOI: 10.1016/j.crohns.2013.09.021]

26 **Solem CA**, Loftus EV, Tremaine WJ, Sandborn WJ. Venous thromboembolism in inflammatory bowel disease. *Am J Gastroenterol* 2004; **99**: 97-101 [PMID: 14687149]

27 **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]

28 **Tinsley A**, Naymagon S, Enomoto LM, Hollenbeak CS, Sands BE, Ullman TA. Rates of pharmacologic venous thromboembolism prophylaxis in hospitalized patients with active ulcerative colitis: results from a tertiary care center. *J Crohns Colitis* 2013; **7**: e635-e640 [PMID: 23706933 DOI: 10.1016/j.crohns.2013.05.002]

29 **Kaptoge S**, Seshasai SR, Gao P, Freitag DF, Butterworth AS, Borglykke A, Di Angelantonio E, Gudnason V, Rumley A, Lowe GD, Jørgensen T, Danesh J. Inflammatory cytokines and risk of coronary heart disease: new prospective study and updated meta-analysis. *Eur Heart J* 2014; **35**: 578-589 [PMID: 24026779 DOI: 10.1093/eurheartj/eht367]

30 **Dagli N**, Poyrazoglu OK, Dagli AF, Sahbaz F, Karaca I, Kobat MA, Bahcecioglu IH. Is inflammatory bowel disease a risk factor for early atherosclerosis? *Angiology* 2010; **61**: 198-204 [PMID: 19398421 DOI: 10.1177/0003319709333869]

31 **Bernstein CN**, Wajda A, Blanchard JF. The incidence of arterial thromboembolic diseases in inflammatory bowel disease: a population-based study. *Clin Gastroenterol Hepatol* 2008; **6**: 41-45 [PMID: 18063423 DOI: 10.1016/j.cgh.2007.09.016]

32 **Singh S**, Singh H, Loftus EV, Pardi DS. Risk of cerebrovascular accidents and ischemic heart disease in patients with inflammatory bowel disease: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2014; **12**: 382-393.e1: quiz e22 [PMID: 23978350 DOI: 10.1016/j.cgh.2013.08.023]

33 **Sridhar AR**, Parasa S, Navaneethan U, Crowell MD, Olden K. Comprehensive study of cardiovascular morbidity in hospitalized inflammatory bowel disease patients. *J Crohns Colitis* 2011; **5**: 287-294 [PMID: 21683298 DOI: 10.1016/j.crohns.2011.01.011]

34 **BERKSON J**. Limitations of the application of fourfold table analysis to hospital data. *Biometrics* 1946; **2**: 47-53 [PMID: 21001024]

35 **Freilinger T**, Riedel E, Holtmannspötter M, Dichgans M, Peters N. Ischemic stroke and peripheral arterial thromboembolism in a patient with Crohn's disease: a case presentation. *J Neurol Sci* 2008; **266**: 177-179 [PMID: 17904158 DOI: 10.1016/j.jns.2007.08.035]

36 **Halliday CE**, Farthing MJ. Arterial thrombosis in Crohn's disease. *Med J Aust* 1988; **149**: 559-560 [PMID: 3185327]

37 **Silverstein A**, Present DH. Cerebrovascular occlusions in relatively young patients with regional enteritis. *JAMA* 1971; **215**: 976-977 [PMID: 5107514]

38 **Younes-Mhenni S**, Derex L, Berruyer M, Nighoghossian N, Philippeau F, Salzmann M, Trouillas P. Large-artery stroke in a young patient with Crohn's disease. Role of vitamin B6 deficiency-induced hyperhomocysteinemia. *J Neurol Sci* 2004; **221**: 113-115 [PMID: 15178225 DOI: 10.1016/j.jns.2004.03.016]

39 **Andersohn F**, Waring M, Garbe E. Risk of ischemic stroke in patients with Crohn's disease: a population-based nested case-control study. *Inflamm Bowel Dis* 2010; **16**: 1387-1392 [PMID: 20014016 DOI: 10.1002/ibd.21187]

40 **Huerta C**, Rivero E, Montoro MA, García-Rodriguez LA. Risk factors for intestinal ischaemia among patients registered in a UK primary care database: a nested case-control study. *Aliment Pharmacol Ther* 2011; **33**: 969-978 [PMID: 21366637 DOI: 10.1111/j.1365-2036.2011.04614.x]

41 **Ekbom A**, Helmick CG, Zack M, Holmberg L, Adami HO. Survival and causes of death in patients with inflammatory bowel disease: a population-based study. *Gastroenterology* 1992; **103**: 954-960 [PMID: 1499945]

42 **Jess T**, Frisch M, Simonsen J. Trends in overall and cause-specific mortality among patients with inflammatory bowel disease from 1982 to 2010. *Clin Gastroenterol Hepatol* 2013; **11**: 43-48 [PMID: 23022699 DOI: 10.1016/j.cgh.2012.09.026]

43 **Bewtra M**, Kaiser LM, TenHave T, Lewis JD. Crohn's disease and ulcerative colitis are associated with elevated standardized mortality ratios: a meta-analysis. *Inflamm Bowel Dis* 2013; **19**: 599-613 [PMID: 23388544 DOI: 10.1097/MIB.0b013e31827f27ae]

44 **Desreumaux P**, Ghosh S. Review article: mode of action and delivery of 5-aminosalicylic acid - new evidence. *Aliment Pharmacol Ther* 2006; **24** Suppl 1: 2-9 [PMID: 16939423 DOI: 10.1111/j.1365-2036.2006.03069.x]

45 **Bozkurt B**, Kribbs SB, Clubb FJ, Michael LH, Didenko VV, Hornsby PJ, Seta Y, Oral H, Spinale FG, Mann DL. Pathophysiologically relevant concentrations of tumor necrosis factor-alpha promote progressive left ventricular dysfunction and remodeling in rats. *Circulation* 1998; **97**: 1382-1391 [PMID: 9577950]

46 **Lecour S**, Smith RM, Woodward B, Opie LH, Rochette L, Sack MN. Identification of a novel role for sphingolipid signaling in TNF alpha and ischemic preconditioning mediated cardioprotection. *J Mol Cell Cardiol* 2002; **34**: 509-518 [PMID: 12056855 DOI: 10.1006/jmcc.2002.1533]

47 **McMurray J**, Abdullah I, Dargie HJ, Shapiro D. Increased concentrations of tumour necrosis factor in "cachectic" patients with severe chronic heart failure. *Br Heart J* 1991; **66**: 356-358 [PMID: 1747295]

48 **Padfield GJ**, Din JN, Koushiappi E, Mills NL, Robinson SD, Cruden Nle M, Lucking AJ, Chia S, Harding SA, Newby DE. Cardiovascular effects of tumour necrosis factor α antagonism in patients with acute myocardial infarction: a first in human study. *Heart* 2013; **99**: 1330-1335 [PMID: 23574969 DOI: 10.1136/heartjnl-2013-303648]

49 **Roubille C**, Martel-Pelletier J, Haraoui B, Tardif JC, Pelletier JP. Biologics and the cardiovascular system: a double-edged sword. *Antiinflamm Antiallergy Agents Med Chem* 2013; **12**: 68-82 [PMID: 23286291]

50 **Bissonnette R**, Tardif JC, Harel F, Pressacco J, Bolduc C, Guertin MC. Effects of the tumor necrosis factor-α antagonist adalimumab on arterial inflammation assessed by positron emission tomography in patients with psoriasis: results of a randomized controlled trial. *Circ Cardiovasc Imaging* 2013; **6**: 83-90 [PMID: 23204039 DOI: 10.1161/CIRCIMAGING.112.975730]

51 **Greenberg JD**, Kremer JM, Curtis JR, Hochberg MC, Reed G, Tsao P, Farkouh ME, Nasir A, Setoguchi S, Solomon DH. Tumour necrosis factor antagonist use and associated risk reduction of cardiovascular events among patients with rheumatoid arthritis. *Ann Rheum Dis* 2011; **70**: 576-582 [PMID: 21109516 DOI: 10.1136/ard.2010.129916]

52 **Andersen NN**, Rungoe C, Andersson M, Munkholm P, Pasternak B, Jess T. Tumor Necrosis Factor-Alpha Antagonists And Cardiovascular Disease In Inflammatory Bowel Disease. European Crohn Colitis Organisation Congress, Vienna, Austria, February 14-16, 2013. Available from: URL: https://www.ecco-ibd.eu/publications/congress-abstract-s/abstracts-2013/item/19-tumor-necrosis-factor-alpha-antagonists-and-cardiovascular-disease-in-inflammatory-bowel-disease.html

53 **Crockett SD**, Hansen RA, Stürmer T, Schectman R, Darter J, Sandler RS, Kappelman MD. Statins are associated with reduced use of steroids in inflammatory bowel disease: a retrospective cohort study. *Inflamm Bowel Dis* 2012; **18**: 1048-1056 [PMID: 21826766 DOI: 10.1002/ibd.21822]

P- Reviewer: Bokemeyer B, Tasci I **S- Editor**: Ma YJ
L- Editor: A **E- Editor:** Lu YJ

World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4291/wjgp.v5.i3.366

World J Gastrointest Pathophysiol 2014 August 15; 5(3): 366-372
ISSN 2150-5330 (online)

RETROSPECTIVE STUDY

Cancer stem cells in *Helicobacter pylori* infection and aging: Implications for gastric carcinogenesis

Edi Levi, Paula Sochacki, Nabiha Khoury, Bhaumik B Patel, Adhip PN Majumdar

Edi Levi, Paula Sochacki, Nabiha Khoury, Bhaumik B Patel, Adhip PN Majumdar, Department of Veterans Affairs, John D Dingell VA Medical Center, Wayne State University, Detroit, MI 48201, United States
Edi Levi, Pathology, Wayne State University, Detroit, MI 48201, United States
Bhaumik B Patel, Adhip PN Majumdar, Karmanos Cancer Center, Wayne State University, Detroit, MI 48201, United States
Adhip PN Majumdar, Departments of Internal Medicine, Wayne State University, Detroit, MI 48201, United States
Author contributions: Levi E and Patel BB performed the experiments and wrote the manuscript; Sochacki P and Khoury N evaluated the slides, verified the diagnoses and scored the immunohistochemical stains, they also participated in the drafting of the manuscript; Majumdar APN participated in the design, evaluation of data and writing the manuscript.
Supported by Grants to Dr. Majumdar from NIH/NIA, No. AG014343; and the Department of Veterans Affairs (VA Merit Review)
Correspondence to: Adhip PN Majumdar, PhD, DSc, Department of Veterans Affairs, John D Dingell VA Medical Center, 4646 John R, Room B-4238, Detroit, MI 48201, United States. majumdar@med.wayne.edu
Telephone: +1-313-5764460 Fax: +1-313-5761112
Received: November 2, 2013 Revised: April 3, 2014
Accepted: May 8, 2014
Published online: August 15, 2014

Abstract

AIM: To demonstrated the combined effects of aging and carcinogen treatment on cancer stem/stem-like cells (CSCs) of gastric mucosa in an animal model.

METHODS: In this study we investigated the effects of aging and *Helicobacter pylori* (*H. pylori*) inflammation as a model for inflammation induced carcinogenesis in human and rat gastric mucosa samples. In aging studies, we compared 4-mo old (young) with 22 mo (aged) old Fischer-344 rats. For human studies, gastric biopsies and resection specimens representing normal mucosa or different stages of *H. pylori* gastritis and gastric adenocarcinomas were used for determining the expression of stem cell markers CD166, ALDH1 and LGR5. In addition we performed immunofluorescent double labeling for B-catenin and Lgr5 in both rat and human gastric tissues to examine the status of Wnt signaling in these cells.

RESULTS: CSC markers ALDH1, LGR5, and CD166 were expressed in very low levels in normal human gastric mucosa or young rat gastric mucosa. In contrast, level of expression for all three markers significantly increased in *H. pylori* gastritis and gastric adenocarcinomas as well as in normal gastric mucosa in aged rats. We also observed cytoplasmic B-catenin staining in both aged rat and human *H. pylori* inflamed gastric mucosa, which were found to be colocalized with Lgr5 immunoreactive cells. The increased number of ALDH1, CD166 and LGR5 positive cells in *H. pylori* gastritis indicates that increased number of stem-like cells in gastric mucosa is an early event, and may constitute an important step in the progression to neoplasia.

CONCLUSION: Our observation of the age-related increase in cancer stem/stem-like cells in the gastric mucosa may explain the increased incidence of gastric cancer during aging. Combination of aging and *H. pylori* infection may have additive effects in progression to neoplasia.

Key words: Cancer stem cells; Aging; CD166; ALDH1; LGR5; Gastric cancer; *Helicobacter pylori*

Core tip: In this study we demonstrated an age-related increase in cancer stem/stem-like cells (CSCs) in normal appearing gastric mucosa with activated Wnt signaling. In addition, we have shown that gastric infection

by *Helicobacter pylori* (*H. pylori*) induces an increase in CSC population in the gastric mucosa. Based on our observations we believe that aging and chronic inflammation with *H. pylori* are two significant factors that overlap and presumably exacerbate each other in gastric carcinogenesis.

Levi E, Sochacki P, Khoury N, Patel BB, Majumdar APN. Cancer stem cells in *Helicobacter pylori* infection and aging: Implications for gastric carcinogenesis. *World J Gastrointest Pathophysiol* 2014; 5(3): 366-372 Available from: URL: http://www.wjgnet.com/2150-5330/full/v5/i3/366.htm DOI: http://dx.doi.org/10.4291/wjgp.v5.i3.366

INTRODUCTION

It has been well established that the incidences of cancer rise sharply with age and the majority of cancer cases are detected in patients over the age of 65 years[1]. Such a direct correlation between cancer incidence and advanced age in most cancers clearly suggests that the phenomenon of aging and cancer are intricately connected. Accumulating evidence also suggests that the increase in tumor incidence with advancing age is preceded in part by chronic disorders including inflammation[1,2]. The etiological causes of inflammation are many folds and include viruses, bacteria, environmental pollutants, and stress as well as food factors. Chronic inflammation as risk factor for most cancers is well recognized[2].

Aging and chronic inflammation are two factors associated with an increased risk for gastric cancer[1,2]. Within the gastrointestinal tract, inflammatory conditions such as gastroesophageal reflux disease, *Helicobacter pylori* infection (*H. pylori*), inflammatory bowel disease, and viral hepatitis are well known to be associated with cancers[1,2]. The possible explanations for the link between cancer and inflammation are accumulating gene mutations, inhibition of apoptosis, increased cell proliferation and pro-inflammatory cytokine release which creates a pro-carcinogenic microenvironment[1,2].

A growing body of evidence supports the contention that cancers, including the gastric cancer are diseases driven by a small set of self renewing cells, termed cancer stem cells (CSC) or cancer-initiating cells, that are distinct from the bulk of the cells in the tumor[3-9]. CSCs are widely believed to arise from the normal stem cells or progenitor cells upon mutations[7].

The putative progenitor/stem cell in the stomach is thought to reside in the isthmic region of the fundic epithelium[6,7,10]. In mice, granule free cells in the isthmus have been shown to act as stem cells[11].

The gastric progenitor/stem cells accomodate to acute and chronic injury to the gastric epithelium and replenish the destroyed epithelium during the lifetime of the organism of interest, which creates the risk of accumulating mutations and giving rise to gastric cancers[7,8,12]. *H. pylori* gastritis which is a known preneoplastic condition, is a good model to study the response of stem cells to chronic injury and mutagenesis[13-15]. A recent study has shown a direct interaction between *H. pylori* organisms and gastric stem cells[15].

We have recently demonstrated the combined effects of aging and carcinogen treatment on the colon CSCs in a rat model[16,17]. In this model, carcinogen treated rats had more dramatic increase in CSCs if they were also aged. Based on these and other relevant observations[1,9,17-21], we hypothesize that, aging and chronic inflammation are two parallel events leading to an increased incidence of cancers in the gastrointestinal tract, including colon and gastric cancers. We further hypothesize that the initiating factor in this scenario is the alteration of the CSC population in the normal appearing mucosa.

To test our hypothesis that combination of the effects of aging and inflammation on CSCs exacerbates cancer development, we made an attempt to identify gastric CSCs by using immunohistochemical (IHC) markers in young and old rat gastric mucosa samples. We then expanded our studies to human gastric mucosa with various degrees of *H. pylori* induced inflammation in order to show the alterations in CSC compartment during the course of *H. pylori*- induced disease.

MATERIALS AND METHODS

Animals

Male Fischer-344 rats, aged 4-6 (young) or 22-24 mo (old) were purchased from the National Institute on Aging (Bethesda, MD). All procedures were performed according to the standards for use of laboratory animals established by the Institute of Laboratory Animal Resources, National academy of Sciences, and were approved by the Animal Investigation Committee at Wayne State University School of Medicine. The details of animal handling have been previously published[16,20].

Human gastric tissues

Formalin fixed-paraffin embedded gastric tissue samples representing normal/uninfected mucosa (n = 10), helicobacter pylori gastritis (n = 12), helicobacter pylori gastritis with intestinal metaplasia (n = 10), dysplasia (n = 6) and gastric cancer (n = 12) were retrieved from the Pathology archives of John D. Dingell VA Medical Center, Detroit MI. The diagnoses were confirmed by three pathologists who are co-authors of this study. The study was approved by the IRB committee of Wayne State University, and the R&D committee of John D. Dingell VA Medical Center.

The mean age of the patients was 46 ± 6 (SD). They were all male, reflecting the population profile of the hospital. The difference of age between the control and the inflamed mucosa samples was not statistically significant (not shown).

Immunohistochemistry

The antibodies utilized for immunohistochemical stains

Table 1 Staining scores for stem cell markers in human gastric mucosa and rat gastric mucosa

	n	ALDH	CD166	LGR5
Normal	10	1-2	0	1-2
H. pylori without IM	12	5-6[a]	3-4[a]	3-4[a]
H. pylori with IM	10	7-8[a]	5-6[a]	5-6[a]
Gastric adenocarcinoma	12	85%	75%	70%
4-mo-old rat	6	1-2	1-2	0
24-mo-old rat	6	3-4	5-6[a]	3-4[a]

The numbers are cells expressing the marker per a crypt counted. We grouped the cell counts as 0; 1-2; 3-4; 5-6; 7-8; *etc*. For evaluation of gastric adenocarcinoma, we expressed the percentage of cells staining with the related marker. [a]$P < 0.05$ *vs* normal human gastric mucosa or 4-mo-old rat gastric mucosa. Gastric adenocarcinoma was not included in statistical analysis.

were LGR5 (dilution at 1:200, ABGENT, San Diego CA), CD166 (dilution at 1:200 RD systems, Minneapolis MN), ALDH1 (dilution at 1:100 BD Biosciences, San Jose CA) and B-catenin (SCBT, Dallas TX at 1:100 dilution).

Immunohistochemistry was performed according to our standard protocol[8,13,19]. Briefly, the paraffin blocks of the fixed colon tissues were cut into 5 μm sections. The slides were deparaffinized. For antigen retrieval, tissues were microwaved for 15 min in Citrate pH = 6.0 buffer, then allowed to cool to room temperature. Endogenous peroxide was quenched by incubation of the sections with 3% hydrogen peroxide. Non specific binding was blocked application of 5% horse serum. Primary antibodies were applied overnight at 4 ℃ and antibody detection was completed utilizing the Vecstatin Elite ABC system detection kit from Vector (Burlingame CA). AEC was used as chromogen.

We defined positivity in normal and *H. pylori* cases as membranous and cytoplasmic staining in number of cells per gland (CPG). For cancer cases we used percentage of tumor cells to define positivity.

Immunofluorescence double labeling of B-catenin and LGR5

We have performed double labeling for B-catenin and Lgr5 on sections from rat gastric mucosa and human gastric epithelium by using immunofluorescent secondary antibodies to demonstrate the co-expression of these markers. For B-catenin primary antibody (Santa Cruz BT) anti-mouse IgG TRITC (Sigma, St Louis MO) secondary antibody was used. For LGR5 (ABGENT) antibody, anti-rabbit IgG FITC (Sigma) antibody was used. The slides were evaluated by a fluorescent microscope with green and red filters. Gastric cancer specimens were used as positive controls. For negative controls, we omitted the primary antibody, and applied only secondary antibody.

Statistical analysis

For statistical analysis we assessed the CSC expression as low *vs* high expression, with 0 and 1-2 CSC considered as low expression and ⩾ 3-4 CSC as high expression. This cut off value was based on the observation that in normal mucosa we rarely encountered more than 1-2 CSC per gland counted. Statistical significance was assessed by χ^2 test.

RESULTS

Rat gastric mucosa

In the 4 mo old (young) rats ALDH1, CD166 and LGR5 staining were rare events. Very few cells in the isthmic region of the fundic mucosa demonstrated cytoplasmic staining for the three markers (Figure 1). The results are summarized in Table 1.

The expression of LGR5 and CD166 was significantly increased in the 24 mo old rats (Figure 1 and Table 1). The staining location was still in the isthmus region. The B-catenin staining was limited to few stem cells and was membranous in the young rat mucosa and cytoplasmic in the aged mucosa (Figure 1).

B-catenin LGR5 double labeling

We also investigated the expression of B-catenin and LGR5 in a double immunofluorescent labeling study. B-catenin is normally expressed in the cell membrane in the inactive state. Activated B-catenin pathway can be detected by nuclear or cytoplasmic staining. We therefore investigated the status of B-catenin signaling in the LGR5 expressing putative stem cells. As shown in Figure 2A, a rare cell with LGR5 expression (green signal) also revealed cytoplasmic B-catenin immunoreactivity (red signal).

Human gastric mucosa

We first investigated the expression of CSC markers in normal mucosa which included both antral and fundic mucosa. The staining of the cells was localized to the isthmic region of the fundic mucosa (Figure 3). In antrum, the staining was present in the base of the glands.

In *H. pylori* infected gastric mucosa, the expression of CSC markers was significantly increased for all three markers examined (Table 1 and Figure 3).

In the *H. pylori* infected gastric mucosa with intestinal metaplasia and also in gastric cancer, the level of staining was also significantly increased (Table 1).

In addition, we performed a double labeling immunofluorescent staining for LGR5 and B-catenin in *H. pylori* infected gastric mucosa. We observed that in the LGR5 expressing cells in the gastric mucosa; the expression of B-catenin is cytoplasmic, implying an activation of B-catenin signaling (Figure 2B).

DISCUSSION

In this study we demonstrated an age-related in CSCs in normal appearing gastric mucosa with activated WNT signaling. In addition, we have shown that, gastric infec-

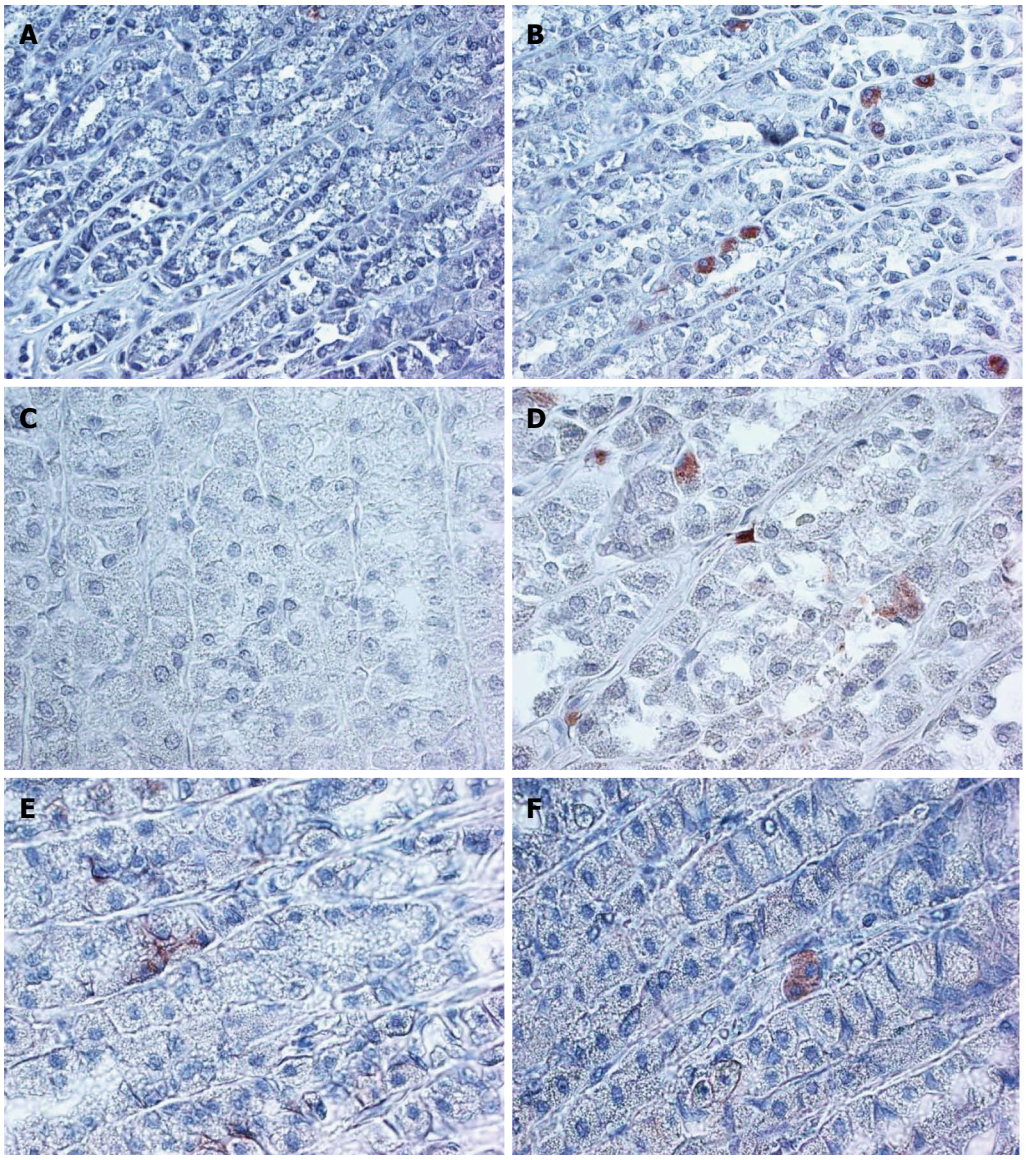

Figure 1 Higher expression of B-catenin, CD166, and LGR5 in normal aged rat gastric mucosa, compared to young rat normal gastric mucosa is demonstrated. × 200 magnification. A: 4 m CD166; B: 24 m CD166; C: 4 m LGR5; D: 24 m LGR5; E: 4 m B-catenin; F: 24 m B-catenin.

tion by *H. pylori* induces an increase in CSC population in the gastric mucosa. Based on our observations we believe that aging and chronic inflammation with *H. pylori* are two significant factors that overlap and presumably exacerbate each other in gastric carcinogenesis.

Studies of the murine gastrointestinal tract have shown that cells from old mice at or near the position of the stem cells within the crypts of Lieberkuhn are more susceptible to apoptosis under stress[18] and exhibit reduced regenerative potential despite an age dependent increase in the number of crypt cells. Similar age related decline in functional properties of stem cells have been shown in other tissues particularly in hematopoietic stem cells[9]. It is very likely that the increase in the number of stem cells is a compensatory event to replenish the destroyed cells in the target tissue and is a reflection of the decreased functional capacity of these cells. This situation is analogous to myelodysplastic syndrome, which is commonly present in the elderly and is characterized by a hypercellular bone marrow despite a peripheral cytopenia.

Stem cells are subject to the similar array of insults as somatic cells and are therefore susceptible to genetic damage. The accumulating damage, unlike that of somatic cells is propagated to the daughter cells and to downstream lineages through the process of self renewal and differentiation. The nature of accumulating mutations and genetic damage determines the fate of the CSCs. The outcome could be senescence, apoptosis, or transformation[9].

In this context, the survival pathways utilized by the stem cells are very critical in their maintenance and possibly transformation. The important signaling pathways involved in gastric CSCs survival include the Wnt/B-catenin, sonic hedgehog (shh), Notch, and fibroblastic growth factor/bone morphogenic protein (FGF/BMP)

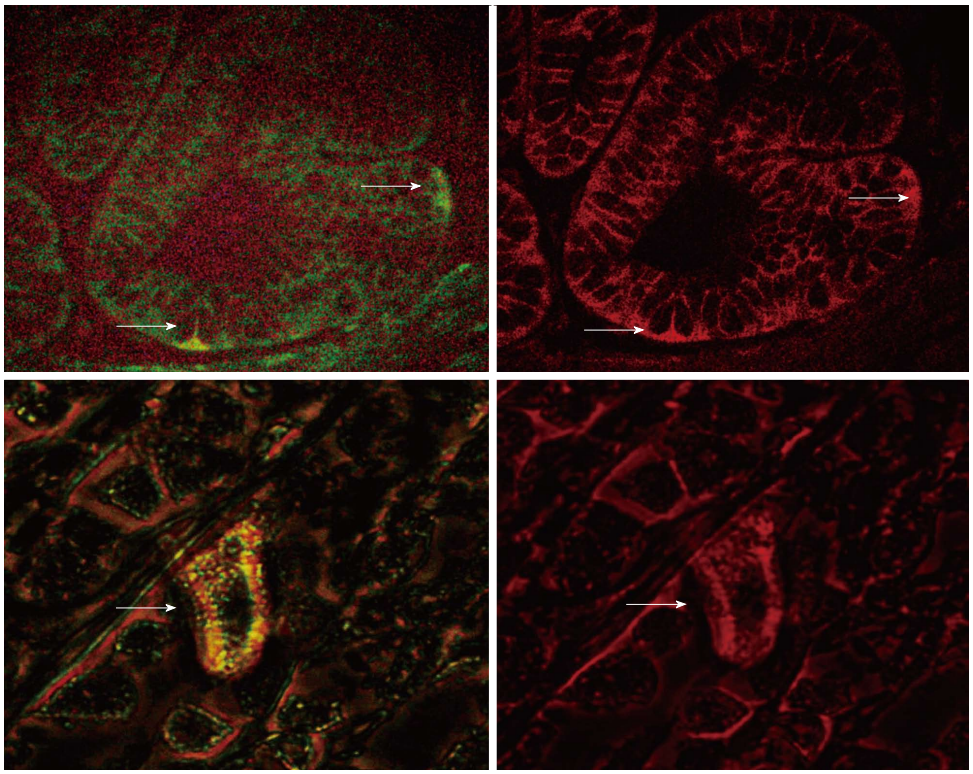

Figure 2 Double labeling for LGR5 (green, left panel) and B-catenin (red, right panel) in aged rat gastric mucosa (Lower Panel, × 600 magnification), *H. pylori* infected human gastric mucosa (Upper Panel, × 400 magnification) shows cytoplasmic localization of B-catenin in an LGR5 expressing cell.

pathways[8,9,12,22].

Wnt signaling pathway releases B-catenin from the AXIN/GSK3b degradation complex. Activation of the Wnt signaling results in the translocation of B-catenin from the cell membrane to the cytoplasm and subsequent translocation to the nucleus. Accumulation of B-catenin in the nucleus results in the transcriptional activation of target genes that play critical roles in regulating cell proliferation[22].

H. pylori infection results in the activation of the stem cell signaling networks such as Wnt, Notch, FGF/BMP, and Hh/SHH oncogenic signaling pathways[2,8,22]. In a previous study, *H. pylori* infection has been shown to be associated with an increased expression of CD44 in gastric mucosa[10]. We also investigated CD44 in the gastric mucosa and found that CD44 expression is markedly increased in *H. pylori* infected mucosa (data not shown).

LGR5 is an orphan G-protein coupled receptor and Wnt target gene, and is a putative marker for gastrointestinal stem cells. Barker *et al*[3] first identified a subpopulation of Lgr5+ stem cells at the base of the crypts in mouse small intestine and colon. Since then, several studies have confirmed the utility of Lgr5 as a putative stem cell/progenitor marker[3-7]. Our studies highlight an Lgr5 positive population in normal human fundic epithelium, localized to the isthmic region, in concordance with the anatomic localization of the progenitor cells. In addition, we demonstrate that Lgr5 expressing cells are increased during aging and in response to *H. pylori* infection.

Our double labeling studies demonstrate that B-catenin is relocated to cytoplasm in the stem cells in aging and *H. pylori* infection. Wnt signaling may preferentially influence the expansion of progenitor cells in the gastrointestinal system and may be the driving force behind the early increase in CSCs in the colon and stomach. However, we do not know whether the activation of B-Catenin is entirely normal or an early phase of neoplastic transformation. Since WNT signaling can support both the normal and CSCs renewal and maintenance, it is a potential target for therapeutic interventions or preventative measures.

Our current findings are very similar to our previous data from colonic mucosa of aged and carcinogen exposed rats[14,16]. We propose that in gastrointestinal cancers, aging and chronic inflammation leading to cytokine activation is a critical factor. The increase in CSCs is probably one of the early events in this process. Further studies are needed to directly observe the link between increase in CSCs and acquisition of cancer phenotype.

The finding of increase in CSCs in otherwise normal appearing mucosa has ramifications for diagnostic, prognostic, preventive, and therapeutic approaches to the gastrointestinal cancers. CSC markers can be used in gastric biopsies from patients with atrophic gastritis and intestinal metaplasia to see the status of CSC population. This can be used as a surrogate for increased risk for cancer. In addition, targeted therapies can be designed to specifically attack the stem cell population in cancers, and response to treatment can be monitored by observ-

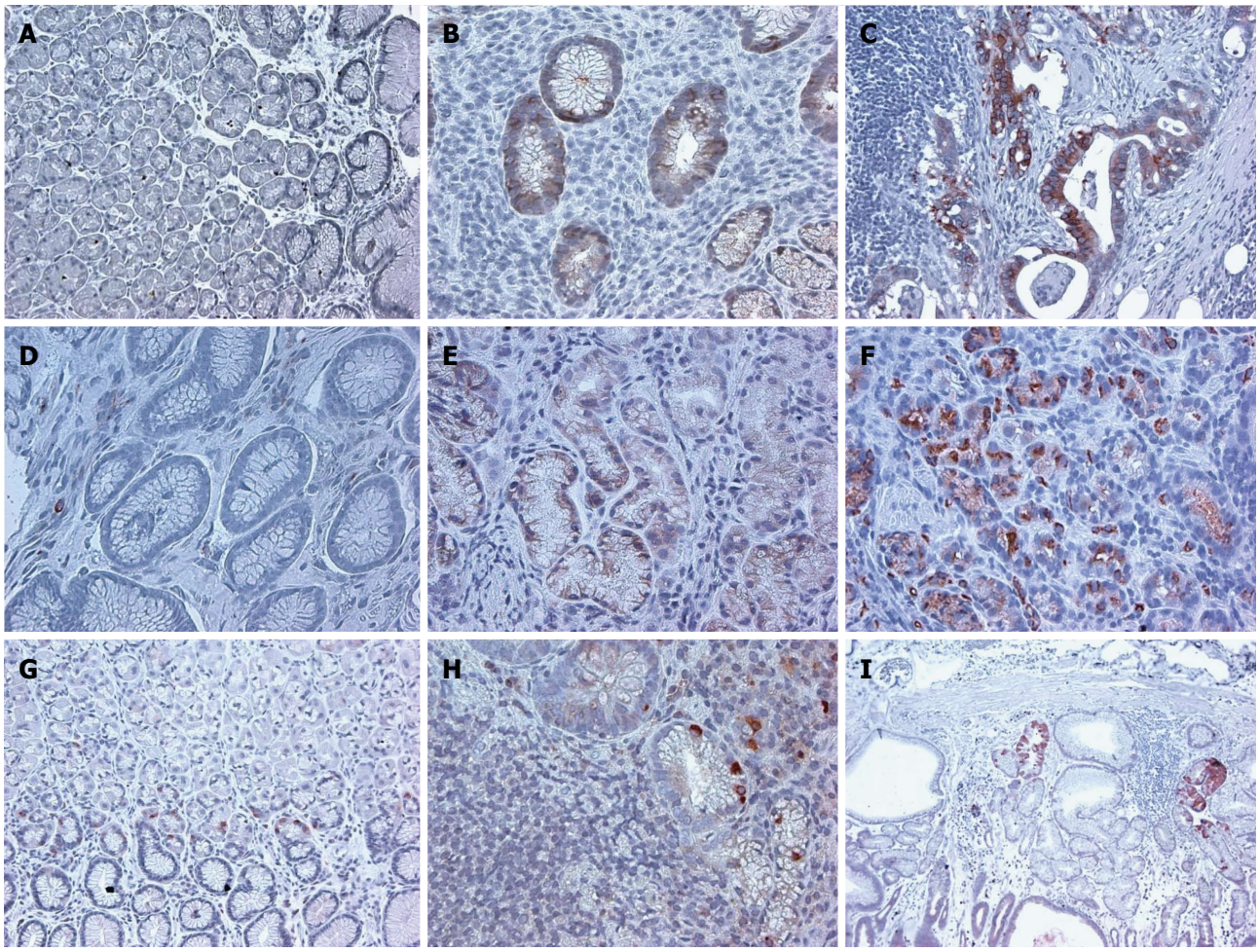

Figure 3 Immunohistochemical staining of stem cell markers ALDH1, CD166, LGR5 in human normal gastric mucosa, gastric mucosa with *H. pylori* gastritis, and gastric adenocarcinoma, demonstrates increased expression of each of the markers over the normal controls. × 200 magnification. A: ALDH1 Normal; B: ALDH1 HP; C: ALDH1 CA; D: CD166 Normal; E: CD166 HP; F: CD166 CA; G: LGR5 Normal; H: LGR5 HP; I: LGR5 CA.

ing the changes in the stem cell population.

COMMENTS

Background

Aging and chronic inflammation are two factors associated with gastric cancer. There is evidence suggesting a link between stem cells in gastric mucosa and increased risk for cancer. Aging and chronic inflammation may cause alterations in stem cells thus causing cancer.

Research frontiers

Cancer stem cells can be detected by using specific markers and demonstrated by immunohistochemistry. This approach allows the authors to demonstrate changes in cancer stem cells associated with aging and inflammation.

Innovations and breakthroughs

In this study the authors demonstrated that aging and chronic inflammation are associated with an increased stem cell population in gastric mucosa.

Applications

Gastric cancer stem cell markers can be utilized as prognostic markers or can be used to monitor response to treatment. They can also help the authors understand tumor pathobiology.

Terminology

Cancer stem cells are thought to be normal resident stem cells or specialized cells which acquire cancer initiating properties. Cancer stem cell hypothesis assumes that cancers arise by alterations in cancer initiating subpopulations of cells in a given tissue.

Peer review

The reviewers think that the study provides data identifying and quantifying stem cells in gastric mucosa of rats and humans. According to their data the number of stem cells is increased by chronic inflammation and aging.

REFERENCES

1 **Majumdar APN,** Basson MD. Effect of Aging on the Gastrointestinal Tract. In: Physiology of the Gastrointestinal tract. Johnson LR, Barrett K, Ghishan F, Merchant JI, Said HM, Wood JD, editors. New York: Academic Press, 2006: 405-433
2 **Quante M**, Wang TC. Inflammation and stem cells in gastrointestinal carcinogenesis. *Physiology* (Bethesda) 2008; **23**: 350-359 [PMID: 19074742 DOI: 10.1152/physiol.00031.2008]
3 **Barker N**, Ridgway RA, van Es JH, van de Wetering M, Begthel H, van den Born M, Danenberg E, Clarke AR, Sansom OJ, Clevers H. Crypt stem cells as the cells-of-origin of intestinal cancer. *Nature* 2009; **457**: 608-611 [PMID: 19092804 DOI: 10.1038/nature07602]
4 **Boman BM**, Huang E. Human colon cancer stem cells: a new paradigm in gastrointestinal oncology. *J Clin Oncol* 2008; **26**: 2828-2838 [PMID: 18539961 DOI: 10.1200/JCO.2008.17.6941]
5 **Boman BM,** Fields JZ, Cavanaugh KL, Guetter A, Runquist OA. How dysregulated colonic crypt dynamics cause stem cell overpopulation and initiate colon cancer. *Cancer Res*

2008; **68**: 3304-3313 [DOI: 10.1158/0008-5472.CAN-07-2061]
6 **Fukuda K**, Saikawa Y, Ohashi M, Kumagai K, Kitajima M, Okano H, Matsuzaki Y, Kitagawa Y. Tumor initiating potential of side population cells in human gastric cancer. *Int J Oncol* 2009; **34**: 1201-1207 [PMID: 19360333]
7 **Jordan CT**, Guzman ML, Noble M. Cancer stem cells. *N Engl J Med* 2006; **355**: 1253-1261 [PMID: 16990388]
8 **Pilpilidis I**, Kountouras J, Zavos C, Katsinelos P. Upper gastrointestinal carcinogenesis: H. pylori and stem cell cross-talk. *J Surg Res* 2011; **166**: 255-264 [PMID: 20452613 DOI: 10.1016/j.jss.2010.02.012]
9 **Rossi DJ,** Jamieson CH, Weissman IL. Stems cells and the pathways to aging and cancer. *Cell* 2008; **132**: 681-696 [PMID: 18295583 DOI: 10.1016/j.cell.2008.01.036]
10 **Takaishi S,** Okumura T, Tu S, Wang SS, Shibata W, Vigneshwaran R, Gordon SA, Shimada Y, Wang TC. Identification of gastric cancer stem cells using the cell surface marker CD44. *Stem Cells* 2009; **27**: 1006-1020 [PMID: 19415765 DOI: 10.1002/stem.30]
11 **Karam SM**, Leblond CP. Dynamics of epithelial cells in the corpus of the mouse stomach. I. Identification of proliferative cell types and pinpointing of the stem cell. *Anat Rec* 1993; **236**: 259-279 [PMID: 8338232 DOI: 10.1002/ar.1092360202]
12 **Saikawa Y**, Fukuda K, Takahashi T, Nakamura R, Takeuchi H, Kitagawa Y. Gastric carcinogenesis and the cancer stem cell hypothesis. *Gastric Cancer* 2010; **13**: 11-24 [PMID: 20373071 DOI: 10.1007/s10120-009-0537-4]
13 **Kato K,** Hasui K, Wang J, Kawano Y, Aikou T, Murata F. Homeostatic mass control in gastric non-neoplastic epithelia under infection of Helicobacter pylori: an immunohistochemical analysis of cell growth, stem cells and programmed cell death. *Acta Histochem Cytochem* 2008; **41**: 23-38 [DOI: 10.1267/ahc.07021]
14 **McDonald SA**, Greaves LC, Gutierrez-Gonzalez L, Rodriguez-Justo M, Deheragoda M, Leedham SJ, Taylor RW, Lee CY, Preston SL, Lovell M, Hunt T, Elia G, Oukrif D, Harrison R, Novelli MR, Mitchell I, Stoker DL, Turnbull DM, Jankowski JA, Wright NA. Mechanisms of field cancerization in the human stomach: the expansion and spread of mutated gastric stem cells. *Gastroenterology* 2008; **134**: 500-510 [PMID: 18242216 DOI: 10.1053/j.gastro.2007.11.035]
15 **Oh JD**, Kling-Bäckhed H, Giannakis M, Engstrand LG, Gordon JI. Interactions between gastric epithelial stem cells and Helicobacter pylori in the setting of chronic atrophic gastritis. *Curr Opin Microbiol* 2006; **9**: 21-27 [PMID: 16406776]
16 **Levi E**, Misra S, Du J, Patel BB, Majumdar AP. Combination of aging and dimethylhydrazine treatment causes an increase in cancer-stem cell population of rat colonic crypts. *Biochem Biophys Res Commun* 2009; **385**: 430-433 [PMID: 19465005 DOI: 10.1016/j.bbrc.2009.05.080]
17 **Nautiyal J,** Du J, Yu Y, Kanwar SS, Levi E, Majumdar APN: EGFR regulation of colon cancer stem-like cells during aging and in response to the colonic carcinogen dimethylhydrazine. *Am J Physiol Gastrointest Liver Physiol* 2012; **302**: G655-63 [PMID: 22281474 DOI: 10.1152/ajpgi.00323.2011]
18 **Martin K**, Potten CS, Roberts SA, Kirkwood TB. Altered stem cell regeneration in irradiated intestinal crypts of senescent mice. *J Cell Sci* 1998; **111** (Pt 16): 2297-2303 [PMID: 9683625]
19 **Patel BB,** Yu Y, Du J, Levi E, Philip PA, Majumdar AP. Age related increase in colorectal cancer stem cells in macroscopically normal mucosa of patients with adenomas: A risk factor for colon cancer. *Biochem Biophys Res Commun* 2009; **378**: 344-347 [DOI: 10.1016/j.bbrc.2008.10.179]
20 **Schmelz EM**, Levi E, Du J, Xu H, Majumdar AP. Age-related loss of EGF-receptor related protein (ERRP) in the aging colon is a potential risk factor for colon cancer. *Mech Ageing Dev* 2004; **125**: 917-922 [PMID: 15563939 DOI: 10.1016/j.mad.2004.03.010]
21 **Xiao ZQ**, Moragoda L, Jaszewski R, Hatfield JA, Fligiel SE, Majumdar AP. Aging is associated with increased proliferation and decreased apoptosis in the colonic mucosa. *Mech Ageing Dev* 2001; **122**: 1849-1864 [PMID: 11557285 DOI: 10.1016/S0047-6374(01)00323-2]
22 **Reya T**, Clevers H. Wnt signalling in stem cells and cancer. *Nature* 2005; **434**: 843-850 [PMID: 15829953]

P- Reviewer: Langner C **S- Editor**: Qi Y **L- Editor**: A
E- Editor: Lu YJ

World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4291/wjgp.v5.i3.373

World J Gastrointest Pathophysiol 2014 August 15; 5(3): 373-379
ISSN 2150-5330 (online)

SYSTEMATIC REVIEWS

Oxidative and nitrosative stress enzymes in relation to nitrotyrosine in *Helicobacter pylori*-infected humans

Anders Elfvin, Anders Edebo, Peter Hallersund, Anna Casselbrant, Lars Fändriks

Anders Elfvin, Anders Edebo, Peter Hallersund, Anna Casselbrant, Lars Fändriks, Department of Gastrosurgical Research and Education, Institute of Clinical Sciences, Sahlgrenska Academy at University of Gothenburg, 416 85 Gothenburg, Sweden
Anders Elfvin, Department of Pediatrics, Institute of Clinical Sciences, Sahlgrenska Academy at University of Gothenburg, 416 85 Gothenburg, Sweden
Author contributions: Elfvin A and Edebo A performed all the gastroscopies; Elfvin A, Hallersund P and Casselbrant A performed the laboratory work, and analysis; Elfvin A and Fändriks L designed the study; Fändriks L coordinated the study and provided financial support; Elfvin A and Hallersund P wrote the manuscript; all authors were involved in editing the manuscript.
Supported by The grants financially from the Swedish Medical Research Council and the Gothenburg Medical Society
Correspondence to: Dr. Anders Elfvin, MD, PhD, Department of Pediatrics, Institute of Clinical Sciences, Sahlgrenska Academy at University of Gothenburg, Diagnosroad 15, 416 85 Gothenburg, Sweden. anders.elfvin@vgregion.se
Telephone: +46-31-3438073 Fax: +46-31-3436696
Received: January 28, 2014 Revised: April 25, 2014
Accepted: June 10, 2014
Published online: August 15, 2014

Abstract

AIM: To compare a possible relation between *Helicobacter pylori* (*H. pylori*) and the oxygen- and nitrogen radical system in humans.

METHODS: Mechanisms for *H. pylori* to interfere with the oxygen and nitrogen radical system is of great importance for understanding of the *H. pylori* persistence and pathogenesis. Biopsies were obtained from the gastric wall of 21 individuals. Ongoing infection with *H. pylori* was detected using direct analyze from the biopsies using campylobacter-like organism test (CLO-test) and/or by using ^{14}C-urea breath test. The individuals were divided in a negative *H. pylori* and a positive *H. pylori* group. Expression in the gastric mucosa of inducible nitric oxide syntase (iNOS), nicotinamide adenine dinucleotide phosphate-oxidase (NADPH-oxidase) myeloperoxidase (MPO), and nitrotyrosine were assessed by Western blotting.

RESULTS: The individuals who undervent gastroscopy were divided in a *H. pylori* neg. [*n* = 13, m/f = 7/6, age (mean) = 39] and a *H. pylori* pos. group [*n*= 8, m/f = 5/3, age (mean) = 53]. Using western blot analysis iNOS was detected as a 130 kDa band. The iNOS expression was upregulated in the antrum of H. pylori infected individuals in comparison to the controls, mean ± SD being 12.6 ± 2.4 *vs* 8.3 ± 3.1, $P < 0.01$. There was a markedly upregulated expression of MPO in the antrum of *H. pylori* infected individuals in comparison to the control group without infection. In several of non-infected controls it was not possible to detect any MPO expression at all, whereas the expression was high in all the infected subjects, mean ± SD being 5.1 ± 3.4 *vs* 2.1 ± 1.9, $P < 0.05$. The NADPH-oxidase expression was analysed by detecting the NADPH-oxidase subunit p47-phox expression. P47-phox was detected as a 47 kDa band using Western blot, and showed a significantly higher expression of p47-phox in the antrum of the *H. pylori* infected individuals compared to the controls, mean ± SD being 3.1 ± 2.2 *vs* 0.3 ± 0.2, $P < 0.01$. Regarding nitrotyrosine formation, Western blot did not show any significant increase or decrease compared to controls, 7.0 ± 0.9 *vs* 6.9 ± 1.1, not significant.

CONCLUSION: iNOS, MPO and NADPH-oxidase was up-regulated among *H. pylori* infected. Regarding nitrotyrosine no difference was found. This support an *H. pylori* related inhibition of radical formation.

Key words: *Helicobacter pylori*; Radical; Myeloperoxidase; Nicotinamide adenine dinucleotide phosphate; Nitrotyrosine; Gastric

Core tip: The present project was performed to compare a possible relation between *Helicobacter pylori* (*H. pylori*) and the oxygen- and nitrogen radical system in humans. Expression of inducible nitric oxide syntase, myeloperoxidase and nicotinamide adenine dinucleotide phosphate-oxidase was upregulted in the antrum of the group with *H. pylori* infection. Regarding nitrotyrosine formation, Western blot did not show any significant increase or decrease compared to controls. The present study illustrates the complex picture of the oxidative stress in relation to *H. pylori* infection. The present study supports the theory of an *H. pylori* related inhibition of the enzymes involved in the oxy- and/or nitro-radical formation pathway.

Elfvin A, Edebo A, Hallersund P, Casselbrant A, Fändriks L. Oxidative and nitrosative stress enzymes in relation to nitrotyrosine in *Helicobacter pylori*-infected humans. *World J Gastrointest Pathophysiol* 2014; 5(3): 373-379 Available from: URL: http://www.wjgnet.com/2150-5330/full/v5/i3/373.htm DOI: http://dx.doi.org/10.4291/wjgp.v5.i3.373

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a pathogen colonizing the human gastric mucosa playing a significant role in the development of gastric ulcer, gastritis, and gastric cancer[1] Until recently there was insufficient knowledge about how *H. pylori* could avoid being eliminated by the acute host defence and establish a chronic infection in the gastric mucosa of humans. Recent studies have shown that *H. pylori* interferes with reactive oxygen species (ROS) such as superoxide anion (O_2^-) that is of importance in the elimination of invading microorganisms[2,3]. At the same time reactive nitrogen intermediates such as nitric oxide (NO) represent another class of oxidants. NO can be formed as a nitrogenous product of nitric oxide synthase (NOS). Peroxynitrite, formed by NO and O_2^-, is a very powerful oxidant. It is unstable with dimensions related to the hydroxyl radical[4]. In neutrophils and macrophages large amounts of reactive oxygen and nitrogen species are presented to the invading microorganism. Neutrophils phagocytose bacteria into the intracellular phagosome, where an eruption of reactive species results in bacterial destruction. During successful conditions the bacteria is eliminated and there is no extracellular oxidant generation[5].

However *H. pylori* persist in the gastric mucosa, causing a chronic infection that increases the risk for pathological changes such as adenocarcinoma. Therefore the mechanisms for *H. pylori* to interfere with the oxygen and nitrogen radical system is of great importance for understanding the persistence and pathogenesis of *H. pylori*.

We and others have pointed out the association between *H. pylori* infection and an increased mucosal expression of iNOS both in humans and in mongolian gerbils[6-8]. Despite what one could expect, the juxtamucosal level of nitric oxide (NO) is lower in the infected than in the uninfected stomach[7,8]. We have shown that there is an inhibition of nitrotyrosine expression, being a reflection of the formation of peroxynitrite, in *H. pylori* infected Mongolian gerbils despite upregulated formation of both myeloperoxidase (MPO) and inducible nitric oxide synthase (iNOS)[6]. Results from *in vivo* registration of NO and hydrogen peroxide (H_2O_2) on Mongolian gerbils substantiates the fact infection with *H. pylori* reduces levels of NO[9]. It is recently suggested that specific proteins contained by *H.pylori* enables the pathogen to cope with the damaging effects of NO. These systems are suggested to be a part in the microbial protection against nitrosative stress[10]. Several traditional anti-inflammatory drugs have been shown to have an effect on epithelial cells infected by *H.pylori* by inhibiting the induction of iNOS by suppressing the activation of NADPH oxidase[11] .

The present project was performed to further compare a possible relation between *H. pylori* and the oxygen- and nitrogen radical system in humans.

Special interest was on the suspected upregulation on the enzymatic oxy- and nitro radical systems, and if this would result in an increased radical formation. To evaluate activity of peroxynitrite, expression of nitrotyrosine was used as an indicator of radical formation.

MATERIALS AND METHODS

Ethics approval

Approval was obtained from the Research Ethics Committee at Sahlgrenska Academy, Gothenburg University and from the Gothenburg Regional Ethical Review Board.

Study groups

Gastric biopsies were obtained from the antral wall of 21 individuals. The individuals were divided in a *H. pylori* neg. [n = 13, m/f = 7/6, age (mean) = 39] and a *H. pylori* pos. group [n = 8, m/f = 5/3, age (mean) = 53]. Gastro-esophageal reflux (GER) was diagnosed in one subject in the *H. pylori* pos group and in four subjects in the *H. pylori* neg group. Ulcer in the duodenum was found in two individuals in the *H. pylori* pos group.

Diagnostic procedures

Ongoing infection with *H. pylori* was detected using direct analyze from the biopsies using campylobacter-like organism test (CLO-test) and/or by using ^{14}C-urea breath test[12].

Western blot

Biopsies were collected during gastroscopy. The samples were snap-frozen in nitrogenum liquidum and kept for further analysis at -70 ℃. Sonication (Sonifier 450/250, Branson Ultrasonics Co. Danbury, United States) or homogenization (Polytron, PT-MR 2100, Kinematica) was performed of all samples at 2 ℃ in a PE-buffer (10

mmol/L potassium Phosphate buffer, pH 6.8, and 1 mmol/L EDTA) containing CHAPS {3-[(3-cholamidopropyl) dimethyl-ammanio] 1-propanesulfonate}, aprotinin (1 μg/mL), leupeptin (10 μg/ mL), pepstatin (10 μg/mL) and Pefablock (1 mg/mL) (Boeringer Mannheim, Mannheim, Germany). All samples were centrifugated at 10.000 *g* for 10 min at 4 ℃. Analysation was performed of the supernatant for protein content using the method of Bradford[12] and then kept at -70 ℃ for future analysis. Samples were diluted in SDS-buffer and heated at 70 ℃ for 10 min before they were loaded on a NuPage 10% BisTris gel (Invitrogen, Carlsbad, CA, United States). One lane of each gel was loaded with prestained molecular weight standards (SeeBlue™, Invitrogen, Carlsbad, CA, United States). Following the electrophoresis the proteins were transferred to a polyvinyldifluoride membrane (Amersham, Buckinghamshire, United Kingdom) which was incubated with antibodies directed against iNOS, MPO, NADPH-oxidase or nitrotyrosine containing proteins. For identifying iNOS the NOS2 (H-174) sc-8310 (Santa Cruz Biotechnology inc) antibody was used. It is a rabbit polyclonal antibody raised against a recombinant protein corresponding to amino acids 2-175 mapping at the amino terminus of iNOS of human origin. Lack of cross-reaction with nNOS or eNOS was reported by manufacturer. Antibody Anti-myeloperoxidase 07-496 lot 24587 (Upstate, Lake Placid, NY, United States) was used for detecting MPO. This is a rabbit antibody that recognizes MPO subunits at 12 and 60 kDa. In the present study the 60 kDa band was used for quantification of the protein. Anti-nitrotyrosine rabbit immunoaffinity purified IgG catalog 06-284, lot 26427 (Upstate, Lake Placid, NY, United States) was used to assess nitrated proteins. For identifying NADPH-oxidase the p47-phox (H-195) sc 14015 (Santa Cruz Biotechnology inc.) was used. This is a rabbit polyclonal antibody raised against amino acids 196-390 of p47-phox of human origin. P47-phox is required for activation of NADPH-oxidase in neutrophils and other phagocytic cells. During activation of NADPH-oxidase, p47-phox migrate to the plasma membrane where it associates with other subunits to form the active complex. Goat anti-rabbit antibodies were used to identify immunoreactive proteins by chemiluminescence [iNOS, NADPH-oxidase (p47-phox) and nitrotyrosine; goat-anti rabbit sc 2007(Santa Cruz, CA, United States)] [MPO; IgG 12-448 (Upstate Lake Placid, NY, United States)]. CDP-Star (Tropix, Bedford, MA, United States) was used as a substrate. Images were captured by a LAS-100 cooled CCD-camera (Fujifilm, Tokyo, Japan) and semi-quantification was performed using the soft ware Gauge 3.3 (Fujifilm, Tokyo, Japan). As positive controls, to confirm the identity of the protein, RAW 264.7 (sc 2212, Santa Cruz Biotech) was used for iNOS, HL60 was used for MPO and NADPH-oxidase (p47-phox).For nitrotyrosine the immunoblotting control (12-354, Upstate) was used.

Statistical analysis

Statistical analysis was performed using non parametric Mann-Whitney *U*-test. *P*-values of < 0.05 were regarded as being of statistical significance.

RESULTS

Inducible nitric oxide synthase

Using western blot analysis iNOS was detected as a 130 kDa band. The iNOS expression was upregulated in the antrum of *H. pylori* infected individuals in comparison to the control group without infection as shown in Figure 1A, mean ± SD being 12.6 ± 2.4 *vs* 8.3 ± 3.1, $P < 0.01$. Western blot detecting iNOS with a band at 130 kDa in RAW 264.7 (pos contr.), and in human antral mucosa retrieved from *H. pylori* pos. and *H. pylori* neg. volunteers during endoscopy is shown in Figure 2.

Myeloperoxidase

As shown in Figure 1B, MPO expression was markedly upregulated in the antrum of the *H. pylori* infected individuals in comparison to the control group without infection, mean ± SD being 5.1 ± 3.4 *vs* 2.1 ± 1.9, $P < 0.05$. In several of the non-infected controls it was not possible to detect any MPO expression at all, whereas the expression was high in all the infected subjects. Western blot of the MPO positive 60 kDa band in the positive HL60 control and in gastric mucosal specimens of *H. pylori* pos. and *H. pylori* neg. volunteers is shown in Figure 2.

NADPH-oxidase

The expression of NADPH-oxidase was analysed by detecting the NADPH-oxidase subunit p47-phox expression. P47-phox was detected as a 47 kDa band using Western blot. Figure 1C shows a significantly higher expression of p47-phox in the the antrum of *H. pylori* infected individuals in comparison to the control group without infection, mean ± SD being 3.1 ± 2.2 *vs* 0.3 ± 0.2, $P < 0.01$. P47-phox was low in all non-infected controls. In the *H. pylori* infected subjects there was a large spreading of the p47-phox expression. A typical Western blot result is shown in Figure 2.

Nitrotyrosine

Western blot analysis did not show any significant increase nor decrease in nitrotyrosine expression the antrum of *H. pylori* infected individuals in comparison to the control group without infection, 7.0 ± 0.9 *vs* 6.9 ± 1.1, not significant (Figure 3). Regarding Western blot representing Nitrotyrosine, several bands of Nitrated proteins could be analysed. Shown in the Figure 2 is a typical 66 kDa band in the positive control and in *H.pylori* pos. and *H. pylori* neg. subjects.

DISCUSSION

The findings of the present investigation can confirm that *H. pylori* infection in humans is related to an up regulation of the expression of MPO, iNOS and NADPH-

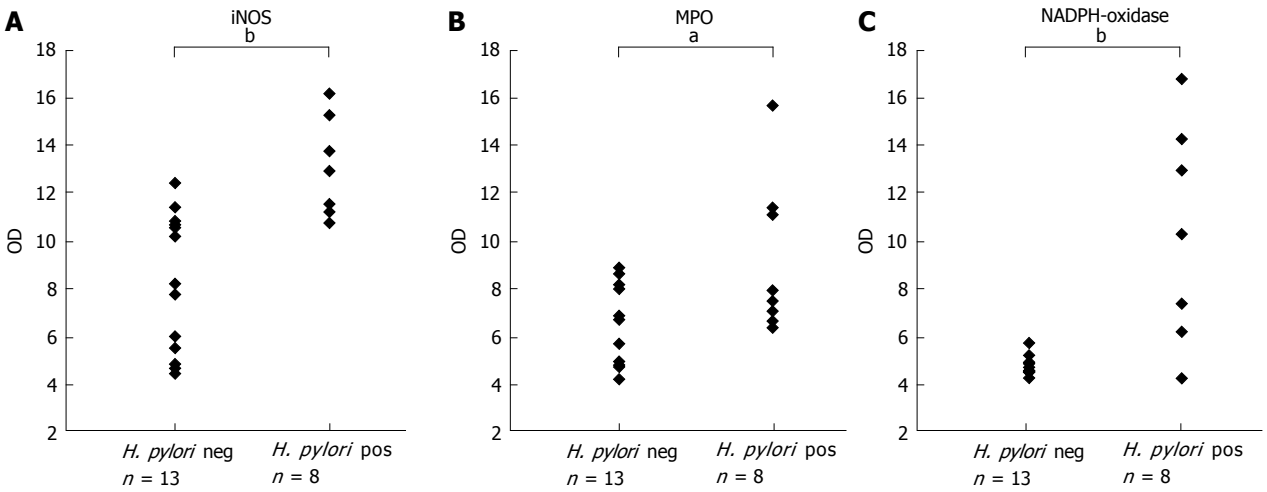

Figure 1 Scatter-plot demonstrating the result of Western blot inducible nitric oxide synthase, myeloperoxidase and nicotinamide adenine dinucleotide phosphate-oxidase protein expression in biopsies from the anrum of the *Helicobacter pylori* neg (*n* = 13) and *Helicobacter pylori* pos (*n* = 8) groups. A: Inducible nitric oxide synthase (iNOS), $^{b}P < 0.01$; B: Myeloperoxidase (MPO), $^{a}P < 0.05$; C: Nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase subunit p47-phox in biopsies from the antrum of the *H. pylori* neg. (*n* = 13) and *H. pylori* pos. (*n* = 8) groups, $^{b}P < 0.01$, OD: Optical density.

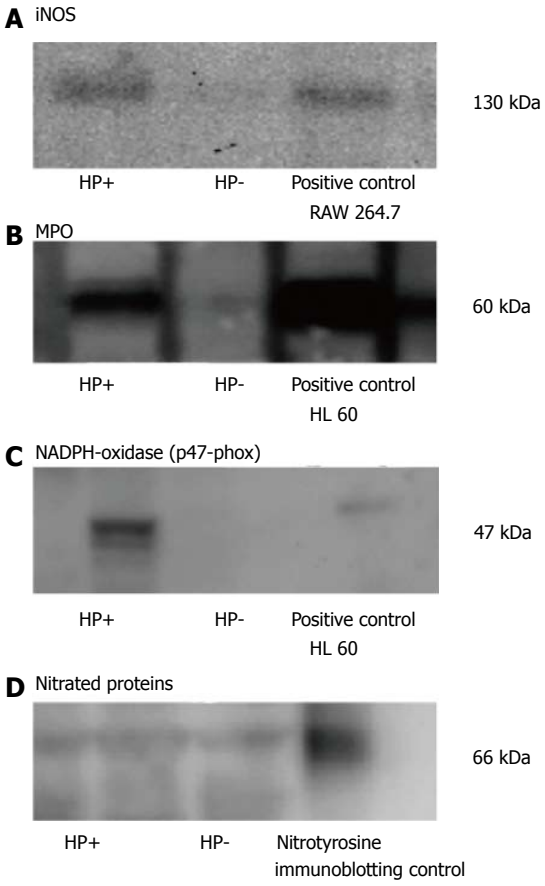

Figure 2 Western blot. A: Western blot detecting inducible nitric oxide syntase (iNOS) with a band at 130 kDa in RAW 264.7 (pos contr.), and in human antral mucosa retrieved from *Helicobacter pylori* (*H. pylori*) pos. and *H. pylori* neg. volunteers during endoscopy; B: Western blot of the MPO positive 60 kDa band in the positive HL60 control and in gastric mucosal specimens of *H.pylori* pos. and *H.pylori* neg. volunteers; C: Western blot of p47-phox, representing NADPH-oxidase with a band at 47 kDa (pos. contr.) HL60, and in HP+ and HP- samples; D: Regarding Western blot representing Nitrotyrosine, several bands of Nitrated proteins could be analysed. Shown in the figure is a typical 66 kDa band in the pos. control and in *H.pylori* pos. and *H.pylori* neg. subjects.

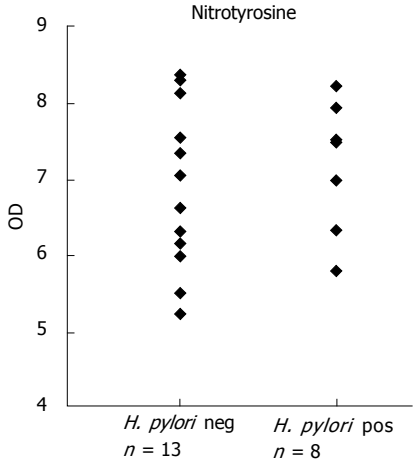

Figure 3 Nitrotyrosine. Scatter-plot demonstrating the result of Western blot analysis of nitrotyrosine formation in biopsies from the antrum of the *Helicobacter pylori* (*H. pylori*) neg. (*n* = 13) and *H. pylori* pos. (*n* = 8) groups. There were no significant changes between groups. OD: Optical density.

oxidase in the human gastric mucosa. Furthermore the study shows that there are no significant changes in levels of proteins containing nitrotyrosine compared to non-infected subjects following this up-regulation. This finding confirms the results from our studies on Mongolian gerbils, and supports the theory of an *H. pylori* related inhibition of radical formation[6,9].

Studying the early stages of *H. pylori* infecting the stomach is important for understanding the evolution of pathology such as carcinogenesis. Using an animal model makes it possible to assess different stages of pathological development in an experimental setting. However it is important to evaluate the experimental findings in a human population before making any conclusions regarding *H. pylori* infection in human gastric mucosa.

The initial host reaction to the *H. pylori* infection is

the same as to any bacterial infection: Phagocytic neutrophils and monocytes are recruited to the infected tissue and consume oxygen that is converted to O_2^- by NADPH-oxidase, and then dismutated to H_2O_2[13]. Activation of neutrophils results in the release of MPO, which catalyzes the oxidation of electron donors by H_2O_2[14]. A complex is formed that is responsible for the production of powerful oxidants with potential to react with a large variety of substances[15,16]. For example, MPO-H_2O_2 reacts with chloride to form hypochlorus acid and subsequently the oxidative chloramines are formed. The MPO-H_2O_2-chloride system is responsible for many biological effects, both beneficial and negative for the host[17].

In general, inflammation results in invading epithelial cells and macrophages leading to a marked expression of iNOS and resulting in generation of NO[17].

Several studies have described an increase in iNOS production following *H. pylori* infection in both humans and animal models[6-8,18-20]. Some have suggested that the up-regulated iNOS production following *H. pylori* infection would lead to an increase in NO production which could result in the increase of DNA damage and apoptosis[18-21]. It has been suggested that classification of iNOS expression in the gastric mucosa could be used clinically to identify patients with a high risk for gastric cancer[22]. The host will try to terminate the infection by activating the mucosal generation of the oxy- and nitro-radical forming enzymes the resulting in formation of the cytotoxic peroxynitrite. In the extracellular space NO released from macrophages can eliminate *H. pylori*[23]. An effective increase of production of NO and oxy-radicals would lead to eradication of the bacteria. However *H. pylori* persist in the host, causing a chronic inflammatory reaction that instead in the long run may be deleterious to the host. The fact that *H. pylori* survives in this hostile environment despite up regulation of iNOS suggests that the pathogen has developed strategies to avoid NO-dependent eradication. An increasing number of studies have reported about the complexity of the *H. pylori* response to oxidative and nitrogen stress[24,25].

H. pylori may also have a direct effect on reduction in gastric mucosal blood flow by inhibiting NO production by iNOS and thereby reducing the vasodilatory and mast cell stabilizing effect of NO[26].

We have by use of electrochemical microelectrodes *in vivo* confirmed reduction of intraluminal NO in Mongolian gerbils following infection with *H.pylori*[9]. Reduced levels of NO could be explained by inhibition of iNOS activity[27]. Helicobacter produced arginase has been proposed as one of the ways for *H. pylori* to inhibit NO production[23,25]. *H. pylori* may also produce asymmetrical dimethyl arginine (ADMA) that can block iNOS by competitive inhibition. ADMA is a methylated form of arginine that has been found to be significantly up-regulated in the human antrum of *H. pylori* positive individuals[8,28]. Another explanation for reduced NO levels could be scavenge of NO by reacting with reactive oxygen species (ROS)[7,18]. A result of this reaction would be an increase in the production of peroxynitrite, and resulting in increased levels of nitrotyrosine. Thus nitrotyrosine can be used to indicate peroxinitrite activity over time. The present investigation as well as previous studies on *H. pylori* infection in Mongolian gerbil demonstrates a significant up regulation of the formation of iNOS and MPO, but no significant changes in the levels of nitrotyrosine[6]. These findings strongly support the theory supports the theory of an *H. pylori* related inhibition of radical formation at an enzymatic level of NO generation.

The present study does not provide data on if *H. pylori* also inhibit the oxy-radical forming enzymes. Oxidative stress could potentially have a negative effect on the capacity of *H. pylori* to infest the human stomach. However it is shown that *H. pylori* produces a number of antioxidative proteins, the most described ones being bacterial produced superoxide dismutase (SOD)[29]. SOD production is described as being of importance for *H. pylori* being able to grow and survive in a situation with oxidative stress, and is regarded as a factor being of importance for of the microbial colonization of the stomach. Catalase and arginase are other examples of antioxidant proteins produced by *H. pylori* that might contribute to bacterial survival under conditions of oxidative stress[23,30,31].

Taken together the present study illustrates the complex picture of the oxidative stress response to *H. pylori* infection. The nitro- and oxy-radical formation systems are up-regulated following infection and inflammation. This up-regulation is to be regarded as an attempt from the host to eradicate the bacteria. However, long standing up-regulation of the reactive oxygen- and nitrogen species will also lead to tissue damage and a risk of carcinogenesis. This study supports the theory supports the theory of an *H. pylori* related inhibition the. The mechanisms behind how the bacteria and the impotent host defence act to induce DNA- and tissue damaging effects need to be further explored.

The results suggest that there is a relationship between inhibition of formation of ROS and reactive nitrogen species and *H. pylori* being able to survive in the human gastric mucosa.

COMMENTS

Background

Helicobacter pylori (H. pylori) colonization of the mucosal space of the stomach causes a chronic infection resulting in the development of pathological changes such as adenocarcinoma. Mechanisms for *H. pylori* to interfere with the oxygen and nitrogen radical system is of great importance for understanding the persistence and pathogenesis of *H. pylori*.

Research frontiers

Several studies have described an increase in inducible nitric oxide syntase (iNOS) production following *H. pylori* infection in both humans and animal models. An effective increase of production of NO and oxy-radicals would lead to eradication of the bacteria. The fact that *H. pylori* survives in this hostile environment despite up regulation of iNOS suggests that the pathogen has developed strategies to avoid NO-dependent eradication. The findings of the present investigation can confirm that *H. pylori* infection in humans is related to an up regulation of the expression of MPO, iNOS and NADPH-oxidase in the human gastric mucosa. Furthermore the study shows that there are no significant

changes in levels of proteins containing nitrotyrosine compared to non-infected subjects following this up-regulation.

Breakthroughs and innovations

The investigation presented here illustrates the complex picture of the oxidative stress response to *H. pylori* infection. The nitro- and oxy-radical formation systems are up-regulated following infection and inflammation. This up-regulation is to be regarded as an attempt from the host to eradicate the bacteria. However, long standing up-regulation of the reactive oxygen- and nitrogen species will also lead to tissue damage and a risk of carcinogenesis. This study supports the theory of an *H.pylori* related inhibition of formation of reactive oxygen species (ROS) and reactive nitrogen species. The mechanisms behind how the bacteria and the impotent host defence act to induce DNA- and tissue damaging effects need to be further explored.

Applications

By understanding how *H. pylori* manages not to be extinguished in the hostile environment by hindering the formation of reactive oxygen species and reactive nitrogen intermediates we will gain a greater understanding of the mechanisms involved in *H. pylori* related disease.

Terminology

Myeloperoxidase (MPO) is an enzyme of importance in the microbicidal role of phagocytes. iNOS was first identified in macrophages. iNOS is involved in the production of NO, but has also many other functions. Nicotinamide adenine dinucleotide phosphate-oxidase (NADPH-oxidase) is a transmembrane electron transport chain involved in the production of different ROS. Nitrotyrosine can be used as marking the activity of peroxynitrite.

Peer review

In this study the authors demonstrated, in human gastric mucosa of *H.pylori* positive patients, an increase of some enzymes belonging to oxidative stress pathway, while the amount of nitrotyrosine rich proteins did not differ from *H.pylori* negative tissues.

REFERENCES

1 **Parsonnet J**, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, Sibley RK. Helicobacter pylori infection and the risk of gastric carcinoma. *N Engl J Med* 1991; **325**: 1127-1131 [PMID: 1891020 DOI: 10.1056/NEJM199110173251603]

2 **Babior BM**. NADPH oxidase: an update. *Blood* 1999; **93**: 1464-1476 [PMID: 10029572]

3 **Kawahara T**, Kohjima M, Kuwano Y, Mino H, Teshima-Kondo S, Takeya R, Tsunawaki S, Wada A, Sumimoto H, Rokutan K. Helicobacter pylori lipopolysaccharide activates Rac1 and transcription of NADPH oxidase Nox1 and its organizer NOXO1 in guinea pig gastric mucosal cells. *Am J Physiol Cell Physiol* 2005; **288**: C450-C457 [PMID: 15469954 DOI: 10.1152/ajpcell.00319.2004]

4 **Nathan C**, Shiloh MU. Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. *Proc Natl Acad Sci USA* 2000; **97**: 8841-8848 [PMID: 10922044 DOI: 10.1073/pnas.97.16.8841]

5 **Thomas MJ**, Hedrick CC, Smith S, Pang J, Jerome WG, Willard AS, Shirley PS. Superoxide generation by the human polymorphonuclear leukocyte in response to latex beads. *J Leukoc Biol* 1992; **51**: 591-596 [PMID: 1319445]

6 **Elfvin A**, Bölin I, Lönroth H, Fändriks L. Gastric expression of inducible nitric oxide synthase and myeloperoxidase in relation to nitrotyrosine in Helicobacter pylori-infected Mongolian gerbils. *Scand J Gastroenterol* 2006; **41**: 1013-1018 [PMID: 16938713 DOI: 10.1080/00365520600633537]

7 **Iguchi M**, Shiotani A, Nishioka S. Helicobacter pylori infection reduces intraluminal nitric oxide. *Scand J Gastroenterol* 2000; **35**: 694-698 [PMID: 10972171 DOI: 10.1080/00365520050023345]

8 **von Bothmer C**, Edebo A, Lönroth H, Olbe L, Pettersson A, Fändriks L. Helicobacter pylori infection inhibits antral mucosal nitric oxide production in humans. *Scand J Gastroenterol* 2002; **37**: 404-408 [PMID: 11989830 DOI: 10.1080/00365520231731602 4]

9 **Elfvin A**, Edebo A, Bölin I, Fändriks L. Quantitative measurement of nitric oxide and hydrogen peroxide in Helicobacter pylori-infected Mongolian gerbils in vivo. *Scand J Gastroenterol* 2007; **42**: 1175-1181 [PMID: 17852850 DOI: 10.1080/00365520701288306]

10 **Justino MC**, Ecobichon C, Fernandes AF, Boneca IG, Saraiva LM.Helicobacter pylori has an unprecedented nitric oxide detoxifying system. *Antioxid Redox Signal* 2012; **17**: 1190200 [DOI: 10.1089/ars.2011.4304]

11 **Cho SO**, Lim JW, Kim H Red ginseng extract inhibits the expression of MCP-1 and iNOS in Helicobacter pylori-infected gastric epithelial cells by suppressing the activation of NADPH oxidase and Jak2/Stat3. *J Ethnopharmacol* 2013; **150**: 761-764 [DOI: 10.1016/j.jep.2013.09.013]

12 **Hamlet AK**, Erlandsson KI, Olbe L, Svennerholm AM, Backman VE, Pettersson AB. A simple, rapid, and highly reliable capsule-based 14C urea breath test for diagnosis of Helicobacter pylori infection. *Scand J Gastroenterol* 1995; **30**: 1058-1063 [PMID: 8578164 DOI: 10.3109/00365529509101607]

13 **Hampton MB**, Kettle AJ, Winterbourn CC. Inside the neutrophil phagosome: oxidants, myeloperoxidase, and bacterial killing. *Blood* 1998; **92**: 3007-3017 [PMID: 9787133]

14 **Krawisz JE**, Sharon P, Stenson WF. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Assessment of inflammation in rat and hamster models. *Gastroenterology* 1984; **87**: 1344-1350 [PMID: 6092199]

15 **Hataishi R**, Kobayashi H, Takahashi Y, Hirano S, Zapol WM, Jones RC. Myeloperoxidase-associated tyrosine nitration after intratracheal administration of lipopolysaccharide in rats. *Anesthesiology* 2002; **97**: 887-895 [PMID: 12357155 DOI: 10.1097/00000542-200210000-00021]

16 **Klebanoff SJ**. Myeloperoxidase: friend and foe. *J Leukoc Biol* 2005; **77**: 598-625 [PMID: 15689384 DOI: 10.1189/jlb.1204697]

17 **Jaiswal M**, LaRusso NF, Gores GJ. Nitric oxide in gastrointestinal epithelial cell carcinogenesis: linking inflammation to oncogenesis. *Am J Physiol Gastrointest Liver Physiol* 2001; **281**: G626-G634 [PMID: 11518674]

18 **Goto T**, Haruma K, Kitadai Y, Ito M, Yoshihara M, Sumii K, Hayakawa N, Kajiyama G. Enhanced expression of inducible nitric oxide synthase and nitrotyrosine in gastric mucosa of gastric cancer patients. *Clin Cancer Res* 1999; **5**: 1411-1415 [PMID: 10389926]

19 **Mannick EE**, Bravo LE, Zarama G, Realpe JL, Zhang XJ, Ruiz B, Fontham ET, Mera R, Miller MJ, Correa P. Inducible nitric oxide synthase, nitrotyrosine, and apoptosis in Helicobacter pylori gastritis: effect of antibiotics and antioxidants. *Cancer Res* 1996; **56**: 3238-3243 [PMID: 8764115]

20 **Son HJ**, Rhee JC, Park DI, Kim YH, Rhee PL, Koh KC, Paik SW, Choi KW, Kim JJ. Inducible nitric oxide synthase expression in gastroduodenal diseases infected with Helicobacter pylori. *Helicobacter* 2001; **6**: 37-43 [PMID: 11328364 DOI: 10.1046/j.1523-5378.2001.00004.x]

21 **Li CQ**, Pignatelli B, Ohshima H. Increased oxidative and nitrative stress in human stomach associated with cagA+ Helicobacter pylori infection and inflammation. *Dig Dis Sci* 2001; **46**: 836-844 [PMID: 11330421 DOI: 10.1023/A:1010764720524]

22 **Naito Y**, Takagi T, Okada H, Nukigi Y, Uchiyama K, Kuroda M, Handa O, Kokura S, Yagi N, Kato Y, Osawa T, Yoshikawa T. Expression of inducible nitric oxide synthase and nitric oxide-modified proteins in Helicobacter pylori-associated atrophic gastric mucosa. *J Gastroenterol Hepatol* 2008; **23** Suppl 2: S250-S257 [PMID: 19120907 DOI: 10.1111/j.1440-1746.2008.05412.x]

23 **Gobert AP**, McGee DJ, Akhtar M, Mendz GL, Newton JC, Cheng Y, Mobley HL, Wilson KT. Helicobacter pylori arginase inhibits nitric oxide production by eukaryotic cells: a strategy for bacterial survival. *Proc Natl Acad Sci USA* 2001; **98**: 13844-13849 [PMID: 11717441 DOI: 10.1073/pnas.241443798]

24 **Nardone G**, Rocco A, Malfertheiner P. Review article: heli-

cobacter pylori and molecular events in precancerous gastric lesions. *Aliment Pharmacol Ther* 2004; **20**: 261-270 [PMID: 15274662 DOI: 10.1111/j.1365-2036.2004.02075.x]

25 **Wang G**, Alamuri P, Maier RJ. The diverse antioxidant systems of Helicobacter pylori. *Mol Microbiol* 2006; **61**: 847-860 [PMID: 16879643 DOI: 10.1111/j.1365-2958.2006.05302.x]

26 **Henriksnäs J**, Atuma C, Phillipson M, Sandler S, Engstrand L, Holm L. Acute effects of Helicobacter pylori extracts on gastric mucosal blood flow in the mouse. *World J Gastroenterol* 2009; **15**: 219-225 [PMID: 19132773 DOI: 10.3748/wjg.15.219]

27 **von Bothmer C**, Bölin I, Pettersson A, Fändriks L. Stimulated murine macrophages as a bioassay for H. pylori-related inhibition of nitric oxide production. *Scand J Gastroenterol* 2003; **38**: 380-386 [PMID: 12739709 DOI: 10.1080/00365520310000816]

28 **Fändriks L**, von Bothmer C, Johansson B, Holm M, Bölin I, Pettersson A. Water extract of Helicobacter pylori inhibits duodenal mucosal alkaline secretion in anesthetized rats. *Gastroenterology* 1997; **113**: 1570-1575 [PMID: 9352859 DOI: 10.1053/gast.1997.v113.pm9352859]

29 **Seyler RW**, Olson JW, Maier RJ. Superoxide dismutase-deficient mutants of Helicobacter pylori are hypersensitive to oxidative stress and defective in host colonization. *Infect Immun* 2001; **69**: 4034-4040 [PMID: 11349073 DOI: 10.1128/IAI.69.6.4034-4040.2001]

30 **Harris AG,** Hazell SL: Localisation of Helicobacter pylori catalase in both the periplasm and cytoplasm, and its dependence on the twin-arginine target protein, KapA, for activity. *FEMS microbiology letters* 2003; **229**: 283-289 [DOI: 10.1016/S0378-1097(03)00850-4]

31 **McGee DJ**, Radcliff FJ, Mendz GL, Ferrero RL, Mobley HL. Helicobacter pylori rocF is required for arginase activity and acid protection in vitro but is not essential for colonization of mice or for urease activity. *J Bacteriol* 1999; **181**: 7314-7322 [PMID: 10572136]

P- Reviewer: Balaban YH, Cardaropoli S, Zhu YL
S- Editor: Ji FF **L- Editor**: A **E- Editor:** Lu YJ

World Journal of Gastrointestinal Pathophysiology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
www.wjgnet.com

World J Gastrointest Pathophysiol 2014 August 15; 5(3): I-V
ISSN 2150-5330 (online)

INSTRUCTIONS TO AUTHORS

GENERAL INFORMATION

World Journal of Gastrointestinal Pathophysiology (*World J Gastrointest Pathophysiol*, *WJGP*, online ISSN 2150-5330, DOI: 10.4291) is a peer-reviewed open access (OA) academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

Aim and scope

WJGP is to report rapidly the most recent results in basic and clinical research on gastrointestinal pathophysiology, including all aspects of normal or abnormal function of the gastrointestinal tract, hepatobiliary system, and pancreas. *WJGP* specifically covers growth and development, digestion, secretion, absorption, metabolism and motility relative to the gastrointestinal organs, as well as immune and inflammatory processes, and neural, endocrine and circulatory control mechanisms that affect these organs. This journal will also report new methods and techniques in gastrointestinal pathophysiological research.

We encourage authors to submit their manuscripts to *WJGP*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

WJGP is edited and published by Baishideng Publishing Group (BPG). BPG has a strong professional editorial team composed of science editors, language editors and electronic editors. BPG currently publishes 43 OA clinical medical journals, including 42 in English, has a total of 15471 editorial board members or peer reviewers, and is a world first-class publisher.

Columns

The columns in the issues of *WJGP* will include: (1) Editorial: The editorial board members are invited to make comments on an important topic in their field in terms of its current research status and future directions to lead the development of this discipline; (2) Frontier: The editorial board members are invited to select a highly cited cutting-edge original paper of his/her own to summarize major findings, the problems that have been resolved and remain to be resolved, and future research directions to help readers understand his/her important academic point of view and future research directions in the field; (3) Diagnostic Advances: The editorial board members are invited to write high-quality diagnostic advances in their field to improve the diagnostic skills of readers. The topic covers general clinical diagnosis, differential diagnosis, pathological diagnosis, laboratory diagnosis, imaging diagnosis, endoscopic diagnosis, biotechnological diagnosis, functional diagnosis, and physical diagnosis; (4) Therapeutics Advances: The editorial board members are invited to write high-quality therapeutic advances in their field to help improve the therapeutic skills of readers. The topic covers medication therapy, psychotherapy, physical therapy, replacement therapy, interventional therapy, minimally invasive therapy, endoscopic therapy, transplantation therapy, and surgical therapy; (5) Field of Vision: The editorial board members are invited to write commentaries on classic articles, hot topic articles, or latest articles to keep readers at the forefront of research and increase their levels of clinical research. Classic articles refer to papers that are included in Web of Knowledge and have received a large number of citations (ranking in the top 1%) after being published for more than years, reflecting the quality and impact of papers. Hot topic articles refer to papers that are included in Web of Knowledge and have received a large number of citations after being published for no more than 2 years, reflecting cutting-edge trends in scientific research. Latest articles refer to the latest published high-quality papers that are included in PubMed, reflecting the latest research trends. These commentary articles should focus on the status quo of research, the most important research topics, the problems that have now been resolved and remain to be resolved, and future research directions. Basic information about the article to be commented (including authors, article title, journal name, year, volume, and inclusive page numbers; (6) Minireviews: The editorial board members are invited to write short reviews on recent advances and trends in research of molecular biology, genomics, and related cutting-edge technologies to provide readers with the latest knowledge and help improve their diagnostic and therapeutic skills; (7) Review: To make a systematic review to focus on the status quo of research, the most important research topics, the problems that have now been resolved and remain to be resolved, and future research directions; (8) Topic Highlight: The editorial board members are invited to write a series of articles (7-10 articles) to comment and discuss a hot topic to help improve the diagnostic and therapeutic skills of readers; (9) Medical Ethics: The editorial board members are invited to write articles about medical ethics to increase readers' knowledge of medical ethics. The topic covers international ethics guidelines, animal studies, clinical trials, organ transplantation, *etc.*; (10) Clinical Case Conference or Clinicopathological Conference: The editorial board members are invited to contribute high-quality clinical case conference; (11) Original Articles: To report innovative and original findings in gastrointestinal pathophysiology; (12) Research Report: To briefly report the novel and innovative findings in gastrointestinal pathophysiology; (13) Meta-Analysis: Covers the systematic review, mixed treatment comparison, meta-regression, and overview of reviews, in order to summarize a given quantitative effect, *e.g.*, the clinical effectiveness and safety of clinical treatments by combining data from two or more randomized controlled trials, thereby providing more precise and externally valid estimates than those which would stem from each individual dataset if analyzed separately from the others; (14) Case Report: To report a rare or typical case; (15) Letters to the Editor: To discuss and make reply to the contributions published in *WJGP*, or to introduce and comment on a controversial issue of general interest; (16) Book Reviews: To introduce and comment on quality monographs of gastrointestinal pathophysiology; and (17) Autobiography: The editorial board members are invited to write their autobiography to provide readers with stories of success or failure in their scientific research career. The topic covers their basic personal information and information about when they started doing research work, where and how they did research work, what they have achieved, and their lessons from success or failure.

Name of journal

World Journal of Gastrointestinal Pathophysiology

ISSN

ISSN 2150-5330 (online)

Launch date

April 15, 2010

Frequency

Quarterly

Instructions to authors
Full instructions are available online at http://www.wjgnet.com/2218-6182/g_info_20100722172951.htm.

Indexed and Abstracted in
PubMed Central, PubMed, Digital Object Identifier, and Directory of Open Access Journals.

SPECIAL STATEMENT

All articles published in journals owned by the BPG represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

Biostatistical editing
Statistical review is performed after peer review. We invite an expert in Biomedical Statistics to evaluate the statistical method used in the paper, including *t*-test (group or paired comparisons), chi-squared test, Ridit, probit, logit, regression (linear, curvilinear, or stepwise), correlation, analysis of variance, analysis of covariance, *etc*. The reviewing points include: (1) Statistical methods should be described when they are used to verify the results; (2) Whether the statistical techniques are suitable or correct; (3) Only homogeneous data can be averaged. Standard deviations are preferred to standard errors. Give the number of observations and subjects (*n*). Losses in observations, such as drop-outs from the study should be reported; (4) Values such as ED50, LD50, IC50 should have their 95% confidence limits calculated and compared by weighted probit analysis (Bliss and Finney); and (5) The word 'significantly' should be replaced by its synonyms (if it indicates extent) or the *P* value (if it indicates statistical significance).

Conflict-of-interest statement
In the interests of transparency and to help reviewers assess any potential bias, *WJGP* requires authors of all papers to declare any competing commercial, personal, political, intellectual, or religious interests in relation to the submitted work. Referees are also asked to indicate any potential conflict they might have reviewing a particular paper. Before submitting, authors are suggested to read "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Ethical Considerations in the Conduct and Reporting of Research: Conflicts of Interest" from International Committee of Medical Journal Editors (ICMJE), which is available at: http://www.icmje.org/ethical_4conflicts.html.

Sample wording: [Name of individual] has received fees for serving as a speaker, a consultant and an advisory board member for [names of organizations], and has received research funding from [names of organization]. [Name of individual] is an employee of [name of organization]. [Name of individual] owns stocks and shares in [name of organization]. [Name of individual] owns patent [patent identification and brief description].

Statement of informed consent
Manuscripts should contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee or it should be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted. Authors should also draw attention to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, as revised in 2004).

Statement of human and animal rights
When reporting the results from experiments, authors should follow the highest standards and the trial should conform to Good Clinical Practice (for example, US Food and Drug Administration Good Clinical Practice in FDA-Regulated Clinical Trials; UK Medicines Research Council Guidelines for Good Clinical Practice in Clinical Trials) and/or the World Medical Association Declaration of Helsinki. Generally, we suggest authors follow the lead investigator's national standard. If doubt exists whether the research was conducted in accordance with the above standards, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study.

Before submitting, authors should make their study approved by the relevant research ethics committee or institutional review board. If human participants were involved, manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and appropriate informed consent of each. Any personal item or information will not be published without explicit consents from the involved patients. If experimental animals were used, the materials and methods (experimental procedures) section must clearly indicate that appropriate measures were taken to minimize pain or discomfort, and details of animal care should be provided.

SUBMISSION OF MANUSCRIPTS

Manuscripts should be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Number all pages consecutively, and start each of the following sections on a new page: Title Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables, Figures, and Figure Legends. Neither the editors nor the publisher are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of BPG, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the ICMJE to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now available, to our knowledge, is http://www.clinicaltrials.gov sponsored by the United States National Library of Medicine and we encourage all potential contributors to register with it. However, in the case that other registers become available you will be duly notified. A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

Authors should retain one copy of the text, tables, photographs and illustrations because rejected manuscripts will not be

returned to the author(s) and the editors will not be responsible for loss or damage to photographs and illustrations sustained during mailing.

Online submissions

Manuscripts should be submitted through the Online Submission System at: http://www.wjgnet.com/esps/. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/2150-5330/g_info_20100316161927.htm) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to bpgoffice@wjgnet.com, or by telephone: +86-10-85381892. If you submit your manuscript online, do not make a postal contribution. Repeated online submission for the same manuscript is strictly prohibited.

MANUSCRIPT PREPARATION

All contributions should be written in English. All articles must be submitted using word-processing software. All submissions must be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Style should conform to our house format. Required information for each of the manuscript sections is as follows:

Title page

Title: Title should be less than 12 words.

Running title: A short running title of less than 6 words should be provided.

Authorship: Authorship credit should be in accordance with the standard proposed by ICMJE, based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

Institution: Author names should be given first, then the complete name of institution, city, province and postcode. For example, Xu-Chen Zhang, Li-Xin Mei, Department of Pathology, Chengde Medical College, Chengde 067000, Hebei Province, China. One author may be represented from two institutions, for example, George Sgourakis, Department of General, Visceral, and Transplantation Surgery, Essen 45122, Germany; George Sgourakis, 2nd Surgical Department, Korgialenio-Benakio Red Cross Hospital, Athens 15451, Greece

Author contributions: The format of this section should be: Author contributions: Wang CL and Liang L contributed equally to this work; Wang CL, Liang L, Fu JF, Zou CC, Hong F and Wu XM designed the research; Wang CL, Zou CC, Hong F and Wu XM performed the research; Xue JZ and Lu JR contributed new reagents/analytic tools; Wang CL, Liang L and Fu JF analyzed the data; and Wang CL, Liang L and Fu JF wrote the paper.

Supportive foundations: The complete name and number of supportive foundations should be provided, e.g. Supported by National Natural Science Foundation of China, No. 30224801

Correspondence to: Only one corresponding address should be provided. Author names should be given first, then author title, affiliation, the complete name of institution, city, postcode, province, country, and email. All the letters in the email should be in lower case. A space interval should be inserted between country name and email address. For example, Montgomery Bissell, MD, Professor of Medicine, Chief, Liver Center, Gastroenterology Division, University of California, Box 0538, San Francisco, CA 94143, United States. montgomery.bissell@ucsf.edu

Telephone and fax: Telephone and fax should consist of +, country number, district number and telephone or fax number, e.g. Telephone: +86-10-85381892 Fax: +86-10-85381893

Peer reviewers: All articles received are subject to peer review. Normally, three experts are invited for each article. Decision on acceptance is made only when at least two experts recommend publication of an article. All peer-reviewers are acknowledged on Express Submission and Peer-review System website.

Abstract

There are unstructured abstracts (no less than 200 words) and structured abstracts. The specific requirements for structured abstracts are as follows:

An informative, structured abstract should accompany each manuscript. Abstracts of original contributions should be structured into the following sections: AIM (no more than 20 words; Only the purpose of the study should be included. Please write the Aim in the form of "To investigate/study/…"), METHODS (no less than 140 words for Original Articles; and no less than 80 words for Brief Articles), RESULTS (no less than 150 words for Original Articles and no less than 120 words for Brief Articles; You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, e.g. 6.92 ± 3.86 *vs* 3.61 ± 1.67, $P < 0.001$), and CONCLUSION (no more than 26 words).

Key words

Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

Core tip

Please write a summary of less than 100 words to outline the most innovative and important arguments and core contents in your paper to attract readers.

Text

For articles of these sections, original articles and brief articles, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both.

Illustrations

Figures should be numbered as 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Keeping all elements compiled is necessary in line-art image. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names should identify the figure and panel. Avoid layering type directly over shaded or textured areas. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes in atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: …*etc.* It is our principle to publish high resolution-figures for the E-versions.

Tables

Three-line tables should be numbered 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each table. Detailed legends should not be included under tables, but rather added into the text where applicable. The information should complement, but not duplicate the text. Use one horizontal line under the title, a second under column heads, and a third below the Table, above any footnotes. Vertical and italic lines should be omitted.

Notes in tables and illustrations

Data that are not statistically significant should not be noted. $^{a}P < 0.05$, $^{b}P < 0.01$ should be noted ($P > 0.05$ should not be noted). If there are other series of *P* values, $^{c}P < 0.05$ and $^{d}P < 0.01$ are used. A third series of *P* values can be expressed as $^{e}P < 0.05$ and $^{f}P < 0.01$. Other notes in tables or under illustrations should be expressed as ^{1}F, ^{2}F, ^{3}F; or sometimes as other symbols with a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve

should be labeled with ●, ○, ■, □, ▲, △, *etc.*, in a certain sequence.

Acknowledgments

Brief acknowledgments of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

REFERENCES

Coding system

The author should number the references in Arabic numerals according to the citation order in the text. Put reference numbers in square brackets in superscript at the end of citation content or after the cited author's name. For citation content which is part of the narration, the coding number and square brackets should be typeset normally. For example, "Crohn's disease (CD) is associated with increased intestinal permeability[1,2]". If references are cited directly in the text, they should be put together within the text, for example, "From references[19,22-24], we know that..."

When the authors write the references, please ensure that the order in text is the same as in the references section, and also ensure the spelling accuracy of the first author's name. Do not list the same citation twice.

PMID and DOI

Pleased provide PubMed citation numbers to the reference list, e.g. PMID and DOI, which can be found at http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed and http://www.crossref.org/SimpleTextQuery/, respectively. The numbers will be used in E-version of this journal.

Style for journal references

Authors: the name of the first author should be typed in bold-faced letters. The family name of all authors should be typed with the initial letter capitalized, followed by their abbreviated first and middle initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR). The title of the cited article and italicized journal title (journal title should be in its abbreviated form as shown in PubMed), publication date, volume number (in black), start page, and end page [PMID: 11819634 DOI: 10.3748/wjg.13.5396].

Style for book references

Authors: the name of the first author should be typed in bold-faced letters. The surname of all authors should be typed with the initial letter capitalized, followed by their abbreviated middle and first initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR) Book title. Publication number. Publication place: Publication press, Year: start page and end page.

Format

Journals

English journal article (list all authors and include the PMID where applicable)

1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood

CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23243641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/2150-5330/g_info_20100312200347.htm.

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*
Biology: *H. pylori*, *E coli*, *etc.*

Examples for paper writing

All types of articles' writing style and requirement will be found in the link: http://www.wjgnet.com/esps/NavigationInfo.aspx?id=15

RESUBMISSION OF THE REVISED MANUSCRIPTS

Authors must revise their manuscript carefully according to the revision policies of BPG. The revised version, along with the signed copyright transfer agreement, responses to the reviewers, and English language Grade A certificate (for non-native speakers of English), should be submitted to the online system *via* the link contained in the e-mail sent by the editor. If you have any questions about the revision, please send e-mail to esps@wjgnet.com.

Language evaluation

The language of a manuscript will be graded before it is sent for revision. (1) Grade A: priority publishing; (2) Grade B: minor language polishing; (3) Grade C: a great deal of language polishing needed; and (4) Grade D: rejected. Revised articles should reach Grade A.

Copyright assignment form

Please download a Copyright assignment form from http://www.wjgnet.com/2150-5330/g_info_20100312200118.htm.

Responses to reviewers

Please revise your article according to the comments/suggestions provided by the reviewers. The format for responses to the reviewers' comments can be found at: http://www.wjgnet.com/2150-5330/g_info_20100312195923.htm.

Proof of financial support

For papers supported by a foundation, authors should provide a copy of the approval document and serial number of the foundation.

STATEMENT ABOUT ANONYMOUS PUBLICATION OF THE PEER REVIEWERS' COMMENTS

In order to increase the quality of peer review, push authors to carefully revise their manuscripts based on the peer reviewers' comments, and promote academic interactions among peer reviewers, authors and readers, we decide to anonymously publish the reviewers' comments and author's responses at the same time the manuscript is published online.

PUBLICATION FEE

WJGP is an international, peer-reviewed, OA online journal. Articles published by this journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium and format, provided the original work is properly cited. The use is non-commercial and is otherwise in compliance with the license. Authors of accepted articles must pay a publication fee. Publication fee: 698 USD per article. All invited articles are published free of charge.

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