ISSN 1007-9327
CN 14-1219/R

# World Journal of Gastroenterology®

**Indexed and Abstracted in:**

Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®) and Journal Citation Reports/Science Edition, *Index Medicus*, MEDLINE and PubMed, Chemical Abstracts, EMBASE/Excerpta Medica, Abstracts Journals, *Nature Clinical Practice Gastroenterology and Hepatology*, CAB Abstracts and Global Health.
ISI JCR 2003-2000 IF: 3.318, 2.532, 1.445 and 0.993.

**Volume 15 Number 1**
**January 7, 2009**
*World J Gastroenterol*
2009 January 7; 15(1): 1-128

**Online Submissions**
wjg.wjgnet.com
www.wjgnet.com
Printed on Acid-free Paper
世界胃腸病学杂志

A Weekly Journal of Gastroenterology and Hepatology

# World Journal of Gastroenterology ®

## Editorial Board

2007-2009

Editorial Office: *World Journal of Gastroenterology*
Room 903, Building D, Ocean International Center
No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China
E-mail: wjg@wjgnet.com http://www.wjgnet.com Telephone: 0086-10-5908-0039 Fax: 0086-10-8538-1893

The World Journal of Gastroenterology Editorial Board consists of 1212 members, representing a team of worldwide experts in gastroenterology and hepatology. They are from 60 countries, including Albania (1), Argentina (4), Australia (39), Austria (10), Belarus (1), Belgium (15), Brazil (2), Bulgaria (1), Canada (29), Chile (1), China (60), Croatia (2), Cuba (1), Czech (2), Denmark (7), Egypt (4), Estonia (1), Finland (4), France (44), Germany (108), Greece (9), Hungary (2), Iceland (1), India (12), Iran (4), Ireland (3), Israel (8), Italy (97), Japan (177), Lebanon (3), Lithuania (1), Macedonia (1), Malaysia (3), Mexico (6), Monaco (1), Morocco (1), The Netherlands (26), New Zealand (1), Nigeria (1), Norway (3), Pakistan (2), Peru (1), Poland (6), Portugal (1), Russia (3), Saudi Arabia (2), Serbia (1), Singapore (4), Slovakia (2), Slovenia (1), South Africa (2), South Korea (15), Spain (38), Sweden (15), Switzerland (13), Turkey (8), United Arab Emirates (1), United Kingdom (83), United States (315) and Uruguay (2).

Andrew V Biankin, *Sydney*
Filip Braet, *Sydney*
Andrew D Clouston, *Sydney*
Graham Cooksley, *Queensland*
Darrell HG Crawford, *Brisbane*
Adrian G Cummins, *Woodville South*
Guy D Eslick, *Sydney*
Michael A Fink, *Melbourne*
Robert JL Fraser, *Daw Park*
Peter Raymond Gibson, *Victoria*
Jacob George, *Westmead*
Mark D Gorrell, *Sydney*
Yik-Hong Ho, *Townsville*
Gerald J Holtmann, *Adelaide*
Michael Horowitz, *Adelaide*
John E Kellow, *Sydney*
Rupert Leong, *Concord*
Geoffrey W McCaughan, *Sydney*
Finlay A Macrae, *Victoria*
Daniel Markovich, *Brisbane*
Phillip S Oates, *Perth*
Jacqui Richmond, *Victoria*
Stephen M Riordan, *Sydney*
Ian C Roberts-Thomson, *Adelaide*
Devanshi Seth, *Camperdown*
Arthur Shulkes, *Melbourne*
Ross C Smith, *Sydney*
Kevin J Spring, *Brisbane*
Huy A Tran, *New South Wales*
Debbie Trinder, *Fremantle*
Martin J Veysey, *Gosford*
Daniel L Worthley, *Bedford*

**Austria**

Peter Ferenci, *Vienna*
Valentin Fuhrmann, *Vienna*
Alfred Gangl, *Vienna*
Christoph Gasche, *Vienna*
Kurt Lenz, *Linz*
Markus Peck-Radosavljevic, *Vienna*
Rudolf E Stauber, *Auenbruggerplatz*
Herbert Tilg, *Innsbruck*
Michael Trauner, *Graz*
Harald Vogelsang, *Vienna*
Guenter Weiss, *Innsbruck*

**Belarus**

Yury K Marakhouski, *Minsk*

**Belgium**

Rudi Beyaert, *Gent*
Bart Rik De Geest, *Leuven*
Inge I Depoortere, *Leuven*
Olivier Detry, *Liège*
Benedicte Y De Winter, *Antwerp*
Karel Geboes, *Leuven*
Thierry Gustot, *Brussels*
Yves J Horsmans, *Brussels*
Geert G Leroux-Roels, *Ghent*
Louis Libbrecht, *Leuven*
Etienne M Sokal, *Brussels*
Marc Peeters, *De Pintelaan*
Gert A Van Assche, *Leuven*
Yvan Vandenplas, *Brussels*
Eddie Wisse, *Keerbergen*

**Brazil**

Heitor Rosa, *Goiania*
Ana Cristina Simões e Silva, *Belo Horizonte*

**Bulgaria**

Zahariy Krastev, *Sofia*

**Canada**

Fernando Alvarez, *Québec*
David Armstrong, *Ontario*
Jeffrey P Baker, *Toronto*
Olivier Barbier, *Québec*
Nancy Baxter, *Toronto*
Matthew Bjerknes, *Toronto*
Frank J Burczynski, *Manitoba*
Michael F Byrne, *Vancouver*
Wang-Xue Chen, *Ottawa*
Chantal Guillemette, *Québec*
Samuel S Lee, *Calgary*
Gary A Levy, *Toronto*
Andrew L Mason, *Alberta*
John K Marshall, *Ontario*
Donna-Marie McCafferty, *Calgary*
Thomas I Michalak, *St. John's*
Gerald Y Minuk, *Manitoba*
Paul Moayyedi, *Hamilton*
Kostas Pantopoulos, *Québec*
William G Paterson, *Kingston*
Eldon Shaffer, *Calgary*
Morris Sherman, *Toronto*
Martin Storr, *Calgary*
Elena F Verdu, *Ontario*
Waliul Khan, *Ontario*
John L Wallace, *Calgary*
Eric M Yoshida, *Vancouver*

**Chile**

Silvana Zanlungo, *Santiago*

**China**

Henry LY Chan, *Hong Kong*
Xiao-Ping Chen, *Wuhan*
Zong-Jie Cui, *Beijing*
Da-Jun Deng, *Beijing*
Er-Dan Dong, *Beijing*
Sheung-Tat Fan, *Hong Kong*
Jin Gu, *Beijing*
Xin-Yuan Guan, *Pokfulam*
De-Wu Han, *Taiyuan*
Ming-Liang He, *Hong Kong*
Wayne HC Hu, *Hong Kong*
Chee-Kin Hui, *Hong Kong*
Ching-Lung Lai, *Hong Kong*
Kam Chuen Lai, *Hong Kong*
James YW Lau, *Hong Kong*
Yuk-Tong Lee, *Hong Kong*
Suet-Yi Leung, *Hong Kong*
Wai-Keung Leung, *Hong Kong*
John M Luk, *Pokfulam*
Chung-Mau Lo, *Hong Kong*
Jing-Yun Ma, *Beijing*
Ronnie Tung Ping Poon, *Hong Kong*
Lun-Xiu Qin, *Shanghai*
Yu-Gang Song, *Guangzhou*
Qin Su, *Beijing*
Wai-Man Wong, *Hong Kong*
Hong Xiao, *Shanghai*
Dong-Liang Yang, *Wuhan*
Winnie Yeo, *Hong Kong*
Yuan Yuan, *Shenyang*
Man-Fung Yuen, *Hong Kong*
Jian-Zhong Zhang, *Beijing*
Xin-Xin Zhang, *Shanghai*
Bo-Jian Zheng, *Hong Kong*
Shu Zheng, *Hangzhou*

**Croatia**

Tamara Cacev, *Zagreb*
Marko Duvnjak, *Zagreb*

**Cuba**

Damian C Rodriguez, *Havana*

**Czech**

Milan Jirsa, *Praha*
Pavel Trunečka, *Prague*

**Denmark**

Peter Bytzer, *Copenhagen*
Asbjørn M Drewes, *Aalborg*
Hans Gregersen, *Aalborg*
Jens H Henriksen, *Hvidovre*
Claus P Hovendal, *Odense*
Fin S Larsen, *Copenhagen*
Søren Møller, *Hvidovre*

**Egypt**

Abdel-Rahman El-Zayadi, *Giza*
Amr M Helmy, *Cairo*
Ayman Yosry, *Cairo*

**Estonia**

Riina Salupere, *Tartu*

**Finland**

Irma E Jarvela, *Helsinki*
Katri M Kaukinen, *Tampere*
Minna Nyström, *Helsinki*
Pentti Sipponen, *Espoo*

**France**

Bettaieb Ali, *Dijon*
Corlu Anne, *Rennes*
Denis Ardid, *Clermont-Ferrand*
Charles P Balabaud, *Bordeaux*
Soumeya Bekri, *Rouen*
Jacques Belghiti, *Clichy*
Jacques Bernuau, *Clichy Cedex*
Pierre Brissot, *Rennes*
Patrice P Cacoub, *Paris*
Franck Carbonnel, *Besancon*
Laurent Castera, *Pessac*
Bruno Clément, *Rennes*
Benoit Coffin, *Colombes*
Jacques Cosnes, *Paris*
Thomas Decaens, *Cedex*

Francoise L Fabiani, *Angers*
Gérard Feldmann, *Paris*
Jean Fioramonti, *Toulouse*
Jean-Noël Freund, *Strasbourg*
Jean-Paul Galmiche, *Nantes*
Catherine Guettier, *Villejuif*
Chantal Housset, *Paris*
Juan L Iovanna, *Marseille*
Rene Lambert, *Lyon*
Patrick Marcellin, *Paris*
Philippe Mathurin, *Lille*
Tamara Matysiak-Budnik, *Paris*
Francis Mégraud, *Bordeaux*
Richard Moreau, *Clichy*
Thierry Piche, *Nice*
Raoul Poupon, *Paris*
Jean Rosenbaum, *Bordeaux*
Dominique Marie Roulot, *Bobigny*
Thierry Poynard, *Paris*
Jean-Philippe Salier, *Rouen*
Didier Samuel, *Villejuif*
Jean-Yves Scoazec, *Lyon*
Alain L Servin, *Châtenay-Malabry*
Khalid A Tazi, *Clichy*
Emmanuel Tiret, *Paris*
Baumert F Thomas, *Strasbourg*
Jean-Pierre H Zarski, *Grenoble*
Jessica Zucman-Rossi, *Paris*

**Germany**

Hans-Dieter Allescher, *G-Partenkirchen*
Martin Anlauf, *Kiel*
Rudolf Arnold, *Marburg*
Max G Bachem, *Ulm*
Thomas F Baumert, *Freiburg*
Daniel C Baumgart, *Berlin*
Hubert Blum, *Freiburg*
Thomas Bock, *Tuebingen*
Katja Breitkopf, *Mannheim*
Dunja Bruder, *Braunschweig*
Markus W Büchler, *Heidelberg*
Christa Buechler, *Regensburg*
Reinhard Buettner, *Bonn*
Elke Cario, *Essen*
Uta Dahmen, *Essen*
Christoph F Dietrich, *Bad Mergentheim*
Arno J Dormann, *Koeln*
Rainer J Duchmann, *Berlin*
Volker F Eckardt, *Wiesbaden*
Paul Enck, *Tuebingen*
Fred Fändrich, *Kiel*
Ulrich R Fölsch, *Kiel*
Helmut Friess, *Heidelberg*
Peter R Galle, *Mainz*
Nikolaus Gassler, *Aachen*
Andreas Geier, *Aachen*
Markus Gerhard, *Munich*
Wolfram H Gerlich, *Giessen*
Dieter Glebe, *Giessen*
Burkhard Göke, *Munich*
Florian Graepler, *Tuebingen*
Axel M Gressner, *Aachen*
Veit Gülberg, *Munich*
Rainer Haas, *Munich*
Eckhart G Hahn, *Erlangen*
Stephan Hellmig, *Kiel*
Martin Hennenberg, *Bonn*
Johannes Herkel, *Hamburg*
Klaus R Herrlinger, *Stuttgart*
Eva Herrmann, *Homburg/Saar*
Eberhard Hildt, *Berlin*
Joerg C Hoffmann, *Berlin*
Ferdinand Hofstaedter, *Regensburg*
Werner Hohenberger, *Erlangen*
Jörg C Kalff, *Bonn*
Ralf Jakobs, *Ludwigshafen*
Jutta Keller, *Hamburg*
Andrej Khandoga, *Munich*
Sibylle Koletzko, *München*
Stefan Kubicka, *Hannover*
Joachim Labenz, *Siegen*
Frank Lammert, *Bonn*
Thomas Langmann, *Regensburg*
Christian Liedtke, *Aachen*
Matthias Löhr, *Mannheim*
Christian Maaser, *Muenster*
Ahmed Madisch, *Dresden*
Peter Malfertheiner, *Magdeburg*
Michael P Manns, *Hannover*
Helmut Messmann, *Augsburg*
Stephan Miehlke, *Dresden*
Sabine Mihm, *Göttingen*
Silvio Nadalin, *Essen*
Markus F Neurath, *Mainz*
Johann Ockenga, *Berlin*
Florian Obermeier, *Regensburg*
Gustav Paumgartner, *Munich*
Ulrich KS Peitz, *Magdeburg*
Markus Reiser, *Bochum*
Emil C Reisinger, *Rostock*
Steffen Rickes, *Magdeburg*
Tilman Sauerbruch, *Bonn*
Dieter Saur, *Munich*
Hans Scherubl, *Berlin*
Joerg Schirra, *Munich*
Roland M Schmid, *München*
Volker Schmitz, *Bonn*
Andreas G Schreyer, *Regensburg*
Tobias Schroeder, *Essen*
Henning Schulze-Bergkamen, *Mainz*
Hans Seifert, *Oldenburg*
Norbert Senninger, *Muenster*
Manfred V Singer, *Mannheim*
Gisela Sparmann, *Rostock*
Christian J Steib, *München*
Jurgen M Stein, *Frankfurt*
Ulrike S Stein, *Berlin*
Manfred Stolte, *Bayreuth*
Christian P Strassburg, *Hannover*
Wolfgang R Stremmel, *Heidelberg*
Harald F Teutsch, *Ulm*
Robert Thimme, *Freiburg*
Hans L Tillmann, *Leipzig*
Tung-Yu Tsui, *Regensburg*
Axel Ulsenheimer, *Munich*
Patrick Veit-Haibach, *Essen*
Claudia Veltkamp, *Heidelberg*
Siegfried Wagner, *Deggendorf*
Henning Walczak, *Heidelberg*
Heiner Wedemeyer, *Hannover*
Fritz von Weizsacker, *Berlin*
Jens Werner, *Heidelberg*
Bertram Wiedenmann, *Berlin*
Reiner Wiest, *Regensburg*
Stefan Wirth, *Wuppertal*
Stefan JP Zeuzem, *Homburg*

**Greece**

Alexandra A Alexopoulou, *Athens*
George N Dalekos, *Larissa*
Christos Dervenis, *Athens*
Melanie Maria Deutsch, *Athens*
Tsianos Epameinondas, *Ioannina*
Elias A Kouroumalis, *Heraklion*
George Papatheodoridis, *Athens*
Spiros Sgouros, *Athens*

**Hungary**

Peter L Lakatos, *Budapest*
Zsuzsa Szondy, *Debrecen*

**Iceland**

Hallgrimur Gudjonsson, *Reykjavik*

**India**

Philip Abraham, *Mumbai*
Rakesh Aggarwal, *Lucknow*
Kunissery A Balasubramanian, *Vellore*
Deepak Kumar Bhasin, *Chandigarh*
Sujit K Bhattacharya, *Kolkata*
Yogesh K Chawla, *Chandigarh*
Radha K Dhiman, *Chandigarh*
Sri Prakash Misra, *Allahabad*
Ramesh Roop Rai, *Jaipur*
Nageshwar D Reddy, *Hyderabad*
Rakesh Kumar Tandon, *New Delhi*

**Iran**

Mohammad Abdollahi, *Tehran*
Seyed-Moayed Alavian, *Tehran*
Reza Malekzadeh, *Tehran*
Seyed A Taghavi, *Shiraz*

**Ireland**

Billy Bourke, *Dublin*
Ronan A Cahill, *Cork*
Anthony P Moran, *Galway*

**Israel**

Simon Bar-Meir, *Hashomer*
Abraham R Eliakim, *Haifa*
Zvi Fireman, *Hadera*
Yaron Ilan, *Jerusalem*
Avidan U Neumann, *Ramat-Gan*
Yaron Niv, *Pardesia*
Ran Oren, *Tel Aviv*
Ami D Sperber, *Beer-Sheva*

**Italy**

Giovanni Addolorato, *Roma*
Luigi E Adinolfi, *Naples*
Domenico Alvaro, *Rome*
Mario Angelico, *Rome*
Vito Annese, *San Giovanni Rotond*
Filippo Ansaldi, *Genoa*
Adolfo F Attili, *Roma*
Giovanni Barbara, *Bologna*
Claudio Bassi, *Verona*
Gabrio Bassotti, *Perugia*
Pier M Battezzati, *Milan*
Stefano Bellentani, *Carpi*
Antonio Benedetti, *Ancona*
Mauro Bernardi, *Bologna*
Livia Biancone, *Rome*
Luigi Bonavina, *Milano*
Flavia Bortolotti, *Padova*
Giuseppe Brisinda, *Rome*
Elisabetta Buscarini, *Crema*
Giovanni Cammarota, *Roma*

Antonino Cavallari, *Bologna*
Giuseppe Chiarioni, *Valeggio*
Michele Cicala, *Rome*
Massimo Colombo, *Milan*
Amedeo Columbano, *Cagliari*
Massimo Conio, *Sanremo*
Dario Conte, *Milano*
Gino R Corazza, *Pavia*
Francesco Costa, *Pisa*
Antonio Craxi, *Palermo*
Silvio Danese, *Milan*
Roberto de Franchis, *Milano*
Roberto De Giorgio, *Bologna*
Maria Stella De Mitri, *Bologna*
Giovanni D De Palma, *Naples*
Fabio Farinati, *Padua*
Giammarco Fava, *Ancona*
Francesco Feo, *Sassari*
Fiorucci Stefano, *Perugia*
Andrea Galli, *Firenze*
Valeria Ghisett, *Turin*
Gianluigi Giannelli, *Bari*
Edoardo G Giannini, *Genoa*
Paolo Gionchetti, *Bologna*
Fabio Grizzi, *Milan*
Salvatore Gruttadauria, *Palermo*
Mario Guslandi, *Milano*
Pietro Invernizzi, *Milan*
Ezio Laconi, *Cagliari*
Giacomo Laffi, *Firenze*
Giovanni Maconi, *Milan*
Lucia Malaguarnera, *Catania*
Emanuele D Mangoni, *Napoli*
Paolo Manzoni, *Torino*
Giulio Marchesini, *Bologna*
Fabio Marra, *Florence*
Marco Marzioni, *Ancona*
Roberto Mazzanti, *Florence*
Giuseppe Mazzella, *Bologna*
Mario U Mondelli, *Pavia*
Giuseppe Montalto, *Palermo*
Giovanni Monteleone, *Rome*
Giovanni Musso, *Torino*
Gerardo Nardone, *Napoli*
Valerio Nobili, *Rome*
Fabio Pace, *Milano*
Luisi Pagliaro, *Palermo*
Francesco Pallone, *Rome*
Fabrizio R Parente, *Milan*
Maurizio Parola, *Torino*
Francesco Perri, *San Giovanni Rotondo*
Raffaele Pezzilli, *Bologna*
Alberto Pilotto, *San Giovanni Rotondo*
Alberto Piperno, *Monza*
Mario Pirisi, *Novara*
Anna C Piscaglia, *Roma*
Paolo Del Poggio, *Treviglio*
Gabriele B Porro, *Milano*
Piero Portincasa, *Bari*
Cosimo Prantera, *Roma*
Bernardino Rampone, *Siena*
Oliviero Riggio, *Rome*
Claudio Romano, *Messina*
Marco Romano, *Napoli*
Gerardo Rosati, *Potenza*
Mario Del Tacca, *Pisa*
Gloria Taliani, *Rome*
Pier A Testoni, *Milan*
Enrico Roda, *Bologna*
Domenico Sansonno, *Bari*
Vincenzo Savarino, *Genova*
Vincenzo Stanghellini, *Bologna*
Giovanni Tarantino, *Naples*
Roberto Testa, *Genoa*
Dino Vaira, *Bologna*
Anna Linda Zignego, *Florence*

**Japan**

Kyoichi Adachi, *Izumo*
Yasushi Adachi, *Sapporo*
Taiji Akamatsu, *Matsumoto*
Sk Md Fazle Akbar, *Ehime*
Takafumi Ando, *Nagoya*
Akira Andoh, *Otsu*
Taku Aoki, *Tokyo*
Masahiro Arai, *Tokyo*
Tetsuo Arakawa, *Osaka*
Yasuji Arase, *Tokyo*
Masahiro Asaka, *Sapporo*
Hitoshi Asakura, *Tokyo*
Takeshi Azuma, *Fukui*
Yoichi Chida, *Fukuoka*
Takahiro Fujimori, *Tochigi*
Jiro Fujimoto, *Hyogo*
Kazuma Fujimoto, *Saga*
Mitsuhiro Fujishiro, *Tokyo*
Yoshihide Fujiyama, *Otsu*
Hiroyuki Fukui, *Tochigi*
Hiroyuki Hanai, *Hamamatsu*
Naohiko Harada, *Fukuoka*
Makoto Hashizume, *Fukuoka*
Tetsuo Hayakawa, *Nagoya*
Toru Hiyama, *Higashihiroshima*
Kazuhide Higuchi, *Osaka*
Keisuke Hino, *Ube*
Keiji Hirata, *Kitakyushu*
Yuji Iimuro, *Nishinomiya*
Kenji Ikeda, *Tokyo*
Toru Ikegami, *Fukuoka*
Kenichi Ikejima, *Bunkyo-ku*
Fumio Imazeki, *Chiba*
Yutaka Inagaki, *Kanagawa*
Yasuhiro Inokuchi, *Yokohama*
Haruhiro Inoue, *Yokohama*
Masayasu Inoue, *Osaka*
Hiromi Ishibashi, *Nagasaki*
Shunji Ishihara, *Izumo*
Toru Ishikawa, *Niigata*
Kei Ito, *Sendai*
Masayoshi Ito, *Tokyo*
Hiroaki Itoh, *Akita*
Ryuichi Iwakiri, *Saga*
Yoshiaki Iwasaki, *Okayama*
Terumi Kamisawa, *Tokyo*
Hiroshi Kaneko, *Aichi-Gun*
Shuichi Kaneko, *Kanazawa*
Takashi Kanematsu, *Nagasaki*
Mitsuo Katano, *Fukuoka*
Junji Kato, *Sapporo*
Mototsugu Kato, *Sapporo*
Shinzo Kato, *Tokyo*
Norifumi Kawada, *Osaka*
Sunao Kawano, *Osaka*
Mitsuhiro Kida, *Kanagawa*
Yoshikazu Kinoshita, *Izumo*
Tsuneo Kitamura, *Chiba*
Seigo Kitano, *Oita*
Kazuhiko Koike, *Tokyo*
Norihiro Kokudo, *Tokyo*
Satoshi Kondo, *Sapporo*
Shoji Kubo, *Osaka*
Masatoshi Kudo, *Osaka*
Shigeki Kuriyama, *Kagawa*[2]
Masato Kusunoki, *Tsu Mie*
Katsunori Iijima, *Sendai*
Shin Maeda, *Tokyo*
Shigeru Marubashi, *Suita*
Masatoshi Makuuchi, *Tokyo*
Osamu Matsui, *Kanazawa*
Yasuhiro Matsumura, *Chiba*
Yasushi Matsuzaki, *Tsukuba*
Kiyoshi Migita, *Omura*
Kenji Miki, *Tokyo*
Tetsuya Mine, *Kanagawa*
Hiroto Miwa, *Hyogo*
Masashi Mizokami, *Nagoya*
Yoshiaki Mizuguchi, *Tokyo*
Motowo Mizuno, *Hiroshima*
Morito Monden, *Suita*
Hisataka S Moriwaki, *Gifu*
Yasuaki Motomura, *Iizuka*
Yoshiharu Motoo, *Kanazawa*
Naofumi Mukaida, *Kanazawa*
Kazunari Murakami, *Oita*
Kunihiko Murase, *Tusima*
Hiroaki Nagano, *Suita*
Masahito Nagaki, *Gifu*
Masaki Nagaya, *Kawasaki*
Yujl Naito, *Kyoto*
Atsushi Nakajima, *Yokohama*
Hisato Nakajima, *Tokyo*
Hiroki Nakamura, *Yamaguchi*
Shotaro Nakamura, *Fukuoka*
Mikio Nishioka, *Niihama*
Shuji Nomoto, *Nagoya*
Susumu Ohmada, *Maebashi*
Hirohide Ohnishi, *Akita*
Masayuki Ohta, *Oita*
Tetsuo Ohta, *Kanazawa*
Kazuichi Okazaki, *Osaka*
Katsuhisa Omagari, *Nagasaki*
Saburo Onishi, *Nankoku*
Morikazu Onji, *Ehime*
Satoshi Osawa, *Hamamatsu*
Masanobu Oshima, *Kanazawa*
Hiromitsu Saisho, *Chiba*
Hidetsugu Saito, *Tokyo*
Yutaka Saito, *Tokyo*
Isao Sakaida, *Yamaguchi*
Michiie Sakamoto, *Tokyo*
Yasushi Sano, *Chiba*
Hiroki Sasaki, *Tokyo*
Iwao Sasaki, *Sendai*
Motoko Sasaki, *Kanazawa*
Chifumi Sato, *Tokyo*
Shuichi Seki, *Osaka*
Hiroshi Shimada, *Yokohama*
Mitsuo Shimada, *Tokushima*
Tomohiko Shimatan, *Hiroshima*
Hiroaki Shimizu, *Chiba*
Ichiro Shimizu, *Tokushima*
Yukihiro Shimizu, *Kyoto*
Shinji Shimoda, *Fukuoka*
Tooru Shimosegawa, *Sendai*
Tadashi Shimoyama, *Hirosaki*
Ken Shirabe, *Iizuka City*
Yoshio Shirai, *Niigata*
Katsuya Shiraki, *Mie*
Yasushi Shiratori, *Okayama*
Masayuki Sho, *Nara*
Yasuhiko Sugawara, *Tokyo*
Hidekazu Suzuki, *Tokyo*
Minoru Tada, *Tokyo*
Tadatoshi Takayama, *Tokyo*
Tadashi Takeda, *Osaka*
Koji Takeuchi, *Kyoto*
Kiichi Tamada, *Tochigi*
Akira Tanaka, *Kyoto*
Eiji Tanaka, *Matsumoto*
Noriaki Tanaka, *Okayama*
Shinji Tanaka, *Hiroshima*
Hideki Taniguchi, *Yokohama*
Kyuichi Tanikawa, *Kurume*
Akira Terano, *Shimotsugagun*
Hitoshi Togash, *Yamagata*
Shinji Togo, *Yokohama*
Kazunari Tominaga, *Osaka*
Takuji Torimura, *Fukuoka*
Minoru Toyota, *Sapporo*

Akihito Tsubota, *Chiba*
Takato Ueno, *Kurume*
Naomi Uemura, *Tokyo*
Shinichi Wada, *Tochigi*
Hiroyuki Watanabe, *Kanazawa*
Toshio Watanabe, *Osaka*
Yuji Watanabe, *Ehime*
Toshiaki Watanabe, *Tokyo*
Chun-Yang Wen, *Nagasaki*
Satoshi Yamagiwa, *Niigata*
Koji Yamaguchi, *Fukuoka*
Takayuki Yamamoto, *Yokkaichi*
Takashi Yao, *Fukuoka*
Masashi Yoneda, *Tochigi*
Hiroshi Yoshida, *Tokyo*
Masashi Yoshida, *Tokyo*
Norimasa Yoshida, *Kyoto*
Hitoshi Yoshiji, *Nara*
Kentaro Yoshika, *Toyoake*
Yasunobu Yoshikai, *Fukuoka*
Masahide Yoshikawa, *Kashihara*
Katsutoshi Yoshizato, *Higashihiroshima*

**Lebanon**
Bassam N Abboud, *Beirut*
Ala I Sharara, *Beirut*
Joseph D Boujaoude, *Beirut*

**Lithuania**
Limas Kupcinskas, *Kaunas*

**Macedonia**
Vladimir C Serafimoski, *Skopje*

**Malaysia**
Andrew Seng Boon Chua, *Ipoh*
Khean-Lee Goh, Kuala *Lumpur*
Jayaram Menon, *Sabah*

**Mexico**
Diego Garcia-Compean, *Monterrey*
Eduardo R Marin-Lopez, *Jesús García*
Nahum Méndez-Sánchez, *Mexico*
Saúl Villa-Treviño, *México*

**Monaco**
Patrick Rampal, *Monaco*

**Morocco**
Abdellah Essaid, *Rabat*

**The Netherlands**
Ulrich Beuers, *Amsterdam*
Gerd Bouma, *Amsterdam*
Lee Bouwman, *Leiden*
J Bart A Crusius, *Amsterdam*
NKH de Boer, *Amsterdam*
Koert P de Jong, *Groningen*
Henrike Hamer, *Maastricht*
Frank Hoentjen, *Haarlem*
Janine K Kruit, *Groningen*
Ernst J Kuipers, *Rotterdam*
CBHW Lamers, *Leiden*
Ton Lisman, *Utrecht*
Yi Liu, *Amsterdam*
Jeroen Maljaars, *Maastricht*
Servaas Morré, *Amsterdam*
Chris JJ Mulder, *Amsterdam*
Michael Müller, *Wageningen*
Amado S Peña, *Amsterdam*
Robert J Porte, *Groningen*
Ingrid B Renes, *Rotterdam*
Andreas Smout, *Utrecht*
Paul E Sijens, *Groningen*
Reinhold W Stockbrugger, *Maastricht*
Luc JW van der Laan, *Rotterdam*
Karel van Erpecum, *Utrecht*
Gerard P VanBerge-Henegouwen,*Utrecht*

**New Zealand**
Ian D Wallace, *Auckland*

**Nigeria**
Samuel B Olaleye, *Ibadan*

**Norway**
Trond Berg, *Oslo*
Tom H Karlsen, *Oslo*
Helge L Waldum, *Trondheim*

**Pakistan**
Muhammad S Khokhar, *Lahore*
Syed MW Jafri, *Karachi*

**Peru**
Hector H Garcia, *Lima*

**Poland**
Tomasz Brzozowski, *Cracow*
Robert Flisiak, *Bialystok*
Hanna Gregorek, *Warsaw*
Dariusz M Lebensztejn, *Bialystok*
Wojciech G Polak, *Wroclaw*
Marek Hartleb, *Katowice*

**Portugal**
Miguel C De Moura, *Lisbon*

**Russia**
Vladimir T Ivashkin, *Moscow*
Leonid Lazebnik, *Moscow*
Vasiliy I Reshetnyak, *Moscow*

**Saudi Arabia**
Ibrahim A Al Mofleh, *Riyadh*
Ahmed Helmy, *Riyadh*

**Serbia**
Dusan M Jovanovic, *Sremska Kamenica*

**Singapore**
Bow Ho, *Singapore*
Khek-Yu Ho, *Singapore*
Fock Kwong Ming, *Singapore*
Francis Seow-Choen, *Singapore*

**Slovakia**
Silvia Pastorekova, *Bratislava*
Anton Vavrecka, *Bratislava*

**Slovenia**
Sasa Markovic, *Ljubljana*

**South Africa**
Rosemar Joyce Burnett, *Pretoria*
Michael C Kew, *Parktown*

**South Korea**
Byung Ihn Choi, *Seoul*
Ho Soon Choi, *Seoul*
Marie Yeo, *Suwon*
Sun Pyo Hong, *Gyeonggi-do*
Jae J Kim, *Seoul*
Jin-Hong Kim, *Suwon*
Myung-Hwan Kim, *Seoul*
Chang Hong Lee, *Seoul*
Jeong Min Lee, *Seoul*
Jong Kyun Lee, *Seoul*
Eun-Yi Moon, *Seoul*
Jae-Gahb Park, *Seoul*
Dong Wan Seo, *Seoul*
Dong Jin Suh, *Seoul*
Byung Chul Yoo, *Seoul*

**Spain**
Juan G Abraldes, *Barcelona*
Agustin Albillos, *Madrid*
Raul J Andrade, *Málaga*
Luis Aparisi, *Valencia*
Fernando Azpiroz, *Barcelona*
Ramon Bataller, *Barcelona*
Josep M Bordas, *Barcelona*
Xavier Calvet, *Sabadell*
Jordi Camps, *Catalunya*
Andres Cardenas, *Barcelona*
Vicente Carreño, *Madrid*
Jose Castellote, *Barcelona*
Antoni Castells, *Barcelona*
Vicente Felipo, *Valencia*
Juan C Garcia-Pagán, *Barcelona*
Jaime B Genover, *Barcelona*
Javier P Gisbert, *Madrid*
Jaime Guardia, *Barcelona*
Isabel Fabregat, *Barcelona*
Mercedes Fernandez, *Barcelona*
Angel Lanas, *Zaragoza*
Juan-Ramón Larrubia, *Guadalajara*
Laura Lladóa, *Barcelona*
María IT López, *Jaén*
Juan R Malagelada, *Barcelona*
José M Mato, *Derio*
Juan F Medina, *Pamplona*
Miguel A Muñoz-Navas, *Pamplona*
Julian Panes, *Barcelona*
Miguel M Perez, *Valencia*
Miguel Perez-Mateo, *Alicante*

Josep M Pique, *Barcelona*
Jesús M Prieto, *Pamplona*
Sabino Riestra, *Pola De Siero*
Luis Rodrigo, *Oviedo*
Manuel Romero-Gómez, *Sevilla*
Joan Roselló-Catafau, *Barcelona*

**Sweden**

Einar S Björnsson, *Gothenburg*
Curt Einarsson, *Huddinge*
Per M Hellström, *Stockholm*
Ulf Hindorf, *Lund*
Elisabeth Hultgren-Hörnquist, *Örebro*
Anders E Lehmann, *Mölndal*
Hanns-Ulrich Marschall, *Stockholm*
Lars C Olbe, *Molndal*
Lars A Pahlman, *Uppsala*
Matti Sallberg, *Stockholm*
Magnus Simrén, *Göteborg*
Xiao-Feng Sun, *Linköping*
Ervin Tóth, *Malmö*
Weimin Ye, *Stockholm*
Christer S von Holstein, *Lund*

**Switzerland**

Chrish Beglinger, *Basel*
Pierre A Clavien, *Zurich*
Jean-Francois Dufour, *Bern*
Franco Fortunato, *Zurich*
Jean L Frossard, *Geneva*
Gerd A Kullak-Ublick, *Zurich*
Pierre Michetti, *Lausanne*
Francesco Negro, *Genève*
Bruno Stieger, *Zurich*
Radu Tutuian, *Zurich*
Stephan R Vavricka, *Zurich*
Gerhard Rogler, *Zurich*
Arthur Zimmermann, *Berne*

**Turkey**

Yusuf Bayraktar, *Ankara*
Figen Gurakan, *Ankara*
Aydin Karabacakoglu, *Konya*
Serdar Karakose, *Konya*
Hizir Kurtel, *Istanbul*
Osman C Ozdogan, *Istanbul*
Özlem Yilmaz, *Izmir*
Cihan Yurdaydin, *Ankara*

**United Arab Emirates**

Sherif M Karam, *Al-Ain*

**United Kingdom**

David H Adams, *Birmingham*
Simon Afford, *Birmingham*
Navneet K Ahluwalia, *Stockport*
Ahmed Alzaraa, *Manchester*
Lesley A Anderson, *Belfast*
Charalambos G Antoniades, *London*
Anthony TR Axon, *Leeds*
Qasim Aziz, *Manchester*
Nicholas M Barnes, *Birmingham*
Jim D Bell, *London*
Mairi Brittan, *London*
Alastair D Burt, *Newcastle*
Simon S Campbell, *Manchester*
Simon R Carding, *Leeds*
Paul J Ciclitira, *London*
Eithne Costello, *Liverpool*
Tatjana Crnogorac-Jurcevic, *London*
Harry Dalton, *Truro*
Amar P Dhillon, *London*
William Dickey, *Londonderry*
James E East, *London*
Emad M El-Omar, *Aberdeen*
Ahmed M Elsharkawy, *Newcastle Upon Tyne*
Annette Fristscher-Ravens, *London*
Elizabeth Furrie, *Dundee*
Daniel R Gaya, *Edinburgh*
Subrata Ghosh, *London*
William Greenhalf, *Liverpool*
Indra N Guha, *Southampton*
Peter C Hayes, *Edinburgh*
Gwo-Tzer Ho, *Edinburgh*
Anthony R Hobson, *Salford*
Lesley A Houghton, *Manchester*
Stefan G Hübscher, *Birmingham*
Robin Hughes, *London*
Pali Hungin, *Stockton*
David P Hurlstone, *Sheffield*
Rajiv Jalan, *London*
Janusz AZ Jankowski, *Oxford*
Brian T Johnston, *Belfast*
David EJ Jones, *Newcastle*
Roger Jones, *London*
Michael A Kamm, *Harrow*
Peter Karayiannis, *London*
Laurens Kruidenier, *Harlow*
Patricia F Lalor, *Birmingham*
Chee Hooi Lim, *Midlands*
Hong-Xiang Liu, *Cambridge*
Yun Ma, *London*
Kenneth E L McColl, *Glasgow*
Stuart AC McDonald, *London*
Dermot P Mcgovern, *Oxford*
Giorgina Mieli-Vergani, *London*
Nikolai V Naoumov, *London*
John P Neoptolemos, *Liverpool*
James Neuberger, *Birmingham*
Philip Noel Newsome, *Birmingham*
Mark S Pearce, *Newcastle Upon Tyne*
Stephen P Pereira, *London*
D Mark Pritchard, *Liverpool*
Sakhawat Rahman, *London*
Stephen E Roberts, *Swansea*
Marco Senzolo, *Padova*
Soraya Shirazi-Beechey, *Liverpool*
Robert Sutton, *Liverpool*
Simon D Taylor-Robinson, *London*
Paris P Tekkis, *London*
Ulrich Thalheimer, *London*
David G Thompson, *Salford*
Nick P Thompson, *Newcastle*
David Tosh, *Bath*
Frank I Tovey, *London*
Chris Tselepis, *Birmingham*
Diego Vergani, *London*
Geoffrey Warhurst, *Salford*
Alastair John Watson, *Liverpool*
Peter J Whorwell, *Manchester*
Roger Williams, *London*
Karen L Wright, *Bath*
Min Zhao, *Foresterhill*

**United States**

Manal F Abdelmalek, *Durham*
Gary A Abrams, *Birmingham*
Maria T Abreu, *New York*
Reid B Adams, *Virginia*
Golo Ahlenstiel, *Bethesda*
BS Anand, *Houston*
Frank A Anania, *Atlanta*
M Ananthanarayanan, *New York*
Gavin E Arteel, *Louisville*
Jasmohan S Bajaj, *Milwaukee*
Subhas Banerjee, *Palo Alto*
Peter A Banks, *Boston*
Jamie S Barkin, *Miami Beach*
Kim E Barrett, *San Diego*
Marc D Basson, *Detroit*
Anthony J Bauer, *Pittsburgh*
Wallace F Berman, *Durham*
Timothy R Billiar, *Pittsburgh*
Edmund J Bini, *New York*
David G Binion, *Milwaukee*
Jennifer D Black, *Buffalo*
Herbert L Bonkovsky, *Charlotte*
Carla W Brady, *Durham*
Andrea D Branch, *New York*
Robert S Bresalier, *Houston*
Alan L Buchman, *Chicago*
Ronald W Busuttil, *Los Angeles*
Alan Cahill, *Philadelphia*
John M Carethers, *San Diego*
David L Carr-Locke, *Boston*
Maurice A Cerulli, *New York*
Ravi S Chari, *Nashville*
Jiande Chen, *Galveston*
Xian-Ming Chen, *Omaha*
Xin Chen, *San Francisco*
Ramsey Chi-man Cheung, *Palo Alto*
William D Chey, *Ann Arbor*
John Y Chiang, *Rootstown*
Parimal Chowdhury, *Arkansas*
Raymond T Chung, *Boston*
James M Church, *Cleveland*
Ram Chuttani, *Boston*
Mark G Clemens, *Charlotte*
Ana J Coito, *Los Angeles*
Vincent Coghlan, *Beaverton*
David Cronin II, *New Haven*
John Cuppoletti, *Cincinnati*
Mark J Czaja, *New York*
Peter V Danenberg, *Los Angeles*
Kiron M Das, New *Brunswick*
Conor P Delaney, *Cleveland*
Jose L del Pozo, *Rochester*
Sharon DeMorrow, *Temple*
Deborah L Diamond, *Seattle*
Douglas A Drossman, *Chapel Hill*
Katerina Dvorak, *Tucson*
Bijan Eghtesad, *Cleveland*
Hala El-Zimaity, *Houston*
Michelle Embree-Ku, *Providence*
Sukru Emre, New *Haven*
Douglas G Farmer, *Los Angeles*
Alessio Fasano, *Baltimore*
Mark A Feitelson, *Philadelphia*
Ariel E Feldstein, *Cleveland*
Alessandro Fichera, *Chicago*
Robert L Fine, *New York*
Chris E Forsmark, *Gainesville*
Glenn T Furuta, *Aurora*
Chandrashekhar R Gandhi, *Pittsburgh*
Susan L Gearhart, *Baltimore*
Xupeng Ge, *Boston*
Xin Geng, *New Brunswick*
M Eric Gershwin, *Suite*
Jean-Francois Geschwind, *Baltimore*
Ignacio Gil-Bazo, *New York*
Shannon S Glaser, *Temple*
Ajay Goel, *Dallas*
Richard M Green, *Chicago*
Julia B Greer, *Pittsburgh*
James H Grendell, *New York*

David R Gretch, *Seattle*
Stefano Guandalini, *Chicago*
Anna S Gukovskaya, *Los Angeles*
Sanjeev Gupta, *Bronx*
David J Hackam, *Pittsburgh*
Stephen B Hanauer, *Chicago*
Gavin Harewood, *Rochester*
Margaret M Heitkemper, *Washington*
Alan W Hemming, *Gainesville*
Samuel B Ho, *San Diego*
Peter R Holt, *New York*
Colin W Howden, *Chicago*
Hongjin Huang, *Alameda*
Jamal A Ibdah, *Columbia*
Atif Iqbal, *Omaha*
Hajime Isomoto, *Rochester*
Hartmut Jaeschke, *Tucson*
Dennis M Jensen, *Los Angeles*
Cheng Ji, *Los Angeles*
Leonard R Johnson, *Memphis*
Michael P Jones, *Chicago*
Peter J Kahrilas, *Chicago*
Anthony N Kalloo, *Baltimore*
Marshall M Kaplan, *Boston*
Neil Kaplowitz, *Los Angeles*
Serhan Karvar, *Los Angeles*
Rashmi Kaul, *Tulsa*
Jonathan D Kaunitz, *Los Angeles*
Ali Keshavarzian, *Chicago*
Miran Kim, *Providence*
Joseph B Kirsner, *Chicago*
Leonidas G Koniaris, *Miami*
Burton I Korelitz, *New York*
Robert J Korst, *New York*
Richard A Kozarek, *Seattle*
Alyssa M Krasinskas, *Pittsburgh*
Michael Kremer, *Chapel Hill*
Shiu-Ming Kuo, *Buffalo*
Paul Y Kwo, *Indianapolis*
Daryl Tan Yeung Lau, *Galvesto*
Stephen J Lanspa, *Omaha*
Joel E Lavine, *San Diego*
Bret Lashner, *Cleveland*
Dirk J van Leeuwen, *Lebanon*
Glen A Lehman, *Indianapolis*
Alex B Lentsch, *Cincinnati*
Andreas Leodolter, *La Jolla*
Gene LeSage, *Houston*
Josh Levitsky, *Chicago*
Cynthia Levy, *Gainesville*
Ming Li, *New Orleans*
Zhiping Li, *Baltimore*
Zhe-Xiong Lian, *Davis*
Lenard M Lichtenberger, *Houston*
Gary R Lichtenstein, *Philadelphia*
Otto Schiueh-Tzang Lin, *Seattle*
Martin Lipkin, *New York*
Chen Liu, *Gainesville*
Edward V Loftus, *Rocheste*
Robin G Lorenz, *Birmingham*
Michael R Lucey, *Madison*
James D Luketich, *Pittsburgh*
Guangbin Luo, *Cheveland*
Henry T Lynch, *Omaha*
Patrick M Lynch, *Houston*
John S Macdonald, *New York*
Bruce V MacFadyen, *Augusta*
Willis C Maddrey, *Dallas*
Ashok Malani, *Los Angeles*
Mercedes Susan Mandell, *Aurora*
Peter J Mannon, *Bethesda*
Charles M Mansbach, *Tennessee*
John F Di Mari, *Texas*
John M Mariadason, *Bronx*
Jorge A Marrero, *Ann Arbor*
Paul Martin, *New York*
Paulo Ney Aguiar Martins, *Boston*
Wendy M Mars, *Pittsburgh*
Laura E Matarese, *Pittsburgh*
Richard W McCallum, *Kansas*
Beth A McCormick, *Charlestown*
Lynne V McFarland, *Washington*
Kevin McGrath, *Pittsburgh*
Harihara Mehendale, *Monroe*
Ali Mencin, *New York*
Fanyin Meng, *Ohio*
Stephan Menne, *New York*
Didier Merlin, *Atlanta*
Howard Mertz, *Nashville*
George W Meyer, *Sacramento*
George Michalopoulos, *Pittsburgh*
James M Millis, *Chicago*
Fabrizio Michelassi, *New York*
Albert D Min, *New York*
Pramod K Mistry, *New Haven*
Emiko Mizoguchi, *Boston*
Smruti R Mohanty, *Chicago*
Satdarshan S Monga, *Pittsburgh*
Timothy H Moran, *Baltimore*
Peter L Moses, *Burlington*
Steven F Moss, *Providence*
Andrew J Muir, *Durham*
Milton G Mutchnick, *Detroit*
Masaki Nagaya, *Boston*
Victor Navarro, *Philadelphia*
Laura E Nagy, *Cleveland*
Hiroshi Nakagawa, *Philadelphia*
Douglas B Nelson, *Minneapolis*
Justin H Nguyen, *Florida*
Patrick G Northup, *Charlottesville*
Christopher O'Brien, *Miami*
Robert D Odze, *Boston*
Brant K Oelschlager, *Washington*
Curtis T Okamoto, *Los Angeles*
Stephen JD O'Keefe, *Pittsburgh*
Dimitry Oleynikov, *Omaha*
Stephen J Pandol, *Los Angeles*
Georgios Papachristou, *Pittsburgh*
Pankaj J Pasricha, *Galveston*
Zhiheng Pei, *New York*
Michael A Pezzone, *Pittsburgh*
CS Pitchumoni, *New Brunswiuc*
Paul J Pockros, *La Jolla*
Jay Pravda, *Gainesville*
Massimo Raimondo, *Jacksonville*
GS Raju, *Galveston*
Raymund R Razonable, *Minnesota*
Murray B Resnick, *Providence*
Adrian Reuben, *Charleston*
Douglas K Rex, *Indianapolis*
Victor E Reyes, *Galveston*
Basil Rigas, *New York*
Yehuda Ringel, *Chapel Hill*
Richard A Rippe, *Chapel Hill*
Maribel Rodriguez-Torres, *Santurce*
Marcos Rojkind, *Washington*
Philip Rosenthal, *San Francisco*
Barry Rosser, *Jacksonville Florida*
Hemant K Roy, *Evanston*
Sammy Saab, *Los Angeles*
Shawn D Safford, *Norfolk*
Dushyant V Sahani, *Boston*
Bruce E Sands, *Boston*
James M Scheiman, *Ann Arbor*
Eugene R Schiff, *Miami*
Nicholas J Shaheen, *Chapel Hill*
Vanessa M Shami, *Charlottesville*
Prateek Sharma, *Kansas City*
Harvey L Sharp, *Minneapolis*
Stuart Sherman, *Indianapolis*
Shivendra Shukla, *Columbia*
Alphonse E Sirica, *Virginia*
Shanthi V Sitaraman, *Atlanta*
Stuart J Spechler, *Dallas*
Subbaramiah Sridhar, *Augusta*
Shanthi Srinivasan, *Atlanta*
Michael Steer, *Boston*
Peter D Stevens, *New York*
Charmaine A Stewart, *Rochester*
Christian D Stone, *Saint Louis*
Gary D Stoner, *Columbus*
R Todd Stravitz, *Richmond*
Liping Su, *Chicago*
Christina Surawicz, *Seattle*
Robert W Summers, *Iowa City*
Wing-Kin Syn, *Durham*
Gyongyi Szabo, *Worcester*
Yvette Taché, *Los Angeles*
Toku Takahashi, *Milwaukee*
Seng-Lai Tan, *Seattle*
Andrzej S Tarnawski, *Orange*
K-M Tchou-Wong, *New York*
Jonathan P Terdiman, *San Francisco*
Neil D Theise, *New York*
Christopher C Thompson, *Boston*
Swan N Thung, *New York*
Michael Torbenson, *Baltimore*
Natalie J Torok, *Sacramento*
RA Travagli, *Baton Rouge*
George Triadafilopoulos, *Stanford*
Chung-Jyi Tsai, *Lexington*
Janet Elizabeth Tuttle-Newhall, *Durham*
Andrew Ukleja, *Florida*
Michael F Vaezi, *Nashville*
Hugo E Vargas, *Scottsdale*
Arnold Wald, *Wisconsin*
Scott A Waldman, *Philadelphia*
Jian-Ying Wang, *Baltimore*
Timothy C Wang, *New York*
Irving Waxman, *Chicago*
Steven A Weinman, *Galveston*
Steven D Wexner, *Weston*
Keith T Wilson, *Baltimore*
Jacqueline L Wolf, *Boston*
Jackie Wood, *Ohio*
George Y Wu, *Farmington*
Jian Wu, *Sacramento*
Samuel Wyllie, *Houston*
Wen Xie, *Pittsburgh*
Vijay Yajnik, *Boston*
Vincent W Yang, *Atlanta*
Francis Y Yao, *San Francisco*
Hal F Yee, *San Francisco*
Xiao-Ming Yin, *Pittsburgh*
Min You, *Tampa*
Zobair M Younossi, *Virginia*
Liqing Yu, *Winston-Salem*
David Yule, *Rochester*
Ruben Zamora, *Pittsburgh*
Michael E Zenilman, *New York*
Zhi Zhong, *Chapel Hill*
Michael A Zimmerman, *Colorado*
Stephen D Zucker, *Cincinnati*

**Uruguay**

Henry Cohen, *Montevideo*

[1]Passed away on October 20, 2007
[2]Passed away on June 11, 2007
[3]Passed away on June 14, 2008

World Journal of
Gastroenterology®
Weekly Established in October 1995
Volume 15 Number 1
January 7, 2009

Contents

**Contents**

*World Journal of Gastroenterology*
**Volume** 15 **Number** 1 **January** 7, 2009

**Contents**

*World Journal of Gastroenterology*
**Volume** 15 **Number** 1 **January** 7, 2009

## INTRODUCTION

*World Journal of Gastroenterology* is an international, open-access, peer-reviewed, and multidisciplinary weekly journal that serves gastroenterologists and hepatologists. The biggest advantage of the open access model is that it provides free, full-text articles in PDF and other formats for experts and the public without registration, which eliminates the obstacle that traditional journals possess and usually delays the speed of the propagation and communication of scientific research results. The open access model has been proven to be a true approach that may achieve the ultimate goal of the journals, i.e. the maximization of the values of the readers, the authors and the society.

Maximization of the value of the readers can be comprehended in two ways. First, the journal publishes articles that can be directly read or downloaded free of charge at any time, which attracts more readers. Second, the readers can apply the knowledge in clinical practice without delay after reading and understanding the information in their fields. In addition, the readers are encouraged to propose new ideas based on those of the authors, or to provide viewpoints that are different from those of the authors. Such discussions or debates among different schools of thought will definitely boost advancements and developments in the fields. Maximization of the value of the authors refers to the fact that these journals provide a platform that promotes the speed of propagation and communication to a maximum extent. This is also what the authors really need. Maximization of the value of the society refers to the maximal extent of the social influences and impacts produced by the high quality original articles published in the journal. This is also the main purpose of many journals around the world.



NAME OF JOURNAL
*World Journal of Gastroenterology*

RESPONSIBLE INSTITUTION
Department of Science and Technology of Shanxi Province

SPONSOR
Taiyuan Research and Treatment Center for Digestive Diseases, 77 Shuangta Xijie, Taiyuan 030001, Shanxi Province, China

EDITING
Editorial Board of *World Journal of Gastroenterology,* Room 903, Building D, Ocean International Center, No.62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China
Telephone: +86-10-59080039
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com
http://www.wjgnet.com

PUBLISHING
The WJG Press and Beijing Baishideng BioMed Scientific Co., Ltd., Editorial Department: Room 903, Building D, Ocean International Center, No.62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China
Telephone: +86-10-59080039
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com
http://www.wjgnet.com

PRINTING
Beijing Kexin Printing House

OVERSEAS DISTRIBUTOR
Beijing Bureau for Distribution of Newspapers and Journals
(Code No. 82-261)
China International Book Trading Corporation PO Box 399, Beijing, China (Code No. M4481)

PUBLICATION DATE
January 7, 2009

EDITOR-IN-CHIEF
Lian-Sheng Ma, *Beijing*

SUBSCRIPTION
RMB 50 Yuan for each issue, RMB 2400 Yuan for one year

CSSN
ISSN 1007-9327
CN 14-1219/R

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.1

*World J Gastroenterol* 2009 January 7; 15(1): 1-16
***World Journal of Gastroenterology*** ISSN 1007-9327


*EDITORIAL*

# Importance of gastrin in the pathogenesis and treatment of gastric tumors

Michael D Burkitt, Andrea Varro, D Mark Pritchard

Michael D Burkitt, D Mark Pritchard, Gastroenterology Unit, School of Clinical Sciences, University of Liverpool, Ashton Street, Liverpool, L69 3GE, United Kingdom
Andrea Varro, The Physiological Laboratory, School of Biomedical Sciences, University of Liverpool, Crown Street, Liverpool, L69 3BX, United Kingdom
Author contributions: The review was commissioned from Pritchard DM, who developed the initial plan for the article; Burkitt MD performed the literature search and wrote the first draft of the manuscript; Varro A and Pritchard DM edited subsequent versions of the manuscript.
Supported by Wellcome Trust Clinical Research Training Fellowship to MDB
Correspondence to: D Mark Pritchard, Gastroenterology Unit, School of Clinical Sciences, University of Liverpool, Ashton Street, Liverpool, L69 3GE,
United Kingdom. mark.pritchard@liv.ac.uk
Telephone: +44-151-7945772 Fax: +44-151-7946825
Received: October 21, 2008 Revised: November 28, 2008
Accepted: December 5, 2008
Published online: January 7, 2009

## Abstract

In addition to regulating acid secretion, the gastric antral hormone gastrin regulates several important cellular processes in the gastric epithelium including proliferation, apoptosis, migration, invasion, tissue remodelling and angiogenesis. Elevated serum concentrations of this hormone are caused by many conditions, particularly hypochlorhydria (as a result of autoimmune or *Helicobacter pylori* (*H pylori*)-induced chronic atrophic gastritis or acid suppressing drugs) and gastrin producing tumors (gastrinomas). There is now accumulating evidence that altered local and plasma concentrations of gastrin may play a role during the development of various gastric tumors. In the absence of *H pylori* infection, marked hypergastrinemia frequently results in the development of gastric enterochromaffin cell-like neuroendocrine tumors and surgery to remove the cause of hypergastrinemia may lead to tumor resolution in this condition. In animal models such as transgenic INS-GAS mice, hypergastrinemia has also been shown to act as a cofactor with *Helicobacter* infection during gastric adenocarcinoma development. However, it is currently unclear as to what extent gastrin also modulates human gastric adenocarcinoma development. Therapeutic approaches targeting hypergastrinemia, such as immunization with G17DT, have been evaluated for the treatment of gastric adenocarcinoma, with some promising results. Although the mild hypergastrinemia associated with proton pump inhibitor drug use has been shown to cause ECL-cell hyperplasia and to increase *H pylori*-induced gastric atrophy, there is currently no convincing evidence that this class of agents contributes towards the development of gastric neuroendocrine tumors or gastric adenocarcinomas in human subjects.



**Key words:** *Helicobacter pylori*; Hypergastrinemia; Neuroendocrine; Gastric carcinoma; Proton pump inhibitor

**Peer reviewers:** Tomasz Brzozowski, Professor, Department of Physiology, Jagiellonian University Medical College, 16 Grzegorzecka Str, Cracow 31-531, Poland; Leonard R Johnson, Professor, Department of Physiology, University Tennessee College of Medicine, 894 Union Ave, Memphis, TN 38163, United States

Burkitt MD, Varro A, Pritchard DM. Importance of gastrin in the pathogenesis and treatment of gastric tumors. *World J Gastroenterol* 2009; 15(1): 1-16 Available from: URL: http://www.wjgnet.com/1007-9327/15/1.asp DOI: http://dx.doi.org/10.3748/wjg.15.1

## INTRODUCTION

Gastric epithelial malignancy represents a significant burden of disease. The commonest lesion is gastric adenocarcinoma, which is the fourth commonest malignancy worldwide, and because it is associated with a high mortality, this tumor remains the second commonest cause of cancer-related death globally. The distribution of gastric epithelial malignancies is not uniform between different populations, with increased prevalence being found in East Asia including Japan and China (where 42% of cases occur), as well as in Eastern Europe and South America[1]. This epidemiology raises significant questions as to the predisposing factors for gastric malignancy. Current data suggest that a number of different variables affect an individual's risk of gastric carcinogenesis, amongst which are environmental

factors such as infection with *Helicobacter pylori* (*H pylori*), smoking and diet, as well as host factors such as achlorhydria and specific cytokine polymorphisms. Another important host factor which may play a role during gastric carcinogenesis is the hormone gastrin.

As well as acting as a potential cofactor during gastric adenocarcinoma development, gastrin is also known to play a major role in the pathogenesis of other gastric tumor types, particularly neuroendocrine (carcinoid) tumors. There is therefore accumulating evidence that gastrin not only influences tumor development, but could also be a potential therapeutic target for various gastric neoplasias. These issues will be the main focus of this editorial.

## GASTRIN BIOCHEMISTRY AND PHYSIOLOGY

### *Synthesis and processing*

The presence of a hormone that stimulated gastric acid secretion in the pyloric mucosa was first demonstrated in 1906[2]. Gastrin was subsequently shown to be secreted from neuroendocrine G cells which are principally located in the antrum of the stomach. The gastrin gene is located on the long arm of chromosome 17 and encodes a 101 amino acid polypeptide, preprogastrin. This gene product is subjected to a series of post translational modifications which result in the synthesis of a number of biologically active peptides[3] (Figure 1). Immediately after translation, preprogastrin is cleaved to form progastrin, which is transported to the Golgi network, where it is packaged into secretory vesicles. Further post-translational modification occurs at this site, firstly to form gastrin-34-Gly, the C-terminal glycine extended form of gastrin-34. This peptide may then undergo further cleavage into gastrin-17-Gly or amidation to generate gastrin-34, which in turn may be cleaved to form gastrin-17. Progastrin, glycine extended gastrins and amidated gastrins are all biologically active and exert different functions within gastric and other mucosae. Progastrin and glycine extended gastrins act particularly on the colonic mucosa as mitogens[4,5], glycine extended and amidated gastrins have been shown to affect the differentiation of gastric oxyntic mucosa, whilst amidated gastrin promotes cell proliferation as well as acid secretion in the stomach. In the human stomach, the conditions for post-translational modification of gastrin are such that there is almost complete amidation of glycine extended forms of gastrin, hence the predominant form of secreted gastrin is gastrin-17[3].

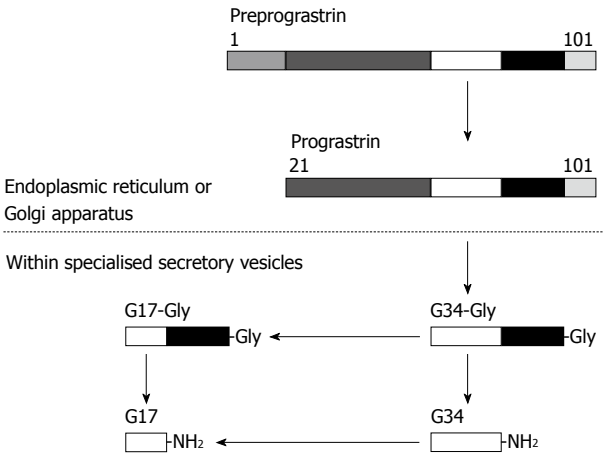


**Figure 1 Biosynthesis of gastrin.**

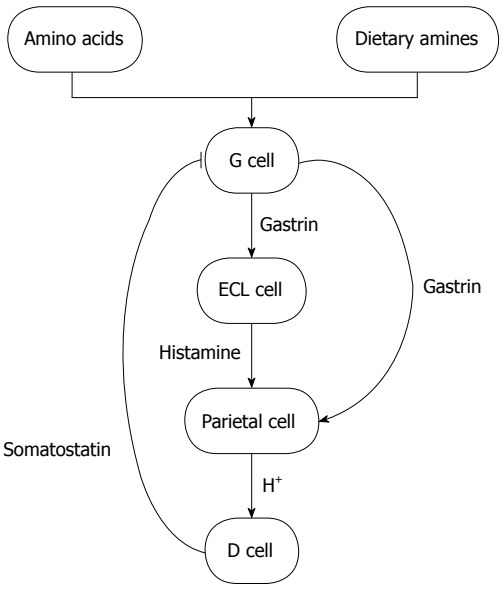


**Figure 2 Mechanism by which gastrin regulates gastric acid secretion.**

### *Cellular effects of gastrin*

**Acid secretion:** Gastrin is secreted in response to a number of luminal stimuli, including the presence of amino acids and dietary amines (reviewed in[3]). Calcium receptors on the surface of the G-cell also sense luminal calcium and modulate the gastrin secretory response[6], with increased calcium resulting in increased gastrin secretion. Following secretion into the gastric vasculature, gastrin binds to CCK-2 receptors, which are expressed on the surface of gastric enterochromaffin-like (ECL) cells and parietal cells[7] (Figure 2). The predominant mode of secretagogue action is *via* ECL cell secretion of histamine and this pathway effectively amplifies the prosecretory signal. Continued gastrin secretion is negatively regulated by the secretion of somatostatin *via* D-cells, which are located in the oxyntic mucosa. In physiological states, these mechanisms maintain appropriate gastric pH.

**Proliferation:** Studies in dogs in 1972 provided the first evidence of mucosal proliferation in response to gastrin[8], however these studies were triggered by earlier clinical observations of increased gastric mucosal proliferation in patients with Zollinger-Ellison syndrome (ZES). Subsequently, increased fundic mucosal proliferation was demonstrated in rodent models including *Mastomys* following the administration of an H2 receptor antagonist which rendered them hypergastrinemic. These animals demonstrated gastric

gland elongation and increased numbers of cells which stained for Ki67, a marker of proliferation[9]. In normal humans, the infusion of gastrin at supraphysiological levels has also been shown to result in increased gastric cell proliferation, as demonstrated by $^{3}$H-thymidine labelling studies[10].

Endocrine cell proliferation in the stomachs of patients with ZES was first reported in 1974[11]. Further evidence that hypergastrinemia provides a proliferative drive to ECL cells emerged from studies in which rats were rendered hypergastrinemic by treatment with either proton pump inhibitor (PPI) or H2 receptor antagonist drugs. ECL cell hyperplasia was not observed in control untreated animals or in rats that had been antrectomized prior to treatment with high-dose PPI[12]. These observations in rats have to some extent been corroborated in other species, as ECL cell hyperplasia has now also been demonstrated following PPI treatment of chickens, hamsters and guinea pigs[13,14]. ECL cell hyperplasia of this magnitude does not however appear to occur in mice or humans treated with acid suppressant drugs.

To investigate the molecular mechanisms by which gastrin promotes proliferation, a number of gastric cancer cell lines that express the CCK-2 receptor have been employed. It has recently been shown that the proliferation of MKN-45 cells, which are derived from a poorly differentiated gastric carcinoma and which have been reported to express the CCK-2 receptor, decreased when treated with the CCK-2 receptor antagonist AG-041R[15]. Several cell lines which have independently been stably transfected with the CCK-2 receptor (but using different expression vectors) have been generated from AGS gastric cancer cells (which do not constitutively express the CCK-2 receptor). The AGS-B cell line (transfected with human full-length CCK-2 receptor using the pcDNA I vector and neomycin selection) was found to proliferate more rapidly in the presence of gastrin, a process that was associated with the upregulation of cyclin D1[16]. In contrast, the effects of gastrin on the proliferation of AGS-G$_R$ cells (transfected with the human full length CCK-2 receptor driven by the EF1$\alpha$ promoter under puromycin selection) were more complex. When cultured in the presence of gastrin-17, AGS-G$_R$ cells showed a reduced rate of proliferation, an effect that was abrogated by the addition of a CCK-2 receptor antagonist. However, when AGS-G$_R$ cells were co-cultured with AGS cells that had been transfected with a green fluorescent protein producing construct (AGS-GFP cells) in the presence of serum-free medium, gastrin exposure caused an increase in the proliferation of the AGS-GFP cells. This suggests that gastrin treatment of AGS-G$_R$ cells results in the secretion of growth factors that are capable of acting in a paracrine manner to stimulate the proliferation of AGS-GFP cells. Further analysis of the underlying mechanisms showed that epidermal growth factor ligands, particularly heparin binding epidermal growth factor (HB-EGF) were involved[17].

HB-EGF promotes cell cycling and is overexpressed by a number of cancer cell lines, including some gastric cancer cell lines and is of particular relevance to this article as it provides a potential molecular link between *H pylori* infection, gastrin and increased cell proliferation[18]. *H pylori* infection of gastric cancer cell lines has been shown to significantly increase HB-EGF levels and this effect is dependent upon the presence of the CCK-2 receptor. The most likely explanation is that *H pylori* induces cells to secrete gastrin, which in turn binds to and activates the CCK-2 receptor, resulting in HB-EGF secretion. This hypothesis is supported by evidence from animal models, as INS-GAS hypergastrinemic mice also overexpress HB-EGF in the premalignant lesions which develop following *Helicobacter felis* (*H felis*) infection[19]. This may partly explain the observed synergy between hypergastrinemia and *Helicobacter* infection during gastric carcinogenesis in this animal model.

**Apoptosis:** Apoptosis is a fundamental cellular process which is often dysregulated during the development of malignancy. There is accumulating evidence that gastrin modulates the apoptosis of both normal and transformed gastric epithelial cells and these mechanisms may contribute towards tumor development.

Several models of carcinogenesis are based upon the somatic mutation hypothesis proposed by Vogelstein in relation to colon cancer[20]. This hypothesis suggests that sequential defects are acquired by a tissue stem cell, eventually leading to the development of dysplastic and malignant phenotypes. This hypothesis suggests that an early failure in targeting mutated stem cells for apoptosis allows them to survive and generate mutated clones which progress to cancer. In the stomach however, an alternative mechanism of adenocarcinoma development has recently been proposed, as the gastric cancers which arise in *H felis* infected mice appear to develop in tissue that was originally derived from bone marrow stem cells. Houghton, Wang and colleagues studied C57BL/6 mice which had undergone bone marrow transplantation from GFP producing mouse strains or ROSA26 mice, thus allowing tissues that originated from the bone marrow rather than preexisting mucosal stem cells to be observed[21]. Following *H felis* infection, gastric cancers developed in these mice at a similar rate to other *H felis*-infected C57BL/6 mice, however, the tumors arose in glands which produced GFP, indicating that the tissue was initially derived from the bone marrow transplant. The authors propose that gastric stem cells undergo apoptosis as a result of *Helicobacter* infection, and that the stem cell niche is replaced by pluripotent stem cells which were originally located in the bone marrow. These cells may be more susceptible to malignant transformation than the previously incumbent gastric epithelial stem cells.

Gastrin signalling *via* the CCK-2 receptor appears to increase the susceptibility of normal gastric epithelial cells *in vivo* to undergo apoptosis. In *Mastomys* treated with H2 antagonist drugs, a two-fold increase in apoptosis was observed in hypergastrinemic animals relative to controls[9]. In addition, when

mice were subjected to 12 Gy gamma irradiation or *Helicobacter* infection, increased numbers of apoptotic cells were observed in the gastric corpus mucosa of hypergastrinemic animals[22,23]. Increased radiation-induced apoptosis was demonstrated both in transgenic INS-GAS mice and also in a model of drug-induced hypergastrinemia involving FVB/N mice treated with omeprazole. Treatment with a CCK-2 receptor antagonist abolished the observed increases in gastric epithelial apoptosis in both cases, suggesting that this response is both gastrin and CCK-2 receptor dependent. In gastric biopsy samples obtained from humans with both *H pylori* infection and hypergastrinemia, similar observations of increased apoptosis were also made[23]. These results suggest that hypergastrinemia may increase the susceptibility of gastric epithelial stem cells to undergo apoptosis, thus permitting the engraftment of bone marrow derived cells as suggested by Houghton and Wang[21].

Antiapoptotic effects of gastrin have however also been described, particularly in transformed cell types. For example, amidated gastrin inhibits the apoptosis of rat pancreatic acinar cancer cells (AR42J), by signalling through a CCK-2 receptor and an AKT-mediated mechanism[24,25]. Similarly, the human gastric cancer cell line MKN-45 has been shown to be more susceptible to apoptosis when treated with a CCK-2 receptor antagonist and this was associated with upregulation of Bax and downregulation of Bcl-2[15]. More recently, gastrin has also been shown to inhibit AGS-G$_R$ cell apoptosis in a manner dependent upon the expression of the anti-apoptotic protein mcl-1. Increased mcl-1 expression was also observed in the type 1 gastric neuroendocrine tumors of hypergastrinemic patients[26].

The effects of gastrin on gastric apoptosis therefore appear to depend upon the underlying physiological and pathological conditions. There are thus a number of important questions which still need to be answered, not only regarding the effects of gastrin on apoptosis in differing circumstances, but also regarding the role of apoptosis during the development of gastric carcinoma and other gastric malignancies.

**Angiogenesis:** Angiogenesis is an essential feature required for tumor survival. In a number of GI malignancies, cyclooxygenase (COX)-2, an important rate-limiting step in the prostaglandin synthesis pathway, has been implicated in enhancing angiogenesis. For example, in human studies, COX-2 expression has been associated with the development of a more dense microvasculature around gastric tumors[27]. Gastrin has been shown to enhance COX-2 secretion in AGS-E cells (which have been stably transfected with the full-length human CCK-2 receptor on a EF1$\alpha$ promoter, but by a different research group from those who produced AGS-G$_R$ cells) *via* an Akt-dependent mechanism[28]. Studies of patients with atrophic gastritis secondary to chronic *Helicobacter* infection have shown that, in addition to elevated levels of gastrin, these patients also have significantly higher levels of COX-2 mRNA compared to unaffected controls, a phenomenon that is reversed after *H pylori* eradication[29]. The mechanisms mediating increased COX-2 transcription in this setting have not been fully investigated to date, and it is not clear whether this effect is solely related to hypergastrinemia associated with *Helicobacter* infection and gastric atrophy or whether additional independent factors also influence COX-2 production.

Case control studies of patients taking aspirin and other COX inhibiting drugs have demonstrated a reduction in relative risk not only of gastric cancer, but also of colon cancer. Aspirin now has an established role in colon cancer prevention, however, its role in preventing diseases of the gastric epithelium is less convincing, due in part to the increased risk of GI bleeding associated with its use. Selective COX-2 inhibitors may have some potential as gastric cancer chemopreventive agents, however the serious cardiovascular side effects associated with the long-term use of the current generation of these drugs makes it unlikely that they will be adopted for this purpose.

In addition to the potential indirect role of gastrin upon angiogenesis *via* COX-2 expression, gastrin has also been suggested to have direct effects upon angiogenesis using an *in vitro* system. When human umbilical vein endothelial cells (HUVECS) were seeded onto fibroblast monolayers, they formed vascular structures in response to various angiogenic stimuli, including both amidated and glycine extended forms of gastrin. This response was associated with increased production of HB-EGF and with elevated levels of matrix metalloproteinase (MMP)2, MMP3 and MMP9[30].

**Migration and invasion:** Other fundamental cellular processes which are involved in promoting carcinogenesis and that are modulated by hypergastrinemia are tissue remodeling and invasion. Gastric cancer development involves extensive remodeling of the gastric mucosa during a hypergastrinemic premalignant phase (atrophic gastritis), in order to institute the conditions required for gastric carcinogenesis. Similar mechanisms are thought to be responsible for the local invasion and metastasis that occur in frankly malignant lesions. These processes are complex and are controlled by numerous different mechanisms, however, gastrin does appear to play a regulatory role. For example, gastrin has been shown to increase levels of MMP-9 *via* an MAPK AP-1 dependent pathway in human patients with gastric cancers and gastric neuroendocrine tumors, resulting in tissue remodelling and invasion[31]. Serum levels of MMP-7 have also been found to be elevated in patients who were hypergastrinemic as a result of either MEN-1 or pernicious anemia, and similar observations have been made both in transgenic hypergastrinemic INS-GAS mice and in gastrin-knockout mice treated with exogenous gastrin[32].

AGS-G$_R$ cells have again been used to investigate the mechanisms responsible for the effects of gastrin upon cellular migration and invasion. In response to gastrin stimulation, AGS-G$_R$ cells undergo a morphological

change, with the induction of a branched phenotype, coupled with extensive remodelling of the cell's actin cytoskeleton. These effects were abrogated by treatment with a CCK-2 receptor antagonist and the alteration in morphology was not seen when the parent AGS cell line was exposed to gastrin. In addition to the morphological changes observed in this cell line, there was also evidence of a change in migration which was again mediated *via* a CCK-2 receptor-dependent pathway. AGS-GR cells, but not AGS cells showed increased migration when cultured in the presence of amidated gastrin and experiments involving the co-culture of AGS-GR and AGS-GFP cells demonstrated that the effects of gastrin on cell migration were at least in part due to paracrine signalling[33].

## INSIGHTS FROM TRANSGENIC ANIMAL MODELS

Generation of a number of transgenic mouse strains over the last 15 years has greatly facilitated understanding of the mechanisms by which members of the gastrin family of peptides regulate the processes involved in gastric epithelial carcinogenesis (summarised in Table 1).

### *Hypergastrinemic mouse models*

**HGAS:** hGas mice transgenically overexpress a complete human gastrin mini gene including the gastrin promoter region in some liver cells. Because the enzymes required for the post-translational processing of preprogastrin are not present in this tissue, hGas mice selectively overexpress human progastrin[4]. These mice show increased colonic proliferation and increased susceptibility to colonic carcinogenesis[4]. However, in contrast to the INS-GAS mice described below, there is no overt gastric phenotype, and no alterations were observed in gastric proliferation[4] or radiation-induced apoptosis[23] in comparison to wild-type. This suggests that the effects of the gastrin family of peptides on gastric mucosa are predominantly due to amidated forms of the hormone[4].

**INS-GAS:** In contrast to the liver, neuroendocrine cells, including those present in pancreatic islets, possess the appropriate enzymatic machinery to allow processing of progastrin into glycine extended and amidated forms of the hormone. Transgenic INS-GAS mice were created by expressing a human gastrin minigene spliced onto the insulin promoter and this resulted in expression of the gene in the pancreatic islets of adult animals. INS-GAS mice therefore have elevated serum concentrations of amidated gastrin[34]. At a young age, these mice have a two-fold increase in plasma gastrin levels compared to wild-type, show increased numbers of gastric parietal cells and ECL cells and secrete up to twice the amount of acid[4]. Beyond 5 mo of age however, there are progressive changes in gastric histology and physiology in these animals, with a reduction in acid secretion, such that at 12 mo they secrete less acid than wild-type controls, and by 20 mo they are essentially achlorhydric. This is associated with a progressive loss of gastric parietal cells and ECL cells over the same time period. Concomitant with these changes in the oxyntic mucosa, there is macroscopic evidence of hypertrophy in the gastric fundus and histological evidence of intestinal type metaplasia, a histological entity that is widely recognized as being premalignant. By 20 mo of age, there is evidence of gastric dysplasia in 100% and frank malignancy in 75% of mice. In comparison, wild-type FVB/N mice maintained under similar conditions did not develop metaplasia, dysplasia or gastric carcinoma[19].

Whilst INS-GAS mice developed gastric malignancies spontaneously over 2 years, it was also shown that this process was accelerated significantly by *H felis* infection. After 6 mo, there was significantly more gastric atrophy in all *H felis*-infected INS-GAS mice relative to both uninfected INS-GAS and *H felis*-infected FVB/N mice. In addition, there was also evidence of malignant progression, with 85% of infected INS-GAS mice being reported as showing at least intramucosal carcinoma after 6 mo of infection, in comparison to 12.5% of the uninfected INS-GAS group and none of the FVB/N groups[19]. Subsequent investigations have demonstrated that *H pylori* as well as *H felis* can induce gastric cancer in these mice, however there was a significant difference in susceptibility between male and female INS-GAS mice. After 7 mo of infection, four of 12 infected males were found to have gastric adenocarcinoma, whilst none of the other groups developed malignancies. In this study, progression to metaplasia and dysplasia was observed in all groups, but more severe changes were present in male than female animals[35].

The *H felis*-infected INS-GAS model has also been used to investigate the effects of administering the CCK-2 receptor inhibitor YF476. When this drug, in conjunction with the H2 receptor antagonist loxtidine, was given to INS-GAS mice infected with *H felis*, it significantly inhibited the development of gastric atrophy, dysplasia, and adenocarcinoma[36].

**MTI/G-Gly and INS-GAS/MTI/G-gly:** A third type of transgenic mouse that produces glycine extended gastrin has also been created by inserting two stop codons into the human gastrin gene after glycine-72. This transgene was spliced with the mouse metallothionein promoter to create transgenic animals that express the transgene in all tested tissue types and which constitutively overexpress glycine extended forms of gastrin[5]. This mouse model does not demonstrate a gastric phenotype histologically, and gastric tumors are not seen in the mice at 1 year of age[5]. In order to investigate whether glycine extended forms of gastrin modulated the effects of amidated gastrin upon the stomach, INS-GAS and MTI/G-Gly mice were crossed to generate doubly transgenic INS-GAS/MTI/G-Gly mice. These mice demonstrated less mucosal atrophy than INS-GAS mice and there was evidence of acid hypersecretion rather than the hypochlorhydria observed in INS-GAS mice. The altered phenotype of

**Table 1 Phenotype of mice with transgenic alterations in members of the gastrin family of peptides**

| Transgenic strain | Transgenic abnormality | Gastric phenotype | Susceptibility to gastric carcinoma | Other relevant phenotype |
|---|---|---|---|---|
| hGAS[4] | Human gastrin minigene expressed in liver-resulting in elevated serum levels of human progastrin | No known gastric phenotype | Not altered | Increased colonic mucosal proliferation[4,113] and susceptibility to azoxymethane-induced colon cancer[114] |
| MTI/G-Gly[5] | Human gastrin gene with two stop codons after glycine-72, spliced with MTI promoter. Transgenic animals have elevated serum levels of glycine extended gastrin | No known gastric phenotype | Not altered | Increased colonic mucosal proliferation[5] |
| INS-GAS[34] | Human gastrin minigene spliced with insulin promoter expressed in pancreatic islets-resulting in elevated serum levels of amidated gastrin | Initial gastric mucosal hypertrophy and excess gastric acid secretion. By 5 mo, gastric atrophy and hypochlorhydria. Increased gastric proliferation and increased susceptibility to apoptosis | Increased (spontaneous tumors at 20 mo and *H Felis*-induced tumors at 6 mo) | Increased colonic mucosal proliferation in proximal and distal colon but not rectum initially observed in 1-year-old animals[4], but no difference in apoptotic or mitotic rates seen in 10-12-wk-old mice[113] and no increase in AOM-induced cancers[114] |
| INS-GAS/ MTI/G-Gly[37] | MTI/G-Gly mice crossed with INS-Gas mice to result in a "double" transgenic mouse that expresses both increased amidated and glycine extended forms of gastrin | Hyperchlorhydric at birth but unlike INS-GAS, no mucosal atrophy at older ages. Reduced apoptosis compared to INS-GAS with similar levels of proliferation. Overall rates of malignant progression comparable to INS-GAS | Increased | |
| GAS-KO[38] | Gastrin knockout mice generated by targeted gene disruption | Achlorhydric with reduced parietal cell numbers (gastric atrophy), clustering of ECL cells at gland bases and increased TFF2-positive cells (spasmolytic polypeptide expressing metaplasia) | Increased | Increased susceptibility to azoxymethane-induced colon carcinogenesis[115] (despite normal untreated proliferation indices[113]) |
| CCK-B-null[49] | Gastrin receptor knockout mice generated by targeted gene disruption | Marked gastric atrophy and achlorhydria. Morphologically abnormal ECL cells with loss of normal secretory vesicles and replacement with dense core granules and microvesicles | Not reported | Increased sensitivity to dopamine[116] and altered behaviour in response to alcohol[117,118] and other stimuli[119,120] |

INS-GAS/MTI/G-Gly mice appeared to result from reduced gastric epithelial apoptosis rather than due to any changes in proliferation[37]. However, these changes in atrophy did not result in a reduction in malignant susceptibility, as at 18 mo all INS/GAS and all INS-GAS/MTI/G-Gly mice had developed gastric malignancies[37].

### Gastrin-deficient mice

The consequences of gastrin deficiency *in vivo* have been investigated by a number of groups by generating gastrin knockout mice. Under normal animal house conditions, these animals develop gastric atrophy, with thinner gastric mucosae, fewer $H^+/K^+$ ATPase-positive parietal cells and impaired acid secretion[38,39]. In addition, the oxyntic mucosa contains fewer chromogranin A immunopositive ECL cells and increased numbers of TFF-2-expressing cells, indicative of spasmolytic peptide expressing metaplasia (SPEM)[40]. Also, ECL cells appear to be clustered towards the bottom of the gastric gland of gastrin knockout mice and there is a reduced rate of parietal cell migration to the base of the gland in these animals[41].

The gastric phenotype of gastrin knockout[42] mice predisposes these animals to colonisation of the stomach with bacteria[43], resulting in inflammation and an initial increase in parietal and G-cell numbers[44]. The long term effect of this chronic inflammatory state may be to promote malignant transformation, and in some laboratories gastric tumors have been found in gastrin knockout mice by 1 year of age[42,45]. When the specific effects of infection with *H pylori* strain 119/95 (a CagA positive, VacA positive strain previously shown to cause gastritis acutely[46], and gastric epithelial lymphomas with chronic infection in C57Bl/6 mice[47]) were investigated in these mice, there was an alteration in acid secretion, thought to be stimulated through a vagal response mechanism, but no increased risk of tumor development was observed at 6 mo[48].

### Transgenic mice resistant to gastrin

CCK-2 receptor null mice have also been produced independently by two groups. These mice demonstrate

**Table 2 Causes of hypergastrinemia in humans**

| Acidic gastric pH | Elevated gastric pH |
|---|---|
| Gastrinoma | Chronic atrophic gastritis |
| Antral predominant *H pylori* infection | Autoimmune |
| Pyloric obstruction | *H pylori* infection |
| Renal failure | Acid-suppressing medication |
| Retained gastric antrum following Billroth Ⅱ gastrectomy | Vagotomy |

marked gastric atrophy and reduced acid secretion as predicted[49-51]. There is also evidence of morphological changes in ECL cells, resulting in cells with loss of normal secretory vesicles and replacement with microvesicles and dense core granules[52]. As far as we are aware, there are no reports of increased susceptibility to gastric carcinogenesis in CCK-2 null animals.

## CAUSES OF HYPERGASTRINEMIA

Persistent hypergastrinemia can occur as a consequence of a number of different pathological states. These can broadly be divided into conditions which cause uncontrolled excess gastrin secretion such as gastrin-secreting tumors, and the normal physiological response to suppressed gastric acid secretion (Table 2). There is evidence that hypergastrinemia, particularly that which results from gastrinomas and chronic atrophic gastritis, may be associated with the development of gastric malignancies.

### *ZES*

Neuroendocrine tumors of the pancreas and duodenum are rare, and are either functional, secreting one of a variety of neuropeptides, or non functioning, where elevated levels of neuropeptides are not detected. Of the functional tumors, the commonest hormone to be secreted is gastrin, accounting for up to 30% of such neoplasms. Gastrinomas have an incidence of 0.5-3 per million population per year[53] and were first described, along with a syndrome of gastric hypersecretion, in 1955 by Zollinger and Ellison[54]. The majority of gastrinomas are sporadic, however, approximately 12% are associated with the multiple endocrine neoplasia syndrome type 1 (MEN1)[55], in association with functional adenomas of the parathyroid (90%), pituitary [e.g. prolactinomas (17%)] and pancreas [e.g. insulinomas (10%)][56].

Unlike the first report by Zollinger and Ellison, in which one patient had radical surgery, but continued to secrete gastric acid and eventually died, and another required a total gastrectomy to control the adverse effects of gastric acid hypersecretion, today the prognosis for patients with gastrinoma is relatively good. The most important prognostic factor is the presence or absence of hepatic metastases. Patients without hepatic metastases at presentation (more than 75% of cases) have a 90%-100% 10-year survival, whilst those with metastatic disease have only a 10%-20% 10-year survival[53]. The advent of H2 receptor antagonists and subsequently PPIs has enabled control of gastric acid hypersecretion in the majority of patients, thereby reducing the risk of peptic ulceration. These therapies have made a significant impact upon the effects of ZES, however they target the consequences of hypergastrinemia rather than the underlying hormone production. Thus, surgery to remove the primary gastrinoma remains the only potentially curative option. This is feasible in at least 20%-45% of patients with sporadic ZES, but in far fewer patients with MEN1/ZES, as they are more likely to have multiple or diffuse tumors that are not amenable to surgery[53].

### *Helicobacter infection*

*H pylori* infection is an important independent risk factor for gastric carcinogenesis and gastric atrophy. Observational data suggest that *H pylori* infection directly causes mild degrees of hypergastrinemia. For example, asymptomatic patients with *H pylori* colonisation have been shown to have elevated serum gastrin concentrations relative to a control population, despite similar gastric acid output[57], while Levi *et al*[58] demonstrated that following *H pylori* eradication, there was a reduction in fasting serum gastrin concentration. It has also been demonstrated that following eradication of *H pylori*, there was an increase in somatostatin mRNA and a concomitant decrease in gastrin mRNA in patients with duodenal ulcers. This was associated with increased numbers of D-cells in the gastric corpus[59], suggesting that the hypergastrinemia caused by *H pylori* infection may result from a loss of somatostatin control over gastrin secretion.

### *Atrophic gastritis*

Gastric atrophy is defined as the loss of parietal cell mass, leading to decreased acid secretion and consequent increased luminal pH. This interrupts the somatostatin negative feedback mechanism and results in hypergastrinemia. The most important causes of gastric atrophy are autoimmune (associated with pernicious anemia) and chronic *H pylori* infection. Both types of atrophic gastritis are associated with hypergastrinemia, although the fasting serum gastrin concentration is usually more markedly elevated in the autoimmune type, due to the more profound loss of parietal cells.

**Autoimmune atrophic gastritis**: The epidemiology of autoimmune atrophic gastritis is similar to that of other autoimmune diseases, with a female to male predominance approaching 2:1 and with individuals in their 7th decade or later being typically affected[60]. This condition is characterised by vitamin B12 deficiency as a result of loss of intrinsic factor and is associated with autoantibodies towards gastric parietal cells and/or intrinsic factor. These autoantibodies are found in the sera of 70% and 55% of patients with pernicious anemia respectively and at least one autoantibody is present in 85% of patients. Although it has been demonstrated that these antibodies can be cytotoxic *in vitro*[61], it is less

**Table 3 Types of gastric neuroendocrine tumor**

| Type | Associated diseases | Proportion of gastric NETs | Typical endoscopic findings | Plasma gastrin | Gastric juice pH | Prognosis |
|---|---|---|---|---|---|---|
| I | Chronic autoimmune atrophic gastritis | 80% | Multiple < 1 cm polyps | High | ~7 | Good |
| II | ZES and MEN1 | 5% | Multiple < 1 cm polyps | High | < 2 | Variable |
| III | None | 15% | Single 2-5 cm polyp | Unchanged | 1-2 | Poor |

clear whether they are the responsible for causing gastric atrophy *in vivo*.

***H pylori*-associated atrophic gastritis:** The second important cause of gastric atrophy is chronic *H pylori* infection. Most commonly, primary infection occurs in childhood, hence many patients have long-term colonization of the stomach. This can have a number of consequences, ranging from increased gastric acid secretion and associated peptic ulcer disease to gastric atrophy with resultant increased luminal pH. The latter is associated with an increased risk of gastric cancer. The factors that influence the clinical outcomes of *H pylori* infection in individual patients remain poorly understood. The site of colonization within the stomach appears to be important however, with antral infections being particularly associated with peptic ulcer disease, whereas gastric corpus colonization is more likely to lead to gastric atrophy.

*H pylori* occupies a niche in the mucus layer of the gastric mucosa and produces urease enzymes which allow the pH of the immediate environment to be raised to physiological levels, thus facilitating prolonged colonization. Various bacterial, host and environmental factors have been suggested to influence the response to *H pylori* infection. For example, mouse models have demonstrated that polarization of the immunological response towards a Th1 type increases the risk of developing gastric atrophy and subsequent gastric cancer[62], while genetic studies have suggested that polymorphisms in immune-response genes may also influence the consequences of infection in human subjects[63].

*H pylori* is associated with gastric autoimmunity, although the specific mechanisms involved are not yet fully understood. Presotto *et al*[64] demonstrated that 58% of 79 asymptomatic patients with detectable anti-parietal cell antibodies had serological or histological evidence of *H pylori* infection compared to 39% of a control population ($P = 0.03$). It has also been proposed that early gastric autoimmunity may be reversible when concomitant *H pylori* infection is treated[65]. The precise interactions between *H pylori* infection and the development of autoimmune gastritis therefore warrant further investigation.

## GASTRIC TUMORS ASSOCIATED WITH HYPERGASTRINEMIA

The hypergastrinemia which results from the causes described above is associated with increased risks of developing various different gastric tumors.

Hypergastrinemia in the absence of *H pylori* infection is most strongly associated with the development of gastric neuroendocrine tumors. In contrast, the hypergastrinemia associated with chronic *H pylori* infection may act as a co-factor during the development of gastric adenocarcinoma. In the following section, we will discuss the role of gastrin in the pathogenesis, diagnosis, and treatment of these specific gastric tumor types.

### *Gastric neuroendocrine tumors*

**Pathogenesis:** Gastric neuroendocrine (carcinoid) tumors are the classical example of gastrin-induced malignancies. These neoplasms are derived from ECL cells, which are the most abundant neuroendocrine cell type in the oxyntic mucosa. Hypergastrinemia alone appears to be sufficient to induce ECL cell hyperplasia, however, for macroscopic neuroendocrine tumor formation, additional triggers are required. For example, lifelong therapy of rats with PPIs resulted in the development of ECL cell tumors[66], whereas the same has not been observed in humans despite induction of hypergastrinemia[13,14]. This suggests that additional host or environmental factors are required for tumor development.

The typical conditions in which ECL tumors develop are either gastric atrophy associated with pernicious anemia (type I gastric neuroendocrine tumors), or the presence of prolonged hypergastrinemia and mutation of the MEN1 gene in ZES associated with MEN type I (type Ⅱ gastric neuroendocrine tumors)[67] (Table 3). The latter condition provides further evidence that hypergastrinemia alone may be insufficient to cause gastric neuroendocrine tumors, as the relative risk for developing such neoplasms is at least 70 fold lower in patients with sporadic ZES compared with those who have ZES associated with MEN1[68].

In pernicious anemia, there is a reduction in the number of gastric parietal cells and subsequent achlorhydria. This affects the somatostatin feedback loop that controls gastrin secretion, thereby rendering the patient hypergastrinemic. Hypergastrinemia then provides a proliferative drive to ECL cells (Figure 3). The achlorhydric environment also provides opportunities for further microenvironmental changes, including the provision of a niche for bacterial colonization. Interest in this aspect of gastric carcinogenensis has been present since the 1980s, when numerous studies demonstrated increased levels of N-nitroso compounds (potential carcinogens that are metabolised by intra gastric bacteria from nitrosamines) in the gastric lumens of patients

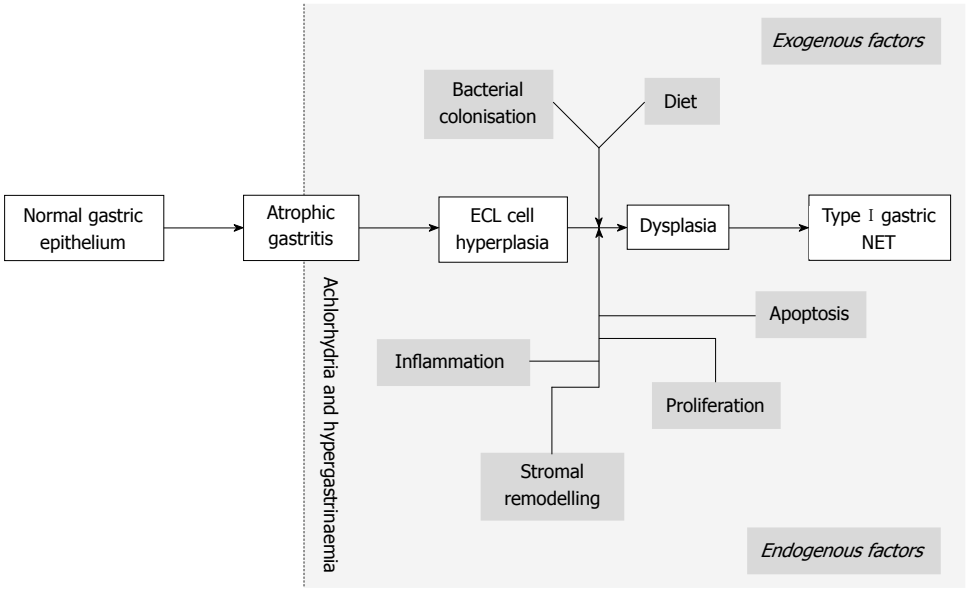


**Figure 3 Pathway of development of type Ⅰ gastric neuroendocrine tumors.**

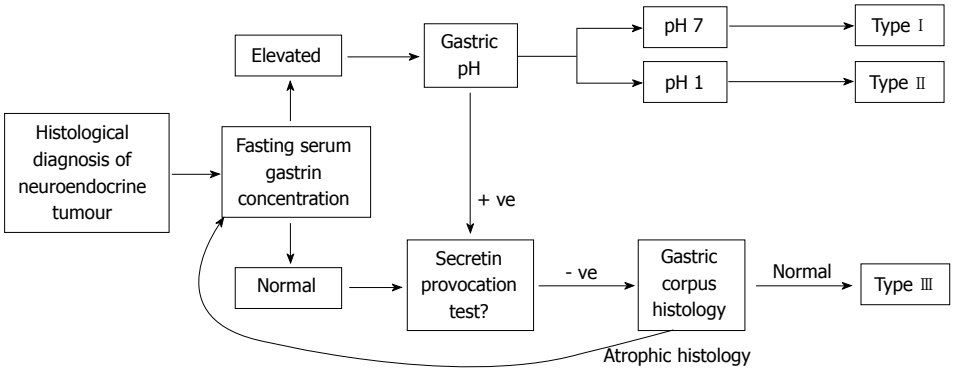


**Figure 4 Diagnostic algorithm for gastric neuroendocrine tumors.**

with both atrophic gastritis and gastric malignancy[69].

The major risk factor in addition to hypergastrinemia that promotes the development of type Ⅱ gastric neuroendocrine tumors is mutation of the *MEN1* gene. Gastric neuroendocrine tumors are seen in less than 1% of patients with sporadic ZES, whilst in those with ZES associated with MEN type Ⅰ, the prevalence is 13%-43%[70]. A recent prospective study of 57 consecutive hypergastrinemic MEN1 patients reported that 100% had abnormal ECL cell distribution and 23% had gastric neuroendocrine tumors.

The *MEN1* gene encodes a 610-amino-acid protein, menin. Menin is expressed in many diverse tissue types and is localized in the nucleus. It binds directly to DNA in a sequence-independent manner and is also capable of binding several other nuclear factors including transcription factors and DNA repair proteins. The physiological function of menin is as a tumor suppressor, although the specific mechanism of action is less clear. The mutations seen in the *MEN1* gene result in either reduced expression of menin or in some cases complete absence of menin[56].

Interestingly, local rather than somatic mutations of menin may also be of significance in the pathogenesis of type I gastric carcinoids and two studies have assessed loss of heterozygosity (LOH) of 11q13, the locus for the *MEN1* gene, in this tumor type. The smaller study assessed three gastric neuroendocrine cell tumors and demonstrated LOH at this locus in two patients and a localized mutation of *MEN1* in one[71]. The larger study investigated 17 type I gastric neuroendocrine tumors, four type Ⅲ gastric neuroendocrine tumors and two histologically defined neuroendocrine carcinomas. 47.1% of type I neuroendocrine tumors had LOH at 11q13 compared to 25% of the type Ⅲ neuroendocrine tumors, while both neuroendocrine carcinomas showed substantial deletions at this locus[72].

**Diagnosis:** Clearly, measurement of fasting serum gastrin concentration is an important component in the diagnostic pathway for patients with gastric neuroendocrine tumors. Once a histological diagnosis of gastric neuroendocrine tumor has been made, it is imperative to ascertain the type of tumor, as well as its stage, as this will influence treatment. Defining the type of tumor can be achieved in most cases by measuring the fasting serum gastrin concentration along with the pH of gastric juice (Figure 4). In the presence of a normal or low fasting serum gastrin concentration, a diagnosis of sporadic or type Ⅲ gastric neuroendocrine tumor is most likely. In the context of an elevated serum gastrin concentration, the gastric juice pH determines whether a type I lesion (neutral pH) or type Ⅱ lesion (acidic pH) is present. This simple algorithm provides

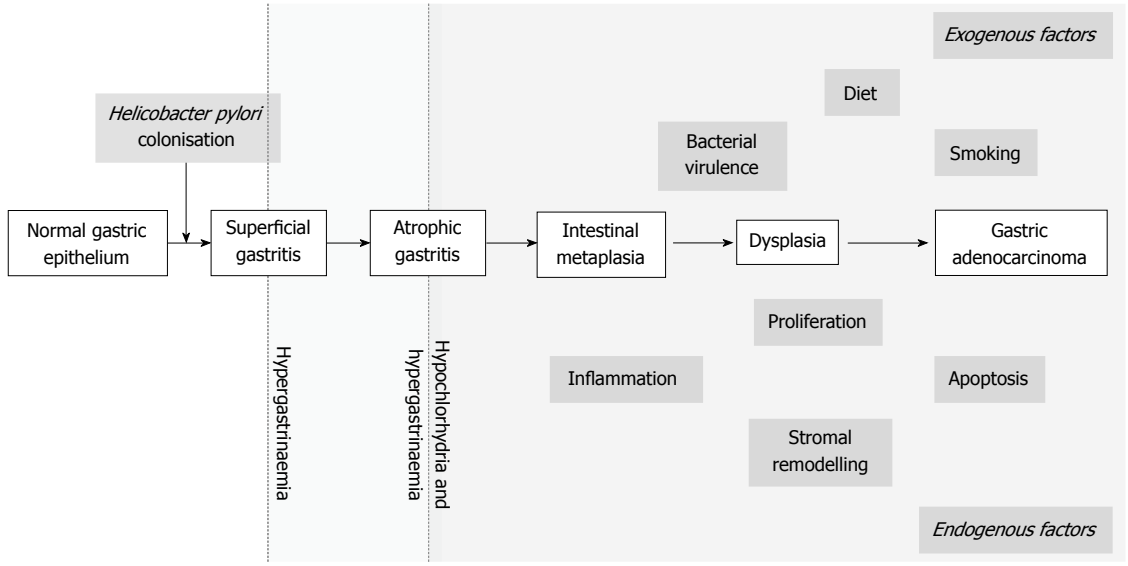


**Figure 5 Pathway of development of gastric adenocarcinoma.**

the initial data that inform future investigations. Many of these lesions are still gastrin sensitive at the time of diagnosis, and in such cases, removal of the source of hypergastrinemia by antrectomy (type Ⅰ) or gastrinoma resection (type Ⅱ) should result in tumor regression. To investigate whether a type Ⅰ gastric neuroendocrine tumor is gastrin sensitive, it may be useful to perform an octreotide suppression test. This involves biopsying the gastric corpus mucosa and neuroendocrine tumors pre and post octreotide infusion and assessing whether there is a reduction in mRNA abundance for the secretory components of ECL cells, such as histidine decarboxylase, following octreotide administration. This surrogate is then used as a marker for gastrin sensitivity[73].

**Treatment:** Those type Ⅰ and Ⅱ gastric neuroendocrine tumors that retain gastrin sensitivity may be amenable to treatment by methods which reduce elevated serum gastrin concentrations. This may involve locating and excising a primary gastrinoma in the case of a type Ⅱ gastric neuroendocrine tumor, or surgical antrectomy in a patient with pernicious anemia and autoimmune chronic atrophic gastritis. Very occasionally, large type Ⅰ gastric carcinoids grow autonomously and are no longer gastrin-sensitive, and such tumors may require gastrectomy. Future treatments for types 1 and 2 neuroendocrine tumors may include the use of CCK-2 receptor antagonists to inhibit the effects of elevated serum gastrin concentrations.

### *Gastric Adenocarcinoma*

**Pathogenesis:** Gastric adenocarcinoma accounts for the greatest mortality and morbidity associated with primary malignancies of the gastric mucosa. It develops through a stereotypical pathological sequence[74] characterized by progression from chronic active gastritis to atrophic gastritis, and *via* metaplastic lesions to dysplasia and malignancy (Figure 5). As described above, gastrin secretion is altered following *Helicobacter* infection. There is accumulating evidence, particularly from the INS-GAS animal model described above, that hypergastrinemia may contribute towards the development of gastric atrophy and remodelling of the gastric mucosa. However, it is currently not clear to what extent inferences about human gastric carcinogenesis can be made from the observations in these transgenic mice.

**Diagnosis:** Measurement of fasting serum gastrin concentration is reasonably simple and therefore several groups have investigated whether such assessment assists in the diagnosis or helps to determine the management of gastric cancer. Although a statistically significant elevation in serum gastrin concentration has been demonstrated in patients with gastric cancer compared to controls[75,76], the clinical usefulness of such assessment is currently limited. Attempts to use fasting serum gastrin concentration either as a marker of prognosis and resectability[77] or as a part of a panel of surrogate markers for initial diagnosis[78] have also been unsuccessful, and such approaches have largely been superceded by advancements in endoscopy and imaging techniques.

More recently, assessment of serum gastrin concentration has been evaluated as part of a panel of potential biomarkers for determining the presence and location of gastric atrophy. A Swedish population of 1000 individuals between the ages of 20-80 years underwent gastroscopy with biopsies taken from the antrum, corpus and fundus to assess for the presence of atrophy and at the same time had blood taken for analysis of gastrin-17, *H pylori* IgG antibody, pepsinogen Ⅰ and Ⅱ. This panel had a relatively high false positive result of 31%, although the authors defended this finding by emphasizing the potential pitfalls of sampling error when relying on histological assessment of small mucosal biopsies. Indeed, they asserted that the finding of serological markers in keeping with atrophy in

4.9% of patients with histological evidence of *H pylori*-associated non-atrophic gastritis, compared to just 0.8% of *H pylori*-negative patients who had no histological evidence of atrophy, potentially demonstrates the strength of serology as a global marker of atrophy rather than being limited to samples obtained from a small geographic area. Taking histology as the gold standard, these biomarkers diagnosed corpus atrophy with a positive predictive value of 69% (CI 95%: 66-72%) and a negative predictive value of 98% (95% CI: 97-99%) in this population[79].

Hansen *et al*[80] recently investigated a cohort of 101 601 patients from whom serum samples were collected in Sweden in the 1970s. They compared 230 patients who developed gastric cancer by 1992 with controls from the same cohort, and demonstrated a significant correlation between elevated serum gastrin concentration and low serum pepsinogen Ⅰ/Ⅱ ratios, and increased risk of developing non-cardia gastric cancer. They also demonstrated that most cases of gastric cardia cancer were not associated with *Helicobacter* infection, and did not show an association with markers of gastric atrophy; however, a minority of cases had serological evidence of past or present *Helicobacter* infection and an association with markers of gastric atrophy. This suggests that gastric cardia cancers develop most commonly through a *Helicobacter*/gastrin-independent mechanism, with a small subset having a similar etiology to non-cardia cancers.

The same panel of biomarkers have also been assessed in other cohorts, including a pediatric population, but in this case, they were found to be insufficiently sensitive to diagnose *Helicobacter* infection or the low incidence of atrophy that is present in this population[81]. Endoscopic evaluation of the upper GI tract therefore remains the accepted gold standard for the assessment of mucosal lesions of the GI tract; however, there may be a role, which needs further investigation, for a panel of biomarkers such as those described above in identifying adults at particularly high risk of gastric atrophy.

**Treatment:** Various groups have assessed whether immunohistochemical analysis of gastric carcinoma tissue for the presence of gastrin and/or the CCK-2 receptor is useful for predicting prognosis. Normal gastric mucosa expresses the CCK-2 receptor on ECL cells and parietal cells. Immunohistochemical studies using archival samples have shown that as the Correa sequence progresses, with development of gastric atrophy and parietal cell loss, an increased percentage of cells in the gastric corpus express the CCK-2 receptor[82]. In normal gastric corpus mucosa, gastrin immunopositive cells are not detectable; however, with progression down the Correa sequence, increased expression of gastrin and its precursors have been observed[82]. An immunohistochemical study based on a tissue array of 304 gastric cancer resection specimens from Korea demonstrated that 56.5% expressed CCK-2 receptors within the malignant tissue and that gastrin was detectable within the tumor mass in 47.7%[83]. A Welsh study has also demonstrated adverse survival in patients whose gastric tumors stained positively for gastrin[84]. These observations suggest that gastrin may represent a potential therapeutic target for the prevention or treatment of gastric carcinoma. Initial attempts at targeting gastrin to improve survival in gastric cancer used proglumide, a weak CCK-2 receptor inhibitor, and a randomized controlled trial demonstrated no survival benefit following treatment with this drug[85]. Subsequent developments have included the development of G17DT, an immunogenic mimic of gastrin 17 that causes the production of anti-gastrin antibodies[86]. Immunization with this agent improved the survival of severe combined immune deficient (SCID) mice xenografted with the human gastric cancer cell line, MGLVA1asc, and this effect was equivalent to combination chemotherapy with 5-FU and leucovorin. Moreover, the use of 5-FU in conjunction with G17DT appeared to have an additive effect[87]. G17DT was well tolerated in phase Ⅱ clinical trials[88], hence a multicenter phase Ⅱ trial of G17DT in conjunction with 5-FU and cisplatin in advanced gastric cancer has recently been performed. 60% of G17DT treated patients successfully developed anti-gastrin-17 antibodies and this subgroup showed significantly improved survival[89].

### *Gastric mucosa-associated lymphoid tissue (MALT) lymphomas*

MALT lymphomas are marginal zone lymphomas derived from the mucosa-associated lymphoid tissue of the stomach. They occur in patients with gastric atrophy and are strongly associated with *H pylori* infection. MALT lymphomas are associated with both hypergastrinemia and overexpression of the CCK-2 receptor in the gastric mucosa. The presence of hypergastrinemia is not surprising in view of the association with gastric atrophy, but the overexpression of CCK-2 receptor suggests a potential mechanism through which gastrin may exert a trophic effect in this tumor type[90,91].

### *Fundic cystic gland polyps*

These lesions are found in the oxyntic mucosa and are described by the World Health Organization as a proliferation of surface foveolar cells lining elongated, distorted pits that extend deep into the stroma. They show hyperplasia of mucous neck cells and variable amounts of cystic dilatation[92]. Although they are not intrinsically dysplastic, progression to gastric malignancy has been reported, particularly when there is underlying familial adenomatous polyposis or juvenile polyposis. Patients with these conditions have fundic cystic gland polyps on endoscopy in up to 90%[93] of cases and of these polyps, up to 40%-50%[93,94] have associated dysplasia.

Patients without inherited polyposis syndromes, however, account for the vast majority of those with fundic cystic gland polyps. In this group, the association between polyps and dysplasia is far less clear cut. Although dysplasia and cancers have been reported in patients who have these lesions[95-97], it is not clear

whether the incidence of malignancy is significantly higher than that of the normal population.

The association between fundic gland polyps and gastrin is derived from the recognition that these lesions occur more commonly in patients who are taking long-term PPIs. However, PPI-induced fundic cystic gland polyps probably have a different etiology compared to the sporadic polyps that occur in patients who are not taking PPIs (which are associated with somatic mutations in β-catenin[92,96,97]) and the polyps associated with FAP or juvenile polyposis, where there are known molecular aberrations. It has been suggested that PPI-induced fundic cystic gland polyps arise as a result of impaired glandular flow after hypergastrinemia-induced parietal cell hyperplasia has caused a mechanical obstruction to the gland[98]. However, this does not appear to be related to the severity of hypergastrinemia, as a small Norwegian study has shown equivalent degrees of hypergastrinemia in patients taking PPIs with and without fundic gland polyps[99].

In the context of PPI usage, the risk of malignant transformation is extremely low and there is at present no recommendation either for endoscopic removal of lesions or for any form of endoscopic surveillance in patients with fundic cystic gland polyps who are taking PPIs, unless they also have a familial polyposis syndrome[98].

## DISCUSSION

Evidence therefore suggests that gastrin may affect the risk of developing various epithelial and possibly lymphoid gastric malignancies by altering key cellular pathways including proliferation, apoptosis, migration, tissue remodelling and possibly angiogenesis. Gastrin is also a potential therapeutic target for the treatment of various gastric tumors. For example, surgical approaches to correct hypergastrinemia may be employed for some gastric neuroendocrine tumors, while agents such as G17DT have shown some promise in phase Ⅱ clinical trials in advanced gastric adenocarcinoma[89]. Such approaches may also have future uses in the prevention of malignancy, for example in patients who have precursor lesions such as ECL cell hyperplasia or gastric atrophy. If such therapies prove effective, there may additionally be a need to reconsider the role of endoscopic surveillance for gastric atrophy, an approach that has lost favor recently.

In population terms, more people are hypergastrinemic than ever before as a result of continued increases in the prescription rates of PPI drugs. In comparison to the numbers of individuals prescribed these drugs, gastrin-associated malignancies are undoubtedly rare, however, there is an ongoing debate about the safety profile of these agents[98,100-102].

Animal studies have shown that ECL cell hyperplasia can occur in response to hypergastrinemia induced by chronic proton pump inhibition[12]. In rats that were rendered achlorhydric for their entire lifespan, ECL-cell-derived neuroendocrine tumors also developed[66]. However, there is no convincing evidence that PPIs cause ECL cell malignancies in humans, possibly because PPIs do not usually induce complete achlorhydria[103]. Although no tumors have been found, there is evidence of diffuse and linear patterns of ECL cell hyperplasia in patients treated for a decade with PPIs[13-14,104].

There has also been concern about whether PPI treatment modulates the consequences of chronic *H pylori* infection. Recent studies have compared patients treated with anti-reflux surgery and those treated with PPIs. In patients who were *H pylori*-negative, treatment for 7 years with a PPI made no difference to mucosal inflammation or atrophy. However, patients who were *H pylori*-positive over the same 7-year period showed increased progression towards mucosal atrophy and increased inflammation if treated with a PPI, in comparison to those treated with surgery[104]. Although this suggests that *H pylori* infection should be eradicated before initiating chronic acid suppression therapy, the observed changes were modest and to date there has been no evidence of progression beyond mucosal atrophy. The studies were not designed to assess whether there was any associated increase in the incidence of gastric cancer, and much larger cohorts would be required to investigate this. Epidemiological database studies have been cited as demonstrating that PPI prescription is associated with an increased risk of gastric cancer[98]. However these data remain unpublished and this type of retrospective analysis cannot reliably distinguish patients who have been prescribed PPIs for the presenting symptoms of gastric cancer from any increase in the incidence of gastric carcinoma as a result of PPI use.

Current evidence therefore suggests that the relatively modest hypergastrinemia induced by PPI drugs is not associated with malignant transformation in the human stomach. When considering the overall safety profile of this class of drugs, gastrin-independent adverse effects such as malabsorption of vitamin B12[105,106] and iron[107], susceptibility to bacterial infection[108-110] and osteoporosis[111,112] should also be considered. Although CCK-2 receptor inhibition or combined PPI and CCK-2 receptor inhibition have been suggested as potential ways of reducing the gastrin-mediated side effects of PPIs, it seems unlikely that this approach will be clinically useful whilst PPIs have such a good safety profile, and especially as some of the adverse effects are probably related to hypochlorhydria rather than hypergastrinemia.

Our understanding of the importance of gastrin in gastric tumorigenesis has therefore increased significantly over recent years and the generation of transgenic animal models has greatly facilitated our understanding of the mechanisms involved. Issues that still need clarification include the precise role of gastrin in the pathogenesis of human gastric adenocarcinoma, whether pharmacological targeting of gastrin or its receptor is beneficial for the treatment and/or prevention of various gastric tumors and whether PPI-induced hypergastrinemia has any long term clinically important consequences, particularly in the context of chronic *H pylori* infection.

## REFERENCES


1 **Parkin DM**, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74-108
2 **Edkins JS**. The chemical mechanism of gastric secretion. *J Physiol* 1906; **34**: 133-144
3 **Dockray GJ**, Varro A, Dimaline R, Wang T. The gastrins: their production and biological activities. *Annu Rev Physiol* 2001; **63**: 119-139
4 **Wang TC**, Koh TJ, Varro A, Cahill RJ, Dangler CA, Fox JG, Dockray GJ. Processing and proliferative effects of human progastrin in transgenic mice. *J Clin Invest* 1996; **98**: 1918-1929
5 **Koh TJ**, Dockray GJ, Varro A, Cahill RJ, Dangler CA, Fox JG, Wang TC. Overexpression of glycine-extended gastrin in transgenic mice results in increased colonic proliferation. *J Clin Invest* 1999; **103**: 1119-1126
6 **Buchan AM**, Squires PE, Ring M, Meloche RM. Mechanism of action of the calcium-sensing receptor in human antral gastrin cells. *Gastroenterology* 2001; **120**: 1128-1139
7 **Schmitz F**, Göke MN, Otte JM, Schrader H, Reimann B, Kruse ML, Siegel EG, Peters J, Herzig KH, Fölsch UR, Schmidt WE. Cellular expression of CCK-A and CCK-B/gastrin receptors in human gastric mucosa. *Regul Pept* 2001; **102**: 101-110
8 **Willems G**, Vansteenkiste Y, Limbosch JM. Stimulating effect of gastrin on cell proliferation kinetics in canine fundic mucosa. *Gastroenterology* 1972; **62**: 583-589
9 **Kidd M**, Tang LH, Modlin IM, Zhang T, Chin K, Holt PR, Moss SF. Gastrin-mediated alterations in gastric epithelial apoptosis and proliferation in a mastomys rodent model of gastric neoplasia. *Digestion* 2000; **62**: 143-151
10 **Hansen OH**, Pedersen T, Larsen JK, Rehfeld JF. Effect of gastrin on gastric mucosal cell proliferation in man. *Gut* 1976; **17**: 536-541
11 **Bordi C**, Cocconi G, Togni R, Vezzadini P, Missale G. Gastric endocrine cell proliferation. Association with Zollinger-Ellison syndrome. *Arch Pathol* 1974; **98**: 274-278
12 **Larsson H**, Carlsson E, Mattsson H, Lundell L, Sundler F, Sundell G, Wallmark B, Watanabe T, Håkanson R. Plasma gastrin and gastric enterochromaffinlike cell activation and proliferation. Studies with omeprazole and ranitidine in intact and antrectomized rats. *Gastroenterology* 1986; **90**: 391-399
13 **Lamberts R**, Brunner G, Solcia E. Effects of very long (up to 10 years) proton pump blockade on human gastric mucosa. *Digestion* 2001; **64**: 205-213
14 **Singh P**, Indaram A, Greenberg R, Visvalingam V, Bank S. Long term omeprazole therapy for reflux esophagitis: follow-up in serum gastrin levels,EC cell hyperplasia and neoplasia. *World J Gastroenterol* 2000; **6**: 789-792
15 **Sun WH**, Zhu F, Chen GS, Su H, Luo C, Zhao QS, Zhang Y, Shao Y, Sun J, Zhou SM, Ding GX, Cheng YL. Blockade of cholecystokinin-2 receptor and cyclooxygenase-2 synergistically induces cell apoptosis, and inhibits the proliferation of human gastric cancer cells in vitro. *Cancer Lett* 2008; **263**: 302-311
16 **Song DH**, Rana B, Wolfe JR, Crimmins G, Choi C, Albanese C, Wang TC, Pestell RG, Wolfe MM. Gastrin-induced gastric adenocarcinoma growth is mediated through cyclin D1. *Am J Physiol Gastrointest Liver Physiol* 2003; **285**: G217-G222
17 **Varro A**, Noble PJ, Wroblewski LE, Bishop L, Dockray GJ. Gastrin-cholecystokinin(B) receptor expression in AGS cells is associated with direct inhibition and indirect stimulation of cell proliferation via paracrine activation of the epidermal growth factor receptor. *Gut* 2002; **50**: 827-833
18 **Dickson JH**, Grabowska A, El-Zaatari M, Atherton J, Watson SA. Helicobacter pylori can induce heparin-binding epidermal growth factor expression via gastrin and its receptor. *Cancer Res* 2006; **66**: 7524-7531
19 **Wang TC**, Dangler CA, Chen D, Goldenring JR, Koh T, Raychowdhury R, Coffey RJ, Ito S, Varro A, Dockray GJ, Fox JG. Synergistic interaction between hypergastrinemia and Helicobacter infection in a mouse model of gastric cancer. *Gastroenterology* 2000; **118**: 36-47
20 **Fearon ER**, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990; **61**: 759-767
21 **Houghton J**, Stoicov C, Nomura S, Rogers AB, Carlson J, Li H, Cai X, Fox JG, Goldenring JR, Wang TC. Gastric cancer originating from bone marrow-derived cells. *Science* 2004; **306**: 1568-1571
22 **Cui G**, Takaishi S, Ai W, Betz KS, Florholmen J, Koh TJ, Houghton J, Pritchard DM, Wang TC. Gastrin-induced apoptosis contributes to carcinogenesis in the stomach. *Lab Invest* 2006; **86**: 1037-1051
23 **Przemeck SM**, Varro A, Berry D, Steele I, Wang TC, Dockray GJ, Pritchard DM. Hypergastrinemia increases gastric epithelial susceptibility to apoptosis. *Regul Pept* 2008; **146**: 147-156
24 **Ramamoorthy S**, Stepan V, Todisco A. Intracellular mechanisms mediating the anti-apoptotic action of gastrin. *Biochem Biophys Res Commun* 2004; **323**: 44-48
25 **Todisco A**, Ramamoorthy S, Witham T, Pausawasdi N, Srinivasan S, Dickinson CJ, Askari FK, Krametter D. Molecular mechanisms for the antiapoptotic action of gastrin. *Am J Physiol Gastrointest Liver Physiol* 2001; **280**: G298-G307
26 **Pritchard DM**, Berry D, Przemeck SM, Campbell F, Edwards SW, Varro A. Gastrin increases mcl-1 expression in type I gastric carcinoid tumors and a gastric epithelial cell line that expresses the CCK-2 receptor. *Am J Physiol Gastrointest Liver Physiol* 2008; **295**: G798-G805
27 **Chen CN**, Hsieh FJ, Cheng YM, Chang KJ, Lee PH. Expression of inducible nitric oxide synthase and cyclooxygenase-2 in angiogenesis and clinical outcome of human gastric cancer. *J Surg Oncol* 2006; **94**: 226-233
28 **Subramaniam D**, Ramalingam S, May R, Dieckgraefe BK, Berg DE, Pothoulakis C, Houchen CW, Wang TC, Anant S. Gastrin-mediated interleukin-8 and cyclooxygenase-2 gene expression: differential transcriptional and posttranscriptional mechanisms. *Gastroenterology* 2008; **134**: 1070-1082
29 **Konturek PC**, Rembiasz K, Konturek SJ, Stachura J, Bielanski W, Galuschka K, Karcz D, Hahn EG. Gene expression of ornithine decarboxylase, cyclooxygenase-2, and gastrin in atrophic gastric mucosa infected with Helicobacter pylori before and after eradication therapy. *Dig Dis Sci* 2003; **48**: 36-46
30 **Clarke PA**, Dickson JH, Harris JC, Grabowska A, Watson SA. Gastrin enhances the angiogenic potential of endothelial cells via modulation of heparin-binding epidermal-like growth factor. *Cancer Res* 2006; **66**: 3504-3512
31 **Wroblewski LE**, Pritchard DM, Carter S, Varro A. Gastrin-stimulated gastric epithelial cell invasion: the role and mechanism of increased matrix metalloproteinase 9 expression. *Biochem J* 2002; **365**: 873-879
32 **Varro A**, Kenny S, Hemers E, McCaig C, Przemeck S, Wang TC, Bodger K, Pritchard DM. Increased gastric expression of MMP-7 in hypergastrinemia and significance for epithelial-mesenchymal signaling. *Am J Physiol Gastrointest Liver Physiol* 2007; **292**: G1133-G1140
33 **Noble PJ**, Wilde G, White MR, Pennington SR, Dockray GJ, Varro A. Stimulation of gastrin-CCKB receptor promotes migration of gastric AGS cells via multiple paracrine pathways. *Am J Physiol Gastrointest Liver Physiol* 2003; **284**: G75-G84
34 **Wang TC**, Bonner-Weir S, Oates PS, Chulak M, Simon B, Merlino GT, Schmidt EV, Brand SJ. Pancreatic gastrin stimulates islet differentiation of transforming growth factor alpha-induced ductular precursor cells. *J Clin Invest* 1993; **92**: 1349-1356
35 **Fox JG**, Rogers AB, Ihrig M, Taylor NS, Whary MT, Dockray G, Varro A, Wang TC. Helicobacter pylori-associated gastric

cancer in INS-GAS mice is gender specific. *Cancer Res* 2003; **63**: 942-950

36 **Takaishi S**, Cui G, Frederick DM, Carlson JE, Houghton J, Varro A, Dockray GJ, Ge Z, Whary MT, Rogers AB, Fox JG, Wang TC. Synergistic inhibitory effects of gastrin and histamine receptor antagonists on Helicobacter-induced gastric cancer. *Gastroenterology* 2005; **128**: 1965-1983

37 **Cui G**, Koh TJ, Chen D, Zhao CM, Takaishi S, Dockray GJ, Varro A, Rogers AB, Fox JG, Wang TC. Overexpression of glycine-extended gastrin inhibits parietal cell loss and atrophy in the mouse stomach. *Cancer Res* 2004; **64**: 8160-8166

38 **Koh TJ**, Goldenring JR, Ito S, Mashimo H, Kopin AS, Varro A, Dockray GJ, Wang TC. Gastrin deficiency results in altered gastric differentiation and decreased colonic proliferation in mice. *Gastroenterology* 1997; **113**: 1015-1025

39 **Friis-Hansen L**, Sundler F, Li Y, Gillespie PJ, Saunders TL, Greenson JK, Owyang C, Rehfeld JF, Samuelson LC. Impaired gastric acid secretion in gastrin-deficient mice. *Am J Physiol* 1998; **274**: G561-G568

40 **Nomura S**, Yamaguchi H, Ogawa M, Wang TC, Lee JR, Goldenring JR. Alterations in gastric mucosal lineages induced by acute oxyntic atrophy in wild-type and gastrin-deficient mice. *Am J Physiol Gastrointest Liver Physiol* 2005; **288**: G362-G375

41 **Kirton CM**, Wang T, Dockray GJ. Regulation of parietal cell migration by gastrin in the mouse. *Am J Physiol Gastrointest Liver Physiol* 2002; **283**: G787-G793

42 **Friis-Hansen L**, Rieneck K, Nilsson HO, Wadström T, Rehfeld JF. Gastric inflammation, metaplasia, and tumor development in gastrin-deficient mice. *Gastroenterology* 2006; **131**: 246-258

43 **Sun FJ**, Kaur S, Ziemer D, Banerjee S, Samuelson LC, De Lisle RC. Decreased gastric bacterial killing and up-regulation of protective genes in small intestine in gastrin-deficient mouse. *Dig Dis Sci* 2003; **48**: 976-985

44 **Zavros Y**, Rieder G, Ferguson A, Samuelson LC, Merchant JL. Genetic or chemical hypochlorhydria is associated with inflammation that modulates parietal and G-cell populations in mice. *Gastroenterology* 2002; **122**: 119-133

45 **Zavros Y**, Eaton KA, Kang W, Rathinavelu S, Katukuri V, Kao JY, Samuelson LC, Merchant JL. Chronic gastritis in the hypochlorhydric gastrin-deficient mouse progresses to adenocarcinoma. *Oncogene* 2005; **24**: 2354-2366

46 **Wang X**, Willén R, Wadström T, Aleljung P. RAPD-PCR, Histopathological and Serological Analysis of Four Mouse Strains Infected with Multiple Strains of Helicobacter pylori. *Microb Ecol Health Dis* 1999; **10**: 148-154

47 **Wang X**, Willén R, Andersson C, Wadström T. Development of high-grade lymphoma in Helicobacter pylori-infected C57BL/6 mice. *APMIS* 2000; **108**: 503-508

48 **Zhao CM**, Wang X, Friis-Hansen L, Waldum HL, Halgunset J, Wadström T, Chen D. Chronic Helicobacter pylori infection results in gastric hypoacidity and hypergastrinemia in wild-type mice but vagally induced hypersecretion in gastrin-deficient mice. *Regul Pept* 2003; **115**: 161-170

49 **Nagata A**, Ito M, Iwata N, Kuno J, Takano H, Minowa O, Chihara K, Matsui T, Noda T. G protein-coupled cholecystokinin-B/gastrin receptors are responsible for physiological cell growth of the stomach mucosa in vivo. *Proc Natl Acad Sci USA* 1996; **93**: 11825-11830

50 **Langhans N**, Rindi G, Chiu M, Rehfeld JF, Ardman B, Beinborn M, Kopin AS. Abnormal gastric histology and decreased acid production in cholecystokinin-B/gastrin receptor-deficient mice. *Gastroenterology* 1997; **112**: 280-286

51 **Rindi G**, Langhans N, Rehfeld JF, Beinborn M, Kopin AS. Abnormal gastric morphology and function in CCK-B/gastrin receptor-deficient mice. *Yale J Biol Med* 1998; **71**: 347-354

52 **Chen D**, Zhao CM, Al-Haider W, Håkanson R, Rehfeld JF, Kopin AS. Differentiation of gastric ECL cells is altered in CCK(2) receptor-deficient mice. *Gastroenterology* 2002; **123**: 577-585

53 **Jensen RT**, Niederle B, Mitry E, Ramage JK, Steinmuller T, Lewington V, Scarpa A, Sundin A, Perren A, Gross D, O'Connor JM, Pauwels S, Kloppel G. Gastrinoma (duodenal and pancreatic). *Neuroendocrinology* 2006; **84**: 173-182

54 **Zollinger RM**, Ellison EH. Primary peptic ulcerations of the jejunum associated with islet cell tumors of the pancreas. *Ann Surg* 1955; **142**: 709-723; discussion, 724-728

55 **Ito T**, Tanaka M, Sasano H, Osamura YR, Sasaki I, Kimura W, Takano K, Obara T, Ishibashi M, Nakao K, Doi R, Shimatsu A, Nishida T, Komoto I, Hirata Y, Imamura M, Kawabe K, Nakamura K. Preliminary results of a Japanese nationwide survey of neuroendocrine gastrointestinal tumors. *J Gastroenterol* 2007; **42**: 497-500

56 **Piecha G**, Chudek J, Wiecek A. Multiple Endocrine Neoplasia type 1. *Eur J Intern Med* 2008; **19**: 99-103

57 **Smith JT**, Pounder RE, Nwokolo CU, Lanzon-Miller S, Evans DG, Graham DY, Evans DJ Jr. Inappropriate hypergastrinaemia in asymptomatic healthy subjects infected with Helicobacter pylori. *Gut* 1990; **31**: 522-525

58 **Levi S**, Beardshall K, Swift I, Foulkes W, Playford R, Ghosh P, Calam J. Antral Helicobacter pylori, hypergastrinaemia, and duodenal ulcers: effect of eradicating the organism. *BMJ* 1989; **299**: 1504-1505

59 **Moss SF**, Legon S, Bishop AE, Polak JM, Calam J. Effect of Helicobacter pylori on gastric somatostatin in duodenal ulcer disease. *Lancet* 1992; **340**: 930-932

60 **Carmel R**. Prevalence of undiagnosed pernicious anemia in the elderly. *Arch Intern Med* 1996; **156**: 1097-1100

61 **Kogawa K**. Parietal cell antibodies. Part II. Cytotoxic activities of parietal cell antibodies. Experiment 1. The observations on cytotoxic activities of parietal cell antibodies to normal human parietal cells in vitro. Experiment 2. The histochemical changes of rat stomach after administrations of rabbit anti-rat gastric mucosa sera. *Gastroenterol Jpn* 1975; **10**: 52-64

62 **Pritchard DM**, Przemeck SM. Review article: How useful are the rodent animal models of gastric adenocarcinoma? *Aliment Pharmacol Ther* 2004; **19**: 841-859

63 **El-Omar EM**, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, Herrera J, Lissowska J, Yuan CC, Rothman N, Lanyon G, Martin M, Fraumeni JF Jr, Rabkin CS. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature* 2000; **404**: 398-402

64 **Presotto F**, Sabini B, Cecchetto A, Plebani M, De Lazzari F, Pedini B, Betterle C. Helicobacter pylori infection and gastric autoimmune diseases: is there a link? *Helicobacter* 2003; **8**: 578-584

65 **Stolte M**, Meier E, Meining A. Cure of autoimmune gastritis by Helicobacter pylori eradication in a 21-year-old male. *Z Gastroenterol* 1998; **36**: 641-643

66 **Havu N**. Enterochromaffin-like cell carcinoids of gastric mucosa in rats after life-long inhibition of gastric secretion. *Digestion* 1986; **35** Suppl 1: 42-55

67 **Burkitt MD**, Pritchard DM. Review article: Pathogenesis and management of gastric carcinoid tumours. *Aliment Pharmacol Ther* 2006; **24**: 1305-1320

68 **Berna MJ**, Annibale B, Marignani M, Luong TV, Corleto V, Pace A, Ito T, Liewehr D, Venzon DJ, Delle Fave G, Bordi C, Jensen RT. A prospective study of gastric carcinoids and enterochromaffin-like cell changes in multiple endocrine neoplasia type 1 and Zollinger-Ellison syndrome: identification of risk factors. *J Clin Endocrinol Metab* 2008; **93**: 1582-1591

69 **Carboni M**, Guadagni S, Pistoia MA, Amicucci G, Lolli D, Palumbo G, Ludovico C, Walters C, Smith P, Viti G. Chronic atrophic gastritis and risk of N-nitroso compounds carcinogenesis. *Langenbecks Arch Chir* 1988; **373**: 82-90

70 **Jensen RT**. Consequences of long-term proton pump blockade: insights from studies of patients with gastrinomas. *Basic Clin Pharmacol Toxicol* 2006; **98**: 4-19

71 **Fujii T**, Kawai T, Saito K, Hishima T, Hayashi Y, Imura J, Hironaka M, Hosoya Y, Koike M, Fukayama M. MEN1 gene mutations in sporadic neuroendocrine tumors of foregut derivation. *Pathol Int* 1999; **49**: 968-973

72 **D'Adda T**, Keller G, Bordi C, Höfler H. Loss of heterozygosity in 11q13-14 regions in gastric neuroendocrine tumors not associated with multiple endocrine neoplasia type 1 syndrome. *Lab Invest* 1999; **79**: 671-677

73 **Higham AD**, Dimaline R, Varro A, Attwood S, Armstrong G, Dockray GJ, Thompson DG. Octreotide suppression test predicts beneficial outcome from antrectomy in a patient with gastric carcinoid tumor. *Gastroenterology* 1998; **114**: 817-822

74 **Correa P**. A human model of gastric carcinogenesis. *Cancer Res* 1988; **48**: 3554-3560

75 **McGuigan JE**, Trudeau WL. Serum and tissue gastrin concentrations in patients with carcinoma of the stomach. *Gastroenterology* 1973; **64**: 22-25

76 **Rakic S**, Milicevic MN. Serum gastrin levels in patients with intestinal and diffuse type of gastric cancer. *Br J Cancer* 1991; **64**: 1189

77 **Soran A**, Aslar AK, Cöl C. Are preoperative serum gastrin levels related to resectability and survival in gastric cancer? *Int J Clin Pract* 2000; **54**: 652-653

78 **Lin JT**, Lee WC, Wu MS, Wang JT, Wang TH, Chen CJ. Diagnosis of gastric adenocarcinoma using a scoring system: combined assay of serological markers of Helicobacter pylori infection, pepsinogen I and gastrin. *J Gastroenterol* 1995; **30**: 156-161

79 **Storskrubb T**, Aro P, Ronkainen J, Sipponen P, Nyhlin H, Talley NJ, Engstrand L, Stolte M, Vieth M, Walker M, Agréus L. Serum biomarkers provide an accurate method for diagnosis of atrophic gastritis in a general population: The Kalixanda study. *Scand J Gastroenterol* 2008; **43**: 1448-1455

80 **Hansen S**, Vollset SE, Derakhshan MH, Fyfe V, Melby KK, Aase S, Jellum E, McColl KE. Two distinct aetiologies of cardia cancer; evidence from premorbid serological markers of gastric atrophy and Helicobacter pylori status. *Gut* 2007; **56**: 918-925

81 **Koivusalo AI**, Pakarinen MP, Kolho KL. Is GastroPanel serum assay useful in the diagnosis of Helicobacter pylori infection and associated gastritis in children? *Diagn Microbiol Infect Dis* 2007; **57**: 35-38

82 **Henwood M**, Clarke PA, Smith AM, Watson SA. Expression of gastrin in developing gastric adenocarcinoma. *Br J Surg* 2001; **88**: 564-568

83 **Hur K**, Kwak MK, Lee HJ, Park DJ, Lee HK, Lee HS, Kim WH, Michaeli D, Yang HK. Expression of gastrin and its receptor in human gastric cancer tissues. *J Cancer Res Clin Oncol* 2006; **132**: 85-91

84 **Stephens MR**, Hopper AN, Lewis WG, Blackshaw G, Edwards P, Osborne B, Thompson IW. Prognostic significance of gastrin expression in patients undergoing R0 gastrectomy for adenocarcinoma. *Gastric Cancer* 2007; **10**: 159-166

85 **Harrison JD**, Jones JA, Morris DL. The effect of the gastrin receptor antagonist proglumide on survival in gastric carcinoma. *Cancer* 1990; **66**: 1449-1452

86 **Watson SA**, Michaeli D, Grimes S, Morris TM, Robinson G, Varro A, Justin TA, Hardcastle JD. Gastrimmune raises antibodies that neutralize amidated and glycine-extended gastrin-17 and inhibit the growth of colon cancer. *Cancer Res* 1996; **56**: 880-885

87 **Watson SA**, Morris TM, Varro A, Michaeli D, Smith AM. A comparison of the therapeutic effectiveness of gastrin neutralisation in two human gastric cancer models: relation to endocrine and autocrine/paracrine gastrin mediated growth. *Gut* 1999; **45**: 812-817

88 **Gilliam AD**, Watson SA, Henwood M, McKenzie AJ, Humphreys JE, Elder J, Iftikhar SY, Welch N, Fielding J, Broome P, Michaeli D. A phase II study of G17DT in gastric carcinoma. *Eur J Surg Oncol* 2004; **30**: 536-543

89 **Ajani JA**, Randolph Hecht J, Ho L, Baker J, Oortgiesen M, Eduljee A, Michaeli D. An open-label, multinational, multicenter study of G17DT vaccination combined with cisplatin and 5-fluorouracil in patients with untreated, advanced gastric or gastroesophageal cancer: the GC4 study. *Cancer* 2006; **106**: 1908-1916

90 **Konturek PC**, Konturek SJ, Starzyska T, Marlicz K, Bielanski W, Pierzchalski P, Karczewska E, Hartwich A, Rembiasz K, Lawniczak M, Ziemniak W, Hahn EC. Helicobacter pylori-gastrin link in MALT lymphoma. *Aliment Pharmacol Ther* 2000; **14**: 1311-1318

91 **Ohashi S**, Segawa K, Okamura S, Urano F, Kanamori S, Hosoi T, Ishikawa H, Kanamori A, Kitabatake S, Sano H, Kobayashi T, Maeda M. Gastrin and Helicobacter pylori in low-grade MALT lymphoma patients. *Scand J Gastroenterol* 2002; **37**: 279-286

92 **Abraham SC**, Nobukawa B, Giardiello FM, Hamilton SR, Wu TT. Sporadic fundic gland polyps: common gastric polyps arising through activating mutations in the beta-catenin gene. *Am J Pathol* 2001; **158**: 1005-1010

93 **Bianchi LK**, Burke CA, Bennett AE, Lopez R, Hasson H, Church JM. Fundic gland polyp dysplasia is common in familial adenomatous polyposis. *Clin Gastroenterol Hepatol* 2008; **6**: 180-185

94 **Attard TM**, Cuffari C, Tajouri T, Stoner JA, Eisenberg MT, Yardley JH, Abraham SC, Perry D, Vanderhoof J, Lynch H. Multicenter experience with upper gastrointestinal polyps in pediatric patients with familial adenomatous polyposis. *Am J Gastroenterol* 2004; **99**: 681-686

95 **Jalving M**, Koornstra JJ, Götz JM, van der Waaij LA, de Jong S, Zwart N, Karrenbeld A, Kleibeuker JH. High-grade dysplasia in sporadic fundic gland polyps: a case report and review of the literature. *Eur J Gastroenterol Hepatol* 2003; **15**: 1229-1233

96 **Jalving M**, Koornstra JJ, Boersma-van Ek W, de Jong S, Karrenbeld A, Hollema H, de Vries EG, Kleibeuker JH. Dysplasia in fundic gland polyps is associated with nuclear beta-catenin expression and relatively high cell turnover rates. *Scand J Gastroenterol* 2003; **38**: 916-922

97 **Abraham SC**, Park SJ, Mugartegui L, Hamilton SR, Wu TT. Sporadic fundic gland polyps with epithelial dysplasia: evidence for preferential targeting for mutations in the adenomatous polyposis coli gene. *Am J Pathol* 2002; **161**: 1735-1742

98 **Kuipers EJ**. Proton pump inhibitors and gastric neoplasia. *Gut* 2006; **55**: 1217-1221

99 **Fossmark R**, Jianu CS, Martinsen TC, Qvigstad G, Syversen U, Waldum HL. Serum gastrin and chromogranin A levels in patients with fundic gland polyps caused by long-term proton-pump inhibition. *Scand J Gastroenterol* 2007; 1-5

100 **Yeomans ND**, Dent J. Personal review: alarmism or legitimate concerns about long-term suppression of gastric acid secretion? *Aliment Pharmacol Ther* 2000; **14**: 267-271

101 **Waldum HL**, Brenna E. Personal review: is profound acid inhibition safe? *Aliment Pharmacol Ther* 2000; **14**: 15-22

102 **Coté GA**, Howden CW. Potential adverse effects of proton pump inhibitors. *Curr Gastroenterol Rep* 2008; **10**: 208-214

103 **Lou HY**, Chang CC, Sheu MT, Chen YC, Ho HO. Optimal dose regimens of esomeprazole for gastric acid suppression with minimal influence of the CYP2C19 polymorphism. *Eur J Clin Pharmacol* 2008

104 **Klinkenberg-Knol EC**, Nelis F, Dent J, Snel P, Mitchell B, Prichard P, Lloyd D, Havu N, Frame MH, Romàn J, Walan A. Long-term omeprazole treatment in resistant gastroesophageal reflux disease: efficacy, safety, and influence on gastric mucosa. *Gastroenterology* 2000; **118**: 661-669

105 **Hirschowitz BI**, Worthington J, Mohnen J. Vitamin B12 deficiency in hypersecretors during long-term acid suppression with proton pump inhibitors. *Aliment Pharmacol Ther* 2008; **27**: 1110-1121

106 **Ruscin JM**, Page RL 2nd, Valuck RJ. Vitamin B(12) deficiency associated with histamine(2)-receptor antagonists and a proton-pump inhibitor. *Ann Pharmacother* 2002; **36**: 812-816
107 **Hutchinson C**, Geissler CA, Powell JJ, Bomford A. Proton pump inhibitors suppress absorption of dietary non-haem iron in hereditary haemochromatosis. *Gut* 2007; **56**: 1291-1295
108 **Kaur S**, Vaishnavi C, Prasad KK, Ray P, Kochhar R. Comparative role of antibiotic and proton pump inhibitor in experimental Clostridium difficile infection in mice. *Microbiol Immunol* 2007; **51**: 1209-1214
109 **Jayatilaka S**, Shakov R, Eddi R, Bakaj G, Baddoura WJ, DeBari VA. Clostridium difficile infection in an urban medical center: five-year analysis of infection rates among adult admissions and association with the use of proton pump inhibitors. *Ann Clin Lab Sci* 2007; **37**: 241-247
110 **Leonard J**, Marshall JK, Moayyedi P. Systematic review of the risk of enteric infection in patients taking acid suppression. *Am J Gastroenterol* 2007; **102**: 2047-2056; quiz 2057
111 **O'Connell MB**, Madden DM, Murray AM, Heaney RP, Kerzner LJ. Effects of proton pump inhibitors on calcium carbonate absorption in women: a randomized crossover trial. *Am J Med* 2005; **118**: 778-781
112 **Cui GL**, Syversen U, Zhao CM, Chen D, Waldum HL. Long-term omeprazole treatment suppresses body weight gain and bone mineralization in young male rats. *Scand J Gastroenterol* 2001; **36**: 1011-1015
113 **Ottewell PD**, Watson AJ, Wang TC, Varro A, Dockray GJ, Pritchard DM. Progastrin stimulates murine colonic epithelial mitosis after DNA damage. *Gastroenterology* 2003; **124**: 1348-1357
114 **Singh P**, Velasco M, Given R, Varro A, Wang TC. Progastrin expression predisposes mice to colon carcinomas and adenomas in response to a chemical carcinogen. *Gastroenterology* 2000; **119**: 162-171
115 **Cobb S**, Wood T, Tessarollo L, Velasco M, Given R, Varro A, Tarasova N, Singh P. Deletion of functional gastrin gene markedly increases colon carcinogenesis in response to azoxymethane in mice. *Gastroenterology* 2002; **123**: 516-530
116 **Rünkorg K**, Veraksits A, Kurrikoff K, Luuk H, Raud S, Abramov U, Matsui T, Bourin M, Kõks S, Vasar E. Distinct changes in the behavioural effects of morphine and naloxone in CCK2 receptor-deficient mice. *Behav Brain Res* 2003; **144**: 125-135
117 **Abramov U**, Raud S, Innos J, Kõks S, Matsui T, Vasar E. Gender specific effects of ethanol in mice, lacking CCK2 receptors. *Behav Brain Res* 2006; **175**: 149-156
118 **Rünkorg K**, Veraksits A, Kurrikoff K, Luuk H, Raud S, Abramov U, Matsui T, Bourin M, Kõks S, Vasar E. Distinct changes in the behavioural effects of morphine and naloxone in CCK2 receptor-deficient mice. *Behav Brain Res* 2003; **144**: 125-135
119 **Abramov U**, Raud S, Kõks S, Innos J, Kurrikoff K, Matsui T, Vasar E. Targeted mutation of CCK(2) receptor gene antagonises behavioural changes induced by social isolation in female, but not in male mice. *Behav Brain Res* 2004; **155**: 1-11
120 **Raud S**, Innos J, Abramov U, Reimets A, Kõks S, Soosaar A, Matsui T, Vasar E. Targeted invalidation of CCK2 receptor gene induces anxiolytic-like action in light-dark exploration, but not in fear conditioning test. *Psychopharmacology* (Berl) 2005; **181**: 347-357

**S- Editor** Li LF **L- Editor** Logan S **E- Editor** Zheng XM

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.17

*World J Gastroenterol* 2009 January 7; 15(1): 17-24
***World Journal of Gastroenterology*** ISSN 1007-9327




**Dr. Peter V Draganov,** *Series Editor*

# Eosinophilic esophagitis

Anand R Gupte, Peter V Draganov

Anand R Gupte, Peter V Draganov, Department of Gastroenterology, Hepatology and Nutrition, University of Florida, 1600 SW Archer Rd, Room HD 602, PO Box 100214 Gainesville, Florida 32610, United States
Author contributions: Gupte AR and Draganov PV have contributed equally.
Correspondence to: Peter V Draganov, MD, Department of Gastroenterology, Hepatology and Nutrition, University of Florida, 1600 SW Archer Rd, Room HD 602, PO Box 100214 Gainesville, Florida 32610,
United States. dragapv@medicine.ufl.edu
Telephone: +1-352-392-2877 Fax: +1-352-392-3618
Received: June 20, 2008 Revised: December 3, 2008
Accepted: December 10, 2008
Published online: January 7, 2009

## Abstract

Eosinophilic esophagitis is increasingly recognized in adults. The diagnosis is based on the presence of both typical symptoms and pathologic findings on esophageal biopsy. Patients usually present with dysphagia, food impaction and/or reflux-like symptoms, and biopsy of the esophagus shows more than 15 eosinophils per high-power field. In addition, it is essential to exclude the presence of known causes of tissue eosinophilia such as gastroesophageal reflux disease, infections, malignancy, collagen vascular diseases, hypersensitivity, and inflammatory bowel disease. There are no standardized protocols for the therapy of eosinophilic esophagitis. A variety of therapeutic approaches including acid suppression, dietary modifications, topical corticosteroids and endoscopic dilation can be used alone or in combination.



**Key words:** Eosinophilic esophagitis; Dysphagia; Endoscopic dilation; Reflux; Gastro esophageal reflux disease

**Peer reviewer:** Kiron M Das, MD, PhD, FACP, FRCP, Chief of Gastroenterology and Hepatology, Professor of Medicine, Director of Crohn's and Colitis Center of NJ, 1 Robert Wood Johnson Place, MEB Rm. 478, New Brunswick, NJ 08903, United States

Gupte AR, Draganov PV. Eosinophilic esophagitis. *World J Gastroenterol* 2009; 15(1): 17-24 Available from: URL: http://www.wjgnet.com/1007-9327/15/17.asp DOI: http://dx.doi.org/10.3748/wjg.15.17

## INTRODUCTION

Eosinophilic esophagitis (EE) in adults is a disease with the following clinicopathological characteristics: (1) symptoms including but not restricted to food impaction and dysphagia; (2) biopsy specimen showing more than 15 eosinophils/high-power field (HPF); and (3) other disorders associated with similar clinical, histological, or endoscopic features have been excluded[1]. EE is increasingly being recognized in adult and pediatric populations, either as a separate entity or as a part of the spectrum of eosinophilic gastroenteritis[2]. It was initially described in the 1970s[3], but subsequently most research focused on gastroesophageal reflux disease (GERD) as being the primary cause of esophageal eosinophilia. It was not until the 1990s that EE came to be regarded as a separate entity[4]. Later on, an allergic component to EE was observed as patients suspected of having EE had improvement of symptoms on either elemental diet[5,6] or on corticosteroids[7]. These features, along with normal pH study results and the relative lack of effectiveness of acid suppression therapy, resulted in EE being regarded as a clinical condition different from GERD.

EE has recently been explored in much detail within various forums. This article aims to review that literature looking at the epidemiological and clinicopathological aspects of EE, with special emphasis on diagnostic approaches and treatment options.

## EPIDEMIOLOGY, ETIOLOGY AND PATHOGENESIS

EE has been studied most extensively in pediatric populations and only recently has further data been compiled in adults. EE can present in the third and fourth decades of life and various studies implicate it to be more predominant in men[8,9]. Among different races and ethnic groups, EE has been seen to be more prevalent in the white population[10]. Geographic distribution is wide, with cases now being reported not only in the United States but also Europe, Canada, Brazil, Japan and Australia[11]. The preponderance of EE in developed nations has unclear etiology and could

either be secondary to the increased prevalence of atopic diseases like asthma[12] or simply because of better reporting and data collection. To probe this further, Cherian *et al*[13] conducted a blind retrospective study of western Australian children investigated for esophageal disease in 1995, 1999 and 2004 and found the prevalence of EE to be indeed increased by 18-fold during this time period. The most recent US study in 74 162 patients used a national pathology database of subjects undergoing upper endoscopy with biopsy. The data confirmed that EE is a male-predominant disorder (74%) and that it can occur at any age. Over the study period (2002-2006), an increasing prevalence was noted. Whether this reflects a true increase in prevalence or increased recognition due to heightened awareness among physicians remains to be determined[14].

Furthermore, because of the past difficulty in diagnosing EE correctly in populations with dysphagia, food impactions or GERD, studies were subsequently conducted in various nations that later found EE to be the primary cause of these symptoms[7,15]. Markowitz *et al*[6] found that 15% of patients initially suspected of having GERD were actually discovered to have EE. They used strict diagnostic criteria for EE such as > 20 eosinophils/HPF in esophageal biopsies, normal esophageal pH monitoring and a lack of response to proton pump inhibitor (PPI) therapy. These findings may warrant changing our diagnostic approach to having an early esophagogastroduodenoscopy (EGD) with subsequent esophageal biopsies if clinical reflux-like symptoms and lack of response to medical treatment continue to be an issue.

Allergens play an important role in the etiology of EE. Kelly *et al*[5] first showed the association of food allergens with EE when they fed an elemental diet to 10 children with unremitting reflux symptoms and found symptomatic and histological improvement. This was later supported in different studies with successful use of either an elemental or a six-food elimination diet[16]. Aero-allergens form another potential cause of EE. Mishra *et al*[17] used a murine model to demonstrate an etiological role for inhaled allergens and eosinophils in gastrointestinal inflammation. A high degree of atopy and polysensitization to several environmental allergens was recently documented in patients with EE, suggesting that sensitization may partly be a response to inhaled allergens[18]. However multiple etiological factors are not mutually exclusive, as was presented by Plaza-Martin *et al*[19] who found evidence of poly-sensitization to aero-allergens and food allergens in their study population of patients who had EE. A familial pattern of inheritance has also been suggested to play a role in the development of EE[20].

The esophagus is normally devoid of eosinophils, however the rest of the gastrointestinal tract is populated with eosinophils beginning from the embryonal stage. Mishra *et al*[21] showed that the peptide eotaxin regulates eosinophil homing to the gastrointestinal (GI) tract during embryonal development. They also showed a connection between allergic hypersensitivity response in lung and esophagus regulated by eotaxin and interleukin (IL)-5[17,22]. It was subsequently shown that IL-13 plays a fundamental role in EE[23]. Once eosinophils have migrated to the esophagus, they release chemoattractants IL-3, IL-5 and granulocyte monocyte-colony stimulating factor (GM-CSF)[24]. Straumann *et al*[25] further confirmed the allergic nature of EE when they showed that a TH2 response, IL-5 and IgE mediated the pathogenesis of EE. However, there are inconsistencies in determining the exact nature and influence of an aero-allergic etiopathogenesis of EE. Balatsinou *et al*[26] observed EE in two patients with anticonvulsant hypersensitivity syndrome, wherein they saw reversal of endoscopic appearance after stopping carbamazepine, suggesting that oral agents could also play a role in the pathogenesis. An association with pollen has also been noted previously[27].

Dysphagia predominantly seen in EE has been attributed to both organic and non-organic (i.e. motility) disorders. Stevoff *et al*[28], in one of the first case reports on EE in octogenarians, showed circumferential but asymmetric thickening of the muscularis propria or a functional constriction related to myenteric plexus infiltration. Various other factors have been implicated in the development of dysphagia. Non-anatomic causes for dysphagia could be related to dysmotility. Nurko *et al*[29] reviewed the different causes of dysmotility that had been proposed in earlier papers, and these included eosinophil-mediated increased contraction of fibroblasts, axonal necrosis or cholinergic pathway interference, all of which contributed to esophageal dysmotility. A caveat to some of these studies is that they were either based on non-allergic models of esophagus or from studies in organ systems other than the esophagus[29].

## CLINICAL FEATURES

EE usually presents with a multitude of symptoms, in part because it is a chronic disease and partly because of the gradual inflammatory involvement of the mucosa and submucosa before symptoms develop[30]. It can, however, present acutely as seen in food impactions[30]. The most common presenting symptom is dysphagia[30,31] but other symptoms such as nausea, vomiting, heartburn, chest pain or abdominal pain can also occur. Symptoms suggestive of esophageal dysmotility may indicate involvement of the muscular layers of the esophagus[32]. Occasionally, presentation of EE has been seen to be more subtle as patients adapt their chewing habits, eating food more slowly and washing down solid food with liquids, thereby decreasing the symptom incidence and leading to a delay in diagnosis[33]. Remedios *et al*[31] found an association of esophageal symptoms with exposure to certain foods even without actual consumption. Not infrequently, patients may also have additional symptoms of asthma[3], allergies or atopic dermatitis. Dauer *et al*[34] and Orenstein *et al*[35] have reported nasal symptoms and rhinosinusitis in about a quarter of patients that have EE. Laryngeal symptoms include hoarseness, cough, croup and sleep-disordered breathing[36]. Ferguson and

**Table 1 Clinical presentation of eosinophilic esophagitis**

| Gastrointestinal symptoms | Other symptoms |
|---|---|
| Dysphagia | Chest pain |
| Food impaction | Rhinitis |
| Nausea and vomiting | Asthma |
| Heartburn | Allergies |
| Abdominal pain | Atopic dermatitis |
| Feeding disorders (pediatric) | Hoarseness |
| Failure to thrive (pediatric) | Croup, cough |
| | Sleep disordered breathing |

**Table 2 Clinical signs in eosinophilic esophagitis**

| Endoscopic features | Histologic features |
|---|---|
| Diminished vascular pattern | Thick epithelium with eosinophilia |
| Mucosal furrows | Abnormally long papillae |
| Thick mucosa | Fibrotic lamina propria |
| Exudates | Microabscesses |
| Strictures | Extracellular Eosinophilic granules |
| Rings | Increased extracellular major basic protein (MBP) |
| Laryngeal edema, vocal cord nodules, laryngeal ventricular obliteration | |

Foxx-Orenstein divided the clinical manifestations according to age groups. Thus feeding disorders and failure to thrive were primarily seen in children below 2 years of age; vomiting, abdominal pain and reflux were seen in pediatric populations up to the age of 12; whereas adults usually present with dysphagia and food impactions[37] (Table 1).

Endoscopically, a normal-appearing esophagus is usually incompatible with a diagnosis of EE, although the findings can be subtle[8]. Typical findings on an EGD that imply the presence of EE include attenuation of subepithelial vascular pattern[38], linear furrowing[39] that may extend along the whole length of the esophagus, surface exudates composed of eosinophils or abscesses or strictures[8]. Presence of mucosal changes suggestive of ulcerations usually implies peptic injury by itself or in association with EE[40]. Schatzki ring has also been previously associated with EE[41] but one of the most characteristic and frequently quoted patterns is that of stacked circular rings or felinization[40], so called because of their presence in the cat esophagus. This has been postulated to be due to lamina propria and dermal papillary fibrosis caused by either the mediators that stimulate eosinophils or through the effect of eosinophils themselves. Previously, Vasilopoulos and Shaker described a small caliber esophagus as a major cause of dysphagia in patients with EE[42]. The esophagus was seen to have a smooth, diffusely narrow lumen shown on barium esophogram or esophagoscopy. Food impactions are also relatively frequent in patients with EE. Fox *et al*[40] have attributed these food impactions to either the strictures themselves or to decreased peristalsis secondary to underlying inflammation. Therefore, in patients with food impaction, it is worthwhile to follow EGD with biopsies for early diagnosis and treatment of this disorder. Airway endoscopy findings in patients with recurrent croup and EE include diffuse laryngeal edema, vocal fold nodules and laryngeal ventricular obliteration[36] (Table 2).

Histopathologically, EE is characterized by the presence of a thick epithelium with a large number of intraepithelial eosinophils lined near the surface, abnormally long papillae and a fibrotic lamina propria containing eosinophils[43]. Cheung *et al*[44], in their retrospective study of 42 children with dysphagia, described the presence of extracellular eosinophilic granules in patients with EE. Major basic protein (MBP) is a byproduct of eosinophil degranulation, and as such, increased deposition of MBP has been observed in pediatric and adult patients with EE[45,46].

Complications arising from EE can either be attributed to the clinical manifestations of the disease itself or to diagnostic and therapeutic interventions. Acute food impaction is one of the main reasons patients present as emergencies to the hospital. In one study, 57% of patients with EE had strictures that were successfully treated with dilatation, with subsequent resolution of symptoms[8]. More severe disease could lead to long segment narrowing which has been postulated to be in two forms[47]. The first form is referred to as trachealization[48], corrugated esophagus[48,49] or feline esophagus[50]. The second form is the small-caliber esophagus mentioned by Vasilopoulos *et al*[51], who found a diffusely narrow esophagus in three out of the five patients referred to them for chronic dysphagia. EE may also predispose to fungal and viral infections in the absence of steroid treatment or immunosuppression[47]. Straumann *et al*[52] conducted a chart review of 251 cases of esophagitis and found a case of Boerhaave's syndrome (spontaneous esophageal rupture). In their report, they recommend that all Boerhaave's cases be evaluated for EE. Chronic inflammation in EE may also lead to dysfunction of the lower esophageal sphincter and cause secondary reflux disease, as was reported by Remedios *et al*[31].

The risk of esophageal perforation is significantly increased during diagnostic or therapeutic endoscopic evaluation in a patient with EE[50]. In their chart and pathology review, Kaplan *et al*[50] found that more than half of their patients with EE had mucosal rents after simple passage of the endoscope, with one patient developing a perforation. Therefore, intense retrosternal pain after endoscopic evaluation in a patient suspected to have EE should particularly raise the suspicion of perforation and appropriate diagnostic evaluation should be undertaken[53]. The esophageal mucosa in EE is very fragile and inelastic, which Straumann *et al*[54] have termed "crepe paper mucosa" as it tore easily even with minor trauma. Consequently therapeutic interventions such as food bolus removal, dilation or biopsy can pose an even higher risk of perforation[52,55,56]. Kaplan *et al*[50] recommend about 8 wk of medical therapy before considering dilation in patients diagnosed with EE because of the high risk of perforation and the good response to medical therapy (Table 3).

**Table 3 Complications in EE related to disease and to the interventions performed for treatment**

| Complications of EE | Complications of therapeutic interventions |
|---|---|
| Acute food impactions | Mucosal rents/tears |
| Long and short segment narrowing | Perforation |
| Stenosis | Infections-due to chronic use of steroids |
| GERD | Nutritional deficiencies |
| Boerhaave's syndrome | |
| Nutritional deficiencies | |

## DIAGNOSIS

EE should always be considered in the following circumstances: (1) history of food impaction; (2) persistent dysphagia especially in young individuals and in patients having a history of atopy; or (3) GERD refractory to medical therapy. Other causes of eosinophilia such as parasitic infestations, malignancy, drug hypersensitivity, collagen vascular diseases and inflammatory bowel disease need to be ruled out[57]. EE is primarily a clinicopathological condition and hence both symptoms and pathological diagnosis form an integral part of the diagnosis. The First International Gastrointestinal Eosinophilic Research Symposium (FIGERS) came up with comprehensive guidelines regarding the diagnostic criteria for EE. Accordingly, an eosinophil count of $\geqslant$ 15/HPF, along with normal gastric and duodenal biopsies, can substantiate the diagnosis of EE. Moreover, patients must have biopsies after 6-8 wk of twice daily acid suppression with PPI or have a negative pH study result[1] in order to correctly diagnose EE. At least five such biopsies must be obtained and preferably from both the proximal and the distal esophagus to account for the heterogeneous nature of the tissue eosinophilia[31,58] (Table 4).

Prasad *et al*[59] did a prospective study of 376 patients and found that mid-esophageal biopsies had a yield rate of about 10%. They thus recommend taking mid-esophageal biopsies in patients with unexplained food dysphagia. However, EE is patchy and hence increasing the number of biopsy specimens would theoretically yield a higher sensitivity and specificity in diagnosing EE[58]. Gonsalves conducted a chart review of 76 patients and found that sensitivity increased from 55% to 100% if the number of biopsies were increased from one to about five[58]. No statistically significant difference was found between the biopsies obtained from either the proximal or the distal esophagus. Thus, Collins in her article has recommended obtaining at least three pieces from two different sites in the esophagus including the distal and either mid or proximal esophagus[43]. If there is a high suspicion of the presence of EE, then biopsies should be obtained even if the esophagus endoscopically appears normal. Liacouras *et al*[60] found in their chart review that about one third of the patients with severe EE had a visually normal esophagus and they recommended that one should not rely only on the endoscopic appearance and rather aim to get biopsies for histological analysis if there is suspicion for EE.

**Table 4 Diagnostic guidelines for eosinophilic esophagitis**

| Eosinophilic esophagitis | |
|---|---|
| Symptoms (adults) | GERD refractory to medical therapy |
| | Dysphagia |
| | Food Impaction |
| | Retrosternal chest pain |
| Endoscopy | Mucosal furrows |
| | Exudates |
| | Esophageal lumen narrowing |
| | Rings |
| Histology | Esophageal biopsies ≥15 eosinophils/HPF |
| | Biopsies obtained after 6-8 wk of *bid* PPI therapy or patients must have a documented negative pH study |
| | Normal biopsies in the rest of the GI tract |

EE is thought to be primarily a TH2 inflammatory process[25,61] together with a possible allergic association, and as such, diagnosis also focuses on interleukins, eotaxin and eosinophils with their degranulation products. Research is ongoing regarding the development of non-invasive markers for EE. Gupta[62] reviewed some biomarkers that could correlate with disease presence, remission, severity and response to therapy. These include serum IgE, CD23, eotaxins, IL-5, MBP, eosinophil cationic protein (ECP), eosinophil peroxidase (EPO) and eosinophil-derived neurotoxin (EDN). Baxi *et al*[63] found the presence of peripheral blood eosinophilia in 67% of EE patients. However, many of these tests are not readily available, are expensive, time consuming and, as yet, have not been recommended for routine diagnosis of EE. More research thus needs to be done to correlate these tests with disease severity and patient demographics and to establish accurate and precise laboratory investigative methods and normal values[62] before they become part of the mainstream diagnostic tool scenario.

## TREATMENT

Treatment modalities for patients with EE include pharmacological, endoscopic or dietary interventions used either singly or in combination. Endpoints of treatment are still not clear regarding whether relief of symptoms or esophageal inflammation need to be resolved. Clinicopathologically, EE involves esophageal eosinophilia and other causes of esophageal eosinophilia such as inflammatory bowel disease, parasitic infestation and GERD need to be ruled out[64].

### *Acid suppression*

The association between GERD and EE is as yet unclear. Persistent reflux disease may cause esophageal eosinophilia, or EE may lead to secondary GERD[47] or simply, they may co-exist[31]. Acid suppression with PPIs helps to exclude GERD because EE is defined by a lack of response to PPI therapy[1]. There is some controversy as to this definition since Molina-Infante *et al*[65] showed that clinical response to PPIs does not completely rule

out quiescent EE. Furthermore, pathological diagnosis of EE should be done after a patient has been on PPI therapy for at least 4 wk. An important role of the use of PPI in patients with EE is symptomatic relief because of the multilayer involvement of the esophagus and the possibility of secondary GERD[64].

### Systemic steroids

Systemic steroids are effective in managing EE. Liacouras *et al*[60] conducted a 10-year retrospective study of 381 patients diagnosed with EE and found that systemic corticosteroids significantly improved clinical symptoms and esophageal histology. Unfortunately EE recurred after withdrawal of steroids. Thus, side effects and recurrence after withdrawal limit their usage in management of EE.

### Topical steroids

Arora *et al* evaluated 21 patients with dysphagia and treated them with swallowed fluticasone. Relief of dysphagia occurred in all and symptom relief lasted at least 4 mo. Schaefer *et al*[66] compared oral prednisone and swallowed fluticasone and found no clinical advantage of prednisone over fluticasone. Symptom relief and histological improvement were observed in both treatment groups. Symptom relapse was seen in both groups upon discontinuation of therapy, thus necessitating the need for long-term maintenance protocols. Because of the higher risk of systemic side effects and the need for maintenance therapy, topical therapy may actually turn out to be a better option. Currently, there are no steroids developed specifically for EE. Aceves *et al*[67] described a case series of two children who benefited from treatment with a viscous suspension of budesonide but not with fluticasone. However, studies to determine the efficacy of different topical steroids, methods of preparation and long-term maintenance need to be performed to recommend any one steroid over another.

### Leukotriene inhibitors

Leukotrienes are eosinophil chemoattractants and hence one would expect that blocking leukotrienes may decrease eosinophilic migration and accumulation. Attwood *et al*[68] studied 12 patients who hitherto had been unresponsive to conventional therapy and started eight of them on montelukast. Six of these patients reported complete subjective improvement. Preceding this, there had been reports of an association between zafirlukast, another leukotriene inhibitor, and Churg-Strauss syndrome[69,70]. This association has not yet been seen with montelukast, but further studies are needed to determine the risks and benefits of using leukotriene inhibitors in patients with EE.

### Biologics

IL-5 is a cytokine that plays a role in eosinophil regulation[71], and as such, inhibiting IL-5 could play a role in decreasing eosinophil-mediated inflammation. Garrett *et al*[72] performed an open label trial of mepolizumab, a humanized blocking monoclonal antibody against IL-5, in four patients with hypereosinophilic syndrome, and found it to be effective and safe with steroid-sparing properties. This was later corroborated by Stein *et al*[73] and anti-IL-5 seems to be a promising new therapy in patients with EE.

### Immunomodulators

Netzer *et al*[74] evaluated three patients with corticosteroid-dependant EE and found that azathioprine and 6-mercaptopurine induced clinical and histological remission in all of them. More studies are indicated in this area, especially since treatment with immunomodulators can potentially help in decreasing the side effects associated with chronic steroid use.

### Elemental and elimination diet

Infiltration of the esophagus with eosinophils forms the hallmark of EE. Because of the close association of EE with other allergic disorders, avoidance of presumed allergens provides a rationale for the use of an elimination diet in patients with EE. An elemental diet is one in which all solid foods are replaced with a nutritionally complete elemental formula and the protein source is comprised entirely of synthetic amino acids[75], whereas an elimination diet attempts to avoid including possible food allergens in a person's daily diet. Kelly *et al*[5] studied 10 children with GERD refractory to standard medications and fed them elemental diet followed by repeat endoscopy and food challenges. Symptomatic improvement with a decrease in eosinophils was seen in all patients. Symptoms relapsed after these patients were exposed to food challenges. This pioneering work formed the basis for many follow-up trials, which reported success with elimination diets. Markowitz *et al*[6] found that patients responded symptomatically and histologically to an elemental diet. Further confirmation of the success of an elemental diet was also confirmed using more formal evaluation with skin prick and atopy patch testing[76,77]. Sugnanam *et al*[78] then analyzed prospectively the sensitization profile of food and inhalant allergens in their cohort of patients with EE, by performing skin prick and patch testing. They found that younger patients showed more IgE and patch sensitization to food allergens. Spergel *et al*[77], in their retrospective study analyzing the relation between skin prick and atopy patch testing and food elimination diet in patients with EE, found that a large number of their patient population had normalization of biopsy results on elimination and reoccurrence on reintroduction. Kagalwalla *et al*[16] used a six-food elimination diet rather than the conventional elemental diet and found it to be associated with good clinical and histological response. The major problem with an elemental diet is the lack of palatability and thus a six-food elimination diet offers the advantage of better acceptability and compliance[16]. Elemental formulae do not contain fiber, and other nutrients may not be available based on the formula used in any particular patient. In these situations, fiber supplementation may be useful, especially in children or those who are prone to constipation, and other nutrients may be provided by other foods[75]. It is also beneficial

to have a registered dietician or a nutritionist involved because elimination diets may have a significant impact on the whole family who will need to be educated on the type of food that the patient can safely eat and on balancing the daily nutritional requirements of the individual. Food reintroduction forms an important aspect of management. Spergel and Shuker, in their article on nutritional management of EE, advocate reintroducing the least allergic foods followed by the most allergic ones. Periodic endoscopies are performed to assure symptomatic and histological improvement. If symptoms reappear, then that food is avoided, but by using this approach, patients can go back to an appropriate diet acceptable to the patient and the family[75].

### *Endoscopic dilatation*

EE is characterized by eosinophilic infiltration that may extend into deeper layers of the esophagus[1] and by subsequent chronic inflammation causing tissue remodeling including subepithelial fibrosis[45]. Endoscopically, this may present as luminal narrowing, stricture formation or decreased tissue compliance[40], wherein, patients typically present with chronic dysphagia or foreign-body impaction. Food impaction is one of the commonest causes of dysphagia, and is considered an alarm symptom warranting immediate evaluation. The push technique has previously been advocated in acute esophageal food impaction[79,80]. Recent reports have suggested a prevalence of EE in at least 50% of patients with esophageal food impaction[81,82]. EE therefore is now increasingly being considered in patients presenting with the above symptoms. However, Kaplan *et al*[50] reported that tearing of the esophagus can occur even with routine passage of the endoscope, and because of this, dilatation was recommended after careful consideration only in those patients non-responsive to medical therapy and having rings obstructing the lumen. Fox in his article reported that longitudinal tearing or splitting of the mucosa is occasionally appreciated only during withdrawal of the endoscope[40], thus extreme care is warranted is selecting patients for endoscopic evaluation and dilatation. In a recent article, Straumann cautions against food bolus removal with rigid endoscopy in patients suspected of having EE, because of the high rate of perforation[52]. Other reports, however, suggest endoscopic dilatation to be a relatively safe procedure. Croese *et al*[8] found that 87% of their patients with EE had tears but none had serious complications, thus indicating dilation to be a safe intervention in patients with strictures. It will be worthwhile to conduct trials to evaluate whether the frequency of endoscopic dilations or the risk of complications with endoscopic maneuvers decrease if patients have prior medical treatment.

## CONCLUSION

Eosinophilic esophagitis is increasingly being recognized in the adult population. It can present with a variety of symptoms including dysphagia and food impaction, along with other nasal and trachea-bronchial symptoms. Long-term sequelae of EE may include secondary malnutrition, weight loss, and acute esophageal perforations. Diagnosis of EE involves clinicopathological criteria and endoscopic biopsies. Because of the absence of a single known factor involved in the pathogenesis of EE, treatment options are multiple and include acid suppression, steroids, leukotriene inhibitors, elemental and elimination diets, and endoscopic dilations. Careful selection of patients must be done before the initiation of therapy because of the inherent risks and acceptability involved in each of them.

## REFERENCES


1 **Furuta GT**, Liacouras CA, Collins MH, Gupta SK, Justinich C, Putnam PE, Bonis P, Hassall E, Straumann A, Rothenberg ME. Eosinophilic esophagitis in children and adults: a systematic review and consensus recommendations for diagnosis and treatment. *Gastroenterology* 2007; **133**: 1342-1363

2 **Rothenberg ME**. Eosinophilic gastrointestinal disorders (EGID). *J Allergy Clin Immunol* 2004; **113**: 11-28; quiz 29

3 **Dobbins JW**, Sheahan DG, Behar J. Eosinophilic gastroenteritis with esophageal involvement. *Gastroenterology* 1977; **72**: 1312-1316

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

5 **Kelly KJ**, Lazenby AJ, Rowe PC, Yardley JH, Perman JA, Sampson HA. Eosinophilic esophagitis attributed to gastroesophageal reflux: improvement with an amino acid-based formula. *Gastroenterology* 1995; **109**: 1503-1512

6 **Markowitz JE**, Spergel JM, Ruchelli E, Liacouras CA. Elemental diet is an effective treatment for eosinophilic esophagitis in children and adolescents. *Am J Gastroenterol* 2003; **98**: 777-782

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

8 **Croese J**, Fairley SK, Masson JW, Chong AK, Whitaker DA, Kanowski PA, Walker NI. Clinical and endoscopic features of eosinophilic esophagitis in adults. *Gastrointest Endosc* 2003; **58**: 516-522

9 **Potter JW**, Saeian K, Staff D, Massey BT, Komorowski RA, Shaker R, Hogan WJ. Eosinophilic esophagitis in adults: an emerging problem with unique esophageal features. *Gastrointest Endosc* 2004; **59**: 355-361

10 **Assa'ad AH**, Putnam PE, Collins MH, Akers RM, Jameson SC, Kirby CL, Buckmeier BK, Bullock JZ, Collier AR, Konikoff MR, Noel RJ, Guajardo JR, Rothenberg ME. Pediatric patients with eosinophilic esophagitis: an 8-year follow-up. *J Allergy Clin Immunol* 2007; **119**: 731-738

11 **Noel RJ**, Putnam PE, Rothenberg ME. Eosinophilic esophagitis. *N Engl J Med* 2004; **351**: 940-941

12 **Chehade M**, Sampson HA. Epidemiology and etiology of eosinophilic esophagitis. *Gastrointest Endosc Clin N Am* 2008; **18**: 33-44; viii

13 **Cherian S**, Smith NM, Forbes DA. Rapidly increasing prevalence of eosinophilic oesophagitis in Western Australia. *Arch Dis Child* 2006; **91**: 1000-1004

14 **Kapel RC**, Miller JK, Torres C, Aksoy S, Lash R, Katzka DA. Eosinophilic esophagitis: a prevalent disease in the United States that affects all age groups. *Gastroenterology* 2008; **134**: 1316-1321

15 **Byrne KR**, Panagiotakis PH, Hilden K, Thomas KL, Peterson KA, Fang JC. Retrospective analysis of esophageal food impaction: differences in etiology by age and gender.

*Dig Dis Sci* 2007; **52**: 717-721
16 **Kagalwalla AF**, Sentongo TA, Ritz S, Hess T, Nelson SP, Emerick KM, Melin-Aldana H, Li BU. Effect of six-food elimination diet on clinical and histologic outcomes in eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2006; **4**: 1097-1102
17 **Mishra A**, Hogan SP, Brandt EB, Rothenberg ME. An etiological role for aeroallergens and eosinophils in experimental esophagitis. *J Clin Invest* 2001; **107**: 83-90
18 **Roy-Ghanta S**, Larosa DF, Katzka DA. Atopic characteristics of adult patients with eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2008; **6**: 531-535
19 **Plaza-Martín AM**, Jiménez-Feijoo R, Andaluz C, Giner-Muñoz MT, Martin-Mateos MA, Piquer-Gibert M, Sierra-Martínez JI. Polysensitization to aeroallergens and food in eosinophilic esophagitis in a pediatric population. *Allergol Immunopathol* (Madr) 2007; **35**: 35-37
20 **Collins MH**, Blanchard C, Abonia JP, Kirby C, Akers R, Wang N, Putnam PE, Jameson SC, Assa'ad AH, Konikoff MR, Stringer KF, Rothenberg ME. Clinical, pathologic, and molecular characterization of familial eosinophilic esophagitis compared with sporadic cases. *Clin Gastroenterol Hepato*l 2008; **6**: 621-629
21 **Mishra A**, Hogan SP, Lee JJ, Foster PS, Rothenberg ME. Fundamental signals that regulate eosinophil homing to the gastrointestinal tract. *J Clin Invest* 1999; **103**: 1719-1727
22 **Mishra A**, Wang M, Pemmaraju VR, Collins MH, Fulkerson PC, Abonia JP, Blanchard C, Putnam PE, Rothenberg ME. Esophageal remodeling develops as a consequence of tissue specific IL-5-induced eosinophilia. *Gastroenterology* 2008; **134**: 204-214
23 **Blanchard C**, Mingler MK, Vicario M, Abonia JP, Wu YY, Lu TX, Collins MH, Putnam PE, Wells SI, Rothenberg ME. IL-13 involvement in eosinophilic esophagitis: transcriptome analysis and reversibility with glucocorticoids. *J Allergy Clin Immunol* 2007; **120**: 1292-1300
24 **Desreumaux P**, Bloget F, Seguy D, Capron M, Cortot A, Colombel JF, Janin A. Interleukin 3, granulocyte-macrophage colony-stimulating factor, and interleukin 5 in eosinophilic gastroenteritis. *Gastroenterology* 1996; **110**: 768-774
25 **Straumann A**, Bauer M, Fischer B, Blaser K, Simon HU. Idiopathic eosinophilic esophagitis is associated with a T(H)2-type allergic inflammatory response. *J Allergy Clin Immunol* 2001; **108**: 954-961
26 **Balatsinou C**, Milano A, Laterza F, Caldarella MP, Angelucci D, Vecchiet J, Zingariello P, Falasca K, Lapenna D, Neri M. Esophagitis and anticonvulsant hypersensitivity syndrome. *Endoscopy* 2006; **38**: 957
27 **Fogg MI**, Ruchelli E, Spergel JM. Pollen and eosinophilic esophagitis. *J Allergy Clin Immunol* 2003; **112**: 796-797
28 **Stevoff C**, Rao S, Parsons W, Kahrilas PJ, Hirano I. EUS and histopathologic correlates in eosinophilic esophagitis. *Gastrointest Endosc* 2001; **54**: 373-377
29 **Nurko S**, Rosen R. Esophageal dysmotility in patients who have eosinophilic esophagitis. *Gastrointest Endosc Clin N Am* 2008; **18**: 73-89; ix
30 **Straumann A**, Spichtin HP, Grize L, Bucher KA, Beglinger C, Simon HU. Natural history of primary eosinophilic esophagitis: a follow-up of 30 adult patients for up to 11.5 years. *Gastroenterology* 2003; **125**: 1660-1669
31 **Remedios M**, Campbell C, Jones DM, Kerlin P. Eosinophilic esophagitis in adults: clinical, endoscopic, histologic findings, and response to treatment with fluticasone propionate. *Gastrointest Endosc* 2006; **63**: 3-12
32 **Lucendo AJ**, Castillo P, Martín-Chávarri S, Carrión G, Pajares R, Pascual JM, Manceñido N, Erdozain JC. Manometric findings in adult eosinophilic oesophagitis: a study of 12 cases. *Eur J Gastroenterol Hepatol* 2007; **19**: 417-424
33 **Katzka DA**. Demographic data and symptoms of eosinophilic esophagitis in adults. *Gastrointest Endosc Clin N Am* 2008; **18**: 25-32; viii
34 **Dauer EH**, Freese DK, El-Youssef M, Thompson DM. Clinical characteristics of eosinophilic esophagitis in children. *Ann Otol Rhinol Laryngol* 2005; **114**: 827-833
35 **Orenstein SR**, Shalaby TM, Di Lorenzo C, Putnam PE, Sigurdsson L, Mousa H, Kocoshis SA. The spectrum of pediatric eosinophilic esophagitis beyond infancy: a clinical series of 30 children. *Am J Gastroenterol* 2000; **95**: 1422-1430
36 **Thompson DM**, Orvidas LJ. Otorhinolaryngologic manifestations of eosinophilic esophagitis. *Gastrointest Endosc Clin N Am* 2008; **18**: 91-98; ix
37 **Ferguson DD**, Foxx-Orenstein AE. Eosinophilic esophagitis: an update. *Dis Esophagus* 2007; **20**: 2-8
38 **Straumann A**, Spichtin HP, Bucher KA, Heer P, Simon HU. Eosinophilic esophagitis: red on microscopy, white on endoscopy. *Digestion* 2004; **70**: 109-116
39 **Müller S**, Pühl S, Vieth M, Stolte M. Analysis of symptoms and endoscopic findings in 117 patients with histological diagnoses of eosinophilic esophagitis. *Endoscopy* 2007; **39**: 339-344
40 **Fox VL**. Eosinophilic esophagitis: endoscopic findings. *Gastrointest Endosc Clin N Am* 2008; **18**: 45-57; viii
41 **Nurko S**, Teitelbaum JE, Husain K, Buonomo C, Fox VL, Antonioli D, Fortunato C, Badizadegan K, Furuta GT. Association of Schatzki ring with eosinophilic esophagitis in children. *J Pediatr Gastroenterol Nutr* 2004; **38**: 436-441
42 **Vasilopoulos S**, Shaker R. Defiant dysphagia: small-caliber esophagus and refractory benign esophageal strictures. *Curr Gastroenterol Rep* 2001; **3**: 225-230
43 **Collins MH**. Histopathologic features of eosinophilic esophagitis. *Gastrointest Endosc Clin N Am* 2008; **18**: 59-71; viii-ix
44 **Cheung KM**, Oliver MR, Cameron DJ, Catto-Smith AG, Chow CW. Esophageal eosinophilia in children with dysphagia. *J Pediatr Gastroenterol Nutr* 2003; **37**: 498-503
45 **Aceves SS**, Newbury RO, Dohil R, Bastian JF, Broide DH. Esophageal remodeling in pediatric eosinophilic esophagitis. *J Allergy Clin Immunol* 2007; **119**: 206-212
46 **Lucendo AJ**, Navarro M, Comas C, Pascual JM, Burgos E, Santamaría L, Larrauri J. Immunophenotypic characterization and quantification of the epithelial inflammatory infiltrate in eosinophilic esophagitis through stereology: an analysis of the cellular mechanisms of the disease and the immunologic capacity of the esophagus. *Am J Surg Pathol* 2007; **31**: 598-606
47 **Straumann A**. The natural history and complications of eosinophilic esophagitis. *Gastrointest Endosc Clin N Am* 2008; **18**: 99-118; ix
48 **Langdon DE**. "Congenital" esophageal stenosis, corrugated ringed esophagus, and eosinophilic esophagitis. *Am J Gastroenterol* 2000; **95**: 2123-2124
49 **Langdon DE**. Corrugated ringed and too small esophagi. *Am J Gastroenterol* 1999; **94**: 542-543
50 **Kaplan M**, Mutlu EA, Jakate S, Bruninga K, Losurdo J, Losurdo J, Keshavarzian A. Endoscopy in eosinophilic esophagitis: "feline" esophagus and perforation risk. *Clin Gastroenterol Hepatol* 2003; **1**: 433-437
51 **Vasilopoulos S**, Murphy P, Auerbach A, Massey BT, Shaker R, Stewart E, Komorowski RA, Hogan WJ. The small-caliber esophagus: an unappreciated cause of dysphagia for solids in patients with eosinophilic esophagitis. *Gastrointest Endosc* 2002; **55**: 99-106
52 **Straumann A**, Bussmann C, Zuber M, Vannini S, Simon HU, Schoepfer A. Eosinophilic esophagitis: analysis of food impaction and perforation in 251 adolescent and adult patients. *Clin Gastroenterol Hepatol* 2008; **6**: 598-600
53 **Liguori G**, Cortale M, Cimino F, Sozzi M. Circumferential mucosal dissection and esophageal perforation in a patient with eosinophilic esophagitis. *World J Gastroenterol* 2008; **14**: 803-804
54 **Straumann A**, Rossi L, Simon HU, Heer P, Spichtin HP, Beglinger C. Fragility of the esophageal mucosa: a pathognomonic endoscopic sign of primary eosinophilic esophagitis? *Gastrointest Endosc* 2003; **57**: 407-412
55 **Cohen MS**, Kaufman AB, Palazzo JP, Nevin D, Dimarino AJ

Jr, Cohen S. An audit of endoscopic complications in adult eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2007; **5**: 1149-1153

56 **Eisenbach C**, Merle U, Schirmacher P, Hansmann J, Stiehl A, Stremmel W, Kulaksiz H. Perforation of the esophagus after dilation treatment for dysphagia in a patient with eosinophilic esophagitis. *Endoscopy* 2006; **38** Suppl 2: E43-E44

57 **Gonsalves N**. Eosinophilic esophagitis: history, nomenclature, and diagnostic guidelines. *Gastrointest Endosc Clin N Am* 2008; **18**: 1-9; vii

58 **Gonsalves N**, Policarpio-Nicolas M, Zhang Q, Rao MS, Hirano I. Histopathologic variability and endoscopic correlates in adults with eosinophilic esophagitis. *Gastrointest Endosc* 2006; **64**: 313-319

59 **Prasad GA**, Talley NJ, Romero Y, Arora AS, Kryzer LA, Smyrk TC, Alexander JA. Prevalence and predictive factors of eosinophilic esophagitis in patients presenting with dysphagia: a prospective study. *Am J Gastroenterol* 2007; **102**: 2627-2632

60 **Liacouras CA**, Spergel JM, Ruchelli E, Verma R, Mascarenhas M, Semeao E, Flick J, Kelly J, Brown-Whitehorn T, Mamula P, Markowitz JE. Eosinophilic esophagitis: a 10-year experience in 381 children. *Clin Gastroenterol Hepatol* 2005; **3**: 1198-1206

61 **Furuta GT**, Straumann A. Review article: the pathogenesis and management of eosinophilic oesophagitis. *Aliment Pharmacol Ther* 2006; **24**: 173-182

62 **Gupta SK**. Noninvasive markers of eosinophilic esophagitis. *Gastrointest Endosc Clin N Am* 2008; **18**: 157-167; xi

63 **Baxi S**, Gupta SK, Swigonski N, Fitzgerald JF. Clinical presentation of patients with eosinophilic inflammation of the esophagus. *Gastrointest Endosc* 2006; **64**: 473-478

64 **Liacouras CA**. Pharmacologic treatment of eosinophilic esophagitis. *Gastrointest Endosc Clin N Am* 2008; **18**: 169-178; xi

65 **Molina-Infante J**, Ferrando-Lamana L, Mateos-Rodríguez JM, Pérez-Gallardo B, Prieto-Bermejo AB. Overlap of reflux and eosinophilic esophagitis in two patients requiring different therapies: a review of the literature. *World J Gastroenterol* 2008; **14**: 1463-1466

66 **Schaefer ET**, Fitzgerald JF, Molleston JP, Croffie JM, Pfefferkorn MD, Corkins MR, Lim JD, Steiner SJ, Gupta SK. Comparison of oral prednisone and topical fluticasone in the treatment of eosinophilic esophagitis: a randomized trial in children. *Clin Gastroenterol Hepatol* 2008; **6**: 165-173

67 **Aceves SS**, Dohil R, Newbury RO, Bastian JF. Topical viscous budesonide suspension for treatment of eosinophilic esophagitis. *J Allergy Clin Immunol* 2005; **116**: 705-706

68 **Attwood SE**, Lewis CJ, Bronder CS, Morris CD, Armstrong GR, Whittam J. Eosinophilic oesophagitis: a novel treatment using Montelukast. *Gut* 2003; **52**: 181-185

69 **Wechsler ME**, Garpestad E, Flier SR, Kocher O, Weiland DA, Polito AJ, Klinek MM, Bigby TD, Wong GA, Helmers RA, Drazen JM. Pulmonary infiltrates, eosinophilia, and cardiomyopathy following corticosteroid withdrawal in patients with asthma receiving zafirlukast. *JAMA* 1998; **279**: 455-457

70 **Churg J**, Churg A. Zafirlukast and Churg-Strauss syndrome. *JAMA* 1998; **279**: 1949-1950

71 **Gleich GJ**. Mechanisms of eosinophil-associated inflammation. *J Allergy Clin Immunol* 2000; **105**: 651-663

72 **Garrett JK**, Jameson SC, Thomson B, Collins MH, Wagoner LE, Freese DK, Beck LA, Boyce JA, Filipovich AH, Villanueva JM, Sutton SA, Assa'ad AH, Rothenberg ME. Anti-interleukin-5 (mepolizumab) therapy for hypereosinophilic syndromes. *J Allergy Clin Immunol* 2004; **113**: 115-119

73 **Stein ML**, Collins MH, Villanueva JM, Kushner JP, Putnam PE, Buckmeier BK, Filipovich AH, Assa'ad AH, Rothenberg ME. Anti-IL-5 (mepolizumab) therapy for eosinophilic esophagitis. *J Allergy Clin Immunol* 2006; **118**: 1312-1319

74 **Netzer P**, Gschossmann JM, Straumann A, Sendensky A, Weimann R, Schoepfer AM. Corticosteroid-dependent eosinophilic oesophagitis: azathioprine and 6-mercaptopurine can induce and maintain long-term remission. *Eur J Gastroenterol Hepatol* 2007; **19**: 865-869

75 **Spergel JM**, Shuker M. Nutritional management of eosinophilic esophagitis. *Gastrointest Endosc Clin N Am* 2008; **18**: 179-194; xi

76 **Spergel JM**, Beausoleil JL, Mascarenhas M, Liacouras CA. The use of skin prick tests and patch tests to identify causative foods in eosinophilic esophagitis. *J Allergy Clin Immunol* 2002; **109**: 363-368

77 **Spergel JM**, Andrews T, Brown-Whitehorn TF, Beausoleil JL, Liacouras CA. Treatment of eosinophilic esophagitis with specific food elimination diet directed by a combination of skin prick and patch tests. *Ann Allergy Asthma Immunol* 2005; **95**: 336-343

78 **Sugnanam KK**, Collins JT, Smith PK, Connor F, Lewindon P, Cleghorn G, Withers G. Dichotomy of food and inhalant allergen sensitization in eosinophilic esophagitis. *Allergy* 2007; **62**: 1257-1260

79 **Longstreth GF**, Longstreth KJ, Yao JF. Esophageal food impaction: epidemiology and therapy. A retrospective, observational study. *Gastrointest Endosc* 2001; **53**: 193-198

80 **Vicari JJ**, Johanson JF, Frakes JT. Outcomes of acute esophageal food impaction: success of the push technique. *Gastrointest Endosc* 2001; **53**: 178-181

81 **Desai TK**, Stecevic V, Chang CH, Goldstein NS, Badizadegan K, Furuta GT. Association of eosinophilic inflammation with esophageal food impaction in adults. *Gastrointest Endosc* 2005; **61**: 795-801

82 **Kerlin P**, Jones D, Remedios M, Campbell C. Prevalence of eosinophilic esophagitis in adults with food bolus obstruction of the esophagus. *J Clin Gastroenterol* 2007; **41**: 356-361

**S- Editor** Tian L **L- Editor** Logan S **E- Editor** Zheng XM

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.25

*World J Gastroenterol* 2009 January 7; 15(1): 25-37
***World Journal of Gastroenterology*** ISSN 1007-9327




**Dr. Peter V Draganov,** *Series Editor*

# Gastroparesis: Current diagnostic challenges and management considerations

Shamaila Waseem, Baharak Moshiree, Peter V Draganov

Shamaila Waseem, Department of Pediatric Gastroenterology, Hepatology and Nutrition, University of Florida, Gainesville, Florida 32610, United States
Baharak Moshiree, Peter V Draganov, Department of Gastroenterology, Hepatology and Nutrition, University of Florida, Gainesville, Florida 32610, United States
Author contributions: Waseem S, Moshiree B and Draganov PV designed the format; Waseem S performed the literature search and wrote the first draft of the paper; Moshiree B and Draganov PV contributed new articles to the literature search and reviewed the article.
Correspondence to: Peter V Draganov, MD, Department of Gastroenterology, Hepatology and Nutrition, University of Florida, 1600 SW Archer Rd, Room HD 602, PO Box 100214 Gainesville, Florida 32610,
United States. dragapv@medicine.ufl.edu
Telephone: +1-352-3922877 Fax: +1-352-3923618
Received: August 5, 2008 Revised: November 25, 2008
Accepted: December 2, 2008
Published online: January 7, 2009

## Abstract

Gastroparesis refers to abnormal gastric motility characterized by delayed gastric emptying in the absence of mechanical obstruction. The most common etiologies include diabetes, post-surgical and idiopathic. The most common symptoms are nausea, vomiting and epigastric pain. Gastroparesis is estimated to affect 4% of the population and symptomatology may range from little effect on daily activity to severe disability and frequent hospitalizations. The gold standard of diagnosis is solid meal gastric scintigraphy. Treatment is multimodal and includes dietary modification, prokinetic and anti-emetic medications, and surgical interventions. New advances in drug therapy, and gastric electrical stimulation techniques have been introduced and might provide new hope to patients with refractory gastroparesis. In this comprehensive review, we discuss gastroparesis with emphasis on the latest developments; from the perspective of the practicing clinician.



**Key words:** Gastroparesis, Nausea; Vomiting; Prokinetic; Therapy

**Peer reviewer:** Andrew Ukleja, MD, Assistant Professor, Clinical Assistant Professor of Medicine, Director of Nutrition Support Team, Director of Esophageal Motility Laboratory, Cleveland Clinic Florida, Department of Gastroenterology, 2950 Cleveland Clinic Blvd., Weston, FL 33331, United States

Waseem S, Moshiree B, Draganov PV. Gastroparesis: Current diagnostic challenges and management considerations. *World J Gastroenterol* 2009; 15(1): 25-37 Available from: URL: http://www.wjgnet.com/1007-9327/15/25.asp DOI: http://dx.doi.org/10.3748/wjg.15.25

## INTRODUCTION

Gastroparesis is a condition of abnormal gastric motility characterized by delayed gastric emptying in the absence of mechanical outlet obstruction. The true prevalence of gastroparesis is unknown; however, it has been estimated that up to 4% of the adult population experiences symptomatic manifestations of this condition. A large study on long-term outcomes of gastroparetic adults revealed that 82% of patients were female[1]. Gastroparesis has a higher prevalence in the patient population of tertiary medical centers than in the community hospital setting. Moreover, a widely available diagnostic test that could be applied in a standard fashion is currently lacking in the primary care setting.

## PATHOPHYSIOLOGY

Gastric motility results from the integration of tonic contractions of the fundus, phasic contractions of the antrum, and inhibitory forces of pyloric and duodenal contractions[2]. These contractions require a complex interaction between gastric smooth muscle, the enteric nervous system and specialized pacemaker cells, the interstitial cells of Cajal (ICC)[3]. Motor dysfunction of the stomach may result from autonomic neuropathy, enteric neuropathy, abnormalities of ICCs, fluctuations in blood glucose and psychosomatic factors[4-6].

The etiology of gastroparesis is multifactorial (Table 1). The three most common etiologies are diabetes, idiopathic, and post-surgical, especially if the vagus nerve is damaged. Other causes include medication, Parkinson's disease, collagen vascular disorders, thyroid dysfunction, liver disease, chronic renal insufficiency, intestinal pseudo-obstruction and

**Table 1 Causes of gastroparesis**

| Causes | |
|---|---|
| Idiopathic | Medications: opiates, anticholinergics, β-adrenergics, Ca-channel blockers, glucagon, THC, alcohol, tobacco, *etc* |
| Surgical causes | Vagotomy and gastric resection/drainage<br>Fundoplication, esophagectomy<br>Gastric bypass surgery<br>Whipple procedure<br>Heart/lung transplant |
| Infections | Viruses-EBV, varicella, parvovirus-like<br>Chagas disease<br>*Clostridium Botulinum* |
| Central nervous system disorders | Cerebrovascular accidents/trauma<br>Tumors<br>Labyrinthine disorders<br>Seizures |
| Peripheral nervous system disorders | Parkinson's disease<br>Guillain-Barre<br>Multiple sclerosis<br>Dysautonomias |
| Neuropsychiatric disorders | Anorexia nervosa/bulimia<br>Rumination syndrome |
| Rheumatologic disease | Scleroderma<br>Systemic lupus erythematosus<br>Polymyositis/dermatomyositis |
| Endocrine and metabolism diseases | Diabetes<br>Hypothyroidism<br>Parathyroid disease<br>Electrolyte disorders<br>Renal failure<br>Pregnancy<br>Neoplastic(para)-breast, small cell lung, pancreas |
| Misc. neuromuscular diseases | Amyloidosis<br>Chronic intestinal pseudoobstruction<br>Myotonic dystrophy |

**Table 2 Proposed classification of gastroparesis severity**

| Classification | |
|---|---|
| Grade 1: Mild gastroparesis | Symptoms relatively easily controlled<br>Able to maintain weight and nutrition on a regular diet or minor dietary modifications |
| Grade 2: Compensated gastroparesis | Moderate symptoms with partial control with pharmacological agents<br>Able to maintain nutrition with dietary and lifestyle adjustments<br>Rare hospital admissions |
| Grade 3: Severe gastroparesis | Refractory symptoms despite medical therapy<br>Inability to maintain nutrition *via* oral route<br>Frequent emergency room visits or hospitalizations |

miscellaneous[1,7].

Originating in the region of ICCs, electrical activity in the form of gastric slow waves sweeps across the stomach toward the pylorus. However, these slow waves do not directly result in contraction of the gastric smooth muscle, but instead cause a simultaneous release of neurotransmitters from the enteric nerve endings, leading to smooth muscle contraction. Although neurohumoral control of gastric emptying is incompletely understood, both motilin and ghrelin are peptides secreted by the gastrointestinal endocrine cells that have been shown to increase gastric motor function[8,9].

In general, several factors affect gastric motility. These include motor dysfunction i.e. hypomotility and pyloric spasm, sensory dysfunction (such as impaired fundic relaxation, accommodation and abnormal sensation), electrical dysfunction (such as gastric arrhythmias and abnormal propagation), CNS effects resulting in nausea and vomiting, and others such as bacterial overgrowth, visceral hyperalgesia and gastrointestinal hormones.

## SYMPTOMS AND EVALUATION

Gastroparesis is diagnosed by the presence of delayed gastric emptying in a symptomatic patient after other potential etiologies such as ulcer disease, mechanical obstruction, gastric cancer or other malignancies are excluded[10,11]. Symptoms of gastroparesis include nausea, vomiting, early satiety, bloating, post-prandial fullness, abdominal pain, weight loss and/or weight gain. These symptoms are non-specific and may mimic other disorders[10]. A simple severity grading scale has been proposed for stratification of symptoms[12] (Table 2). Also, a patient-based symptom instrument, the gastroparesis cardinal symptom index (GCSI) has been developed to assess severity of gastroparesis[13]. The GCSI total scores are based on three subscales of nausea/vomiting, post-prandial fullness/early satiety, and bloating. The GCSI scale is used to rate symptom change by either physicians or by the patient's own self-evaluations. In 146 patients with gastroparesis, nausea was present in 92%, vomiting in 84%, abdominal bloating in 75%, and early satiety in 60%. Abdominal pain or discomfort was present in 46%-89% of patients but was not the predominant symptom[1]. Abdominal pain in gastroparesis responds poorly to treatment[14]. Constipation may also be associated with gastroparesis. Treatment of constipation with an osmotic laxative has shown to improve dyspeptic symptoms as well as gastric emptying delay[15]. Complications of gastroparesis include esophagitis, Mallory-Weiss tear from chronic nausea/vomiting, malnutrition, volume depletion with acute renal failure (secondarily), electrolyte disturbances and bezoar formation[16,17].

## DIAGNOSTIC TESTS

### *Radiographic tests*

**Gastric scintigraphy**: Gastric emptying scintigraphy of a radiolabeled solid meal is the gold standard for the diagnosis of gastroparesis. This test provides a physiological, non-invasive and quantitative measure of gastric emptying. Measurement of emptying of solids is more sensitive by scintigraphy. This is due to the fact that liquid emptying may remain normal despite advanced disease. A variety of foods including chicken, liver, eggs, egg whites, oatmeal, or pancakes are used as meals. The content of the meal is one of the most important

variables in gastric emptying. Solids versus liquids, indigestible residue, fat content, calories and volume of the test meal, can all alter gastric emptying time. Consensus recommendations for a standardized gastric emptying procedure have recommended a universally acceptable 99-m technetium sulfur-colloid labeled low fat, egg-white meal[18]. Medications that alter gastric emptying may be discontinued 48-72 h in advance, blood glucose in diabetics should be < 275 mg/dL on the day of the test and scinti-scanning at a minimum of 1, 2 and 4 h after test meal ingestion is performed in the upright position. This periodic measurement of radiolabeled solid meal has a specificity of 62% and a sensitivity of 93% when compared to continuous scinti-scanning[19]. Emptying of solids exhibits a lag phase followed by a prolonged linear emptying phase. The results of this test can be reported in two ways. The simplest approach is to report percent retention at defined times (minimum 1, 2, and 4 h). Half-times ($T_{½}$ values) may also be calculated but may potentially be less accurate, particularly in patients with very long emptying for whom extrapolation is needed to calculate the half-time if it was not actually reached during the test. Retention of over 10% of the solid meal after 4 h is abnormal. A grading of severity based on 4 h values might be used: grade 1 (mild), 11%-20% retention at 4 h; grade 2 (moderate), 21%-35% retention at 4 h; grade 3 (severe), 36%-50% retention at 4 h; and grade 4 (very severe), > 50% retention at 4 h[18]. Prokinetics may also be administered intravenously after the last measurement (i.e. 4 h) to evaluate if the patient is a "responder" or "non-responder" to the agent. Again, percent retained or extrapolated $T_{½}$ times can be calculated to assess the response. The drawbacks of the test include lack of standardization in different academic institutions, despite the current consensus recommendations, and radiation exposure, which is equivalent to about 1/3 of the average annual radiation exposure in the US from natural sources.

**Radiopaque markers:** After ingestion of indigestible markers, i.e. 10 small pieces of nasogastric tubing, none of the markers should remain in the stomach on an X-ray taken 6 h after ingestion with a meal[20]. This simple test correlates with clinical gastroparesis and is readily available and inexpensive. The drawbacks of the test include lack of standardization of the meal and size of markers and difficultly of determining if the markers are located in the stomach or other regions that overlap with the stomach (e.g. proximal small bowel, transverse colon).

**Ultrasonography:** Transabdominal ultrasound has been used to measure emptying of a liquid meal by serially evaluating cross-sectional changes in the volume remaining in the gastric antrum over time[21-23]. Emptying is considered complete when the antral area/volume returns to the fasting baseline. Some studies have revealed gastric emptying measurements similar to those seen with scintigraphy[24]. Three-dimensional ultrasound is a newly-developed technique that has recently been reported to be useful in determining stomach function[25,26] and duplex sonography can quantify transpyloric flow of liquid gastric contents. These techniques may be preferred over scintigraphy in patients such as pregnant women or children, in order to minimize radiation exposure. Drawbacks of the test include the fact that it is somewhat operator dependent, has proven reliable only for measurements of liquid emptying rates[24], and is less reliable when the patient is obese or when excessive gastric air is present. Moreover, liquid emptying is rarely impaired in patients with severe gastroparesis.

**Magnetic resonance imaging:** MRI using gadolinium has been found to accurately measure semi-solid gastric emptying and accommodation using sequential transaxial abdominal scans[27]. MRI provides excellent special resolution with a high sensitivity. It is also non-invasive and radiation free. Antral propagation waves can be observed and their velocity calculated. In gastroparesis, a significant reduction is seen in the velocity of these waves[28]. MRI can also differentiate gastric meal volume and total gastric volume, allowing gastric secretory rates to be calculated. New rapid techniques allow careful measurements of wall motion in both the proximal and distal stomach during emptying, and solid markers now permit measurement of solid meal emptying[29,30]. The drawback of this test is the expense and lack of availability.

**Single-photon emission CT:** This technique uses intravenously administered 99-Tc pertechnetate that accumulates within the gastric wall rather than the lumen and provides a three-dimensional outline of the stomach[31]. Measurement of regional gastric volumes in real-time to assess fundic accommodation and intragastric distribution can be made. The drawback of this test is the need of large radiation doses, and wide unavailability.

### *Stable isotope breath tests*

The non-invasive 13-C-labeled octanoate breath test is an indirect means of measuring gastric emptying. It is a medium chain triglyceride which is bound to a solid meal such as a muffin. After ingestion and stomach emptying, 13-C octanoate is rapidly absorbed in the small intestine and metabolized to 13 $CO_2$ which is expelled from the lungs during expiration. The rate limiting step for the signal appearing in the breath is the rate of gastric emptying. Compared to detailed scintigraphy done over a period of 4 h, the breath test has a specificity of 80% and sensitivity of 86%[32]. The test assumes normal small bowel, pancreas, liver and pulmonary functions. Some studies have demonstrated a strong correlation between the carbon-labeled breath test and gastric scintigraphy[33,34]. The drawback of this test is the need for normal small intestinal absorption, liver metabolism, and pulmonary excretion to validate the test results.

### *Swallowed capsule telemetry*

The ingestible "SmartPill®" (VA Boston Healthcare

System, MA, USA), or telemetry capsule, offers a promising new non-radioactive method for assessing gastric emptying. This capsule measures pH, pressure and temperature using miniaturized wireless sensor technology. This has been developed for ambulatory assessment of GI transit. The time taken for the pill to be expelled from the stomach into the duodenum is measured by monitoring the time point at which the acid readings of the stomach are replaced by the dramatic increase in pH as the capsule enters the duodenum. It has been shown that gastric transit time calculated using the SmartPill correlates well with gastric scintigraphy with good sensitivity (82%) and specificity (83%)[35]. The frequencies and amplitudes of antral contractions can be used to calculate motility indices. A current drawback is the cost of the pill and lack of widespread availability.

### *Antroduodenal manometry*

In antroduodenal manometry, a water-perfused or solid-state manometric catheter is passed from the nares or mouth and placed fluoroscopically into the stomach and small bowel to measure actual gastroduodenal contractile activity. The frequency and amplitude of fasting, interdigestive and post-prandial contractions can be recorded, and the response to prokinetic agents can be assessed. Distinct patterns characterize the fasting and fed phases. During the fasting period, three cyclical phases known as migrating motor complex (MMC) recur approximately every 2 h: Phase Ⅰ, Phase Ⅱ and Phase Ⅲ. Phase Ⅰ is a period of motor quiescence followed by Phase Ⅱ, a period of intermittent phasic contractions. Phase Ⅲ, considered the "intestinal housekeeper", consists of an integrated peristaltic wave, initiated in the antrum, that sweeps indigestible solids from the stomach into the duodenum and beyond. Feeding disrupts the MMC and replaces it with a fed motor pattern of more regular antral contractions of variable amplitude that are either segmental or propulsive in character.

Gastroparesis is characterized by loss of normal fasting MMC's and reduced postprandial antral contractions and, in some cases pylorospasm[36]. Small intestinal motor dysfunction is detected in 17%-85% of patients with gastroparesis[37]. Manometry can also distinguish between myopathic and neuropathic small intestinal dysmotilities. However only in approximately 20%-25% of patients diagnosed with dysmotility syndromes by antroduodenal manometry, is clinical management influenced[38]. Antroduodenal manometry is usually reserved for the refractory gastroparesis patient evaluated at tertiary referral centers with the benefit of provocative testing to assess manometric response to treatment[39]. Drawbacks are that it is an invasive procedure, it needs motility expertise to perform and interpret the results, giving rise to problems with over interpretation in the unskilled hands.

### *Electrogastrography (EGG)*

EGG measures gastric slow-wave myoelectrical activity *via* serosal, mucosal or cutaneous electrodes. It is most conveniently recorded with cutaneous electrodes positioned along the long axis of the stomach. Initially a pre-prandial recording for 45-60 min is captured. Patients are given a 500 kcal cheese or turkey sandwich and an equivalent postprandial recording is captured. The recorded signals are amplified and filtered to exclude contamination by noise from cardiorespiratory activity and patient movement. Computer analysis converts raw EGG signals to a three-dimensional plot. In healthy persons, EGG recordings exhibit uniform waveforms of 3 cycles/min, which increase in amplitude after ingestion of a meal. Abnormality of EGG is defined by rhythm disruption of more than 30% of the recording time including tachygastria (frequency of > 4 cycles/min) and bradygastria (< 2 cycles/min) and a lack of signal amplitude with eating[40]. EGG abnormalities are present in 75% of patients with gastroparesis[40]. EGG is considered by some authors as more of an adjunct to gastric emptying measurement for a comprehensive evaluation of patients with refractory symptoms[40]. Drawbacks are the little documented utility of EGG in the management of patients with suspected gastric dysmotility and movement artifacts that make recordings difficult to interpret.

### *Other tests*

The gastric barostat test consists of a high compliance balloon device placed into the stomach to measure pressure-volume relationships and visceral sensation[41]. The drawback of this test is that it is invasive and is used therefore only as a research tool in a few tertiary centers.

The satiety test involves ingestion of water or a liquid nutrient until the patient reports maximal fullness. This test is not frequently performed and its main drawback is that results are subjective.

A common misconception is the use of barium upper gastrointestinal testing in the diagnosis of gastroparesis. Although this test can be used to evaluate anatomic abnormalities such as gastric outlet obstruction, it is not a functional study for the diagnosis of gastroparesis and other lesions such as malignancy may still be missed.

## TREATMENT

The general principles of treatment of symptomatic gastroparesis are to: (1) correct fluid, electrolyte, and nutritional deficiencies; (2) identify and rectify the underlying cause of gastroparesis if possible; and (3) reduce symptoms[12,42].

In addition, patient education and explanation of the condition is an integral part of treatment. The disabling chronic symptoms of gastroparesis impact profoundly on the patient's sense of wellbeing, mental state, behavior and social life. Sensitive caring from the clinical team and professional counseling might be necessary to help the patient cope with the disability. Patients should be informed that a number of drugs might be tried in an attempt to discover the optimal therapeutic regimen and that the aim of treatment is to control rather than cure the disorder[43].

The patient's drug list should be reviewed to eliminate drugs that can cause gastric dysmotility. Management can be tailored to the severity of the gastroparesis. For grade 1 (mild) gastroparesis, dietary modifications should be tried. Low doses of antiemetic or prokinetic medications can be taken on an as-needed basis. Grade 2 (compensated) gastroparesis is treated by combination of antiemetic and prokinetic medications given at scheduled regular intervals. These agents relieve the more chronic symptoms of nausea, vomiting, early satiety and bloating. They frequently have no effect on abdominal pain. In grade 3 (severe) gastroparesis or gastric failure, more aggressive treatments including hospitalizations for i.v. hydration and medications, enteral or parenteral nutritional support and endoscopic or surgical therapy may be needed[12].

### *Dietary manipulation*

Dietary recommendations rely on measures that promote gastric emptying or, at least theoretically, do not retard gastric emptying. At the outset, it is advisable to introduce an experienced dietician who can discuss and explore the patient's tolerance of solids, semi-solids and liquids, as well as dietary balance, meal size and timing of meals. Fats and fiber tend to retard emptying, thus their intake should be minimized. This should be stressed as many of these patients who often concomitantly also have constipation, have been told to take fiber supplementation for treatment of their constipation. Multiple small low fat meals about four or five times each day should be recommended. Carbonated liquids should be avoided to limit gastric distention. Patients are instructed to take fluids throughout the course of the meal and to sit or walk for 1-2 h after meals. If the above measures are ineffective, the patient may be advised to consume the bulk of their calories as liquid since liquid emptying is often preserved in patients with gastroparesis. Poor tolerance of a liquid diet is predictive of a future poor success[12].

### *Correction of glycemic control*

Patients with diabetes should be counseled to achieve optimal glycemic control. Hyperglycemia itself delays gastric emptying, even in the absence of neuropathy or myopathy, which is likely to be mediated by reduced phasic antral contractility and induction of pyloric pressure waves. Hyperglycemia can inhibit the accelerating effects of prokinetic agents[44]. Measures more likely to be effective include more aggressive glucose monitoring, with frequent dosing of short acting insulin preparations to prevent post-prandial hyperglycemia. Prevention of wide fluctuations of hyperglycemia may be more important than maintenance of a given steady-state blood glucose level[45]. Improvement of glucose control increases antral contractility, corrects gastric dysrhythmias and accelerates emptying.

### *Pharmacological therapy*

The pharmacotherapy of gastroparesis is stepwise, incremental and long term. The most commonly used drug classes include pro-motility and anti-emetic agents. There has been little in the way of randomized controlled investigations directly comparing the different agents. Consequently, a selection of drugs is used by trial and error.

**Prokinetic agents**: Prokinetic medications enhance the contractility of the GI tract, correct gastric dysrhythmias, and promote the movement of luminal contents in the antegrade direction. Prokinetics may improve predominantly symptoms of nausea, vomiting and bloating. They do not seem to relieve abdominal pain and early satiety associated with gastroparesis. They should be administered 30 min before meals to elicit maximal clinical effects. Bedtime doses are often added to facilitate nocturnal gastric emptying of indigestible solids. The response to treatment is usually judged clinically rather than with serial gastric emptying tests because symptom improvements correlate poorly with the acceleration of gastric emptying[46]. A meta-analysis assessing benefits of four different drugs in 514 patients in 36 clinical trials reported that the macrolide antibiotic erythromycin is the most potent stimulant of gastric emptying, while erythromycin and the dopamine receptor antagonist, domperidone, are best at reducing symptoms of gastroparesis[47]. Several factors must be considered when choosing a prokinetic drug for patients with gastroparesis, including efficacy, toxicity, regional availability and cost.

**( I ) Erythromycin.** Erythromycin is a macrolide antibiotic that is also a motilin receptor agonist[48]. The intravenous form is the most potent stimulant of solid and liquid gastric emptying[49,50]. Motilin is a polypeptide hormone present in the distal stomach and duodenum that increases lower esophageal sphincter pressure and is responsible for initiating the MMC in the antrum of the stomach[51,52]. Erythromycin binds to motilin receptors and hence increases the amplitude of antral peristalsis, triggers premature MMC phase Ⅲ activity, and stimulates gastric emptying[53]. Interestingly, erythromycin has also been shown to accelerate emptying in post-vagotomy and antrectomy patients[54]. This may be due to its stimulatory effects on the fundus.

Erythromycin should be started at a low dose (200 mg per 5 mL) and is most rapidly absorbed when administered as a suspension[55]. However, tachyphylaxis develops in patients on chronic erythromycin therapy, due to down-regulation of motilin receptors which can develop as early as a few days of initiating therapy[53]. If tachyphylaxis develops, erythromycin can be discontinued for 2 wk and then restarted again. Intravenous erythromycin is used occasionally for inpatients with severe refractory gastroparesis[55]. Common side effects include skin rashes, nausea, cramping and abdominal pain. A large cohort reported that erythromycin increases the risk of sudden cardiac arrest by 2.01 times when compared to control population[56]. The risk for death was further increased in those patients who also were on CYP3A (cytochrome P-450 3A) inhibitors such as selected antipsychotics, cardiac antiarrythmics, antifungals, calcium antagonists,

antidepressants, and anti-emetics. Therefore, prior to initiating EES therapy for treatment of gastroparesis, all these factors need to be considered. Although this has not undergone formal testing, in our institution, a QTc of 450 ms in men and 460 ms in women has been used as the cut-off value over which EES is not administered due to risk of QT prolongation.

**(Ⅱ) Metoclopramide.** Metoclopramide is a substituted benzamide with several prokinetic actions, which include combined serotonin 5-hydroxytryptamine (HT) 4 receptor agonism, dopamine D2 receptor antagonism, and direct stimulation of gut smooth muscle contraction. The drug also has anti-emetic effects *via* brainstem D2 receptor antagonism, vagal and brainstem 5-HT3 receptor antagonism. The prokinetic properties of metoclopramide are limited to the proximal gut. Metoclopramide increases esophageal, fundic and antral contractile amplitudes, elevates lower esophageal sphincter pressure, and improves antropyloroduodenal coordination. Metoclopramide is administered orally in pill or liquid suspension form. Intravenous forms commonly are reserved for inpatients that cannot retain oral medications. Subcutaneous administration has also been reported to provide symptom control[57]. At least five controlled trails and four open label series have studied the efficacy of metoclopramide in gastroparesis[10]. In these nine trials, symptoms improved in seven studies, but improvement in gastric emptying was noted in only five. Patients may develop tolerance to the prokinetic action of metoclopramide over time; however, its antiemetic effects are sustained[58]. Metoclopramide is effective for the short-term treatment of gastroparesis for up to several weeks[59,60]. The long-term utility of metoclopramide has not been proven[61]. Side effects of metoclopramide occur in up to 30% of patients and result from antidopaminergic effects on the CNS. Acute dystonic reactions such as facial spasm, oculogyric crisis, trismus, and torticollis occur in 0.2%-6% of patients and are often observed in patients less than 30 years of age and within 48 h of initiating therapy[62]. Drowsiness, fatigue, and lassitude are reported by 10% of patients. Metoclopramide can worsen depression. Other side effects include restlessness, agitation, irritability, akathisia and hyperprolactinemic effects. Prolonged treatment with metoclopramide can produce extrapyramidal symptoms. These symptoms usually subside with 2-3 mo of discontinuation of the drug. Irreversible tardive dyskinesia is a catastrophic consequence that occurs in 1% to 10% of cases when metoclopramide is taken for more than 3 mo[62]. This condition is disabling and can develop without warning, therefore, it should be discussed in detail with the patients or their families with documentation of the discussion in their medical record. The current standard has been to sign an informed consent to document communicating the risks of metoclopramide.

**(Ⅲ) Domperidone.** Domperidone, a benzimidazole derivative, is a peripheral dopamine D2 receptor antagonist with benefits similar to those of metoclopramide. Domperidone does not cross the blood-brain barrier and consequently it has fewer central side effects. Brainstem structures regulating vomiting are outside the blood-brain barrier, therefore, domperidone has potent central anti-emetic action. At least five controlled trials and four open case series have assessed domperidone in patients with gastroparesis and diabetic gastropathy[63]. Symptoms improved in all studies, but accelerated gastric emptying was not uniformly observed. Domperidone may show tachphylaxis on repeated administration[64]. Adverse reactions to domperidone are commonly related to hyperprolectinemia due to the porous blood-brain barrier in the anterior pituitary[65]. These include menstrual irregularities, breast engorgement, and galactorrhea. An intravenous formulation of domperidone was removed in 1980 due to generation of cardiac arrhythmias[66]. Domperidone is not approved by the FDA for prescription in the United States, although it can be obtained in Canada, Mexico, New Zealand, Europe, and Japan. It is available in the US with approval of local institutional review boards, through an FDA investigational new drug application (IND) to patients with gastroparesis refractory to other therapies.

**(Ⅳ) Tegaserod.** This is a 5-HT4 receptor partial agonist used in the treatment of constipation predominant irritable bowel syndrome. In healthy volunteers, the drug stimulates small-intestinal motility and post-prandial antral and intestinal motility. Tegaserod has been shown to accelerate gastric emptying in some[67] but not all studies of healthy volunteers[68]. Tegaserod was completely withdrawn form the US market in April 2008 due to a reported increase in the risk of cardiovascular adverse effects.

**(Ⅴ) Cisapride.** Cisapride is a 5-HT4 receptor agonist with weak 5-HT3 antagonist properties that once was widely used for gastroparesis. This drug was withdrawn from the market in the United States in 2000 because of numerous reports of sudden death from cardiac arrhythmias[69]. Although the drug is still available overseas in numerous countries and obtainable from overseas websites, a recent consensus document did not recommend its use in gastroparesis[12].

**(Ⅵ) Bethanechol.** Bethanechol is an approved smooth muscle muscarinic agonist that increases lower esophageal sphincter pressure and evokes fundoantral contractions but does not induce propulsive contractions or accelerate gastric emptying[70]. Rarely, the drug may be used as an adjunct with other prokinetic medications in patients refractory to standard treatment with prokinetics and anti-emetic drugs. Prominent adverse effects include abdominal cramps, skin flushing, diaphoresis, lacrimation, salivation, nausea, vomiting, bronchoconstriction, urinary urgency, and miosis. Dangerous cardiovascular effects include abrupt decreases in blood pressure in hypertensive patients and atrial fibrillation in patients with hyperthyroidism.

**(Ⅶ) Drugs in research.** (1) Motilin receptor agonists. (a) Azithromycin is a macrolide antibiotic similar to erythromycin. It has been postulated that azithromycin

is also a motilin receptor agonist. In preliminary studies, intravenous administration of azithromycin improves antroduodenal contractions as measured by manometry[71]. However, there are no data available revealing an improvement in gastric emptying rates or patient symptoms after the administration of i.v. or oral azithromycin. The potential benefit of azithromycin is the longer half-life (68 h) as compared to erythromycin (1.5-2 h) and thus the less frequent dosing may help improve compliance with the medication (once a day *versus* four times a day). Furthermore, azithromycin is not metabolized, and elimination is largely in the feces, following excretion into the bile, with less than 10% excreted in the urine. Thus, it does not utilize the P-450 pathway in the liver and has less adverse effects due to drug interactions. It also appears that azithromycin has lower pro-arrhythmic potential compare with erythromycin but nevertheless cardiac adverse events have been reported[72-74]. From that prospective, it seems prudent to check the length of the QTc interval prior to initiating azithromycin therapy as well. (b) Mitemcinal is also a macrolide derived motilin receptor agonist with prokinetic properties. It does not have any antimicrobial actions. It produced symptom benefit in patients with diabetic gastropathy who had a body mass index of $< 35$ kg/m$^2$ and with hemoglobin A1C values $< 10\%$[75]. In addition, tachyphylaxis was not observed during the study period. (c) Atilmotin is another motilin receptor agonist, which, when given i.v., has been shown to accelerate gastric emptying of liquids and solids in healthy subjects[76]. It is not known whether atilmotin has significant effects on symptoms in patients with gastroparesis. (d) Ghrelin is a neurohumoral transmitter secreted by the stomach and is believed to play a physiological role as a stimulant of food intake and is also structurally related to motilin. Ghrelin has prokinetic properties, and has been shown to accelerate gastric emptying of a test meal in diabetic patients with slow gastric emptying[77], as well as improve gastric emptying and decreased meal-related symptoms in patients with idiopathic gastroparesis[78]. (2) Dopamine antagonists and serotonin agonists. (a) Itopride is a new D2 antagonist with anti-acetylcholinesterase effects. This drug showed prokinetic properties in animal models as well as promising effects in functional dyspepsia[79]. However, in healthy subjects, itopride had no effect on gastric emptying[80]. (b) Sulpiride is a dopamine blocker used for psychiatric disorders. Initial studies have shown that oral levosulpiride is superior to placebo[81], and may be as effective as cisapride in relieving nausea and vomiting in patients with gastroparesis[82,83]. Although this drug is not new, further studies are of interest to see whether it deserves a more established position for these gastrointestinal indications. (c) Mosapride is a 5-HT4 receptor agonist that accelerates gastric emptying in healthy volunteers and patients with diabetic gastroparesis[84]. In contrast to cisapride, mosapride has little effect on potassium-channel activity and seems to exhibit a significantly lower cardiac dysrhythmogenic potential[85]. (d) Renzapride is a combined 5-HT4 receptor agonist and 5-HT3-receptor antagonist. Future studies are needed to determine if renzapride exhibits efficacy in gastroparesis[12]. (3) Miscellaneous. (a) Physiostigmine and neostigmine are muscarinic receptor activators that stimulate gut motor activity by increasing acetylcholine levels. These drugs increase gastric contractions but have limited action in accelerate gastric emptying. However, pyridostigmine has been recently noted to reduce symptoms in a patient with gastroparesis secondary to underlying autoimmune disease[86]. (b) Nizatidine is a H2-receptor antagonist which exhibits anticholinesterase activity and stimulates gastric emptying but its efficacy in long-term treatment of gastroparesis is unknown[87]. (c) Cholecystokinin receptor antagonists such as loxiglumide and dexloxiglumide accelerate gastric emptying in some studies. The utility of such agents in gastroparesis remains to be determined[88]. (d) Sildenafil is a phosphodiesterase 5 inhibitor which has been shown to restore gastric emptying of liquids in an animal model of diabetes[89]. Sildenafil also reduced the dysrhythmias of the stomach induced experimentally by hyperglycemia in humans[90]. On the other hand, a thorough study of the effects of sildenafil on human gastric sensimotor functions showed that the drug significantly increases postprandial gastric volume and slows liquid (though not solid) emptying rate[91]. Sildenafil has also been found to inhibit interdigestive motor activity of the antrum and duodenum[92]. Clinical trials are clearly needed before this medication can be considered for the treatment of gastroparesis.

**Anti-emetic medications:** It is likely that a component of the clinical benefits observed with some of the available prokinetic drugs, such as metoclopramide and domperidone, stem from their anti-emetic actions on brain-stem nuclei. Nausea and vomiting are the most disabling symptom of gastroparesis and anti-emetic agents without stimulatory activity are often used alone or in concert with prokinetic drugs to treat gastroparesis. Antiemetic medications act on a broad range of distinct receptors subtypes in the peripheral and central nervous systems. Like prokinetics, the choice of antiemetic is empirical and it is reasonable to try the less expensive therapies initially.

(Ⅰ) Phenothiazines. These are the most commonly prescribed traditional antiemetics which include prochlorperazine and tiethyperazine. These drugs are both dopamine and cholinergic receptor antagonists acting on the area postrema (chemoreceptor trigger zone) in the brainstem. Prochlorperazine can be administered in the tablet form, liquid suspension, suppository and by injection. Side effects include sedation and extra-pyramidal effects such as drowsiness, dry mouth, constipation, skin rashes and Parkinsonian-like tardive dyskinesia.

(Ⅱ) Serotonin 5-HT3 receptor antagonist. These medications include ondansetron, granisetron, and dolasetron and are useful for prophylaxis of

chemotherapy induced nausea and vomiting, as well as symptoms occurring post operatively or during radiation therapy. These drugs may act on the chemoreceptor trigger zone as well as on peripheral afferent nerve fibers within the vagus nerve[42]. Ondansetron has no effect on gastric emptying in healthy volunteers and patients with gastroparesis and moreover can cause constipation[93,94]. This class of drugs maybe helpful when all other drugs have failed to provide symptom relief and are best given on an as-needed basis.

(Ⅲ) Anti-histamines. Antihistamines acting on H1 receptors exhibit central antiemetic effects[42]. Commonly prescribed antiemetics include diphenhydramine, dimenhydrinate and meclizine. These agents are most useful to treat symptoms related to motion sickness. The mechanism of action is poorly understood but is likely to involve both labyrinthine and chemoreceptor trigger zones. Side effects include drowsiness, dry mouth, blurred vision, difficulty urinating, constipation, palpitations, dizziness, insomnia and tremor.

(Ⅳ) Low-dose tricyclic antidepressants. Tricyclic antidepressants (TCAs) impair gastrointestinal motility through their anticholinergic activity but have been shown to relieve nausea, vomiting and pain in functional dyspepsia[95,96]. In a recent publication, 88% of diabetic patients with nausea and vomiting reported benefits with TCAs[97], of which one third had delayed gastric emptying, suggesting that these agents may have utility in gastroparesis. However, formal prospective trials of these antidepressants for the treatment of gastroparesis have not been performed, thus their use is still considered off-label. Side effects of low-dose TCAs are uncommon, excessive sedation and dry mouth occasionally limits use.

(Ⅴ) Other antiemetics. (1) Cannabinoids. Cannabinoid drugs such as dronabinol have been studied for improvement of gastrointestinal symptoms from chemotherapy and appear to have potency similar to standard antidopaminergics. Their benefit for gastroparesis has not been evaluated and they may also delay gastric emptying. (2) Benzodiazepines. These are useful for anticipatory nausea and vomiting before chemotherapy, but their efficacy in gastroparesis is unknown. These drugs maybe useful for their sedating effects in those patients with associated anxiety. (3) Neurokinin NK1-receptor antagonists. These are new antiemetics which treat both acute and delayed chemotherapy-induced nausea and vomiting[98,99], but their actions on gastric motor activity and symptoms in gastroparesis are uninvestigated. (4) Corticosteriods. Corticosteriods are employed as antiemetics in the postoperative setting or in the prevention of chemotherapy-induced emesis. One individual with idiopathic myenteric ganglionitis exhibited improvement with corticosteroid therapy, confirming the inflammatory basis of some cases of upper gut dysmotility[100].

**Complementary and alternative therapies:** Ginger, a traditional Chinese antiemetic agent, has weak 5-HT3 receptor antagonist properties and has gastric slow wave antidysrhythmic effects in humans[101,102]. These therapies are often given for treatment of nausea and vomiting of diverse etiologies. Acupressure and electrical acustimulation on the P6 acupuncture point reduce nausea postoperatively, after chemotherapy, and during nausea of pregnancy. One group observed benefits with acupuncture in 35 diabetic gastroparesis patients[103].

**Medications for control of symptoms other than nausea and vomiting:** (1) Early satiety. Early satiety has been related to defects in fundic accommodation in patients with functional dyspepsia[104]. Nitrates, buspirone, sumitriptan, and selective serotonin reuptake inhibitors promote fundic relaxation in this condition[105,106]. The use of fundic relaxants in managing early satiety in gastroparesis has not been investigated; (2) Abdominal pain. Epigastric pain is disabling in some individuals with gastroparesis and can result in excessive utilization of healthcare resources. The pathogenesis of pain is poorly understood and treatments for this symptom are largely unsatisfactory. Pain in gastroparesis has been postulated to be due to sensory rather than motor dysfunction, and therapies to reduce afferent dysfunction may be more effective for this symptom[107]. However, this hypothesis has not been tested. Although, non-steroidal anti-inflammatory drugs (NSAID's) have been shown to ameliorate gastric slow wave dysrhythmias in several healthy subjects[108], their adverse effects including renal dysfunction and ulcerogenic properties, limit their usage on a chronic basis. Antidepressant medications may help with gastroparesis associated neuropathic pain[109]. These include low dose tricyclic antidepressants (TCA), selective serotonin reuptake inhibitors (SSRIs), selective noradrenaline reuptake inhibitors (SNRIs) and combined serotonin/noradrenaline reuptake inhibitors. Paroxetine, an SSRI, may selectively accelerate small intestinal transit[110,111]. Opiates, including milder agents such as tramadol, should be avoided because of their inhibitory effects on motility as well as risk of addiction. (3) Nutritional support, enteral and parenteral. Some patients with refractory gastroparesis benefit from enteral or parenteral nutrition intermittently for symptom flares or for permanent support. Patients with chronic symptoms of gastroparesis may develop dehydration, electrolyte abnormalities and/or extreme malnutrition. The choice of nutritional support and its administration route depends on the severity of disease. The indications for supplementation of enteral nutrition include unintentional loss of 10% or more of the usual body weight during a period of 3 to 6 mo, inability to achieve the recommended weight by the oral route, repeated hospitalizations for refractory symptoms, interference with delivery of nutrients and medications, need for nasogastric intubation to relieve symptoms, and nausea and vomiting resulting in poor quality of life[12].

Except in cases of profound malnutrition or electrolyte disturbance, enteral feeding are preferable to chronic parenteral nutrition because of the significant risks of infection and liver disease in the latter treatment. On the other hand, short-term total parenteral nutrition (TPN) can reverse rapid weight decline and ensure adequate fluid delivery. Home intravenous TPN may be needed for individuals who cannot tolerate enteral feeding. Several options for enteral access and feeding are available and no data exists favoring one approach over the other. However, nasogastric tubes and gastrostomy tubes are not encouraged due to the possibility of worsening gastroparesis and risk of pulmonary aspiration. Jejunostomy tubes are preferred in order to bypass the gastroparetic stomach except if the patient has small bowel dysmotility. Short-term nasojejunal feeding is often used to help determine if the patient will tolerate chronic small bowel feeding through a permanent enteral access. Formulas that are low in osmolarity (e.g. Peptamen, Isocal) and with a caloric density of 1.0-1.5 cal/mL are recommended. A dietician should be consulted early on. Initially, infusion rates should be low and then advanced every 4-12 h as tolerated to meet caloric needs. Eventually, infusions can be converted to nocturnal feedings to free up daytime h for optional oral intake and to participate in normal daily activities.

### *Endoscopic treatment*

Therapeutic endoscopy with pyloric injection of botulinum toxin A may provide benefit in some patient with gastroparesis. Botulinum toxin A is a bacterial toxin that inhibits acetylcholine release, causing muscle paralysis. Manometric studies in patients with diabetic gastroparesis have shown evidence of prolonged pylorospasm producing a functional outlet obstruction[36]. Several uncontrolled case series have reported reduced symptoms and acceleration of gastric emptying after botulinum toxin treatment[112-114]. The largest series reported 63 highly selected patients with primary idiopathic gastroparesis, 43% of whom responded symptomatically with mean response duration of 5 mo[115]. A double-blind controlled trial found no efficacy of botulinum toxin over placebo[116]. However, this report was underpowered to detect the effect of the drug. Another recent double-blind placebo-controlled trial revealed that intrapyloric injection of botulinum toxin improved gastric emptying in patients with gastroparesis, although this benefit was not superior to placebo at one month. Also, in comparison to placebo, symptoms did not improve significantly after 1 mo of injection[117]. The use of botulinum toxin for gastroparesis is considered off-label and should be considered when other accepted therapies have failed or produced unacceptable side effects. To date, few adverse effects have been reported with botulinum toxin therapy.

### *Surgical treatment*

Surgical intervention is increasingly used to treat medically refractory/severe gastroparesis. Limited data are available concerning surgical treatment of gastroparesis[118]. The most common procedure is gastric electrical stimulation (GES). Other procedures offered include venting/feeding gastrostomy and jejunostomy tubes, surgical pyloroplasy, gastrectomy and surgical drainage procedures and pancreatic transplantation in diabetic patients. Apart from GES and feeding tubes, other surgical procedures are performed as a last resort in carefully evaluated patients with profound gastric stasis.

**GES:** Over the past decade, GES has been used for treatment of medically refractory gastroparesis[10,12,119]. Paced GES using an implantable stimulator (Enterra therapy, by Medtronic Inc.) has been approved by the FDA through a humanitarian device exemption. Electrical stimulation is delivered by two electrodes usually placed laproscopically on to the serosal surface of the stomach overlying the pacemaker area in the body of the stomach. Leads from the electrodes connect to a pulse generator that resembles a cardiac pacemaker that is implanted in a subcutaneous pocket of the anterior abdominal wall. The pulse generator delivers low energy 0.1-s trains of pulses at a frequency of 12 cycles/min. Within each pulse train, individual pulses oscillate at a frequency of 14 cycles/s[12]. Although the exact mechanism of action of the GES is unknown, the clinical effect is believed to be mediated by local neurostimulation. The stimulation impulses used are able to excite nerves but are too weak to excite gastric smooth muscles. Furthermore, poor correlation is observed between patients' symptoms and gastric emptying rates[119,120]. It has been hypothesized that the mechanism may stem from a vagal and cerebral pathway[121]; however, GES has been shown to work well even in patients with vagotomy[122]. Multiple uncontrolled studies in diabetic, idiopathic and post-surgical gastroparesis have shown efficacy of GES. In one uncontrolled multicenter trial, 35 of 38 patients experienced > 80% reductions in nausea and vomiting which persisted for 2.9-15.6 mo, with an associated 5.5% increase in weight and reduced requirement of supplemental nutrition[123]. Other studies reported similar long-term symptom benefits, which may persist for at least 10 years with improvements in body mass index, serum albumin and glycemic control[124,125]. In the only controlled trial of GES, 33 patients with idiopathic or diabetic gastroparesis completed a 2-mo double-blind, crossover, sham stimulation-controlled phase followed by 12 mo uncontrolled observation, with the device activated[119]. During the blinded phase, frequency of weekly vomiting in all patients was 6.8 times when the device was ON as opposed to 13.5 times when it was OFF. Although there was not a significant reduction in the total symptom score (TSS) in the ON *vs* OFF state, 21 patients preferred the stimulation ON, whereas seven preferred OFF and five had no preference. Symptom reductions were more impressive during the unblinded phase where median vomiting frequency decreased by > 80% for 50% of all patients. TSS was also significantly

improved in all patients from a score of 16.8 at baseline to 11.1 and 11.4 at 6 and 12 mo, respectively. The major adverse effect of GES is infection resulting in removal of the device in approximately 10% of patients[119,123]. The frequency of such infections seems to be decreasing during recent years. This may be explained by more careful surgical technique and the increasing use of laparoscopy instead of open surgery. The second concern is of the non-responder issue. In the earlier mentioned randomized trial[119] 13% of the patients were non-responders with < 25% symptom reduction. There seems to be a higher non-responder rate in idiopathic gastroparesis[125,126]. Abell and colleagues have applied temporary mucosal GES with endoscopically placed electrodes and used the effects on symptoms after ⩾ 3 d as a measure of response[127].

**Other surgical options:** In refractory patients with severe nausea and vomiting, placement of a gastrostomy tube for intermittent decompression by venting or suctioning may provide symptom relief, especially of interdigestive fullness and bloating secondary to retained intragastric gas and liquids. Pyloroplasty may be considered as another option but limited data are available on the efficacy of this procedure. There are limited controlled data concerning gastrectomy in gastroparesis[118]. A study of patients with near-total gastrectomy revealed long-term symptom relief in 43% patients with postsurgical gastroparesis[128]. The literature is sparse concerning correction of diabetic gastroparesis status post-pancreas and pancreas-kidney transplant in patients with type 1 diabetes[129,130].

## REFERENCES


1 **Soykan I**, Sivri B, Sarosiek I, Kiernan B, McCallum RW. Demography, clinical characteristics, psychological and abuse profiles, treatment, and long-term follow-up of patients with gastroparesis. *Dig Dis Sci* 1998; **43**: 2398-2404
2 **Park MI**, Camilleri M. Gastroparesis: clinical update. *Am J Gastroenterol* 2006; **101**: 1129-1139
3 **Huizinga JD**. Neural injury, repair, and adaptation in the GI tract. IV. Pathophysiology of GI motility related to interstitial cells of Cajal. *Am J Physiol* 1998; **275**: G381-G386
4 **Vinik AI**, Maser RE, Mitchell BD, Freeman R. Diabetic autonomic neuropathy. *Diabetes Care* 2003; **26**: 1553-1579
5 **Ziegler D**, Schadewaldt P, Pour Mirza A, Piolot R, Schommartz B, Reinhardt M, Vosberg H, Brosicke H, Gries FA. [13C]octanoic acid breath test for non-invasive assessment of gastric emptying in diabetic patients: validation and relationship to gastric symptoms and cardiovascular autonomic function. *Diabetologia* 1996; **39**: 823-830
6 **Ordog T**, Takayama I, Cheung WK, Ward SM, Sanders KM. Remodeling of networks of interstitial cells of Cajal in a murine model of diabetic gastroparesis. *Diabetes* 2000; **49**: 1731-1739
7 **Ali T**, Hasan M, Hamadani M, Harty RF. Gastroparesis. *South Med J* 2007; **100**: 281-286
8 **Peeters TL**. New motilin agonists: a long and winding road. *Neurogastroenterol Motil* 2006; **18**: 1-5
9 **Murray CD**, Martin NM, Patterson M, Taylor SA, Ghatei MA, Kamm MA, Johnston C, Bloom SR, Emmanuel AV. Ghrelin enhances gastric emptying in diabetic gastroparesis: a double blind, placebo controlled, crossover study. *Gut* 2005; **54**: 1693-1698
10 **Parkman HP**, Hasler WL, Fisher RS. American Gastroenterological Association technical review on the diagnosis and treatment of gastroparesis. *Gastroenterology* 2004; **127**: 1592-1622
11 **Parkman HP**, Schwartz SS. Esophagitis and gastroduodenal disorders associated with diabetic gastroparesis. *Arch Intern Med* 1987; **147**: 1477-1480
12 **Abell TL**, Bernstein RK, Cutts T, Farrugia G, Forster J, Hasler WL, McCallum RW, Olden KW, Parkman HP, Parrish CR, Pasricha PJ, Prather CM, Soffer EE, Twillman R, Vinik AI. Treatment of gastroparesis: a multidisciplinary clinical review. *Neurogastroenterol Motil* 2006; **18**: 263-283
13 **Revicki DA**, Rentz AM, Dubois D, Kahrilas P, Stanghellini V, Talley NJ, Tack J. Gastroparesis Cardinal Symptom Index (GCSI): development and validation of a patient reported assessment of severity of gastroparesis symptoms. *Qual Life Res* 2004; **13**: 833-844
14 **Hoogerwerf WA**, Pasricha PJ, Kalloo AN, Schuster MM. Pain: the overlooked symptom in gastroparesis. *Am J Gastroenterol* 1999; **94**: 1029-1033
15 **Boccia G**, Buonavolonta R, Coccorullo P, Manguso F, Fuiano L, Staiano A. Dyspeptic symptoms in children: the result of a constipation-induced cologastric brake? *Clin Gastroenterol Hepatol* 2008; **6**: 556-560
16 **Parkman HP**, Schwartz SS. Esophagitis and gastroduodenal disorders associated with diabetic gastroparesis. *Arch Intern Med* 1987; **147**: 1477-1480
17 **Blam ME**, Lichtenstein GR. A new endoscopic technique for the removal of gastric phytobezoars. *Gastrointest Endosc* 2000; **52**: 404-408
18 **Abell TL**, Camilleri M, Donohoe K, Hasler WL, Lin HC, Maurer AH, McCallum RW, Nowak T, Nusynowitz ML, Parkman HP, Shreve P, Szarka LA, Snape WJ Jr, Ziessman HA. Consensus recommendations for gastric emptying scintigraphy: a joint report of the American Neurogastroenterology and Motility Society and the Society of Nuclear Medicine. *Am J Gastroenterol* 2008; **103**: 753-763
19 **Camilleri M**, Zinsmeister AR, Greydanus MP, Brown ML, Proano M. Towards a less costly but accurate test of gastric emptying and small bowel transit. *Dig Dis Sci* 1991; **36**: 609-615
20 **Feldman M**, Smith HJ, Simon TR. Gastric emptying of solid radiopaque markers: studies in healthy subjects and diabetic patients. *Gastroenterology* 1984; **87**: 895-902
21 **Bateman DN**, Whittingham TA. Measurement of gastric emptying by real-time ultrasound. *Gut* 1982; **23**: 524-527
22 **Bolondi L**, Bortolotti M, Santi V, Calletti T, Gaiani S, Labo G. Measurement of gastric emptying time by real-time ultrasonography. *Gastroenterology* 1985; **89**: 752-759
23 **Holt S**, Cervantes J, Wilkinson AA, Wallace JH. Measurement of gastric emptying rate in humans by real-time ultrasound. *Gastroenterology* 1986; **90**: 918-923
24 **Gentilcore D**, Hausken T, Horowitz M, Jones KL. Measurements of gastric emptying of low- and high-nutrient liquids using 3D ultrasonography and scintigraphy in healthy subjects. *Neurogastroenterol Motil* 2006; **18**: 1062-1068
25 **Gilja OH**, Detmer PR, Jong JM, Leotta DF, Li XN, Beach KW, Martin R, Strandness DE Jr. Intragastric distribution and gastric emptying assessed by three-dimensional ultrasonography. *Gastroenterology* 1997; **113**: 38-49
26 **Gilja OH**. Ultrasound of the stomach--the EUROSON lecture 2006. *Ultraschall Med* 2007; **28**: 32-39
27 **Kim DY**, Myung SJ, Camilleri M. Novel testing of human gastric motor and sensory functions: rationale, methods, and potential applications in clinical practice. *Am J Gastroenterol* 2000; **95**: 3365-3373
28 **Ajaj W**, Goehde SC, Papanikolaou N, Holtmann G, Ruehm SG, Debatin JF, Lauenstein TC. Real time high resolution magnetic resonance imaging for the assessment of gastric motility disorders. *Gut* 2004; **53**: 1256-1261
29 **Kunz P**, Feinle C, Schwizer W, Fried M, Boesiger P.

Assessment of gastric motor function during the emptying of solid and liquid meals in humans by MRI. *J Magn Reson Imaging* 1999; **9**: 75-80
30 **Feinle C**, Kunz P, Boesiger P, Fried M, Schwizer W. Scintigraphic validation of a magnetic resonance imaging method to study gastric emptying of a solid meal in humans. *Gut* 1999; **44**: 106-111
31 **Kuiken SD**, Samsom M, Camilleri M, Mullan BP, Burton DD, Kost LJ, Hardyman TJ, Brinkmann BH, O'Connor MK. Development of a test to measure gastric accommodation in humans. *Am J Physiol* 1999; **277**: G1217-G1221
32 **Viramontes BE**, Kim DY, Camilleri M, Lee JS, Stephens D, Burton DD, Thomforde GM, Klein PD, Zinsmeister AR. Validation of a stable isotope gastric emptying test for normal, accelerated or delayed gastric emptying. *Neurogastroenterol Motil* 2001; **13**: 567-574
33 **Ghoos YF**, Maes BD, Geypens BJ, Mys G, Hiele MI, Rutgeerts PJ, Vantrappen G. Measurement of gastric emptying rate of solids by means of a carbon-labeled octanoic acid breath test. *Gastroenterology* 1993; **104**: 1640-1647
34 **Braden B**, Adams S, Duan LP, Orth KH, Maul FD, Lembcke B, Hor G, Caspary WF. The [13C]acetate breath test accurately reflects gastric emptying of liquids in both liquid and semisolid test meals. *Gastroenterology* 1995; **108**: 1048-1055
35 **Kuo B**, McCallum RW, Koch KL, Sitrin MD, Wo JM, Chey WD, Hasler WL, Lackner JM, Katz LA, Semler JR, Wilding GE, Parkman HP. Comparison of gastric emptying of a nondigestible capsule to a radio-labelled meal in healthy and gastroparetic subjects. *Aliment Pharmacol Ther* 2008; **27**: 186-196
36 **Mearin F**, Camilleri M, Malagelada JR. Pyloric dysfunction in diabetics with recurrent nausea and vomiting. *Gastroenterology* 1986; **90**: 1919-1925
37 **Camilleri M**, Malagelada JR. Abnormal intestinal motility in diabetics with the gastroparesis syndrome. *Eur J Clin Invest* 1984; **14**: 420-427
38 **Soffer E**, Thongsawat S. Clinical value of duodenojejunal manometry. Its usefulness in diagnosis and management of patients with gastrointestinal symptoms. *Dig Dis Sci* 1996; **41**: 859-863
39 **Camilleri M**, Hasler WL, Parkman HP, Quigley EM, Soffer E. Measurement of gastrointestinal motility in the GI laboratory. *Gastroenterology* 1998; **115**: 747-762
40 **Parkman HP**, Hasler WL, Barnett JL, Eaker EY. Electrogastrography: a document prepared by the gastric section of the American Motility Society Clinical GI Motility Testing Task Force. *Neurogastroenterol Motil* 2003; **15**: 89-102
41 **Azpiroz F**, Malagelada JR. Gastric tone measured by an electronic barostat in health and postsurgical gastroparesis. *Gastroenterology* 1987; **92**: 934-943
42 **Quigley EM**, Hasler WL, Parkman HP. AGA technical review on nausea and vomiting. *Gastroenterology* 2001; **120**: 263-286
43 **Patrick A**, Epstein O. Review article: gastroparesis. *Aliment Pharmacol Ther* 2008; **27**: 724-740
44 **Petrakis IE**, Vrachassotakis N, Sciacca V, Vassilakis SI, Chalkiadakis G. Hyperglycaemia attenuates erythromycin-induced acceleration of solid-phase gastric emptying in idiopathic and diabetic gastroparesis. *Scand J Gastroenterol* 1999; **34**: 396-403
45 **Gentilcore D**, O'Donovan D, Jones KL, Horowitz M. Nutrition therapy for diabetic gastroparesis. *Curr Diab Rep* 2003; **3**: 418-426
46 **Talley NJ**. Diabetic gastropathy and prokinetics. *Am J Gastroenterol* 2003; **98**: 264-271
47 **Sturm A**, Holtmann G, Goebell H, Gerken G. Prokinetics in patients with gastroparesis: a systematic analysis. *Digestion* 1999; **60**: 422-427
48 **Farrugia G**, Macielag MJ, Peeters TL, Sarr MG, Galdes A, Szurszewski JH. Motilin and OHM-11526 activate a calcium current in human and canine jejunal circular smooth muscle. *Am J Physiol* 1997; **273**: G404-G412
49 **DiBaise JK**, Quigley EM. Efficacy of prolonged administration of intravenous erythromycin in an ambulatory setting as treatment of severe gastroparesis: one center's experience. *J Clin Gastroenterol* 1999; **28**: 131-134
50 **Kendall BJ**, Chakravarti A, Kendall E, Soykan I, McCallum RW. The effect of intravenous erythromycin on solid meal gastric emptying in patients with chronic symptomatic post-vagotomy-antrectomy gastroparesis. *Aliment Pharmacol Ther* 1997; **11**: 381-385
51 **Peeters TL**. Agonist effect of erythromycin and its analogues on motilin receptors. A new family of prokinetics? Clinical interest. *Acta Gastroenterol Belg* 1993; **56**: 257-260
52 **Parkman HP**, Pagano AP, Vozzelli MA, Ryan JP. Gastrokinetic effects of erythromycin: myogenic and neurogenic mechanisms of action in rabbit stomach. *Am J Physiol* 1995; **269**: G418-G426
53 **Richards RD**, Davenport K, McCallum RW. The treatment of idiopathic and diabetic gastroparesis with acute intravenous and chronic oral erythromycin. *Am J Gastroenterol* 1993; **88**: 203-207
54 **Ramirez B**, Eaker EY, Drane WE, Hocking MP, Sninsky CA. Erythromycin enhances gastric emptying in patients with gastroparesis after vagotomy and antrectomy. *Dig Dis Sci* 1994; **39**: 2295-2300
55 **Ehrenpreis ED**, Zaitman D, Nellans H. Which form of erythromycin should be used to treat gastroparesis? A pharmacokinetic analysis. *Aliment Pharmacol Ther* 1998; **12**: 373-376
56 **Ray WA**, Murray KT, Meredith S, Narasimhulu SS, Hall K, Stein CM. Oral erythromycin and the risk of sudden death from cardiac causes. *N Engl J Med* 2004; **351**: 1089-1096
57 **McCallum RW**, Valenzuela G, Polepalle S, Spyker D. Subcutaneous metoclopramide in the treatment of symptomatic gastroparesis: clinical efficacy and pharmacokinetics. *J Pharmacol Exp Ther* 1991; **258**: 136-142
58 **Malagelada JR**, Rees WD, Mazzotta LJ, Go VL. Gastric motor abnormalities in diabetic and postvagotomy gastroparesis: effect of metoclopramide and bethanechol. *Gastroenterology* 1980; **78**: 286-293
59 **Perkel MS**, Moore C, Hersh T, Davidson ED. Metoclopramide therapy in patients with delayed gastric emptying: a randomized, double-blind study. *Dig Dis Sci* 1979; **24**: 662-666
60 **Snape WJ Jr**, Battle WM, Schwartz SS, Braunstein SN, Goldstein HA, Alavi A. Metoclopramide to treat gastroparesis due to diabetes mellitus: a double-blind, controlled trial. *Ann Intern Med* 1982; **96**: 444-446
61 **Lata PF**, Pigarelli DL. Chronic metoclopramide therapy for diabetic gastroparesis. *Ann Pharmacother* 2003; **37**: 122-126
62 **Ganzini L**, Casey DE, Hoffman WF, McCall AL. The prevalence of metoclopramide-induced tardive dyskinesia and acute extrapyramidal movement disorders. *Arch Intern Med* 1993; **153**: 1469-1475
63 **Soykan I**, Sarosiek I, McCallum RW. The effect of chronic oral domperidone therapy on gastrointestinal symptoms, gastric emptying, and quality of life in patients with gastroparesis. *Am J Gastroenterol* 1997; **92**: 976-980
64 **Horowitz M**, Harding PE, Chatterton BE, Collins PJ, Shearman DJ. Acute and chronic effects of domperidone on gastric emptying in diabetic autonomic neuropathy. *Dig Dis Sci* 1985; **30**: 1-9
65 **Brogden RN**, Carmine AA, Heel RC, Speight TM, Avery GS. Domperidone. A review of its pharmacological activity, pharmacokinetics and therapeutic efficacy in the symptomatic treatment of chronic dyspepsia and as an antiemetic. *Drugs* 1982; **24**: 360-400
66 **Bruera E**, Villamayor R, Roca E, Barugel M, Tronge J, Chacon R. Q-T interval prolongation and ventricular fibrillation with i.v. domperidone. *Cancer Treat Rep* 1986; **70**: 545-546

67 **Degen L**, Matzinger D, Merz M, Appel-Dingemanse S, Osborne S, Luchinger S, Bertold R, Maecke H, Beglinger C. Tegaserod, a 5-HT4 receptor partial agonist, accelerates gastric emptying and gastrointestinal transit in healthy male subjects. *Aliment Pharmacol Ther* 2001; **15**: 1745-1751

68 **Prather CM**, Camilleri M, Zinsmeister AR, McKinzie S, Thomforde G. Tegaserod accelerates orocecal transit in patients with constipation-predominant irritable bowel syndrome. *Gastroenterology* 2000; **118**: 463-468

69 **Griffin JP**. Prepulsid withdrawn from UK & US markets. *Adverse Drug React Toxicol Rev* 2000; **19**: 177

70 **McCallum RW**, Fink SM, Lerner E, Berkowitz DM. Effects of metoclopramide and bethanechol on delayed gastric emptying present in gastroesophageal reflux patients. *Gastroenterology* 1983; **84**: 1573-1577

71 **Moshiree B**, Gupta V, Verne GN, Toskes PP. Azithromycin: a new therapy for gastroparesis. *Gastroenterology* 2005; **128**: A547 (Abstract)

72 **Huang BH**, Wu CH, Hsia CP, Yin Chen C. Azithromycin-induced torsade de pointes. *Pacing Clin Electrophysiol* 2007; **30**: 1579-1582

73 **Kezerashvili A**, Khattak H, Barsky A, Nazari R, Fisher JD. Azithromycin as a cause of QT-interval prolongation and torsade de pointes in the absence of other known precipitating factors. *J Interv Card Electrophysiol* 2007; **18**: 243-246

74 **Milberg P**, Eckardt L, Bruns HJ, Biertz J, Ramtin S, Reinsch N, Fleischer D, Kirchhof P, Fabritz L, Breithardt G, Haverkamp W. Divergent proarrhythmic potential of macrolide antibiotics despite similar QT prolongation: fast phase 3 repolarization prevents early afterdepolarizations and torsade de pointes. *J Pharmacol Exp Ther* 2002; **303**: 218-225

75 **McCallum RW**, Cynshi O. Clinical trial: effect of mitemcinal (a motilin agonist) on gastric emptying in patients with gastroparesis - a randomized, multicentre, placebo-controlled study. *Aliment Pharmacol Ther* 2007; **26**: 1121-1130

76 **Park MI**, Ferber I, Camilleri M, Allenby K, Trillo R, Burton D, Zinsmeister AR. Effect of atilmotin on gastrointestinal transit in healthy subjects: a randomized, placebo-controlled study. *Neurogastroenterol Motil* 2006; **18**: 28-36

77 **Murray CD**, Martin NM, Patterson M, Taylor SA, Ghatei MA, Kamm MA, Johnston C, Bloom SR, Emmanuel AV. Ghrelin enhances gastric emptying in diabetic gastroparesis: a double blind, placebo controlled, crossover study. *Gut* 2005; **54**: 1693-1698

78 **Tack J**, Depoortere I, Bisschops R, Verbeke K, Janssens J, Peeters T. Influence of ghrelin on gastric emptying and meal-related symptoms in idiopathic gastroparesis. *Aliment Pharmacol Ther* 2005; **22**: 847-853

79 **Holtmann G**, Talley NJ, Liebregts T, Adam B, Parow C. A placebo-controlled trial of itopride in functional dyspepsia. *N Engl J Med* 2006; **354**: 832-840

80 **Choung RS**, Talley NJ, Peterson J, Camilleri M, Burton D, Harmsen WS, Zinsmeister AR. A double-blind, randomized, placebo-controlled trial of itopride (100 and 200 mg three times daily) on gastric motor and sensory function in healthy volunteers. *Neurogastroenterol Motil* 2007; **19**: 180-187

81 **Mansi C**, Savarino V, Vigneri S, Sciaba L, Perilli D, Mele MR, Celle G. Effect of D2-dopamine receptor antagonist levosulpiride on diabetic cholecystoparesis: a double-blind crossover study. *Aliment Pharmacol Ther* 1995; **9**: 185-189

82 **Mansi C**, Borro P, Giacomini M, Biagini R, Mele MR, Pandolfo N, Savarino V. Comparative effects of levosulpiride and cisapride on gastric emptying and symptoms in patients with functional dyspepsia and gastroparesis. *Aliment Pharmacol Ther* 2000; **14**: 561-569

83 **Mearin F**, Rodrigo L, Perez-Mota A, Balboa A, Jimenez I, Sebastian JJ, Patan C. Levosulpiride and cisapride in the treatment of dysmotility-like functional dyspepsia: a randomized, double-masked trial. *Clin Gastroenterol Hepatol* 2004; **2**: 301-308

84 **Kanaizumi T**, Nakano H, Matsui Y, Ishikawa H, Shimizu R, Park S, Kuriya N. Prokinetic effect of AS-4370 on gastric emptying in healthy adults. *Eur J Clin Pharmacol* 1991; **41**: 335-337

85 **Potet F**, Bouyssou T, Escande D, Baro I. Gastrointestinal prokinetic drugs have different affinity for the human cardiac human ether-a-gogo K(+) channel. *J Pharmacol Exp Ther* 2001; **299**: 1007-1012

86 **Pasha SF**, Lunsford TN, Lennon VA. Autoimmune gastrointestinal dysmotility treated successfully with pyridostigmine. *Gastroenterology* 2006; **131**: 1592-1596

87 **Ueki S**, Seiki M, Yoneta T, Aita H, Chaki K, Hori Y, Morita H, Tagashira E, Itoh Z. Gastroprokinetic activity of nizatidine, a new H2-receptor antagonist, and its possible mechanism of action in dogs and rats. *J Pharmacol Exp Ther* 1993; **264**: 152-157

88 **Scarpignato C**, Kisfalvi I, D'Amato M, Varga G. Effect of dexloxiglumide and spiroglumide, two new CCK-receptor antagonists, on gastric emptying and secretion in the rat: evaluation of their receptor selectivity in vivo. *Aliment Pharmacol Ther* 1996; **10**: 411-419

89 **Watkins CC**, Sawa A, Jaffrey S, Blackshaw S, Barrow RK, Snyder SH, Ferris CD. Insulin restores neuronal nitric oxide synthase expression and function that is lost in diabetic gastropathy. *J Clin Invest* 2000; **106**: 373-384

90 **Coleski R**, Gonlachanvit S, Owyang C, Hasler WL. Selective reversal of hyperglycemia-evoked gastric myoelectric dysrhythmias by nitrergic stimulation in healthy humans. *J Pharmacol Exp Ther* 2005; **312**: 103-111

91 **Sarnelli G**, Sifrim D, Janssens J, Tack J. Influence of sildenafil on gastric sensorimotor function in humans. *Am J Physiol Gastrointest Liver Physiol* 2004; **287**: G988-G992

92 **Bortolotti M**, Mari C, Lopilato C, La Rovere L, Miglioli M. Sildenafil inhibits gastroduodenal motility. *Aliment Pharmacol Ther* 2001; **15**: 157-161

93 **Netzer P**, Gaia C, Lourens ST, Reber P, Wildi S, Noelpp U, Ritter EP, Ledermann H, Luscher D, Varga L, Kinser JA, Buchler MW, Scheurer U. Does intravenous ondansetron affect gastric emptying of a solid meal, gastric electrical activity or blood hormone levels in healthy volunteers? *Aliment Pharmacol Ther* 2002; **16**: 119-127

94 **Nielsen OH**, Hvid-Jacobsen K, Lund P, Langoholz E. Gastric emptying and subjective symptoms of nausea: lack of effects of a 5-hydroxytryptamine-3 antagonist ondansetron on gastric emptying in patients with gastric stasis syndrome. *Digestion* 1990; **46**: 89-96

95 **Loldrup D**, Langemark M, Hansen HJ, Olesen J, Bech P. Clomipramine and mianserin in chronic idiopathic pain syndrome. A placebo controlled study. *Psychopharmacology* (Berl) 1989; **99**: 1-7

96 **Mertz H**, Fass R, Kodner A, Yan-Go F, Fullerton S, Mayer EA. Effect of amitriptyline on symptoms, sleep, and visceral perception in patients with functional dyspepsia. *Am J Gastroenterol* 1998; **93**: 160-165

97 **Sawhney MS**, Prakash C, Lustman PJ, Clouse RE. Tricyclic antidepressants for chronic vomiting in diabetic patients. *Dig Dis Sci* 2007; **52**: 418-424

98 **Dando TM**, Perry CM. Aprepitant: a review of its use in the prevention of chemotherapy-induced nausea and vomiting. *Drugs* 2004; **64**: 777-794

99 **Diemunsch P**, Schoeffler P, Bryssine B, Cheli-Muller LE, Lees J, McQuade BA, Spraggs CF. Antiemetic activity of the NK1 receptor antagonist GR205171 in the treatment of established postoperative nausea and vomiting after major gynaecological surgery. *Br J Anaesth* 1999; **82**: 274-276

100 **De Giorgio R**, Barbara G, Stanghellini V, Cogliandro RF, Arrigoni A, Santini D, Ceccarelli C, Salvioli B, Rossini FP, Corinaldesi R. Idiopathic myenteric ganglionitis underlying intractable vomiting in a young adult. *Eur J Gastroenterol Hepatol* 2000; **12**: 613-616

101 **Gupta YK**, Sharma M. Reversal of pyrogallol-induced delay in gastric emptying in rats by ginger (Zingiber officinale). *Methods Find Exp Clin Pharmacol* 2001; **23**: 501-503

102 **Gonlachanvit S**, Chen YH, Hasler WL, Sun WM, Owyang C.

Ginger reduces hyperglycemia-evoked gastric dysrhythmias in healthy humans: possible role of endogenous prostaglandins. *J Pharmacol Exp Ther* 2003; **307**: 1098-1103
103 **Wang L**. Clinical observation on acupuncture treatment in 35 cases of diabetic gastroparesis. *J Tradit Chin Med* 2004; **24**: 163-165
104 **Tack J**, Piessevaux H, Coulie B, Caenepeel P, Janssens J. Role of impaired gastric accommodation to a meal in functional dyspepsia. *Gastroenterology* 1998; **115**: 1346-1352
105 **Tack J**, Broekaert D, Coulie B, Fischler B, Janssens J. Influence of the selective serotonin re-uptake inhibitor, paroxetine, on gastric sensorimotor function in humans. *Aliment Pharmacol Ther* 2003; **17**: 603-608
106 **Coulie B**, Tack J, Sifrim D, Andrioli A, Janssens J. Role of nitric oxide in fasting gastric fundus tone and in 5-HT1 receptor-mediated relaxation of gastric fundus. *Am J Physiol* 1999; **276**: G373-G377
107 **Hasler WL**. Gastroparesis: symptoms, evaluation, and treatment. *Gastroenterol Clin North Am* 2007; **36**: 619-647, ix
108 **Hasler WL**, Soudah HC, Dulai G, Owyang C. Mediation of hyperglycemia-evoked gastric slow-wave dysrhythmias by endogenous prostaglandins. *Gastroenterology* 1995; **108**: 727-736
109 **Gorelick AB**, Koshy SS, Hooper FG, Bennett TC, Chey WD, Hasler WL. Differential effects of amitriptyline on perception of somatic and visceral stimulation in healthy humans. *Am J Physiol* 1998; **275**: G460-G466
110 **Chial HJ**, Camilleri M, Ferber I, Delgado-Aros S, Burton D, McKinzie S, Zinsmeister AR. Effects of venlafaxine, buspirone, and placebo on colonic sensorimotor functions in healthy humans. *Clin Gastroenterol Hepatol* 2003; **1**: 211-218
111 **Gorard DA**, Libby GW, Farthing MJ. 5-Hydroxytryptamine and human small intestinal motility: effect of inhibiting 5-hydroxytryptamine reuptake. *Gut* 1994; **35**: 496-500
112 **Lacy BE**, Crowell MD, Schettler-Duncan A, Mathis C, Pasricha PJ. The treatment of diabetic gastroparesis with botulinum toxin injection of the pylorus. *Diabetes Care* 2004; **27**: 2341-2347
113 **Arts J**, van Gool S, Caenepeel P, Verbeke K, Janssens J, Tack J. Influence of intrapyloric botulinum toxin injection on gastric emptying and meal-related symptoms in gastroparesis patients. *Aliment Pharmacol Ther* 2006; **24**: 661-667
114 **Gupta P**, Rao SS. Attenuation of isolated pyloric pressure waves in gastroparesis in response to botulinum toxin injection: a case report. *Gastrointest Endosc* 2002; **56**: 770-772
115 **Bromer MQ**, Friedenberg F, Miller LS, Fisher RS, Swartz K, Parkman HP. Endoscopic pyloric injection of botulinum toxin A for the treatment of refractory gastroparesis. *Gastrointest Endosc* 2005; **61**: 833-839
116 **Arts J**, Holvoet L, Caenepeel P, Bisschops R, Sifrim D, Verbeke K, Janssens J, Tack J. Clinical trial: a randomized-controlled crossover study of intrapyloric injection of botulinum toxin in gastroparesis. *Aliment Pharmacol Ther* 2007; **26**: 1251-1258
117 **Friedenberg FK**, Palit A, Parkman HP, Hanlon A, Nelson DB. Botulinum toxin A for the treatment of delayed gastric emptying. *Am J Gastroenterol* 2008; **103**: 416-423
118 **Jones MP**, Maganti K. A systematic review of surgical therapy for gastroparesis. *Am J Gastroenterol* 2003; **98**: 2122-2129
119 **Abell T**, McCallum R, Hocking M, Koch K, Abrahamsson H, Leblanc I, Lindberg G, Konturek J, Nowak T, Quigley EM, Tougas G, Starkebaum W. Gastric electrical stimulation for medically refractory gastroparesis. *Gastroenterology* 2003; **125**: 421-428
120 **Lin Z**, Forster J, Sarosiek I, McCallum RW. Treatment of diabetic gastroparesis by high-frequency gastric electrical stimulation. *Diabetes Care* 2004; **27**: 1071-1076
121 **McCallum RW**, Dusing RW, Sarosiek I, Cocjin J, Forster J, Lin Z. Mechanisms of high-frequency electrical stimulation of the stomach in gastroparetic patients. *Conf Proc IEEE Eng Med Biol Soc* 2006; **1**: 5400-5403
122 **McCallum R**, Lin Z, Wetzel P, Sarosiek I, Forster J. Clinical response to gastric electrical stimulation in patients with postsurgical gastroparesis. *Clin Gastroenterol Hepatol* 2005; **3**: 49-54
123 **Abell TL**, Van Cutsem E, Abrahamsson H, Huizinga JD, Konturek JW, Galmiche JP, VoelIer G, Filez L, Everts B, Waterfall WE, Domschke W, Bruley des Varannes S, Familoni BO, Bourgeois IM, Janssens J, Tougas G. Gastric electrical stimulation in intractable symptomatic gastroparesis. *Digestion* 2002; **66**: 204-212
124 **Abell T**, Lou J, Tabbaa M, Batista O, Malinowski S, Al-Juburi A. Gastric electrical stimulation for gastroparesis improves nutritional parameters at short, intermediate, and long-term follow-up. *JPEN J Parenter Enteral Nutr* 2003; **27**: 277-281
125 **Lin Z**, Sarosiek I, Forster J, McCallum RW. Symptom responses, long-term outcomes and adverse events beyond 3 years of high-frequency gastric electrical stimulation for gastroparesis. *Neurogastroenterol Motil* 2006; **18**: 18-27
126 **Maranki JL**, Lytes V, Meilahn JE, Harbison S, Friedenberg FK, Fisher RS, Parkman HP. Predictive factors for clinical improvement with Enterra gastric electric stimulation treatment for refractory gastroparesis. *Dig Dis Sci* 2008; **53**: 2072-2078
127 **Ayinala S**, Batista O, Goyal A, Al-Juburi A, Abidi N, Familoni B, Abell T. Temporary gastric electrical stimulation with orally or PEG-placed electrodes in patients with drug refractory gastroparesis. *Gastrointest Endosc* 2005; **61**: 455-461
128 **Forstner-Barthell AW**, Murr MM, Nitecki S, Camilleri M, Prather CM, Kelly KA, Sarr MG. Near-total completion gastrectomy for severe postvagotomy gastric stasis: analysis of early and long-term results in 62 patients. *J Gastrointest Surg* 1999; **3**: 15-21, discussion 21-23
129 **Murat A**, Pouliquen B, Cantarovich D, Lucas B, Bizais Y, Vecchierini MF, Charbonnel B, Galmiche JP, Soulillou JP. Gastric emptying improvement after simultaneous segmental pancreas and kidney transplantation. *Transplant Proc* 1992; **24**: 855
130 **Hathaway DK**, Abell T, Cardoso S, Hartwig MS, el Gebely S, Gaber AO. Improvement in autonomic and gastric function following pancreas-kidney versus kidney-alone transplantation and the correlation with quality of life. *Transplantation* 1994; **57**: 816-822

**S- Editor** Cheng JX **L- Editor** Stewart GJ **E- Editor** Yin DH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.38

*World J Gastroenterol* 2009 January 7; 15(1): 38-47
***World Journal of Gastroenterology*** ISSN 1007-9327


*GUIDELINES CLINICAL PRACTICE*

**Dr. Peter V Draganov,** *Series Editor*

# Pancreatic pseudocyst

Samir Habashi, Peter V Draganov

Samir Habashi, Peter V Draganov, Department of Gastroenterology Hepatology and Nutrition, University of Florida, Gainesville, Florida 32610, United States
Author contributions: Habashi S and Draganov PV contributed equally to this work.
Correspondence to: Peter V Draganov, MD, Department of Gastroenterology, Hepatology and Nutrition, University of Florida, 1600 SW Archer Rd, Room HD 602, PO Box 100214 Gainesville, Florida 32610,
United States. dragapv@medicine.ufl.edu
Telephone: +1-352-3922877 Fax: +1-352-3923618
Received: June 8, 2008 Revised: October 2, 2008
Accepted: October 9, 2008
Published online: January 7, 2009

## Abstract

Pancreatic pseudocysts are complications of acute or chronic pancreatitis. Initial diagnosis is accomplished most often by cross-sectional imaging. Endoscopic ultrasound with fine needle aspiration has become the preferred test to help distinguish pseudocyst from other cystic lesions of the pancreas. Most pseudocysts resolve spontaneously with supportive care. The size of the pseudocyst and the length of time the cyst has been present are poor predictors for the potential of pseudocyst resolution or complications, but in general, larger cysts are more likely to be symptomatic or cause complications. The main two indications for some type of invasive drainage procedure are persistent patient symptoms or the presence of complications (infection, gastric outlet or biliary obstruction, bleeding). Three different strategies for pancreatic pseudocysts drainage are available: endoscopic (transpapillary or transmural) drainage, percutaneous catheter drainage, or open surgery. To date, no prospective controlled studies have compared directly these approaches. As a result, the management varies based on local expertise, but in general, endoscopic drainage is becoming the preferred approach because it is less invasive than surgery, avoids the need for external drain, and has a high long-term success rate. A tailored therapeutic approach taking into consideration patient preferences and involving multidisciplinary team of therapeutic endoscopist, interventional radiologist and pancreatic surgeon should be considered in all cases.



**Key words:** Pancreatic pseudocyst; Cyst; Pancreatititis; Endoscopic ultrasound; Therapy

**Peer reviewer:** Luis Bujanda, Professor, Donostia Hospital, Avda. Sancho El Sabio 21-3C, San Sebastián, 20010, Spain

Habashi S, Draganov PV. Pancreatic pseudocyst. *World J Gastroenterol* 2009; 15(1): 38-47 Available from: URL: http://www.wjgnet.com/1007-9327/15/38.asp DOI: http://dx.doi.org/10.3748/wjg.15.38

## INTRODUCTION

Pseudocyst of the pancreas is a localized fluid collection that is rich in amylase and other pancreatic enzymes and is surrounded by a wall of fibrous tissue that is not lined by epithelium[1]. Pseudocysts are connected with the pancreatic duct system, either as a direct communication or indirectly *via* the pancreatic parenchyma. They are caused by pancreatic ductal disruption following increased pancreatic ductal pressure, either due to stenosis, calculi or protein plugs obstructing the main pancreatic ductal system, or as a result of pancreatic necrosis following an attack of acute pancreatitis[2,3]. Pseudocysts are a common clinical problem and complicate the course of chronic pancreatitis in 30% to 40% of patients[4].

## ETIOLOGY

The occurrence of pseudocyst parallels that of pancreatitis and the etiology of pseudocysts resembles the causes of pancreatitis closely, although pseudocyst formation is less common after acute compared to chronic pancreatitis, and it is more common after alcohol-induced than after non-alcohol-related pancreatitis. Alcohol-related pancreatitis appears to be the major cause in studies from countries where alcohol consumption is high and accounts for 59%-78% of all pseudocysts[5].

Walt *et al*[6] reported data collected from Wayne State University Hospital in Detroit, USA. The causative factors in the 357 admissions for pancreatic pseudocysts included alcohol use in 251 cases (70%), biliary tract disease in 28 (8%), blunt trauma in 17 (5%), penetrating trauma in four (1%), operative trauma in one (0.3%),

and idiopathic in 56 (16%). Most of the patients in the idiopathic group were thought to have been alcohol-related, but no definite evidence was recorded[6].

## CLASSIFICATION

D'Egidio and Schein, in 1991, described a classification of pancreatic pseudocyst based on the underlying etiology of pancreatitis (acute or chronic), the pancreatic ductal anatomy, and the presence of communication between the cyst and the pancreatic duct[7]. They define three distinct types of pseudocysts[7]. Type Ⅰ, or acute "post-necrotic" pseudocysts, that occur after an episode of acute pancreatitis and are associated with normal duct anatomy, and rarely communicate with the pancreatic duct. Type Ⅱ, also post-necrotic pseudocysts, which occurs after an episode of acute-on-chronic pancreatitis (the pancreatic duct is diseased, but not strictured, and there is often a duct-pseudocyst communication). Type Ⅲ, defined as "retention" pseudocysts, occur with chronic pancreatitis and are uniformly associated with duct stricture and pseudocystduct communication.

Another classification, based entirely on pancreatic duct anatomy, is proposed by Nealon and Walser[8]. Type Ⅰ: normal duct/no communication with the cyst. Type Ⅱ: normal duct with duct-cyst communication. Type Ⅲ: otherwise normal duct with stricture and no duct-cyst communication. Type Ⅳ: otherwise normal duct with stricture and duct-cyst communication. Type Ⅴ: otherwise normal duct with complete cut-off. Type Ⅵ: chronic pancreatitis, no duct-cyst communication. Type Ⅶ: chronic pancreatitis with duct-cyst communication[8].

## INCIDENCE

Regardless of the etiology of pseudocyst, the incidence is low, 1.6%-4.5%, or 0.5-1 per 100 000 adults per year[9,10]. In a study by Imrie, pseudocysts developed after emergency hospital admission for an episode of acute pancreatitis in 86 patients[11]. Sixty-two of the 86 pseudocysts consequent to acute pancreatitis were derived from the local hospital population area, in which 879 patients with acute pancreatitis were admitted to hospital during the same time period. This resulted in a 7% overall incidence of pseudocysts as a complication of acute pancreatitis[11].

In a series of 926 patients with non-alcoholic acute pancreatitis, fluid collections were observed in 83 (9%). At the end of 6 wk, 48 (5%) still had a fluid collection consistent with a pseudocyst[12].

Kourtesis *et al*[13] followed prospectively with computed tomography (CT) 128 consecutive patients with acute pancreatitis (mostly alcohol-induced). Forty-eight patients (37%) developed fluid collection in the pancreatic region. The majority of these resolved spontaneously. In 15 (12%) patients, symptomatic pseudocysts developed.

Pseudocysts tend to be more common in chronic as compared to acute pancreatitis. Incidence figures of 30% to 40% have been reported in the literature[4]. However, there is a lack of precise data based on the long-term follow-up of patients with chronic pancreatitis, in contrast to acute pseudocysts where the patient with chronic pancreatitis may have had the disease for 10, 20 or more years giving him a high risk of developing a pseudocyst at least once over a long period of sickness[14].

**Table 1 Differential diagnosis of pancreatic pseudocyst**

| Pancreatic diseases | Extrapancreatic diseases |
|---|---|
| Acute & chronic pancreatitis | Peptic ulcer disease & gastric cancer |
| Pancreatic necrosis & abscess | Acute cholecystitis & gallstones |
| Adenocarcinoma of the pancreas | Abdominal aortic aneurysm |
| Pancreatic cystic neoplasms | Intestinal ischemia |
| Pancreatic artery pseudoaneurysm | Ovarian cysts & cancers |
| | Bowel obstruction |
| | Acute myocardial infarction |
| | Pneumonia |

## PATHOGENESIS

The pathogenesis of pseudocysts seems to stem from disruptions of the pancreatic duct due to pancreatitis or trauma followed by extravasation of pancreatic secretions. Two thirds of patients with pseudocysts have demonstrable connections between the cyst and the pancreatic duct. In the other third, an inflammatory reaction most likely sealed the connection so that it is not demonstrable.

In case of pseudocyst following an episode of acute pancreatitis, only if the acute fluid collection persists more than 4-6 wk, and is well-defined by a wall of fibrous or granulation tissue, can one say that an acute pseudocyst has appeared. Such a pseudocyst usually contains enzymatic fluid and necrotic debris[1,5].

The pathogenesis of pseudocyst formation in chronic pancreatitis is less well understood but, at least two mechanisms may be involved, the cyst may develop as a consequence of an acute exacerbation of the underlying disease and/or blockage of a major branch of the pancreatic duct by a protein plug, calculus or localized fibrosis[15].

## CLINICAL PRESENTATION, DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

The clinical presentation of pancreatic pseudocyst can range from asymptomatic patient to major abdominal catastrophe due to complications[16-18]. Acute complications include bleeding (usually from splenic artery pseudoaneurysm), infection, and rupture.

Chronic complications include gastric outlet obstruction, biliary obstruction and thrombosis of the splenic or portal vein with development of gastric varices[18].

A variety of diseases can mimic the clinical presentation of pancreatic pseudocyst (Table 1). Once pancreatic cyst is identified by an imaging modality, the most important question is to differentiate pseudocyst from other cystic lesions of the pancreas (Table 2).

**Table 2 Differential diagnosis of cystic pancreatic lesions**

| | SCA | MCN | IPMN | SPN | Pseudocyst |
|---|---|---|---|---|---|
| Prevalent age | Middle age | Middle age | Elderly | Young | Variable |
| Sex | Mostly female | Mostly female | Male > female | Mostly female | Male > female |
| Presentation | Mass/pain | Mass/pain | Pancreatitis | Mass/pain | Pain |
| Location | Evenly | Body/tail | Head | Evenly | Evenly |
| Malignant potential | Very low | Moderate to high | Low to high | Low | None |

SCA: Serous cystadenoma; MCN: Mucinous cystic neoplasm; IPMN: Intraductal papillary mucinous neoplasia; SPN: Solid pseudopapillary neoplasm.

## HISTORY AND PHYSICAL EXAMINATION

No specific set of symptoms is specific for pseudocysts; however, one should consider the possibility of a pseudocyst in a patient who has persistent abdominal pain, anorexia, or abdominal mass after a case of pancreatitis. Rarely, patients present with jaundice or sepsis from an infected pseudocyst[16]. Occasionally, even patients with large pancreatic pseudocyst are asymptomatic. In patients presenting with pancreatic cyst incidentally discovered on imaging, a crucial point is to define whether the patient has had prior history of pancreatitis. The sensitivity of physical examination findings is limited. Patients frequently have a tender abdomen. They can occasionally have a palpable abdominal mass. Peritoneal signs suggest rupture of the cyst or infection. Other possible findings include fever, scleral icterus or pleural effusion[17].

## LABORATORY EVALUATIONS

Serum tests have limited utility. Amylase and lipase levels are often elevated, but may be within reference ranges. The serum bilirubin and liver chemistries may be elevated if the bile duct is obstructed from stone, extrinsic compression from the pseudocyst or from underlying liver disorder (e.g. alcoholic hepatitis). Some laboratory tests may provide clues to the underlying etiology of pancreatitis (e.g. elevated triglycerides or calcium level). Elevated liver chemistries raise the suspicion for biliary pancreatitis.

## IMAGING MODALITIES

### *Transabdominal ultrasound (US)*

Pancreatic pseudocyst appears as an echoic structure associated with distal acoustic enhancement on US examination. They are well defined and round or oval, and they are contained within a smooth wall. During the early phases of their development, pseudocysts can appear more complex, with varying degrees of internal echoes. Usually, this appearance results from the presence of necrotic debris and is more common in pseudocysts that form as a result of acute necrotizing pancreatitis than in chronic pancreatitis related pseudocysts. The debris is cleared over time in most cases. The pseudocyst can appear more complex in two other instances: when hemorrhage occurs into the cyst or when infection of the cyst complicates the clinical course. Color Doppler or duplex scanning should always be performed in cystic lesions to ensure that the lesion in question is not a giant pseudoaneurysm. Sensitivity rates for US in the detection of pancreatic pseudocysts are 75% to 90%. Therefore, US is inferior to CT, which has a sensitivity of 90% to 100%. US has several limitations, as compared with CT, in the initial diagnosis of a pseudocyst: the presence of overlying bowel gas decreases the sensitivity of US, and unlike CT, US examinations are highly operator dependent[19].

### *CT*

The identification of a thick-walled, rounded, fluid-filled mass adjacent to the pancreas on an abdominal CT scan in a patient with a history of acute or chronic pancreatitis is virtually pathognomonic for pancreatic pseudocyst. Positive CT findings in this clinical situation do not require confirmation with another diagnostic modality. In the acute setting, a CT scan is the better choice because significant amounts of bowel gas resulting from ileus or obstruction decrease the sensitivity of US. In addition, CT scans provide more detailed information regarding the surrounding anatomy and can demonstrate additional pathology, including pancreatic duct dilatation and calcifications, common bile duct dilatation, and extension of the pseudocyst outside the lesser sac. The major weakness of CT scanning is the relative inability to differentiate pseudocyst from cystic neoplasm, especially mucinous cystadenomas and intraductal papillary mucinous neoplasm (IPMN)[20]. Furthermore, the intravenous contrast administered at the time of CT can precipitate or worsen kidney dysfunction.

### *Magnetic resonance imaging (MRI)*

MRI and magnetic resonance cholangiopancreatography (MRCP) are sensitive diagnostic modalities for pancreatic pseudocysts. They are generally not routinely used because CT scanning typically offers all the diagnostic information that is required. However, the increased contrast provides for better characterization of fluid collections. MRI or MRCP is superior to CT in depicting debris within fluid collections and pseudocysts. On T2-weighted images, a fluid-filled cystic mass produces high signal intensity and appears bright. The pancreatic duct and biliary systems are easily visualized in detail, although interpreting the status of pancreatic duct integrity may be difficult[21].

The ability of MRI/MRCP to depict choledocholithiasis

**Table 3 Cystic fluid analysis in cystic pancreatic diseases**

| | SCA | MCN | MCAC | Pseudocyst |
|---|---|---|---|---|
| CEA | Low | High | High | Low |
| CA125 | Variable | Variable | High | Low |
| CA19-9 | Variable | Variable-high | Variable-high | Variable |
| Amylase | Low-high | Low-high | Low-high | High |
| Lipase | Low | Low | Low | High |

SCA: Serous cystadenoma; MCN: Mucinous cystic neoplasm; MCAC: Mucinous cystadenocarcinoma

is far superior to that of CT or US. Furthermore, MRCP techniques can also depict subtle branch-chain dilatation in chronic pancreatitis. MRI is also highly sensitive to detect bleeding with complex fluid collections.

### *Endoscopic retrograde cholangiopancreatography (ERCP)*

ERCP is not necessary in diagnosing pseudocysts, but can provide definitive therapy in some cases. It also can be useful in planning possible drainage strategy. A study by Nealon *et al*[22] investigated the use of ERCP and the treatment of pseudocysts and acute pancreatitis and reported that ERCP findings may influence the treatment plan. Some authors, therefore, recommend performing an ERCP before contemplated surgical procedures. We believe that with the advent of alternative imaging technology [(CT, MRI, MRCP and endoscopic ultrasound (EUS)] ERCP is not necessary in most cases, but this has not been formally tested in a prospective study.

### *EUS*

EUS is usually used as a secondary test to further evaluate pancreatic cyst detected by other imaging modality (US, CT or MRI). EUS is the test of choice when attempting to distinguish pancreatic pseudocyst from other cystic lesions of the pancreas. Visualization of the pancreas *via* EUS provides high quality images due to the close proximity of the ultrasound transducer to the area of interest. Criteria suggestive of cystic neoplasm include a cyst wall thickness of greater than 3 mm, macroseptation (all cystic components more than 10 mm), the presence of a mass or nodule, and cystic dilation of the main pancreatic duct[23-25]. Fine needle aspiration (FNA) of the cyst can be performed at the time of EUS and cyst fluid obtained for laboratory evaluation (see laboratory evaluation above). EUS can also be used to guide therapeutic endoscopic drainage.

Analysis of the cyst fluid may help differentiate pseudocysts from cystic tumors of the pancreas (Table 3). The preferred modality to obtain cystic fluid for analysis is EUS. Carcinoembryonic antigen (CEA) level in the cystic fluid is the marker most commonly used. It is low in pseudocysts and serous cystadenomas and elevated in mucinous cystadenomas. A CEA level of greater than 400 ng/mL within the cyst fluid strongly suggests mucinous lesion[23,24,26]. Amylase levels are usually high in pseudocysts and low in serous cystadenoma. Cytology is occasionally helpful, but a negative result does not exclude malignancy.

Hammel *et al*[27] published a study to assess the reliability of preoperative biochemical and tumor marker analysis in cyst fluids obtained by FNA for pathological diagnosis. Cyst fluid was obtained preoperatively by FNA, and biochemical and tumoral marker values were measured. The diagnosis of cystic tumors (seven serous cystadenomas and 12 mucinous tumors) was established by surgical specimen analysis. Thirty-one pancreatic pseudocysts complicating well-documented chronic pancreatitis were also studied. The results showed that carbohydrate antigen 19-9 levels of > 50 000 U/mL had a 75% sensitivity and a 90% specificity for distinguishing mucinous tumors from other cystic lesions. CEA levels of < 5 ng/mL had a 100% sensitivity and an 86% specificity for distinguishing serous cystadenomas from other cystic lesions. Amylase levels of > 5000 U/mL had a 94% sensitivity and a 74% specificity for distinguishing pseudocysts from other cystic lesions. His conclusion was: high carbohydrate antigen 19-9, low CEA, and high amylase levels in cyst fluid are very indicative of mucinous tumors, serous cystadenomas, and pseudocysts, respectively[27].

Sperti *et al*[28] published a study that was performed to evaluate the utility of serum and cyst fluid analysis for enzymes (amylase and lipase) and tumor markers (CEA, CA 19-9, CA 125, and CA 72-4) in the differential diagnosis of cystic pancreatic lesions. In the study, serum and cyst fluid were obtained from 48 patients with pancreatic cysts (21 pseudocysts, 14 mucinous cystic neoplasms, six ductal carcinomas, and seven serous cystadenomas), observed between 1989 and 1994. The results showed that serum CA 19-9 levels were significantly higher in ductal carcinomas (all > 100 U/mL) and mucinous cystic neoplasms ($P < 0.05$). CA 72-4 cyst fluid levels were significantly higher in mucinous cystic tumors ($P < 0.005$), with 95% specificity and 80% sensitivity in detecting mucinous or malignant cysts. A combined assay of serum CA 19-9 and cyst fluid CA 72-4 correctly identified 19 of 20 (pre-) malignant lesions (95%), with only one false-positive result (3.6%). Cytology showed a sensitivity of 48% and specificity of 100%. Their conclusion was that any pancreatic cyst with high serum CA 19-9 values, positive cytology, or high CA 72-4 in the fluid should be considered for resection[28].

Khalid *et al*[29] published a prospective study of the utility of molecular analysis of the pancreatic pseudocyst. In the study, endoscopic ultrasound-guided pancreatic cyst aspirates were prospectively collected during a period of 19 mo and studied for cytology, CEA level, and molecular analysis. Molecular evaluation incorporated DNA quantification (amount and quality), *κ-ras* point mutation, and broad panel tumor suppressor linked microsatellite marker allelic loss analysis by using fluorescent capillary electrophoresis. The sequence of mutation acquisition was also calculated on the basis of a clonal expansion model, and comparison was made to the final pathology. Thirty-six cysts with confirmed histology were analyzed. There were 11 malignant, 15

premalignant, and 10 benign cysts. Malignant cysts could be differentiated from premalignant cysts on the basis of fluid CEA level ($P = 0.034$), DNA quality ($P = 0.009$), number of mutations ($P = 0.002$), and on the sequence of mutations acquired ($P < 0.001$). Early *κ-ras* mutation followed by allelic loss was the most predictive of a malignant cyst (sensitivity, 91%; specificity, 93%). The study concluded that malignant cyst fluid contains adequate DNA to allow mutational analysis. A first hit *κ-ras* mutation followed by allelic loss is most predictive of the presence of malignancy in a pancreatic cyst. This approach should serve as an ancillary tool to the conventional work-up of pancreatic cysts. Cumulative amount and timing of detectable mutational damage can assist in diagnosis and clinical management[29].

## TREATMENT OF PANCREATIC PSEUDOCYST

### Supportive medical care

Intravenous fluids, analgesics and antiemetics are routinely given. For patients that can tolerate oral intake, low fat diet is recommended. In patients that cannot tolerate oral nutrition, support can be provided *via* naso-enteral feeding or total parenteral nutrition (TPN). To date, no studies have compared these two approaches in the seating of pancreatic pseudocyst and choice is based on availability and local preferences. If one can extrapolate from studies comparing the two modalities in the seating of acute necrotizing pancreatitis, one can expect that jejunal feeding will be related with fewer complications (infection), but may not be able to provide as much calories as TPN.

The rationale of using octreotide as a therapy for pancreatic pseudocyst is that it will decrease pancreatic secretions and aid in pseudocyst resolution. Unfortunately, this strategy has not been rigorously tested and only a handful of case series have been published[30,31].

Most pseudocysts resolve with supportive medical care. Vitas *et al*[32] followed over a period of 5 years 114 patients with the diagnosis of pancreatic pseudocyst. Forty-six patients underwent primary operative therapy, with 13% undergoing emergency operations for pseudocyst-related complications. Although no operative deaths occurred, significant morbidity occurred in 26% of patients (emergency operations, 67%; elective procedures, 10%). The remaining 68 patients were initially treated with a nonoperative, expectant approach. Severe, life-threatening complications in this group (follow-up for a mean of 46 mo) occurred in only six patients (9%); 19 patients eventually underwent elective operation directed at either the pseudocyst or other complications related to pancreatitis. Overall, in patients managed by a nonoperative approach, resolution of the pseudocyst occurred in 57% of the 24 patients with satisfactory radiographic follow-up, with 38% resolving more than 6 mo after diagnosis. Although patients eventually undergoing operation tended to have larger pancreatic pseudocysts than the patients managed successfully nonoperatively (6.9 cm *vs* 4.9 cm), no serious complications occurred in seven patients with pancreatic pseudocysts greater than 10 cm who were treated expectantly[32].

Several studies have indicated that the size of the cyst and the length of time the cyst has been present are poor predictors of potential for pseudocyst resolution or complications, but in general, larger cysts are more likely to become symptomatic or cause complications[33]. However, some patients with larger collections do well; therefore, size of the pseudocyst alone is not an indication for drainage[34,35]. The two main indications for invasive intervention are the presence of symptoms or the presence of complications (infection, bleeding, gastric outlet or biliary obstruction).

## DRAINAGE PROCEDURES

Symptomatic pseudocysts or the presence of some complications (infected pseudocyst, gastric outlet or biliary obstruction) are the main two indications for some type of drainage procedure. To date, no prospective controlled studies have compared directly percutaneous, surgical and endoscopic drainage approaches. As a result, the management varies based on local expertise but in general endoscopic drainage is becoming the preferred approach.

### Percutaneous drainage

External drainage can be achieved using CT or US guidance. With this technique, a drainage pigtail catheter is placed percutaneously into the fluid cavity and fluid is drained. Three-dimensional ultrasonography has been reported useful for the guidance of catheters into cyst cavities and avoiding vessels[36]. The fluid is collected over several weeks into an external collection system. When the drainage output becomes minimal, the catheter is removed. Contrast injection into the cyst cavity will demonstrate the size of the remaining cavity and this finding can be used to monitor the progress. This technique is successful at resolving pseudocysts, but it has a high risk of infections. The external drain tends to create significant patient discomfort. Furthermore, the catheter tends to clog and may require repositioning and exchange. The reported long-term success rate for pseudocyst resolution for US-guided pseudocyst drainage is around 50%. Unsuccessful drainages are usually caused by large ductal leaks or obstruction of the main pancreatic duct. Percutaneous catheter drainage is contraindicated in patients who are poorly compliant and cannot manage a catheter at home. It is also contraindicated in patients with strictures of the main pancreatic duct and in patients with cysts containing bloody or solid material[37,38].

### Surgical drainage

Surgical drainage of pseudocysts is accomplished by providing a communication between the pseudocyst

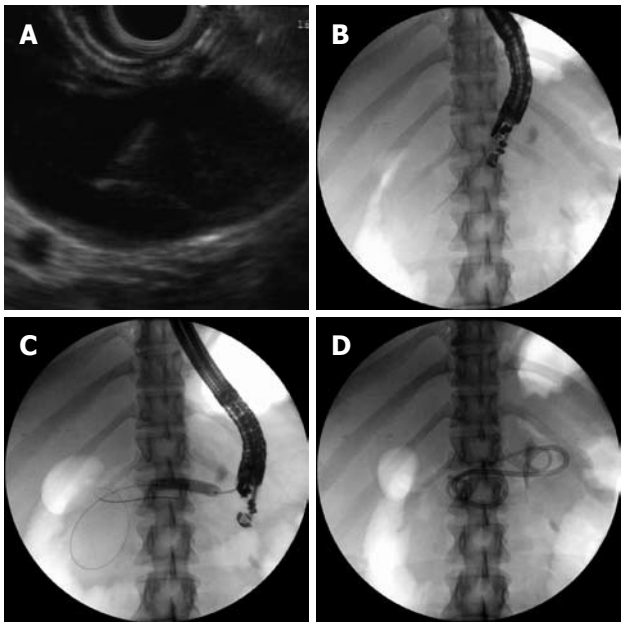


**Figure 1 EUS and fluoroscopic image.** A: EUS image of pseudocyst with FNA needle; B: Fluoroscopy image of pseudocyst with FNA needle; C: Fluoroscopy image of balloon dilating the cyst gastrostomy tract; D: Fluoroscopic image of two double pigtail stents draining the pseudocyst cavity.

cavity and the stomach or small bowel. This approach to drainage is often reserved for those patients that cannot tolerate or have failed percutaneous or endoscopic drainage. The surgical stoma should be placed in the most dependent portion of the cystic cavity in order to maximize the chances of complete drainage. The stoma usually remains patent and functional for several months.

Adams and Anderson published findings from a retrospective analysis of 94 patients[39]. The study population consisted of 42 patients undergoing internal surgical drainage and 52 patients undergoing percutaneous pseudocyst drainage. Significant complications occurred in 16.7% of the patients undergoing surgery and in 7.7% of the patients undergoing percutaneous drainage ($P > 0.05$). A subsequent operation was required in 9.5% of the surgical group and 19.2% of the percutaneous drainage group ($P > 0.05$). A significantly higher mortality rate was associated with surgical therapy (9%) than with percutaneous therapy (1%) ($P < 0.05$)[39].

### *Endoscopic drainage*

Endoscopic drainage of pseudocysts is becoming the preferred therapeutic approach because it is less invasive than surgery, avoids the need for external drain and has a high long-term success rate. Drainage is accomplished with either a transpapillary approach with ERCP or direct drainage across the stomach or duodenal wall. A transpapillary approach is used when the pseudocyst communicates with the main pancreatic duct, usually in the genue of the pancreatic duct. This approach is also successful for patients with pancreatic duct disruption.

A transgastric or transduodenal approach is used when the pseudocyst is directly adjacent to the gastro-duodenal wall. To determine the size and location of the pseudocyst, and to measure the thickness of the pseudocyst wall, EUS has become the test of choice. A distance between the gastric or duodenal wall and cyst wall of more than 1 cm or the presence of large intervening vessels or varices are relative contraindications for endoscopic drainage[40,41]. Transgastric or transduodenal stenting of pseudocysts may be performed using an endoscopic approach under fluoroscopic guidance or using EUS to introduce the guidewire into the pseudocyst cavity.

The endoscopic approach is dependent upon the presence of a bulge into the lumen of the stomach or duodenum in order to determine the entry site for catheterization. This approach has several inherent risks, including missing the pseudocyst, injuring intervening vessels, and sub-optimal placement of the drainage catheter[42]. Therapeutic echoendoscopes now make it possible to treat pseudocysts with EUS-guided transmural stenting[43]. Several series have described the deployment of a 7 Fr stent that is introduced with a needle knife catheter[44]. A new large-channel echoendoscope allows the use of 10 Fr stents across the stomach or duodenum[45].

The exact technique for transmural pseudocyst drainage has not been standardized. In our institution, we prefer a combined EUS/fluoroscopy guided technique. The linear therapeutic channel EUS endoscope is used to detect an optimal site of apposition of pseudocyst and gut wall, free of intervening vascular structures (Figure 1A). The 19 Fr gauge EUS FNA needle is then advanced into the cyst cavity under real-time ultrasound guidance. The needle position is then located under fluoroscopy (Figure 1B). After the pseudocyst cavity has been entered, fluid is aspirated and a floppy-tip 0.035 guide wire is advanced *via* the needle and under fluoroscopic control is curled few times into the cyst cavity. The cyst-gastrostomy (duodenostomy) fistula tract is then pneumatically dilated, with 8 to 15 mm biliary balloon dilators (Figure 1C). The size of the balloon used for dilation is arbitrarily determined based on the size of the cyst, proximity of vessels, presence of necrotic debris in the cyst cavity, viscosity of the aspirated pseudocyst fluid and the presence of infection. In an attempt to decrease the risk of bleeding we try to avoid using electrocautery to create the fistulous tract. In a rare occasion, when the pseudocyst wall is very tick and the balloon dilator cannot be advanced, we use the Cystotome (Cook Medical, Winston-Salem, NC, USA). We will then stent the tract with two or more double pigtail stents (7F-10F) *via* the EUS scope (Figure 1D).

In a small series, the EUS approach has resulted in a success rate of more than 90% in patients with chronic pseudocysts[46]. The recurrence rate after endoscopic drainage is low, 4%, and the complication rate is less than 16%[47].

EUS is also capable of guiding the drainage of infected pseudocysts using naso-cystic drains[48]. It may even be possible to drain infected necrotic pancreatic tissue using EUS and endoscopic techniques[49].

Hookey *et al*[50] published a chart review and prospective follow-up for 116 patients with attempted

endoscopic drainage of symptomatic pancreatic-fluid collections (pseudocysts and organized pancreatic necrosis). A total of 116 patients presented with fluid collections classified as acute fluid collection ($n$ = 5), necrosis ($n$ = 8), acute pseudocyst ($n$ = 30), chronic pseudocyst ($n$ = 64), and pancreatic abscess ($n$ = 9). The median diameter of the collection drained was 60 mm (15-275 mm). Median follow-up after drainage was 21 mo. The drainage technique was transpapillary in 15 patients, transmural in 60, and both in 41. Successful resolution of symptoms and collection occurred in 87.9% of cases. No difference in success rates was observed between patients with acute pancreatitis and those with chronic pancreatitis. However, drainage of organized necrosis was associated with a significantly higher failure rate than other collections. No significant differences were observed regarding success when disease, drainage technique, or site of drainage was considered. Complications occurred in 13 patients (11%), and there were six deaths in the 30 d after drainage, including one that was procedure related. He concluded that endoscopic drainage of pancreatic-fluid collections is successful in the majority of patients and is accompanied by an acceptable complication rate[50].

Muscatiello *et al*[51] published a case report of alcohol use for the treatment of a pancreatic pseudocyst. In his report, aspiration of the pancreatic pseudocyst was started, and after an apparent reduction in the volume of the pseudocyst by about 30%, 30 mL of absolute ethanol diluted 1:1 with saline was injected and maintained for about 10 min. Aspiration then continued until EUS imaging showed that the cyst was completely empty. CT 24 h later demonstrated no complications and confirmed that the procedure had been successful. Culture of the aspiration fluid identified a *Pseudomonas aeruginosa* and *Citrobacter freundii* complex. Cytological examination did not show any neoplastic cells. The patient was discharged on the seventh day with no symptoms and with normal laboratory tests. It seems that, in addition to causing sclerosis of the cystic wall, ethanol contributes to sterilizing the infected fluid collection. In that case, a long follow-up period (18 mo) in which there was no recurrence of the pseudocyst confirms that this procedure may be useful in the treatment of organized necrotic abscesses and pancreatic abscesses when there is no communication with the pancreatic duct[51].

In a large retrospective analysis of 603 patients who were undergoing EUS-FNA of pancreatic cysts, possible infection developed in only a single patient. The majority of patients in this series (90%) received antibiotic prophylaxis, most commonly a fluoroquinolone given for 3 d after the procedure, and this may possibly explain the low infection rate. The benefit of prophylactic antibiotics before an FNA of cystic lesions has not been evaluated by prospective randomized studies[52].

The ASGE, in 2008, published the guidelines for prophylactic use of antibiotics for GI endoscopy. According to these guidelines, prophylaxis with an antibiotic, such as a fluoroquinolone administered before EUS-FNA of cystic lesions along the GI tract including pancreatic cyst. Antibiotics may be continued for 3 to 5 d after the procedure (supported by observational studies). When antibiotic prophylaxis is administered, a fluoroquinolone administered before the procedure and continued for 3 d after the procedure is a reasonable regimen[53].

Cahen *et al*[54] published a retrospective study to evaluates the short-term and long-term results with the endoscopic drainage of pancreatic pseudocyst and aimed to identify procedural modifications that may improve its safety and efficacy. A total of 92 patients were included (66 men, 26 women; median age 49 years). The technical success rate of the drainage procedure was 97% and the mortality rate was 1%. Complications occurred in 31 patients (34%), eight of which (9%) were major and required surgery: hemorrhage in four cases (three of which were caused by erosion of a straight endoprosthesis through the cyst wall), secondary infection in three, and perforation in one. During a median follow-up period of 43 mo, 10 patients (11%) underwent additional (nonendoscopic) treatment for a persistent cyst and five (5%) for a recurrent cyst. Overall, endoscopic drainage was successful in 65 patients (71%). He concluded that endoscopic drainage is an effective treatment for pancreatic pseudocysts and offers a definitive solution in almost three-quarters of the cases. The majority of major complications might have been prevented by using pigtail stents instead of straight stents and by taking a more aggressive approach to the prevention and treatment of secondary cyst infection[54].

## COMPLICATIONS OF PANCREATIC PSEUDOCYST

### *Splenic complications*

Splenic complications of pseudocyst include massive hemorrhage into the pseudocyst, sepsis with splenic infarction, and splenic vein thrombosis. The diagnosis of intrasplenic pseudocyst, based on clinical findings alone, is difficult to arrive at but should be suggested by the presence of a mass in the left upper quadrant. Sonography and computerized axial tomography may be particularly helpful in confirming splenic involvement. Selective celiac arteriography should be performed whenever splenic involvement is suggested in order to confirm the diagnosis and to search for pseudoaneurysm formation. Urgent surgical intervention is usually warranted in view of the high incidence of serious complications and the propensity toward rapid clinical deterioration. Resection of the pseudocyst by splenectomy and distal pancreatectomy is the treatment of choice[55].

### *Rupture*

Rupture of a pseudocyst can have either a favorable or an unfavorable outcome and this depends on whether it ruptures into the gastrointestinal tract, into the general peritoneal cavity or into the vascular system[56,57]. Rupture into the gastrointestinal tract either results

in no symptoms or leads to melaena or hematemesis that usually requires urgent measures. Rupture into the general peritoneal cavity results in features of peritonitis and occasionally hemorrhagic shock. Emergent surgical exploration is usually required. While an internal drainage should always be aimed for, usually a thorough abdominal lavage and external drainage is all that can be achieved safely.

### *Hemorrhage*

Hemorrhage can greatly complicate the course of a pseudocyst[58]. The morbidity and mortality is very high because it can appear without warning and is usually due to erosion of a major vessel in the vicinity of the pseudocyst. Interventional radiology can play an invaluable role both in locating the source of bleeding and in embolisation of the bleeding vessel[59]. Without prior information of the bleeding point, surgical exploration can be hazardous and challenging.

### *Infection*

Infection occurs either spontaneously or after therapeutic or diagnostic manipulations. While infected pseudocyst can initially be treated with conservative means, a majority of patients will require intervention. Traditionally surgery has been the preferred modality but endoscopic treatment is gaining acceptance[48,60]. An external drainage may be necessary in selected situations such as when there is evidence of gross sepsis and the patient is too unstable to undergo surgical or endoscopic drainage.

### *Biliary complications*

Biliary complications occur due to a large cyst in the pancreatic head region obstructing the common bile duct and resulting in obstructive jaundice[61,62]. Therapeutic endoscopy with short-term biliary stenting is valuable in this situation. It can be retained until either the pseudocyst resolves or is treated by intervention.

### *Portal hypertension*

Portal hypertension can result from compression or obstruction of the splenic vein/portal vein either by the cyst alone or in conjunction with underlying chronic pancreatitis[63]. In this situation, surgery appears to be the only treatment modality available and an appropriate surgical procedure can effectively treat this form of portal hypertension.

## CONCLUSION

Pancreatic pseudocysts are the result of acute or chronic pancreatitis and are the most common cystic lesions of the pancreas, accounting for 75%-80% of such lesions. The most common symptoms are abdominal pain, nausea and vomiting, although they can be asymptomatic. Abdominal CT is an excellent choice for initial imaging. EUS plays an important role in differentiating pseudocyst from other cystic lesions of the pancreas and can greatly assist in transmural endoscopic drainage. Initial management consists of supportive care. Persistent symptoms and the development of complications warrant invasive intervention. The surgical, percutaneous and endoscopic pseudocyst drainage procedures have not been directly compared in high quality prospective randomized studies and the preferred approach varies based on patient preferences and local expertise. In recent years, the endoscopic approach has gained popularity with surgery reserved for patients who had failed endoscopic or percutaneous drainage. A tailored therapeutic approach taking into consideration patient preferences and involving multidisciplinary team of therapeutic endoscopist, interventional radiologist and pancreatic surgeon should be considered in all cases.

## REFERENCES


1 **Bradley EL 3rd**. 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
2 **Sanfey H**, Aguilar M, Jones RS. Pseudocysts of the pancreas, a review of 97 cases. *Am Surg* 1994; **60**: 661-668
3 **Gumaste VV**, Pitchumoni CS. Pancreatic pseudocyst. *Gastroenterologist* 1996; **4**: 33-43
4 **Boerma D**, Obertop H, Gouma DJ. Pancreatic pseudocysts in chronic pancreatitis. Surgical or interventional drainage? *Ann Ital Chir* 2000; **71**: 43-50
5 **Pitchumoni CS**, Agarwal N. Pancreatic pseudocysts. When and how should drainage be performed? *Gastroenterol Clin North Am* 1999; **28**: 615-639
6 **Walt AJ**, Bouwman DL, Weaver DW, Sachs RJ. The impact of technology on the management of pancreatic pseudocyst. Fifth annual Samuel Jason Mixter Lecture. *Arch Surg* 1990; **125**: 759-763
7 **D'Egidio A**, Schein M. Pancreatic pseudocysts: a proposed classification and its management implications. *Br J Surg* 1991; **78**: 981-984
8 **Nealon WH**, Walser E. Main pancreatic ductal anatomy can direct choice of modality for treating pancreatic pseudocysts (surgery versus percutaneous drainage). *Ann Surg* 2002; **235**: 751-758
9 **Sandy JT**, Taylor RH, Christensen RM, Scudamore C, Leckie P. Pancreatic pseudocyst. Changing concepts in management. *Am J Surg* 1981; **141**: 574-576
10 **Wade JW**. Twenty-five year experience with pancreatic pseudocysts. Are we making progress? *Am J Surg* 1985; **149**: 705-708
11 **Imrie CW**, Buist LJ, Shearer MG. Importance of cause in the outcome of pancreatic pseudocysts. *Am J Surg* 1988; **156**: 159-162
12 **Maringhini A**, Uomo G, Patti R, Rabitti P, Termini A, Cavallera A, Dardanoni G, Manes G, Ciambra M, Laccetti M, Biffarella P, Pagliaro L. Pseudocysts in acute nonalcoholic pancreatitis: incidence and natural history. *Dig Dis Sci* 1999; **44**: 1669-1673
13 **Kourtesis G**, Wilson SE, Williams RA. The clinical significance of fluid collections in acute pancreatitis. *Am Surg* 1990; **56**: 796-799
14 **Ammann RW**, Akovbiantz A, Largiader F, Schueler G. Course and outcome of chronic pancreatitis. Longitudinal study of a mixed medical-surgical series of 245 patients. *Gastroenterology* 1984; **86**: 820-828
15 **Grace PA**, Williamson RC. Modern management of pancreatic pseudocysts. *Br J Surg* 1993; **80**: 573-581
16 **Zdanyte E**, Strupas K, Bubnys A, Stratilatovas E. [Difficulties

of differential diagnosis of pancreatic pseudocysts and cystic neoplasms] *Medicina* (Kaunas) 2004; **40**: 1180-1188
17 **O'Malley VP**, Cannon JP, Postier RG. Pancreatic pseudocysts: cause, therapy, and results. *Am J Surg* 1985; **150**: 680-682
18 **Gouyon P**, Levy P, Ruszniewski P, Zins M, Hammel P, Vilgrain V, Sauvanet A, Belghiti J, Bernades P. Predictive factors in the outcome of pseudocysts complicating alcoholic chronic pancreatitis. *Gut* 1997; **41**: 821-825
19 **Pitchumoni CS**, Agarwal N. Pancreatic pseudocysts. When and how should drainage be performed? *Gastroenterol Clin North Am* 1999; **28**: 615-639
20 **Siegelman SS**, Copeland BE, Saba GP, Cameron JL, Sanders RC, Zerhouni EA. CT of fluid collections associated with pancreatitis. *AJR Am J Roentgenol* 1980; **134**: 1121-1132
21 **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
22 **Nealon WH**, Walser E. Surgical management of complications associated with percutaneous and/or endoscopic management of pseudocyst of the pancreas. *Ann Surg* 2005; **241**: 948-957; discussion 957-960
23 **Lewandrowski KB**, Southern JF, Pins MR, Compton CC, Warshaw AL. Cyst fluid analysis in the differential diagnosis of pancreatic cysts. A comparison of pseudocysts, serous cystadenomas, mucinous cystic neoplasms, and mucinous cystadenocarcinoma. *Ann Surg* 1993; **217**: 41-47
24 **Linder JD**, Geenen JE, Catalano MF. Cyst fluid analysis obtained by EUS-guided FNA in the evaluation of discrete cystic neoplasms of the pancreas: a prospective single-center experience. *Gastrointest Endosc* 2006; **64**: 697-702
25 **Sedlack R**, Affi A, Vazquez-Sequeiros E, Norton ID, Clain JE, Wiersema MJ. Utility of EUS in the evaluation of cystic pancreatic lesions. *Gastrointest Endosc* 2002; **56**: 543-547
26 **Brugge WR**, Lewandrowski K, Lee-Lewandrowski E, Centeno BA, Szydlo T, Regan S, del Castillo CF, Warshaw AL. Diagnosis of pancreatic cystic neoplasms: a report of the cooperative pancreatic cyst study. *Gastroenterology* 2004; **126**: 1330-1336
27 **Hammel P**, Levy P, Voitot H, Levy M, Vilgrain V, Zins M, Flejou JF, Molas G, Ruszniewski P, Bernades P. Preoperative cyst fluid analysis is useful for the differential diagnosis of cystic lesions of the pancreas. *Gastroenterology* 1995; **108**: 1230-1235
28 **Sperti C**, Pasquali C, Guolo P, Polverosi R, Liessi G, Pedrazzoli S. Serum tumor markers and cyst fluid analysis are useful for the diagnosis of pancreatic cystic tumors. *Cancer* 1996; **78**: 237-243
29 **Khalid A**, McGrath KM, Zahid M, Wilson M, Brody D, Swalsky P, Moser AJ, Lee KK, Slivka A, Whitcomb DC, Finkelstein S. The role of pancreatic cyst fluid molecular analysis in predicting cyst pathology. *Clin Gastroenterol Hepatol* 2005; **3**: 967-973
30 **Gullo L**, Barbara L. Treatment of pancreatic pseudocysts with octreotide. *Lancet* 1991; **338**: 540-541
31 **Suga H**, Tsuruta O, Okabe Y, Saitoh F, Noda T, Yoshida H, Ono N, Kinoshita H, Toyonaga A, Sata M. A case of mediastinal pancreatic pseudocyst successfully treated with somatostatin analogue. *Kurume Med J* 2005; **52**: 161-164
32 **Vitas GJ**, Sarr MG. Selected management of pancreatic pseudocysts: operative versus expectant management. *Surgery* 1992; **111**: 123-130
33 **Yeo CJ**, Bastidas JA, Lynch-Nyhan A, Fishman EK, Zinner MJ, Cameron JL. The natural history of pancreatic pseudocysts documented by computed tomography. *Surg Gynecol Obstet* 1990; **170**: 411-417
34 **Cheruvu CV**, Clarke MG, Prentice M, Eyre-Brook IA. Conservative treatment as an option in the management of pancreatic pseudocyst. *Ann R Coll Surg Engl* 2003; **85**: 313-316
35 **Andersson B**, Nilsson E, Willner J, Andersson R. Treatment and outcome in pancreatic pseudocysts. *Scand J Gastroenterol* 2006; **41**: 751-756
36 **Gumaste VV**, Pitchumoni CS. Pancreatic pseudocyst. *Gastroenterologist* 1996; **4**: 33-43
37 **Criado E**, De Stefano AA, Weiner TM, Jaques PF. Long term results of percutaneous catheter drainage of pancreatic pseudocysts. *Surg Gynecol Obstet* 1992; **175**: 293-298
38 **Heider R**, Meyer AA, Galanko JA, Behrns KE. Percutaneous drainage of pancreatic pseudocysts is associated with a higher failure rate than surgical treatment in unselected patients. *Ann Surg* 1999; **229**: 781-787; discussion 787-789
39 **Adams DB**, Anderson MC. Percutaneous catheter drainage compared with internal drainage in the management of pancreatic pseudocyst. *Ann Surg* 1992; **215**: 571-576; discussion 576-578
40 **Weckman L**, Kylanpaa ML, Puolakkainen P, Halttunen J. Endoscopic treatment of pancreatic pseudocysts. *Surg Endosc* 2006; **20**: 603-607
41 **Deviere J**, Bueso H, Baize M, Azar C, Love J, Moreno E, Cremer M. Complete disruption of the main pancreatic duct: endoscopic management. *Gastrointest Endosc* 1995; **42**: 445-451
42 **Lo SK**, Rowe A. Endoscopic management of pancreatic pseudocysts. *Gastroenterologist* 1997; **5**: 10-25
43 **Chak A**. Endosonographic-guided therapy of pancreatic pseudocysts. *Gastrointest Endosc* 2000; **52**: S23-S279
44 **Giovannini M**, Bernardini D, Seitz JF. Cystogastrotomy entirely performed under endosonography guidance for pancreatic pseudocyst: results in six patients. *Gastrointest Endosc* 1998; **48**: 200-203
45 **Wiersema MJ**, Baron TH, Chari ST. Endosonography-guided pseudocyst drainage with a new large-channel linear scanning echoendoscope. *Gastrointest Endosc* 2001; **53**: 811-813
46 **Norton ID**, Clain JE, Wiersema MJ, DiMagno EP, Petersen BT, Gostout CJ. Utility of endoscopic ultrasonography in endoscopic drainage of pancreatic pseudocysts in selected patients. *Mayo Clin Proc* 2001; **76**: 794-798
47 **Libera ED**, Siqueira ES, Morais M, Rohr MR, Brant CQ, Ardengh JC, Ferrari AP. Pancreatic pseudocysts transpapillary and transmural drainage. *HPB Surg* 2000; **11**: 333-338
48 **Giovannini M**, Pesenti C, Rolland AL, Moutardier V, Delpero JR. Endoscopic ultrasound-guided drainage of pancreatic pseudocysts or pancreatic abscesses using a therapeutic echo endoscope. *Endoscopy* 2001; **33**: 473-477
49 **Fuchs M**, Reimann FM, Gaebel C, Ludwig D, Stange EF. Treatment of infected pancreatic pseudocysts by endoscopic ultrasonography-guided cystogastrostomy. *Endoscopy* 2000; **32**: 654-657
50 **Hookey LC**, Debroux S, Delhaye M, Arvanitakis M, Le Moine O, Deviere J. Endoscopic drainage of pancreatic-fluid collections in 116 patients: a comparison of etiologies, drainage techniques, and outcomes. *Gastrointest Endosc* 2006; **63**: 635-643
51 **Muscatiello N**, Pietrini L, Gentile M, Tonti P, Ricciardelli C, Sorrentini I, Ierardi E. Endoscopic ultrasound-guided ethanol lavage of a pancreatic fluid collection. *Endoscopy* 2006; **38**: 951
52 **Lee LS**, Saltzman JR, Bounds BC, Poneros JM, Brugge WR, Thompson CC. EUS-guided fine needle aspiration of pancreatic cysts: a retrospective analysis of complications and their predictors. *Clin Gastroenterol Hepatol* 2005; **3**: 231-236
53 **Banerjee S**, Shen B, Baron TH, Nelson DB, Anderson MA, Cash BD, Dominitz JA, Gan SI, Harrison ME, Ikenberry SO, Jagannath SB, Lichtenstein D, Fanelli RD, Lee K, van Guilder T, Stewart LE. Antibiotic prophylaxis for GI endoscopy. *Gastrointest Endosc* 2008; **67**: 791-798
54 **Cahen D**, Rauws E, Fockens P, Weverling G, Huibregtse K, Bruno M. Endoscopic drainage of pancreatic pseudocysts: long-term outcome and procedural factors associated with safe and successful treatment. *Endoscopy* 2005; **37**: 977-983

55 **Sitzmann JV**, Imbembo AL. Splenic complications of a pancreatic pseudocyst. *Am J Surg* 1984; **147**: 191-196
56 **Yamamoto T**, Hayakawa K, Kawakami S, Nishimura K, Katsuma Y, Hayashi N, Maeda M, Ishii Y. Rupture of a pancreatic pseudocyst into the portal venous system. *Abdom Imaging* 1999; **24**: 494-496
57 **Lesur G**, Bernades P. [Pseudocysts of the pancreas. Diagnosis, course and principles of treatment] *Presse Med* 1996; **25**: 939-943
58 **Ungania S**, Panocchia N. [Splenic artery rupture in pancreatic pseudocyst] *Ann Ital Chir* 2000; **71**: 251-255
59 **Gambiez LP**, Ernst OJ, Merlier OA, Porte HL, Chambon JP, Quandalle PA. Arterial embolization for bleeding pseudocysts complicating chronic pancreatitis. *Arch Surg* 1997; **132**: 1016-1021
60 **Boerma D**, van Gulik TM, Obertop H, Gouma DJ. Internal drainage of infected pancreatic pseudocysts: safe or sorry? *Dig Surg* 1999; **16**: 501-505
61 **Noda T**, Ueno N, Tamada K, Ichiyama M, Fukuda M, Tomiyama T, Nishizono T, Tano S, Aizawa T, Iwao T. A case of chronic pancreatitis with pseudocysts complicated by infection and obstructive jaundice. *Am J Gastroenterol* 1994; **89**: 2066-2069
62 **Maema A**, Kubota K, Bandai Y, Makuuchi M. Proximal bile duct stricture caused by a pancreatic pseudocyst: intra-operative placement of a metallic stent. *Hepatogastroenterology* 1999; **46**: 2020-2023
63 **Bernades P**, Baetz A, Levy P, Belghiti J, Menu Y, Fekete F. Splenic and portal venous obstruction in chronic pancreatitis. A prospective longitudinal study of a medical-surgical series of 266 patients. *Dig Dis Sci* 1992; **37**: 340-346

**S- Editor** Xiao LL **L- Editor** Rippe RA **E- Editor** Yin DH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.48

*World J Gastroenterol* 2009 January 7; 15(1): 48-54
***World Journal of Gastroenterology*** ISSN 1007-9327


*GUIDELINES CLINICAL PRACTICE*

**Dr. Peter V Draganov,** *Series Editor*

# Cystic neoplasms of the pancreas: A diagnostic challenge

Grant F Hutchins, Peter V Draganov

Grant F Hutchins, Peter V Draganov, Department of Gastroenterology, Hepatology and Nutrition, University of Florida, 1600 SW Archer Rd, Room HD 602, PO Box 100214 Gainesville, Florida 32610, United States
Author contributions: Hutchins GF and Draganov PV contributed equally to this work.
Correspondence to: Peter V Draganov, MD, Department of Gastroenterology, Hepatology and Nutrition, University of Florida, 1600 SW Archer Rd, Room HD 602, PO Box 100214 Gainesville, Florida 32610,
United States. dragapv@medicine.ufl.edu
Telephone: +1-352-3922877 Fax: +1-352-3923618
Received: May 14, 2008 Revised: July 14, 2008
Accepted: July 21, 2008
Published online: January 7, 2009

## Abstract

Cystic neoplasms of the pancreas are increasingly recognized due to the expanding use and improved sensitivity of cross-sectional abdominal imaging. Major advances in the last decade have led to an improved understanding of the various types of cystic lesions and their biologic behavior. Despite significant improvements in imaging technology and the advent of endoscopic-ultrasound (EUS)-guided fine-needle aspiration, the diagnosis and management of pancreatic cystic lesions remains a significant clinical challenge. The first diagnostic step is to differentiate between pancreatic pseudocyst and cystic neoplasm. If a pseudocyst has been effectively excluded, the cornerstone issue is then to determine the malignant potential of the pancreatic cystic neoplasm. In the majority of cases, the correct diagnosis and successful management is based not on a single test but on incorporating data from various sources including patient history, radiologic studies, endoscopic evaluation, and cyst fluid analysis. This review will focus on describing the various types of cystic neoplasms of the pancreas, their malignant potential, and will provide the clinician with a comprehensive diagnostic approach.



**Key words:** Cystic neoplasm; Endoscopic ultrasound; Pancreas; Pancreatic cyst; Pancreatitis

**Peer reviewer:** Claudio Bassi, MD, Professor, Department of Surgery and Gastroenterology, Hospital GB Rossi, University of Verona, Piazza LA Scuro 37134 Verona, Italy

Hutchins GF, Draganov PV. Cystic neoplasms of the pancreas: A diagnostic challenge. *World J Gastroenterol* 2009; 15(1): 48-54 Available from: URL: http://www.wjgnet.com/1007-9327/15/48.asp DOI: http://dx.doi.org/10.3748/wjg.15.48

## INTRODUCTION

Pancreatic cystic neoplasms, despite increased recognition, remain rare and represent approximately 10%-15% of primary cystic masses of the pancreas[1-3]. Many pancreatic cystic masses are discovered incidentally during the work-up for abdominal pain, diarrhea, and other non-specific gastrointestinal symptoms and represent a frequent clinical referral in tertiary academic centers with pancreatic expertise. Not surprisingly, the increase in the diagnosis of a pancreatic cystic mass parallels that of the improved number and type as well as the improved overall sensitivity of cross-sectional imaging studies used in routine practice today[4]. It is important for today's practicing physician to be aware of these increasingly recognized neoplasms on radiological imaging, and more importantly, to understand the potential for the presence or development of pancreatic malignancy in a certain subset of these lesions.

## CLASSIFICATION

The classification of cystic pancreatic neoplasms has its roots in the surgical, radiological, and perhaps most importantly in the clinical pathological literature, and dates from the mid to late 1970s[5,6]. The distinction between serous and mucinous cystic neoplasms (MCNs) was first realized at that time and despite many modifications and attempts at radiological[7], endoscopic[8], and more recently with newer laboratory-based analysis using techniques such as mass spectrometry[9], remains intact and a solid initial clinical approach to these neoplastic lesions even today. Importantly, our understanding of MCNs has evolved and since the early 1980s, the clinical entity we now recognize as intraductal papillary mucinous neoplasm (IPMN) was first described in the literature[10]. IPMN remains a very important "lesion of clinical distinction" when evaluating pancreatic cystic neoplasms and is recognized as a distinct histopathological entity as evidenced by the World Health Organization histological classification system[11] (Table 1).

**Table 1 Histological classification of pancreatic cysts**

| | Histologic classification |
|---|---|
| Serous cystic tumors | SCA |
| | Serous cystadenocarcinoma (rare) |
| | Mucinous cystadenoma |
| | Mucinous cystadenoma with moderate dysplasia |
| Mucinous cystic tumors | Mucinous cystadenocarcinoma |
| | Noninfiltrating |
| | Infiltrating |
| Intraductal papillary mucinous tumors | Intraductal papillary mucinous adenoma |
| | IPMN with moderate dysplasia |
| | Intraductal papillary mucinous carcinoma |
| | Noninfiltrating |
| | Infiltrating |
| Solid pseudopapillary tumors | |

## MALIGNANT POTENTIAL OF PANCREATIC CYSTIC NEOPLASMS

The incidentally discovered pancreatic cystic neoplasm not only represents an alarming clinical discovery, but for the affected patient, in many instances, represents a pre-cancerous condition with a great deal of uncertainty regarding management. The discussion regarding malignant potential focuses mainly on the distinction between IPMN and MCNs. Serous cystadenomas (SCAs) are largely benign lesions although case reports of malignant transformation do exist and as such are often managed non-surgically. Solid pseudopapillary tumors have a fairly well defined behavior and malignant risk and are often managed surgically.

The distinction between IPMN lesions and MCN lesions remains a controversial topic and relies on several clinical and pathological factors. Clinical factors include patient age, location of the cyst, cyst characteristics, and relationship to the main pancreatic duct. As is described in more detail below, IPMNs are found most often in male patients in their 60s or 70s, and are more often than not found in the pancreatic head/neck region. IPMNs appear "grape-like" on imaging, including on endoscopic ultrasound (EUS) and appear as cysts side by side one another rather than the "Cyst within a cyst" characteristically seen in MCNs. IPMN lesions also communicate with the pancreatic duct, a feature not seen in MCNs. MCNs in comparison, are often seen in females in the 40 to 50-year age range and are located most often in the pancreatic body and tail regions.

Pathologically, the best studied differentiation criteria involve the presence of ovarian-type stroma on histological analysis[5,12]. The presence of ovarian-type stroma is strongly suggestive of an MCN lesion, although non-ovarian stroma MCNs have been reported in the literature. The distinctions between MCN and IPMN lesions are clinically important as the malignant potential and resultant management are often times based on these differences and permit an individualized care plan, rather than pursuing a "remove all mucinous neoplastic process" management style.

The malignant potential of the various cystic neoplasms of the pancreas are important for the given clinician and are best understood by dividing IPMNs into main-branch *vs* side-branch lesions and comparing/contrasting these with the MCN. Main branch IPMN lesions carry the highest percentage of malignancy, ranging in most studies between 60% and 92%[13-16]. Invasive malignancy defined as non-carcinoma-in-situ is also more common in these lesions and approaches 60% in some studies. Side-branch IPMN lesions in comparison are less often malignant, with a range of malignancy in reported studies between 6% and 46% and are less likely to be invasive, with the highest reported percentage in the 30% range[17,18]. In comparison to IPMN lesions, MCNs have a malignant potential ranging from as low as 6% to as high as 36%[19-21]. A better understanding of the malignant potential of MCN lesions is likely to improve with further acceptance of the ovarian-type stroma as diagnostic criteria regarding these lesions.

## PRESENTATION/EPIDEMIOLOGY

The exact prevalence of pancreatic cysts is difficult to measure because many patients will be entirely asymptomatic, but it has been estimated to be approximately 20% in patients undergoing radiological imaging for non-pancreatic diseases/indications[22]. The asymptomatic nature of these cystic lesions (estimated at 40%-75%) in some studies[23] make further epidemiological studies a clinically difficult task. An autopsy series from Japan estimated the prevalence of pancreatic cysts to be 25%, with an increasing prevalence paralleling advanced patient age[24]. Regardless, the proportion of pancreatic cysts felt to be primary cystic neoplasms is well documented and in the range of 10%-15% with the remaining majority of cysts found to be pseudocysts[25]. This percentage draws attention to the importance of ruling out the presence of a pancreatic pseudocyst using a combination of historical questioning, and in many cases of cystic sampling, usually done *via* EUS.

## DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS OF PANCREATIC CYSTIC LESIONS

Once the presence of a pancreatic cyst has been established by an imaging modality, the cornerstone of management is to differentiate between a pseudocyst and a cystic neoplasm. If a pseudocyst has been effectively excluded, a prudent clinical strategy regarding pancreatic cysts is the division into serous *vs* mucinous neoplasms. During the evaluation of a pancreatic cyst, it is important for the clinician to have an understanding of the different cyst types, their typical location in the pancreas, and their biological behavior. Serous cystic neoplasms (SCNs) represent approximately 30% of primary cystic neoplasms of the pancreas[26], with the largest subset being SCAs. The mucinous neoplasms

**Table 2 Typical characteristics of pancreatic cystic lesions**

| Cyst type | Pseudocyst | SCA | MCN | IPMN | SPN |
|---|---|---|---|---|---|
| Age | Variable | Middle-aged | Middle-aged | Elderly | Young |
| Sex | M > F | F > M | Female | M > F | Female |
| Pancreatitis history[1] | Yes | No | No | Yes[2] | No |
| Location | Evenly | Evenly | Body/tail | Head | Evenly |
| Malignant potential | None | Rarely | Moderate to high | Low to high | Low |
| Biliary obstruction | Yes, Uncommon | No | No | Yes, Uncommon | No |

SCA: Serous cystadenoma; MCN: Mucinous cystic neoplasm; IPMN: Intraductal papillary mucinous neoplasm; SPN: Solid pseudopapillary neoplasm. [1]A history of pancreatitis episodes and pancreatic risk factors including alcohol abuse, gallstones and complications, or family history of pancreatitis is often given; [2]Pancreatitis due to IPMN is predominately of the main pancreatic duct subtype.

are primarily subdivided into MCNs which represent approximately 45%-50% of primary cystic neoplasms of the pancreas[26], and IPMNs which make up approximately 25% of primary cystic neoplasms[27].

It is of great clinical importance at this point of the work-up to consider the clinical background of the patient with a newly discovered pancreatic cystic neoplasm. Remembering that a large proportion of pancreatic cysts are found to be pseudocystic in nature, a thorough review of the history for episodes of definable pancreatitis in conjunction with risk factors for pancreatitis, such as chronic alcohol ingestion, family history of "pancreatic diseases" as often described by patients and their families, and autoimmune disease is always a good clinical starting point. A clear history of a well documented episode of pancreatitis strongly suggests that the cystic pancreatic lesion is a pseudocyst, but occasionally an attack of pancreatitis will be the clinical presentation of a neoplastic cystic lesion particularly an IPMN[28]. Patient demographics including age, sex, and presence or absence of symptoms and the location of the cyst are important considerations while a diagnosis is being sought. For example, MCNs tend to be a middle-age, female-predominant disease with most, but not all, lesions located in the pancreatic body or tail[20]. Serous cystic adenomas (SCAs) in contrast, while present most often in middle-aged females are evenly distributed throughout the pancreatic gland, while IPMNs have an elderly male predominance and are usually located, but not confined to the pancreatic head region[29,30]. Solid pseudopapillary tumors of the pancreas (SPNs) remain a pathologically distinct, rare clinical entity occurring predominately in young females[31]. A comparative index involving the different pancreatic cystic neoplasms as well as the pancreatic pseudocysts is shown in Table 2, and the usual location of pancreatic cystic lesions in Figure 1[32].

The discovery of a lesion thought to represent a possible pancreatic cystic neoplasm is often made incidentally by computed tomograghy (CT) scanning performed for other clinical reasons. With this in mind,

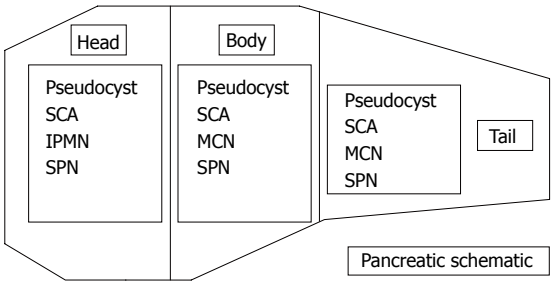


**Figure 1 Typical location for pancreatic cystic lesions.**

a thorough understanding of the different imaging modalities, both radiological and if available endoscopic, is needed to best construct a diagnostic algorithm for optimum care for these patients. The availability of endoscopic retrograde cholangiopancreatography (ERCP) and perhaps most importantly, EUS plus/minus fine-needle aspirate (FNA) and cystic fluid analysis, has led to a much improved understanding and characterization of these lesions.

## RADIOLOGICAL IMAGING STUDIES

Traditionally, three imaging modalities have been used to evaluate pancreatic lesions: trans-abdominal ultrasound (US), CT scanning, and magnetic resonance imaging (MRI)/magnetic resonance cholangiopancreatography (MRCP). Trans-abdominal US, while having the advantage of being inexpensive and readily available, is very operator-dependent, and is limited in its ability to visualize the entire pancreas. Furthermore, the presence of significant bowel gas limits the sensitivity of US for characterization of pancreatic cystic processes.

CT scanning, particularly with intravenous contrast enhancement, is a widely available, relatively inexpensive imaging modality and is often the first imaging procedure ordered when a diagnosis of a pancreatic cystic neoplasm is considered. A review of the diagnostic accuracy of CT scanning has recently been performed[32] with a reported range between 20% and 90%. Differences in study design, characterization of lesions, especially those with atypical features[33,34], and the ultimate study goal, i.e. specific cyst type[34-37] *vs* differentiation of benign *vs* malignant cyst types[8] all were felt to contribute to the wide range in diagnostic accuracy.

The typical appearance of a given cystic neoplasm is reported in many ways *via* CT. Size (i.e. microcystic (< 2 cm) *vs* macrocystic (> 2 cm), uni- *vs* multilocularity, pancreatic duct communication and/or dilation, and the presence of a mass or mural nodule remain the most important imaging characteristics seen on routine CT. SCAs are characteristically microcystic with many small cysts within the larger cyst creating a "honeycomb" type pattern. A central stellate scar is often seen at the center of an SCA and is considered pathognomonic. Pancreatic duct communication is rarely seen and dilation of the pancreatic duct also remains uncommon. MCNs are in comparison, most often macrocystic, although

microcystic lesions do occur and characteristically are multilocular with an "orange fruit" type appearance. Dilation of the pancreatic duct is uncommon as is communication with the main pancreatic duct. IPMN lesions in contrast, are often described as a "bag of grapes" and contain numerous smaller cysts. Pancreatic duct communication is common, and in main branch IPMN lesions, pancreatic duct dilation is seen and predictive of an invasive nature. Associated mural nodules and/or masses are most often observed in IPMN lesions and to a lesser extent in MCNs. The presence of a mural nodule is significant, as this is often predictive of an invasive cystic neoplasm.

MRI of the abdomen when combined with MRCP is a rapidly emerging imaging modality with widespread availability, and has great potential to add to our understanding of pancreatic cystic lesions. MRI/MRCP comparatively, in relation to other imaging modalities, is rivaled only by EUS in its ability to obtain quality images of not only the pancreatic parenchyma, but also of the pancreatic and biliary ductal structures[38-41]. MRCP does remain inferior to ERCP in terms of diagnostic accuracy, but the gap is narrowing and MRCP offers a non-invasive means of diagnosis compared with ERCP and its complications, most notably post-ERCP pancreatitis.

## ENDOSCOPIC STUDIES

The role of endoscopy, specifically ERCP and EUS, in the evaluation and diagnosis of pancreatic cystic neoplasms is a study in evolution that continues today. ERCP remains the most sensitive diagnostic modality for detecting communication between the main pancreatic duct and a given cystic lesion[42,43]. Additionally, in a minority of cases an endoscopic diagnosis of an IPMN can be established if a patulous papilla with mucin extrusion, also sometimes referred to as the "fish-eye" ampulla is visualized[30]. The use of ERCP as a primary diagnostic tool in pancreatic cystic neoplasms is not routinely recommended. In most cases, the correct diagnosis can be achieved with a higher yield, less invasive test.

EUS, since its introduction as an endoscopic technology in the late 1970s and early 1980s, has become an increasingly available tool in the diagnosis, management, and in some cases, therapy of pancreatic cystic neoplasms. The ability to better describe/characterize pancreatic cystic neoplasms, in particular those lesions thought to be pre-malignant or frankly malignant, make the use of EUS, both with and without FNA, an attractive option in the cystic neoplastic work up. EUS criteria for mucinous/malignant neoplasms is still evolving, but include size greater than 2 cm, pancreatic duct dilation, the presence of wall calcifications, and perhaps most importantly the presence of a frank mass or mural nodule. Despite initial enthusiasm, however[44], numerous studies[45-49] have demonstrated a wide range of diagnostic accuracy for EUS imaging alone ranging from 40%-96%. While many factors including study design, number of patients enrolled, goals of a particular study, and interobserver EUS agreement contribute to this discrepancy, it is important to note that a single, prospective study[49] achieved a diagnostic accuracy of approximately 51%. Clearly, larger, prospective, multi-center studies are needed to better define the role of EUS in the diagnostic work up of a pancreatic cystic neoplasm.

EUS, in addition to its imaging capabilities outlined above, allows direct sampling of cystic contents and the cyst wall in an effort to better determine the type of cyst present. The performance of FNA does, however, remain limited to larger, tertiary centers with extensive experience in EUS. In addition, analysis of cystic fluid is often subject to local cytological and laboratory expertise, with a definite learning curve present for accurate analysis of cystic contents and in some cases, by a small volume of aspirate obtained at FNA.

Ideally, an aspirated pancreatic cystic neoplasm should be evaluated for both cytological diagnosis and for the presence of specific intracystic proteins such as amylase and carcinoembryonic antigen (CEA). The cytological evaluation includes specific testing for the presence of columnar epithelial cells which stain for mucin (MCNs, IPMNs), or cuboidal epithelial cells which stain for glycogen (SCAs). Several studies have appeared in the literature regarding the analysis of pancreatic cystic fluid. Several larger studies involving cystic fluid cytological analysis[32,50,51] reflect a sensitivity of approximately 50%, a low but reproducible percentage, while a more recent study by Moparty *et al*[52] revealed a cytological sensitivity of approximately 93% in the differentiation of mucinous and non-mucinous pancreatic neoplasms. The cytological analysis of cystic fluid continues to be an area of intense research.

Amylase level is routinely checked in the cyst fluid aspirate and may be of some diagnostic value. It is uniformly elevated in pseudocysts and IPMNs and frequently elevated in MCNs, but consistently low in SCAs. The analysis of specific intracystic, aspirated proteins continues to be an evolving process. Several proteins including CA19-9, CEA, CA-125, and CA72-4 have been studied. The best studied and currently used most often in routine practice is the level of CEA. The basic differentiation involving CEA level is between the lesions which are mucinous (usually, but not always elevated CEA levels) and those which are serous (low CEA levels)[51-54]. A low CEA level (i.e. < 5 ng/mL) has been shown in pooled data[44,48,51,55], to have a sensitivity between 50%-100% and a specificity of 77%-95% to differentiate between mucinous and serous lesions. The CEA level required to best distinguish a mucinous from a serous lesion continues to be debated in the pancreatic literature, with CEA cutoff levels deemed diagnostically sensitive in the range 20 to 800[48,49,55-57]. The wide range of reported CEA levels lends confusion to the analysis of cystic pancreatic fluid. It must be remembered, however, that by increasing the cutoff value of the CEA level considered diagnostic for mucinous lesions, the specificity of the test increases at the expense of decreased sensitivity. Currently, no standardized cutoff

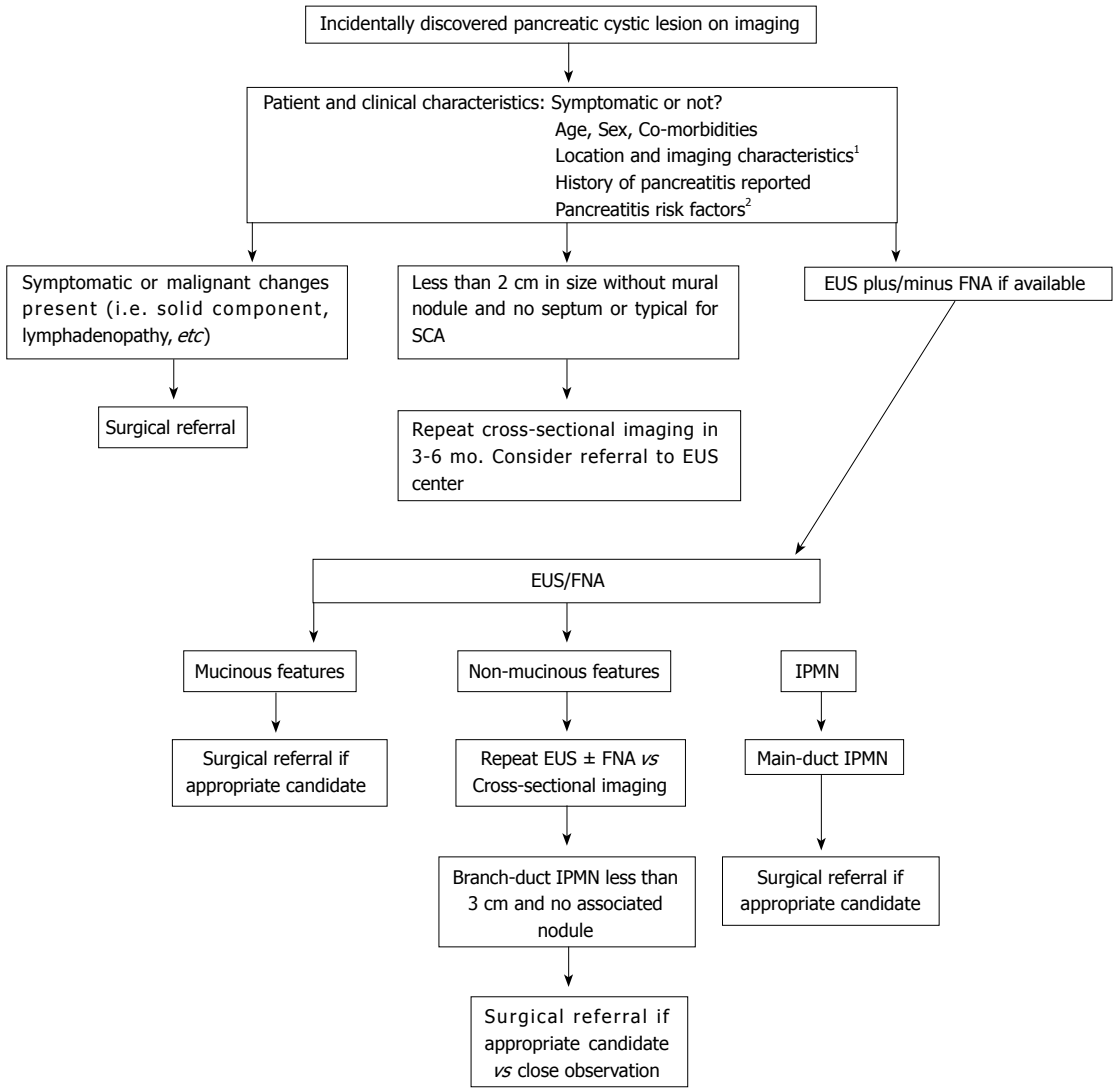


**Figure 2 Diagnostic algorithm.** [1]Imaging characteristics include size > 2 cm, mural nodule presence/absence, total cyst number, pancreatic duct communication. [2]Pancreatitis risk factors include alcohol use, family history of pancreatitis, autoimmune diseases.

level for CEA exists, however, many centers, particularly in the US, use a CEA level of 192 ng/mL, as established by Brugge *et al*[49] as diagnostically sensitive (75%) and specific (84%). At present, aspirated cystic fluid should be evaluated for cytological and biochemical analysis. The biochemical tests which should be routinely ordered are CEA level and amylase. If insufficient fluid is available (e.g. small cyst or very viscous fluid), CEA level should be obtained first with cytology and amylase level ordered only if there is a sufficient amount of fluid left for analysis.

## DIAGNOSTIC ALGORITHM

As outlined above, the diagnosis and prospective management of a pancreatic cystic neoplasm involves coordination on several levels, ranging from the initial discovery to possible surgical referral if a frankly malignant or pre-malignant pancreatic cystic neoplasm is suspected. A proposed diagnostic algorithm beginning with the incidentally discovered pancreatic lesion is presented in Figure 2.

## CONCLUSION

The evaluation of cystic lesions of the pancreas remains a process in evolution. Significant advances have been made in expanding our understanding of these lesions and in the refinement of our diagnostic approach. A comprehensive diagnostic strategy which incorporates data from patient history, lesion imaging, EUS, and cyst fluid analysis will provide an accurate diagnosis in most cases.

## REFERENCES

1 **Fernandez-del Castillo C**, Warshaw AL. Cystic neoplasms of the pancreas. *Pancreatology* 2001; **1**: 641-647
2 **Mulkeen AL**, Yoo PS, Cha C. Less common neoplasms of the pancreas. *World J Gastroenterol* 2006; **12**: 3180-3185
3 **Sakorafas GH**, Sarr MG. Cystic neoplasms of the pancreas; what a clinician should know. *Cancer Treat Rev* 2005; **31**: 507-535
4 **Megibow AJ**, Lombardo FP, Guarise A, Carbognin G, Scholes J, Rofsky NM, Macari M, Balthazar EJ, Procacci C. Cystic pancreatic masses: cross-sectional imaging observations and serial follow-up. *Abdom Imaging* 2001; **26**:

640-647
5 **Compagno J**, Oertel JE. Mucinous cystic neoplasms of the pancreas with overt and latent malignancy (cystadenocarcinoma and cystadenoma). A clinicopathologic study of 41 cases. *Am J Clin Pathol* 1978; **69**: 573-580
6 **Compagno J**, Oertel JE. Microcystic adenomas of the pancreas (glycogen-rich cystadenomas): a clinicopathologic study of 34 cases. *Am J Clin Pathol* 1978; **69**: 289-298
7 **Sahani DV**, Kadavigere R, Saokar A, Fernandez-del Castillo C, Brugge WR, Hahn PF. Cystic pancreatic lesions: a simple imaging-based classification system for guiding management. *Radiographics* 2005; **25**: 1471-1484
8 **Gerke H**, Jaffe TA, Mitchell RM, Byrne MF, Stiffler HL, Branch MS, Baillie J, Jowell PS. Endoscopic ultrasound and computer tomography are inaccurate methods of classifying cystic pancreatic lesions. *Dig Liver Dis* 2006; **38**: 39-44
9 **Scarlett CJ**, Samra JS, Xue A, Baxter RC, Smith RC. Classification of pancreatic cystic lesions using SELDI-TOF mass spectrometry. *ANZ J Surg* 2007; **77**: 648-653
10 **Ohhashi K**, Murakami Y, Maruyama M, Takekoshi T, Ohta H, Ohhashi I, Takagi K, Kato Y. Four cases of mucin-producing cancer of the pancreas on specific findings of the papilla of Vater. *Prog Dig Endosc* 1982; **20**: 348-351
11 **Klöppel G**, Solcia E, Longnecker DS, Capella C, Sobin LH. World Health Organization International Histologic Classification of Tumours 2. Histologic typing of tumours of the exocrine pancreas. Berlin: Springer-Verlag, 1996
12 **Izumo A**, Yamaguchi K, Eguchi T, Nishiyama K, Yamamoto H, Yonemasu H, Yao T, Tanaka M, Tsuneyoshi M. Mucinous cystic tumor of the pancreas: immunohistochemical assessment of "ovarian-type stroma". *Oncol Rep* 2003; **10**: 515-525
13 **Kobari M**, Egawa S, Shibuya K, Shimamura H, Sunamura M, Takeda K, Matsuno S, Furukawa T. Intraductal papillary mucinous tumors of the pancreas comprise 2 clinical subtypes: differences in clinical characteristics and surgical management. *Arch Surg* 1999; **134**: 1131-1136
14 **Terris B**, Ponsot P, Paye F, Hammel P, Sauvanet A, Molas G, Bernades P, Belghiti J, Ruszniewski P, Flejou JF. Intraductal papillary mucinous tumors of the pancreas confined to secondary ducts show less aggressive pathologic features as compared with those involving the main pancreatic duct. *Am J Surg Pathol* 2000; **24**: 1372-1377
15 **Doi R**, Fujimoto K, Wada M, Imamura M. Surgical management of intraductal papillary mucinous tumor of the pancreas. *Surgery* 2002; **132**: 80-85
16 **Kitagawa Y**, Unger TA, Taylor S, Kozarek RA, Traverso LW. Mucus is a predictor of better prognosis and survival in patients with intraductal papillary mucinous tumor of the pancreas. *J Gastrointest Surg* 2003; **7**: 12-18; discussion 18-19
17 **Sugiyama M**, Izumisato Y, Abe N, Masaki T, Mori T, Atomi Y. Predictive factors for malignancy in intraductal papillary-mucinous tumours of the pancreas. *Br J Surg* 2003; **90**: 1244-1249
18 **Sohn TA**, Yeo CJ, Cameron JL, Hruban RH, Fukushima N, Campbell KA, Lillemoe KD. Intraductal papillary mucinous neoplasms of the pancreas: an updated experience. *Ann Surg* 2004; **239**: 788-797; discussion 797-799
19 **Reddy RP**, Smyrk TC, Zapiach M, Levy MJ, Pearson RK, Clain JE, Farnell MB, Sarr MG, Chari ST. Pancreatic mucinous cystic neoplasm defined by ovarian stroma: demographics, clinical features, and prevalence of cancer. *Clin Gastroenterol Hepatol* 2004; **2**: 1026-1031
20 **Thompson LD**, Becker RC, Przygodzki RM, Adair CF, Heffess CS. Mucinous cystic neoplasm (mucinous cystadenocarcinoma of low-grade malignant potential) of the pancreas: a clinicopathologic study of 130 cases. *Am J Surg Pathol* 1999; **23**: 1-16
21 **Tanaka M**, Chari S, Adsay V, Fernandez-del Castillo C, Falconi M, Shimizu M, Yamaguchi K, Yamao K, Matsuno S. International consensus guidelines for management of intraductal papillary mucinous neoplasms and mucinous cystic neoplasms of the pancreas. *Pancreatology* 2006; **6**: 17-32
22 **Zhang XM**, Mitchell DG, Dohke M, Holland GA, Parker L. Pancreatic cysts: depiction on single-shot fast spin-echo MR images. *Radiology* 2002; **223**: 547-553
23 **Sarr MG**, Murr M, Smyrk TC, Yeo CJ, Fernandez-del-Castillo C, Hawes RH, Freeny PC. Primary cystic neoplasms of the pancreas. Neoplastic disorders of emerging importance-current state-of-the-art and unanswered questions. *J Gastrointest Surg* 2003; **7**: 417-428
24 **Kimura W**, Nagai H, Kuroda A, Muto T, Esaki Y. Analysis of small cystic lesions of the pancreas. *Int J Pancreatol* 1995; **18**: 197-206
25 **Warshaw AL**, Rutledge PL. Cystic tumors mistaken for pancreatic pseudocysts. *Ann Surg* 1987; **205**: 393-398
26 **Fernandez-del Castillo C**, Warshaw AL. Cystic tumors of the pancreas. *Surg Clin North Am* 1995; **75**: 1001-1016
27 **Loftus EV Jr**, Olivares-Pakzad BA, Batts KP, Adkins MC, Stephens DH, Sarr MG, DiMagno EP. Intraductal papillary-mucinous tumors of the pancreas: clinicopathologic features, outcome, and nomenclature. Members of the Pancreas Clinic, and Pancreatic Surgeons of Mayo Clinic. *Gastroenterology* 1996; **110**: 1909-1918
28 **Salvia R**, Fernandez-del Castillo C, Bassi C, Thayer SP, Falconi M, Mantovani W, Pederzoli P, Warshaw AL. Main-duct intraductal papillary mucinous neoplasms of the pancreas: clinical predictors of malignancy and long-term survival following resection. *Ann Surg* 2004; **239**: 678-685; discussion 685-687
29 **Scheiman JM**. Cystic lesion of the pancreas. *Gastroenterology* 2005; **128**: 463-469
30 **Azar C**, Van de Stadt J, Rickaert F, Deviere M, Baize M, Kloppel G, Gelin M, Cremer M. Intraductal papillary mucinous tumours of the pancreas. Clinical and therapeutic issues in 32 patients. *Gut* 1996; **39**: 457-464
31 **Pettinato G**, Di Vizio D, Manivel JC, Pambuccian SE, Somma P, Insabato L. Solid-pseudopapillary tumor of the pancreas: a neoplasm with distinct and highly characteristic cytological features. *Diagn Cytopathol* 2002; **27**: 325-334
32 **Oh HC**, Kim MH, Hwang CY, Lee TY, Lee SS, Seo DW, Lee SK. Cystic lesions of the pancreas: challenging issues in clinical practice. *Am J Gastroenterol* 2008; **103**: 229-239; quiz 228, 240
33 **Johnson CD**, Stephens DH, Charboneau JW, Carpenter HA, Welch TJ. Cystic pancreatic tumors: CT and sonographic assessment. *AJR Am J Roentgenol* 1988; **151**: 1133-1138
34 **Curry CA**, Eng J, Horton KM, Urban B, Siegelman S, Kuszyk BS, Fishman EK. CT of primary cystic pancreatic neoplasms: can CT be used for patient triage and treatment? *AJR Am J Roentgenol* 2000; **175**: 99-103
35 **Procacci C**, Biasiutti C, Carbognin G, Accordini S, Bicego E, Guarise A, Spoto E, Andreis IA, De Marco R, Megibow AJ. Characterization of cystic tumors of the pancreas: CT accuracy. *J Comput Assist Tomogr* 1999; **23**: 906-912
36 **Le Borgne J**, de Calan L, Partensky C. Cystadenomas and cystadenocarcinomas of the pancreas: a multiinstitutional retrospective study of 398 cases. French Surgical Association. *Ann Surg* 1999; **230**: 152-161
37 **Bassi C**, Salvia R, Molinari E, Biasutti C, Falconi M, Pederzoli P. Management of 100 consecutive cases of pancreatic serous cystadenoma: wait for symptoms and see at imaging or vice versa? *World J Surg* 2003; **27**: 319-323
38 **Koito K**, Namieno T, Nagakawa T, Shyonai T, Hirokawa N, Morita K. Solitary cystic tumor of the pancreas: EUS-pathologic correlation. *Gastrointest Endosc* 1997; **45**: 268-276
39 **Gress F**, Gottlieb K, Cummings O, Sherman S, Lehman G. Endoscopic ultrasound characteristics of mucinous cystic neoplasms of the pancreas. *Am J Gastroenterol* 2000; **95**: 961-965
40 **Koito K**, Namieno T, Ichimura T, Yama N, Hareyama M, Morita K, Nishi M. Mucin-producing pancreatic tumors: comparison of MR cholangiopancreatography with endoscopic retrograde cholangiopancreatography. *Radiology*

1998; **208**: 231-237
41 **Sahani D**, Prasad S, Saini S, Mueller P. Cystic pancreatic neoplasms evaluation by CT and magnetic resonance chol angiopancreatography. *Gastrointest Endosc Clin N Am* 2002; **12**: 657-672
42 **Fukukura Y**, Fujiyoshi F, Hamada H, Takao S, Aikou T, Hamada N, Yonezawa S, Nakajo M. Intraductal papillary mucinous tumors of the pancreas. Comparison of helical CT and MR imaging. *Acta Radiol* 2003; **44**: 464-471
43 **Yamao K**, Nakamura T, Suzuki T, Sawaki A, Hara K, Kato T, Okubo K, Matsumoto K, Shimizu Y. Endoscopic diagnosis and staging of mucinous cystic neoplasms and intraductal papillary-mucinous tumors. *J Hepatobiliary Pancreat Surg* 2003; **10**: 142-146
44 **O'Toole D**, Palazzo L, Hammel P, Ben Yaghlene L, Couvelard A, Felce-Dachez M, Fabre M, Dancour A, Aubert A, Sauvanet A, Maire F, Levy P, Ruszniewski P. Macrocystic pancreatic cystadenoma: The role of EUS and cyst fluid analysis in distinguishing mucinous and serous lesions. *Gastrointest Endosc* 2004; **59**: 823-829
45 **Sedlack R**, Affi A, Vazquez-Sequeiros E, Norton ID, Clain JE, Wiersema MJ. Utility of EUS in the evaluation of cystic pancreatic lesions. *Gastrointest Endosc* 2002; **56**: 543-547
46 **Ahmad NA**, Kochman ML, Brensinger C, Brugge WR, Faigel DO, Gress FG, Kimmey MB, Nickl NJ, Savides TJ, Wallace MB, Wiersema MJ, Ginsberg GG. Interobserver agreement among endosonographers for the diagnosis of neoplastic versus non-neoplastic pancreatic cystic lesions. *Gastrointest Endosc* 2003; **58**: 59-64
47 **Hernandez LV**, Mishra G, Forsmark C, Draganov PV, Petersen JM, Hochwald SN, Vogel SB, Bhutani MS. Role of endoscopic ultrasound (EUS) and EUS-guided fine needle aspiration in the diagnosis and treatment of cystic lesions of the pancreas. *Pancreas* 2002; **25**: 222-228
48 **Frossard JL**, Amouyal P, Amouyal G, Palazzo L, Amaris J, Soldan M, Giostra E, Spahr L, Hadengue A, Fabre M. Performance of endosonography-guided fine needle aspiration and biopsy in the diagnosis of pancreatic cystic lesions. *Am J Gastroenterol* 2003; **98**: 1516-1524
49 **Brugge WR**, Lewandrowski K, Lee-Lewandrowski E, Centeno BA, Szydlo T, Regan S, del Castillo CF, Warshaw AL. Diagnosis of pancreatic cystic neoplasms: a report of the cooperative pancreatic cyst study. *Gastroenterology* 2004; **126**: 1330-1336
50 **Walsh RM**, Henderson JM, Vogt DP, Baker ME, O'malley CM Jr, Herts B, Zuccaro G Jr, Vargo JJ, Dumot JA, Conwell DL, Biscotti CV, Brown N. Prospective preoperative determination of mucinous pancreatic cystic neoplasms. *Surgery* 2002; **132**: 628-633; discussion 633-634
51 **van der Waaij LA**, van Dullemen HM, Porte RJ. Cyst fluid analysis in the differential diagnosis of pancreatic cystic lesions: a pooled analysis. *Gastrointest Endosc* 2005; **62**: 383-389
52 **Moparty B**, Logrono R, Nealon WH, Waxman I, Raju GS, Pasricha PJ, Bhutani MS. The role of endoscopic ultrasound and endoscopic ultrasound-guided fine-needle aspiration in distinguishing pancreatic cystic lesions. *Diagn Cytopathol* 2007; **35**: 18-25
53 **Federle MP**, McGrath KM. Cystic neoplasms of the pancreas. *Gastroenterol Clin North Am* 2007; **36**: 365-376, ix
54 **Ryu JK**, Woo SM, Hwang JH, Jeong JB, Yoon YB, Park IA, Han JK, Kim YT. Cyst fluid analysis for the differential diagnosis of pancreatic cysts. *Diagn Cytopathol* 2004; **31**: 100-105
55 **Hammel P**, Voitot H, Vilgrain V, Levy P, Ruszniewski P, Bernades P. Diagnostic value of CA 72-4 and carcinoembryonic antigen determination in the fluid of pancreatic cystic lesions. *Eur J Gastroenterol Hepatol* 1998; **10**: 345-348
56 **Sperti C**, Pasquali C, Pedrazzoli S, Guolo P, Liessi G. Expression of mucin-like carcinoma-associated antigen in the cyst fluid differentiates mucinous from nonmucinous pancreatic cysts. *Am J Gastroenterol* 1997; **92**: 672-675
57 **Hammel PR**, Forgue-Lafitte ME, Levy P, Voitot H, Vilgrain V, Flejou JF, Molas G, Gespach C, Ruszniewski P, Bernades P, Bara J. Detection of gastric mucins (M1 antigens) in cyst fluid for the diagnosis of cystic lesions of the pancreas. *Int J Cancer* 1997; **74**: 286-290

**S- Editor** Li DL **L- Editor** Webster JR **E- Editor** Yin DH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.55

*World J Gastroenterol* 2009 January 7; 15(1): 55-60
***World Journal of Gastroenterology*** ISSN 1007-9327




**Dr. Peter V Draganov,** *Series Editor*

# ERCP wire systems: The long and the short of it

Shilpa Chandrupatla Reddy, Peter V Draganov

Shilpa Chandrupatla Reddy, Department of Medicine, University of Florida College of Medicine, Jacksonville, Florida 32610, United States
Peter V Draganov, Department of Gastroenterology, Hepatology and Nutrition, University of Florida, 1600 SW Archer Rd, Room HD 602, PO Box 100214 Gainesville, Florida 32610, United States
Author contributions: Reddy SC and Draganov PV contributed equally to this work.
Correspondence to: Peter V Draganov, MD, Department of Gastroenterology, Hepatology and Nutrition, University of Florida, 1600 SW Archer Rd, Room HD 602, PO Box 100214 Gainesville, Florida 32610, United States. dragapv@medicine.ufl.edu
Telephone: +1-352-392-2878 Fax: +1-352-392-3618
Received: October 11, 2008 Revised: November 6, 2008
Accepted: November 13, 2008
Published online: January 7, 2009

## Abstract

Guidewires are routinely used at the time of endoscopic retrograde cholangiopancreatography (ERCP) to gain and maintain access to the desired duct and aid in the advancement of various devices. Limitations of the traditional long-wire systems have led to the introduction of three proprietary short-wire systems. These systems differ in many respects but share two main principles: They lock a shorter wire in position to allow advancement or removal of various devices without displacement of the wire and they all allow for physician control of the wire. In this comprehensive review, we describe the key features of the three currently available short-wire systems: RX, Fusion and V systems. We also focus on the potential benefits and drawbacks that accompany the short-wire concept as a whole and each specific system in particular. Although the available data are limited, it appears that the use of the short-wire systems lead to reduced procedure, fluoroscopy and device exchange times, decreased sedation requirements, improved wire stability and increased endoscopist control of the wire. Furthermore, the physician-controlled wire-guided cannulation has the potential to decrease ampullary trauma and the rate of post-ERCP pancreatitis. The short guidewire systems appear to be an improvement over the traditional long-wire systems but further studies directly comparing the two approaches are needed.



**Key words:** Endoscopic retrograde cholangiopancreatography; Guidewires; V-system; RX system; Fusion system

**Peer reviewers:** Jose Sahel, Professor, Hepato-gastroenterology, Hospital sainti Marevenite, I270 Boolevard AE Sainti Margrenise, Marseille 13009, France; Atsushi Nakajima, Professor, Division of Gastroenterology, Yokohama City University Graduate School of Medicine, 3-9 Fuku-ura, Kanazawa-ku, Yokohama 236-0004, Japan

Reddy SC, Draganov PV. ERCP wire systems: The long and the short of it. *World J Gastroenterol* 2009; 15(1): 55-60 Available from: URL: http://www.wjgnet.com/1007-9327/15/55.asp DOI: http://dx.doi.org/10.3748/wjg.15.55

## INTRODUCTION

Guidewires are an essential part of both diagnostic and therapeutic endoscopy. They are used to gain or maintain access into a lumen or cavity. In addition, they have an integral role in the advancement of a variety of devices[1]. Guidewires vary in material, length, diameter, and design to aid in specific situations encountered by the endoscopist during a procedure.

The construction of guidewires is designed for use depending on their individual qualities. Monofilament wires are made with stainless steel and are used for their rigidity[1]. Coiled wires have an inner monofilament core which has the quality of stiffness accompanied by an outer spiral coil encompassing durability and flexibility[1]. Lastly, coated or sheathed wires have a monofilament core made of stainless steel, nitinol, or other alloys, whereas the outer sheath may be made of teflon (DE), polyurethane, or another polymer[1]. Many wires have platinum or tungsten dipped cores to enhance visualization during fluoroscopy. Tips have various designs such as straight, angled, J-shaped, or tapered. Wires can range from 150-650 cm in length and from 0.46-0.97 mm in diameter. A common source of confusion pertaining to endoscopic retrograde cholangiopancreatography (ERCP) wires is the fact that it is customary to display the length of the guidewire in cm and the diameter in inches.

Traditional guidewire usage requires advancement under direct visualization through the endoscope with or without fluoroscopy. Maintenance of the guidewire

position is essential to the safety of dilating procedures and tube placements. Assistance with printed markers and movement guides on the wire can decrease the risk of displacement[2].

Applications for guidewire use in the gastrointestinal (GI) tract are vast. These include advancement of dilators, esophageal stents, manometry catheters, feeding tubes, colonic stents, stricture dilatation and endoscopic ultrasound (EUS)-guided biliary and pancreatic access[1]. Guidewires are an indispensable part of ERCP and are used to gain and maintain access to the desired duct, and provide a platform for insertion or withdrawal of various devices.

## CHALLENGES WITH LONG GUIDEWIRE ERCP SYSTEMS

Traditionally, long wires were exclusively used at the time of ERCP. The long length is dictated by the need to exchange various devices over the wire. The length of a typical long wire used for ERCP ranges from 420 to 480 cm. This excessive length creates a number of problems. Since the assistant is in control of the wire and the physician is in control of the ERCP device, excellent communication between the physician and assistant is required. Failure to do so can lead to loss of access, more difficult cannulation, and problems with advancing the wire to the desired target. Furthermore, it is not uncommon for the end of the long wire to touch the floor leading to contamination. Finally, the assistant is challenged to perform multiple tasks at the same time including advancing or retracting the wire, injecting dye, operating the device (inflating/deflating the balloon, flexing/relaxing the sphincterotome *etc*) and doing all of that while making sure that the end of the wire does not touch the floor. Hydrophilic wires are even more prone to displacement from ducts and strictures, which may further contribute to difficulties with catheter exchanges[1]. Repositioning or loss of access to either the bile or pancreatic duct may lead to increases in procedure duration, radiation exposure to patients and staff, failure to place a stent and even perforation[3].

## BACKGROUND OF SHORT GUIDEWIRE ERCP SYSTEMS

At the time of ERCP the use of long guidewires is dictated by the need to exchange various devices over the wire. Therefore, the length of the wire should be, at a minimum, twice the length of the device. Recently, advances in catheter technology combined with wire locking systems provided for the development of short-wire ERCP systems. The main feature of all short-wire ERCP systems is the ability to lock the short-wire in position to allow advancement or removal of various devices without displacement of the wire. In a single-center pilot study, Beilstein *et al*[3] have shown that using a prototype duodenoscope (XTJF-140V2F; Olympus America Corp., Melville, NY., USA) led to a shorter exchange time when compared to standard duodenoscopes, and attributed the benefit to the guidewire locking system and fewer instances of repositioning. This milestone study demonstrated that fixation of the guidewire by the endoscope elevator can substantially improve device exchanges over a shorter wire length. However, additional goals of this new innovation included maintaining ductal access, decreasing procedure time, and reducing sedation and fluoroscopy exposure[3]. Endoscopy assistants are and have been essential to the successful use of ERCP, but the ability of the endoscopist to independently manage the guidewire and the scope was considered an advantage during these procedures.

The emergence of short-wire systems evolved in order to counter limitations of the traditional long wires. All short-wire systems share three independent elements including a means of locking the wire in position during a device exchange, exchanging devices over short-wires while maintaining access and decreasing wire lengths between 185 and 270 cm[4]. Device exchanges over a short guidewire are possible by either fixation of the wire externally at the biopsy port or internally at the elevator. Both external and internal lock designs allow physician control of the guidewire at or near the biopsy port. However, the external and internal lock designs differ in many ways. The external lock design uses suction port caps and allows fixation of the wire with all maneuvers except the limited insertion or withdrawal of the leading short-track portion of the device past the biopsy port[4]. In contrast, the internal lock system can be used with either short or long wire devices. When locking occurs at the level of the ERCP scope elevator, assistant control of the wire is needed when devices are passed beyond the elevator.

## TYPES OF SHORT GUIDEWIRE ERCP SYSTEMS

There are three available short-wire ERCP proprietary systems. These systems integrate cannulation, sphincterotomy, balloon extraction, balloon dilation and biliary stenting devices. The same short-wire devices can be used with traditional long wires if needed.

The RX system, (Boston Scientific Corporation, Natick, MA, USA) was the first short-wire system introduced in 1999. This system incorporates three components. The RX Locking Device has an external lock that may accommodate fixation of one or two wires. A special biopsy port cap minimizes any air or bile leakage during the procedure. RX Compatible Biliary Devices include both open "tear away" channel monorail designs used with sphincterotomes and catheters, as well as short-track designs used with cytology catheters, stone extraction balloons, dilating balloons and stents. The last component includes the 260-cm long 0.035-inch or 0.025-inch wide Jagwire with a coated firm shaft, flexible hydrophilic leading tip and two colored markings, which aid in detection of wire movement[4]. Use of the 0.035-inch Jagwire, in conjunction with an ultra slim upper endoscope (GIF-XP 160, Olympus America,

**Table 1 Differences between the three short-wire systems**

| Characteristics | RX system | Fusion system | V-system |
|---|---|---|---|
| Type of endoscope | Standard | Standard | V-scope |
| Type of lock | External at the biopsy port | External at the biopsy port | Internal lock design |
| Type of device | Open channel tear-away | Close channel breakthrough | Close lumen device |
| Short–track technology | Yes | Yes | No |
| Wire length | 260 cm | 185 cm | 270 cm |
| Can be used with standard guidewires | Yes | Yes | Yes |
| Can be used with 0.025″ or 0.018″ or angled wires | No | Yes | Yes |
| Can be used with hydrophilic Glidewire | No | Yes | Yes |
| Ability to flush wire channel | No | Yes | Yes |
| Intraductal exchange ability | No | Yes | No |
| Insertion of multiple stents without the need to recannulate | No | Yes | No |
| Physician control of wire | Yes | Yes | Yes |
| Pushability of short-wire devices[1] | ++ | +++ | +++ |

[1]author own experience.

Center Valley, PA., USA), for maintaining access allowed for direct visualization of the biliary tree to aid in intraductal diagnosis and treatment[5]. The RX system does allow for long wire conversion in appropriate cases with a 200-cm wire attachment. The 0.025-inch and 0.035-inch diameter wire should be used with their respective devices which are not interchangeable.

The Fusion system (Cook Endoscopy; Winston Salem, NC., USA) was introduced in 2004. As in the RX Biliary system, the Fusion system incorporates both short-track and tear-away capabilities. The external wire lock fits on the biopsy port, which enables the locking of one or two wires. A key feature of this system includes a side port that has been placed at 6 cm from the distal tip of any catheter and a closed tear-away channel running the length of the catheter (as opposed to the open tear-away channel of the RX). The availability of a side port near the device tip allows for a true intraductal exchange. With the intraductal exchange, the wire can be disengaged from the device while both are still within the biliary or pancreatic ducts. The device then can be withdrawn while the wire remains in place. Short-track Fusion push catheters are available for both 5F and 7F stents. The Fusion Guidewire, although not extendable, is 0.035 inches in diameter and 185 cm in length with similar features to the Jagwire[4]. Studies from Europe and the US have shown improved placement of multiple stents into the bile duct, or pseudocyst cavity minimizing the number of guidewires used and shortening procedure duration[6,7]. The ability to move from the short-wire system where the physician has control of the wire to the long wire system where there is reliance on an assistant at any point during the procedure is a real advantage of the Fusion system. This system also provides compatibility with all other systems including all hydrophilic wires such as the Glidewire (Boston Scientific Corporation) available commercially.

The V-system (Olympus, Tokyo, Japan) was introduced in 2005. This is the first modification of a duodenoscope for facilitation of wire exchanges[8]. The V-system scope elevator lever includes a V-shaped groove and an increased angle of articulation in comparison to the standard Olympus TJF-160 series endoscope. This design promotes securing and locking of the short guidewire at the elevator level to reduce repositioning of the guidewire during accessory exchanges[8]. The groove described above acts as the internal wire lock allowing use of a catheter without a short-wire track. The V-system devices are similar to the traditional long wire devices at the leading end but have a different design component at the external end[4]. Device manipulation may be simplified by the LinearGuideV, a 0.035-inch diameter, 270 cm long wire with a hydrophilic coating over the leading 50 cm[4]. Spiral markings have been placed starting at 130 mm from the distal end, extending to the proximal end for easier attachment of the LinearGuideV into the V-Groove. The C-Hook allows the device handle to be attached to the V-Scope. This enables the endoscopist to maneuver the guidewire, inject contrast and manipulate the device handle while keeping a grip on the device control section. The main advantage of the C-Hook is that it is very easy for the endoscopist to relinquish control of the guidewire back to the assistant if needed. The main differences between the three available short-wire ERCP systems are summarized in Table 1.

## BENEFITS OF SHORT GUIDEWIRE SYSTEMS

One of the main benefits of the short-wire systems is clearly associated with the ability to permit physician-controlled guidewire cannulation of the desired duct. Cannulation is the essential initial step during ERCP and can be challenging for the endoscopist. Median time to successful cannulation was shown to be shorter in a wire-assisted cannulation compared to cannulation achieved after first injecting contrast (120 s *versus* 150 s) ($P = 0.73$)[9]. When used by an experienced endoscopist, Katsinelos *et al*[10] showed that use of a 0.035-inch Jagwire provided an 81.4% success rate for deep common bile duct cannulation *versus* 53.9% using a standard catheter ($P < 0.001$). Although rates of successful cannulation were similar between the two groups (hydrophilic guidewire 83.8% *versus* standard catheter 84%) if instrument crossover occurred[10].

Development of the RX Biliary system in 1999 has led to increased control of the guidewire and exchange by the physician, decreased hand and wrist force used during contrast injection, and in return improved physician stress, efficiency, and speed. The changes in guidewire design and physician control of the wire can be expected to reduce ampullary trauma and lead to decreased complication rates and post-ERCP pancreatitis (PEP) in particular[11].

The studies to date have yielded conflicting results regarding the role of guidewire cannulation and prevention of PEP. Lella *et al*[12] conducted a prospective study with 400 patients randomized to either Group A with a guidewire used to access the pancreatic duct and endoscopic sphincterotomes, and Group B with a traditional catheter plus injection technique used. The rate of PEP was 0% in Group A *versus* 4.1% in Group B. One study which randomized patients to either primary contrast or guidewire-assisted cannulation (Jagwire; Boston Scientific Corporation) showed improvements in rates of cannulation, however, there was no reduction in the incidence of PEP (7.9% with guidewire and 6.2% with contrast). Increased attempts at cannulating the papilla demonstrated increased rates of PEP with > 10% after four or more attempts[9]. No difference in the rates of post-procedural pancreatitis after cannulation was shown by Katsinelos *et al*[10] (standard catheter 7.8% *versus* hydrophilic Jagwire 0.035-inch guidewire 5.4%).

Guidewire manipulation of the ampullary surface has been suggested to be less traumatic than contrast injection or forceful manipulation with a catheter. Double wire use is helpful in cannulation of the common bile duct. Pancreatic duct guidewire placement can be used to facilitate cannulation into the choledochus portion of the common bile duct by maintenance of orientation for the endoscopist[13-15].

A major advantage of the short-wire system is the potential for shorter procedure and fluoroscopy time. Papachristou *et al*[16] showed that using the V-system endoscope and accessories with a short hydrophilic wire (Glidewire; Boston Scientific Corporation) can lead to rapid and reliable device exchanges with only a 5% chance of wire loss. In some exchanges the authors used the so called "hydraulic technique". The hydraulic technique uses standard techniques to achieve access with the Glidewire and catheter until all available wire is inserted into the catheter. Then, water is flushed under pressure into the catheter, keeping the wire in place, while the catheter is removed by the endoscopist after confirmation of the wire position[16,17]. Over half of the instances of wire loss were either unrelated to the exchange or required minimal adjustment due to partial loss. All endoscopists regained wire access and one endoscopist was able to reduce his average number of guidewires used per case from two to one[16]. The use of continuous fluoroscopy was also avoided with maintenance of the Glidewire position. The technique described above can permit access to the pancreaticobiliary tree and allow stent insertion in complicated cases with less difficulty than standard methods. The ability of the V-scope to hold the Glidewire varied between exchanges regardless of endoscopic position, but still resulted in faster exchanges than a regular duodenoscope with no change in wire loss rates[16].

A prospective multicenter, randomized and controlled trial was conducted by Joyce *et al*[8] to compare the V-system (Olympus XTJF-140V2F) with the traditional duodenoscope and accessories. The V system scope elevator lever includes a V-shaped groove and an increased angle of articulation in comparison to the standard Olympus TJF-160. The V-system was found to have both reduced rates of guidewire adjustments and time needed to complete accessory exchanges over a guidewire when compared to the traditional system. Reduction in exchange time between the V-system and the conventional system was 19.4 s *versus* 31.7 s ($P < 0.001$)[8], whereas the need to reposition the guidewire was required less often with the V-system, 9.4% *versus* 35.7% ($P = 0.0005$)[8]. In contrast, the reduced procedure and fluoroscopy times were not found to be statistically significant[8]. Failure to secure the guidewire leading to loss of access occurred in 11% of cases[8]. Reasons for loss of access varied from unfamiliarity with the system to nuances with the use of the guidewire and the elevator.

The intraductal exchange technology offered by the Fusion system allows the guidewire to be detached from the catheter within the bile or pancreatic duct. When aggressive endoscopic management is necessary for drainage of pancreatic pseudocysts, this system allows for placement of a number of plastic stents with less effort than traditional methods[6]. There is elimination of both exchange outside the endoscope and multiple cannulations for reentry into the ducts or the pseudocyst cavity. Use of a second guidewire through a cystotome and the intracystic wire exchange technique secures access to the pseudocyst[6].

Preliminary data from a prospective randomized single-blinded trial with 46 patients that compared performance characteristics of the short-wire ERCP system (Fusion) and a standard long wire system (DASH), showed a trend towards shorter procedure times and shorter time to perform various ERCP maneuvers with the short-wire system[7]. A statistically significant reduction in stent insertion times were also observed during this study ($P = 0.001$).

Sai *et al*[18] used the Fusion system for placement of double plastic stents for the palliation of lower biliary obstruction associated with unresectable pancreatic cancer. Successful stenting was accomplished in 94% (15) of patients with two requiring balloon dilatation of the stricture. No complications related to stent insertion and retrieval occurred. Mean patency duration was 151.1 d.

Johlin *et al*[17] at the University of Iowa described the use of a standard catheter-type device in combination with a short 0.035-inch guidewire (240 cm in length). The authors used a 3-mL syringe and sterile water to perform hydraulic exchanges as described earlier. They documented that the entire hydraulic ERCP catheter

**Table 2 Short-wire system potential advantages**

| Advantages |
|---|
| Reduced exchange times |
| Reduced stent insertion times |
| Maintenance of ductal access |
| Reduction of sedation and fluoroscopy time |
| Increased endoscopist control of cannulation |
| Locking of wire in position to increase stability |
| Decreased rates of post-ERCP pancreatitis |
| Decreased trauma when ampullary surface is manipulated |
| Reduced rates of wire adjustments |
| Aids in stricture access |
| Allows placements of multiple stents (Fusion system only) |

**Table 3 Short-wire system potential drawbacks**

| Drawbacks |
|---|
| Only preliminary studies have documented the potential benefit of the short-wire systems (all systems) |
| Hydraulic exchange technique not plausible with RX system |
| Decreased pushability with the open channel design of the RX system |
| Inability to flush channel for hydrophilic wire use (RX system) |
| Inability to use smaller than 0.035 inch or angled wires when channel is torn after first device exchange (unless device is preloaded) (RX system) |
| Deterioration of the device after multiple exchanges (RX, Fusion systems) |
| No easy method for insertion of pancreatic stents (all systems) |
| No reliable method of locking wire (V-system) |
| Looping of wire between the biopsy port and the external locking device (RX, Fusion systems) |
| Poor guidewire visibility (V-system) |
| Air and bile leakage causing increased soiling of operators (RX, Fusion systems) |
| Wires may suspend freely in air after being locked jeopardizing operators (all systems) |
| Loss of visibility due to decreased distention of the duodenum (RX, Fusion systems) |

exchange took less than 30 s. Over the past 10 years this system was shown to save time, save money, maintain capacity to aspirate bile and pancreatic fluid and decrease contamination (if a short-wire is accidentally dropped it will not touch the floor). Tables 2 and 3 summarize the potential benefits and drawbacks of the use of short-wire systems.

## DRAWBACKS

Although there are many benefits to the short-wire system, there have been some inefficiencies associated with them. One study showed that by using a dedicated short-wire monorail catheter with an accessory system (the RX system), slower exchange times by an average of 4 s were observed when compared to standard accessories. The RX system is not amenable to the hydraulic exchange technique[16]. In contrast to other studies, one prospective study showed that the time required for primary selective common bile duct cannulation was increased in the hydrophilic guidewire group at 4.48 ± 0.32 min *versus* the standard catheter group at 3.53 ± 0.32 min[10]. Other potential problems: decreased pushability due to the open channel design (RX), inability to flush the channel to facilitate use with a hydrophilic wire (RX), inability to use smaller than 0.035 inch or angled wires after the channel is torn with the first device exchange (unless the device is preloaded) (RX), deterioration of the device after multiple exchanges (RX, Fusion), not being able to insert pancreatic stents easily (all), no reliable locking of the wire (V), looping of the wire between the biopsy port and the external locking device (RX, Fusion), poor guidewire visibility (V), air and bile leakages which may lead to soiling of the operator and loss of visibility due to decreased distention of the duodenum.

## SAFETY

The safety of short-wire systems has not been addressed exclusively in any published studies. Damage to the guidewire may occur with external locking of the wires. In addition, the proximal end of the shortest wire freely suspends in air after being locked and can present a risk to the operator, assistant, or patient[4]. Antileak caps should consistently retain air and bile preventing any spray of secretions but failure of this feature may lead to adverse outcomes.

When using the internal locking endoscopes, inappropriate locking of the wire leading to access loss may require repeated cannulation of the guidewire. As devices are introduced when using the internal locking endoscopes it is important that the V-shaped elevator is engaged properly[4]. If there is difficulty, it is important to note if there is damage to the tip of the device or catching of the guidewire in the space between the V-groove and the working channel[4].

Guidewire insertion into the bile duct improves the safety margin of a sphincterotomy by ensuring the incision of the biliary sphincter as intended. As mentioned before, this allows for repeated cannulation decreasing any risk of papillary injury if the papillotome becomes dislodged[12].

A search of the Maude database for all three short-wire system manufacturers was carried out and only adverse events regarding the Wallstent RX Biliary (Boston Scientific, Galway, Ireland) were listed. Adverse events included distal biliary duct stent occlusion 7 d after placement, hyperplasia at the site of the distal common bile duct stent 10 mo after placement during a stricture revision procedure, and a stent was found to have "foreshortened and proximally migrated into the bile duct" 1 year after placement. These complications were most likely related to the Wallstent design rather than to the use of the RX system.

## COST

Using the short hydrophilic 0.035-inch biliary guidewire as the sole guidewire during a procedure decreases the need for a second wire, which may minimize cost during ERCP[16].

## CONCLUSION

The practice of using ERCP short guidewire systems was developed to improve procedural outcomes. Although the use of traditional guidewires is vast amongst endoscopists, exposure to these systems may aid physicians to reduce exchange times, increase endoscopist control, reduce sedation exposure, reduce fluoroscopy time, increase stability with integrated lock systems and decrease rates of trauma during the procedure.

## REFERENCES


1 **Somogyi L**, Chuttani R, Croffie J, Disario J, Liu J, Mishkin D, Shah R, Tierney W, Wong Kee Song LM, Petersen BT. Guidewires for use in GI endoscopy. *Gastrointest Endosc* 2007; **65**: 571-576

2 **Cantor DS**. Simple method for maintaining guide wire position. *Gastrointest Endosc* 1987; **33**: 464-465

3 **Beilstein MC**, Ahmad NA, Kochman ML, Long WB, Shah JN, Ginsberg GG. Initial evaluation of a duodenoscope modified to allow guidewire fixation during ERCP. *Gastrointest Endosc* 2004; **60**: 284-287

4 **Shah RJ**, Somogyi L, Petersen BT, Tierney WM, Adler DG, Chand B, Conway JD, Croffie JM, Disario JA, Mishkin DS, Wong Kee Song LM. Short-wire ERCP systems. *Gastrointest Endosc* 2007; **66**: 650-657

5 **Larghi A**, Waxman I. Endoscopic direct cholangioscopy by using an ultra-slim upper endoscope: a feasibility study. *Gastrointest Endosc* 2006; **63**: 853-857

6 **Jansen JM**, Hanrath A, Rauws EA, Bruno MJ, Fockens P. Intracystic wire exchange facilitating insertion of multiple stents during endoscopic drainage of pancreatic pseudocysts. *Gastrointest Endosc* 2007; **66**: 157-161

7 **Fazel A,** Kowalczyk L, Moezardalan K, Forsmark C, Draganov P. Preliminary results from prospective randomized single blinded trial comparing the performance characteristics of a short-wire (FUSION) and standard long-wire (DASH) ERCP device systems. *Gastrointest Endosc* 2006; **63**: AB242

8 **Joyce AM**, Ahmad NA, Beilstein MC, Kochman ML, Long WB, Baron T, Sherman S, Fogel E, Lehman GA, McHenry L Jr, Watkins J, Ginsberg GG. Multicenter comparative trial of the V-scope system for therapeutic ERCP. *Endoscopy* 2006; **38**: 713-716

9 **Bailey AA**, Bourke MJ, Williams SJ, Walsh PR, Murray MA, Lee EY, Kwan V, Lynch PM. A prospective randomized trial of cannulation technique in ERCP: effects on technical success and post-ERCP pancreatitis. *Endoscopy* 2008; **40**: 296-301

10 **Katsinelos P**, Paroutoglou G, Kountouras J, Chatzimavroudis G, Zavos C, Pilpilidis I, Tzelas G, Tzovaras G. A comparative study of standard ERCP catheter and hydrophilic guide wire in the selective cannulation of the common bile duct. *Endoscopy* 2008; **40**: 302-307

11 **Farrell RJ**, Howell DA, Pleskow DK. New technology for endoscopic retrograde cholangiopancreatography: improving safety, success, and efficiency. *Gastrointest Endosc Clin N Am* 2003; **13**: 539-559

12 **Lella F**, Bagnolo F, Colombo E, Bonassi U. A simple way of avoiding post-ERCP pancreatitis. *Gastrointest Endosc* 2004; **59**: 830-834

13 **Gyökeres T**, Duhl J, Varsányi M, Schwab R, Burai M, Pap A. Double guide wire placement for endoscopic pancreaticobiliary procedures. *Endoscopy* 2003; **35**: 95-96

14 **Maeda S**, Hayashi H, Hosokawa O, Dohden K, Hattori M, Morita M, Kidani E, Ibe N, Tatsumi S. Prospective randomized pilot trial of selective biliary cannulation using pancreatic guide-wire placement. *Endoscopy* 2003; **35**: 721-724

15 **Draganov P**, Devonshire DA, Cunningham JT. A new technique to assist in difficult bile duct cannulation at the time of endoscopic retrograde cholangiopancreatography. *JSLS* 2005; **9**: 218-221

16 **Papachristou GI**, Baron TH, Gleeson F, Levy MJ, Topazian MD. Endoscopic retrograde cholangiopancreatography catheter and accessory exchange using a short hydrophilic guide wire: a prospective study. *Endoscopy* 2006; **38**: 1133-1136

17 **Johlin FC**, Silverman WB. Hydraulic ERCP catheter and accessory exchange using a short guidewire. *Am J Gastroenterol* 2001; **96**: 3040

18 **Sai JK**, Suyama M, Kubokawa Y, Watanabe S. Clinical results of double stenting for the palliation of lower biliary obstruction associated with unresectable pancreatic cancer. *Gastrointestinal Endosc* 2007; **65**: AB233


**S- Editor** Li LF **L- Editor** Webster JR **E- Editor** Zheng XM

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.61

*World J Gastroenterol* 2009 January 7; 15(1): 61-66
***World Journal of Gastroenterology*** ISSN 1007-9327




**Dr. Peter V Draganov,** *Series Editor*

# Colorectal cancer surveillance in inflammatory bowel disease: The search continues

Anis Ahmadi, Steven Polyak, Peter V Draganov

Anis Ahmadi, Steven Polyak, Peter V Draganov, Department of Gastroenterology, Hepatology and Nutrition, University of Florida, 1600 SW Archer Rd, Room HD 602, PO Box 100214 Gainesville, Florida 32610, United States
Author contributions: Ahmadi A, Polyak S and Draganov PV designed the format; Ahmadi A performed the literature search and wrote the first draft of the paper; Polyak S and Draganov PV contributed new articles to the literature search and reviewed the article.
Correspondence to: Peter V Draganov, MD, Department of Gastroenterology, Hepatology and Nutrition, University of Florida, 1600 SW Archer Rd, Room HD 602, PO Box 100214 Gainesville, Florida 32610,
United States. dragapv@medicine.ufl.edu
Telephone: +1-352-392-2877 Fax: +1-352-392-3618
Received: August 8, 2008 Revised: October 17, 2008
Accepted: October 24, 2008
Published online: January 7, 2009

## Abstract

Patients with inflammatory bowel disease (IBD) are at increased risk for colorectal cancer (CRC). Risk factors for the development of CRC in the setting of IBD include disease duration, anatomic extent of disease, age at time of diagnosis, severity of inflammation, family history of colon cancer, and concomitant primary sclerosing cholangitis. The current surveillance strategy of surveillance colonoscopy with multiple random biopsies most likely reduces morbidity and mortality associated with IBD-related CRC. Unfortunately, surveillance colonoscopy also has severe limitations including high cost, sampling error at time of biopsy, and interobserver disagreement in histologically grading dysplasia. Furthermore, once dysplasia is detected there is disagreement about its management. Advances in endoscopic imaging techniques are already underway, and may potentially aid in dysplasia detection and improve overall surveillance outcomes. Management of dysplasia depends predominantly on the degree and focality of dysplasia, with the mainstay of management involving either proctocolectomy or continued colonoscopic surveillance. Lastly, continued research into additional chemopreventive agents may increase our arsenal in attempting to reduce the incidence of IBD-associated CRC.



**Key words:** Colorectal cancer; Crohn's disease; Inflammatory bowel disease; Surveillance colonoscopy; Ulcerative colitis

**Peer reviewer:** Rupert Leong, Associate Professor, Director of Endoscopy, Concord Hospital, ACE Unit, Level 1 West, Hospital Rd, Concord NSW 2139, Australia

Ahmadi A, Polyak S, Draganov PV. Colorectal cancer surveillance in inflammatory bowel disease: The search continues. *World J Gastroenterol* 2009; 15(1): 61-66 Available from: URL: http://www.wjgnet.com/1007-9327/15/61.asp DOI: http://dx.doi.org/10.3748/wjg.15.61

## INTRODUCTION

Patients with both ulcerative colitis (UC) and Crohn's disease (CD) are at an increased risk of developing colorectal cancer (CRC). It is believed that this increased risk is a result of persistent inflammation of the colon. The exact mechanism as to how chronic inflammation results in carcinogenesis is unclear, but it is postulated that the same genetic mutations that result in sporadic CRC are also responsible for its development in inflammatory bowel disease (IBD). Surveillance guidelines employing colonoscopy as a tool for screening this high-risk population are available. Unfortunately, the guidelines have their limitations. Additionally, no consensus exists regarding the management of low-grade dysplasia (LGD) in the setting of IBD. This article will review the CRC risk factors in IBD, current surveillance guidelines, management of dysplasia in IBD, and lastly chemoprevention.

## EPIDEMIOLOGY OF CRC IN PATIENTS WITH IBD

It has been known for nearly a century that UC is associated with an increased risk of CRC[1,2]. However, the association between CRC and CD has only recently been recognized[3-5]. The mean duration from the time of diagnosis of UC to the development of CRC is 17 years, with the mean age at diagnosis of CRC being 51 years in men and 54 years in women[6]. In a large meta-analysis

involving 54478 patients and 116 studies, Eaden *et al*[7] calculated the cumulative risk of CRC in UC at 8.3% at 20 years and 18.4% at 30 years. These results, however, have not been replicated, with more recent studies showing a lower annual incidence (0.06%-0.2%) of CRC in patients with UC[8,9]. Interestingly, two separate population-based studies from Denmark and the Mayo Clinic found no increased risk between CRC and UC when compared to the general population[10,11]. The differences among these studies have been postulated to be the result of improved medical therapies with unforeseen chemoprevention, increased colonoscopic surveillance, and improved surgical treatments.

Although there is less data regarding the risk of CRC in CD, it is well established that a similar association exists to that of UC[3-5]. However, the development of dysplasia in CD can involve both the small bowel as well as the colon. In a population-based study from the Mayo Clinic, only a slight increased risk was found between CD and CRC, which is in stark contrast to a 40-fold increased risk of developing small bowel malignancy[10]. Interestingly, an earlier large population-based study from Sweden reported a relative risk of 5.6 for the development of CRC in patients with Crohn's colitis[3].

## PATHOGENESIS OF CRC IN PATIENTS WITH IBD

The exact mechanism by which chronic inflammation results in carcinogenesis is unclear. Persistent inflammation is believed to result in increased cell proliferation as well as oxidative stress, and ultimately the development of dysplasia[12-15]. It is postulated that similar genetic mutations that result in sporadic CRC in the general population are also responsible for its development in IBD, but the sequence of events and frequency are altered[16,17]. These events include microsatellite instability, inhibition of regulatory genes *via* hypermethylation of the promoter regions, and loss of adenomatous polyposis coli (APC), p53, and K-ras tumor suppressor function[12,18]. In sporadic CRC among the general population, loss of APC function generally occurs early and is frequent, whereas p53 mutations occur late and are less frequent. In contrast, loss of APC function generally occurs late and is infrequent, whereas p53 mutations occur early and are more frequent in IBD-associated CRC[18].

## RISK FACTORS FOR CRC IN PATIENTS WITH IBD

Multiple risk factors for the development of CRC in the setting of IBD have been identified. It is well established that greater disease duration and anatomic extent of colitis are important risk factors[19]. Generally, patients with pancolitis develop CRC a decade prior to patients with left-sided colitis. Of note, disease extent is defined as the furthest extent of inflammation, either microscopically or macroscopically[20]. Recent studies have also demonstrated that the degree of colonic inflammation is associated with an increased risk of dysplasia and CRC. This was first introduced by the group at St. Mark's Hospital[21], and further confirmed by two recent studies[22,23]. Additionally, other markers of inflammation, including the presence of pseudopolyps, strictures, and backwash ileitis have been found to be independently associated with an increased risk of developing CRC[24-26].

Independent of a family history of IBD, a family history of sporadic CRC imparts a two-fold higher risk of CRC in IBD patients[27,28]. Also, IBD diagnosis at an earlier age increases the risk of CRC, independent of disease duration[19]. In fact, a Swedish population-based cohort of 3117 patients diagnosed with UC between 1922 and 1983, found a four-fold increase in CRC in patients diagnosed with UC prior to the age of 15 compared with those diagnosed between 15-29 years of age[19]. Lastly, the concomitant presence of primary sclerosing cholangitis (PSC) confers a significantly increased risk of CRC in patients with UC[29]. Among patients with PSC, Kornfeld *et al*[30] calculated a cumulative risk of developing CRC of 33% at 20 years and 40% at 30 years after the diagnosis of UC.

## DEVELOPMENT OF DYSPLASIA IN IBD

Unlike sporadic CRC in the general population, the development of carcinogenesis in IBD does not always follow a sequential progression from LGD, to high-grade dysplasia (HGD), and ultimately to cancer[18]. In fact, Ullman *et al*[31] revealed that cancer can arise in patients with no prior dysplasia, or without first progressing from LGD to HGD. Additionally, they found that patients undergoing colectomy for flat LGD were found to have much more advanced pathology on surgical specimens. Also, CRC arising in the background of IBD is often multifocal and more aggressive than sporadic CRC[32-34]. This unpredictable sequence of events coupled with its more aggressive nature highlights the importance of increased vigilance and surveillance for CRC in this high-risk population.

Dysplasia is defined as the unequivocal neoplastic alteration of the epithelium without invasion into the lamina propria[35]. Macroscopically, dysplastic lesions in the setting of colitis can range from flat lesions (not endoscopically visible) to plaque-like lesions, to raised localized or multifocal lesions[36,37]. Raised, endoscopically visible lesions noted within areas of active colitis are referred to as dysplasia-associated lesions or masses (DALMs)[36]. These are further categorized into adenoma-like lesions and non-adenoma-like lesions, which ultimately results in different management implications[38]. Microscopically, per the IBD Dysplasia Morphology Study Group, dysplasia is divided into three categories: (1) negative for dysplasia; (2) indefinite for dysplasia; and (3) positive for dysplasia, which is further divided into LGD and HGD[35]. Unfortunately, inter-observer agreement and intra-observer reliability among

pathologists is variable in the diagnosis of dysplasia, especially when diagnosing indeterminate and LGD[39,40]. Therefore, it is traditionally recommended that any diagnosis of dysplasia be confirmed by a separate GI pathologist[41].

## CRC SURVEILLANCE IN IBD

Recommendations for CRC screening/surveillance in IBD is aimed at early detection and mortality reduction from CRC. Although a multitude of studies exist demonstrating the need for secondary prevention[42-45], a 2004 Cochrane Database review did not show "clear evidence that surveillance colonoscopy prolonged survival." However, this analysis indirectly revealed a reduced mortality risk in IBD-associated CRC[46].

The most recent surveillance strategy, published in 2005 by the Crohn's and Colitis Foundation of America (CCFA), is based on expert consensus of past surveillance guidelines as well as more recent studies on IBD-associated dysplasia[41]. Per the consensus guidelines, screening colonoscopies should begin 8-10 years after the onset of IBD symptoms in patients with UC pancolitis/left-sided colitis and Crohn's colitis involving at least one third of the colon. The exception to this rule is that patients with concomitant PSC begin yearly surveillance colonoscopies at the time PSC is diagnosed. The timing of follow-up surveillance colonoscopies depends on the presence of dysplasia. If the initial colonoscopy in UC pancolitis/left-sided colitis (and Crohn's colitis) is negative for dysplasia, repeat surveillance colonoscopy should be performed in 1-2 years. Once a patient has two negative surveillance colonoscopies, further surveillance colonoscopies should be performed every 1-3 years. After 20 years of disease duration, surveillance colonoscopies should be repeated every 1-2 years. Management of dysplasia is described below. The recommended surveillance biopsies are four-quadrant biopsies every 10 cm with jumbo forceps. Of note, patients with only proctosigmoiditis of < 35 cm disease extent should follow the standard CRC screening guidelines for the general population, as they are not at an increased risk of developing CRC[41].

## MANAGEMENT OF DYSPLASIA

All dysplastic and indefinite-for-dysplasia biopsies should be confirmed by an expert GI pathologist. Once confirmed, patients with HGD or multifocal flat LGD should be referred for prophylactic total proctocolectomy. Patients with indefinite dysplasia should undergo a more aggressive surveillance plan, with repeat surveillance in 3-6 mo. Controversy exists regarding the management of unifocal flat LGD, as studies have calculated a wide progression rate of 2%-50% from LGD to HGD[31,47-49]. Therefore, it is imperative that an open dialogue regarding the risks and benefits of both surgical and intense colonoscopic surveillance (repeat surveillance colonoscopies every 3-6 mo) be discussed with all patients found to have unifocal flat LGD. If intense surveillance is chosen by the patient, the CCFA consensus strongly recommends a proctocolectomy if multifocal flat LGD, repetitive flat LGD, or more advanced dysplasia is found during subsequent surveillance colonoscopies[41].

All non-adenoma-like DALMs should be referred for a complete proctocolectomy, as up to 50% of these patients can have cancer at the time of surgery[35,50]. Per the CCFA consensus guidelines, adenoma-like DALMs should be resected in their entirety, with the base and surrounding mucosa biopsied separately. Patients should be referred for surgery if the base or surrounding mucosa contains dysplasia. However, if no dysplasia is found in the base or surrounding mucosa, a repeat colonoscopy should be performed in 6 mo[41]. A summary of currently accepted algorithm for surveillance of CRC and management of dysplasia is presented in Figure 1.

Lastly, special attention should be applied to all colonic strictures in IBD. In UC patients, strictures should prompt surgical referral, given the high association with underlying malignancy[41]. Although the risk of malignancy is not as high in Crohn's-related colonic strictures, efforts should be made to visualize the colon proximal to the stricture, since an increased risk of CRC does exist. In fact, a 6.8% frequency rate of colon cancer after 20 years disease duration was found among a review of 175 Crohn's colon strictures[51]. Surgical resection should therefore be considered in longstanding CD, or if the colon proximal to the stricture cannot be visualized[41,51].

Although guidelines are available for CRC surveillance in IBD, multiple limitations exist. Per the current four-quadrant biopsies every 10 cm guideline, only less than 1% of the entire mucosal surface of the colon is sampled, leaving a very high sampling error[18]. Additionally, pathologists do not universally agree on the diagnosis of low-grade or indefinite-for-dysplasia, especially in a field of active inflammation[39,40]. Lastly, the management of flat LGD is often debated, specifically whether to proceed directly to surgery or follow a more aggressive colonoscopic surveillance plan.

## CHEMOPREVENTION

The use of pharmacotherapy as a potential chemopreventive measure to reduce the risk of developing CRC has been studied extensively. The most widely studied agent for chemoprevention is 5-aminosalicylate (5-ASA). Unfortunately, no prospective studies evaluating 5-ASA as a chemopreventive agent exist, and the data is otherwise conflicting. The most compelling data from a meta-analysis of nine observational studies demonstrated a reduced risk of developing CRC or dysplasia with 5-ASA (OR = 0.51, 95% CI 0.38-0.69)[52]. This is in contrast to a retrospective case-controlled study of 25 patients with IBD and CRC, which demonstrated no association between 5-ASA

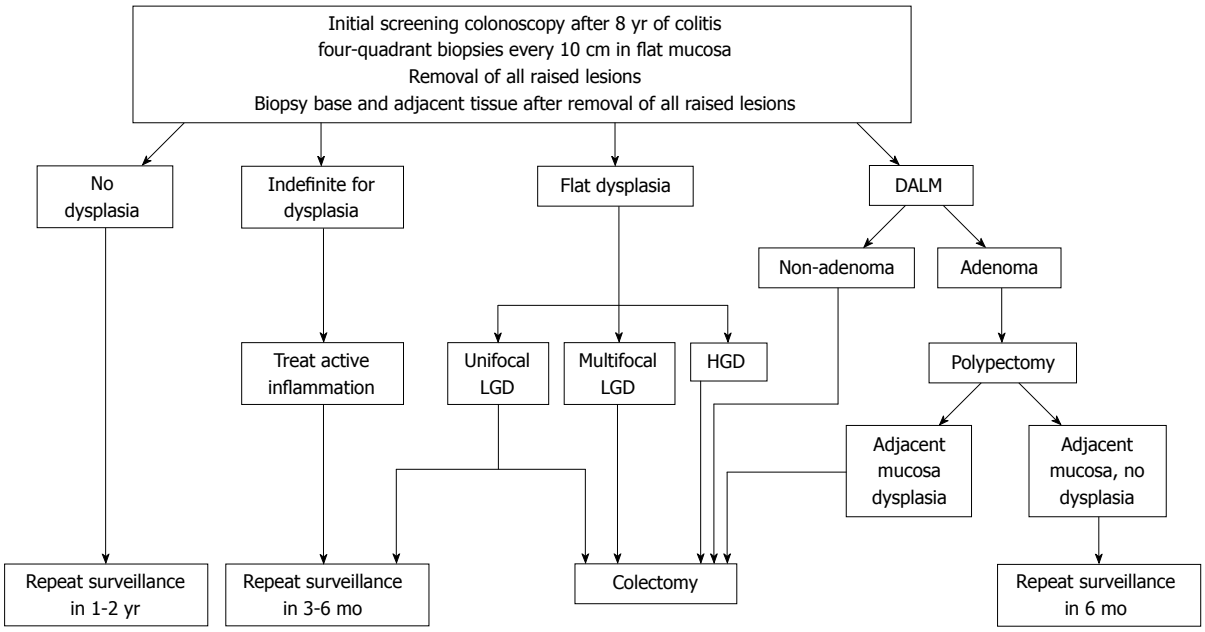


**Figure 1 Algorithm for screening/surveillance for CRC in IBD and management of dysplasia.** CRC: Colorectal cancer; IBD: Inflammatory bowel disease; LGD: Low grade dysplasia; HGD: High grade dysplasia; DALM: Dysplasia-associated lesions or masses.

use and reduction in CRC risk[53]. Nonetheless, with its relatively low side-effect profile, 5-ASA appears to be protective against CRC in IBD.

Additional agents studied for their chemopreventive effects include ursodeoxycholic acid (UDCA), corticosteroids, NSAIDs, folate, statins, calcium, and immunomodulators[18]. Two separate studies have demonstrated that the use of UDCA in patients with concomitant PSC and UC lowers the incidence of developing dysplasia or CRC (OR = 0.18 and 0.26, respectively)[54,55]. Thus, the use of UDCA for chemoprevention in patients with both UC and PSC is to be encouraged. However, the role of UDCA as a chemopreventive agent in UC without PSC is unknown. Currently, insufficient data or inadequate evidence precludes the use of folate, corticosteroids, NSAIDs, calcium, statins, and immunomodulators as chemoprotective agents against CRC[24,56-59].

## ADVANCES IN DYSPLASIA DETECTION

As a result of the difficulty in identifying flat dysplastic lesions, the use of magnification chromoendoscopy was introduced. Magnification chromoendoscopy enhances mucosal contrast *via* the application of stains/dyes. Ideally, this would highlight mucosal changes that would otherwise not be seen by traditional white light endoscopy[60]. Methylene blue and indigo carmine are the most commonly studied dyes in UC surveillance[61,62]. In addition to stains/dyes, advances in imaging technique, including narrow band imaging and confocal laser endomicroscopy may further enhance the ability to target abnormal areas of the colonic mucosa, potentially even at the subcellular level. Currently, data on these new techniques are limited, and are not included, at this time, in surveillance guidelines[63].

## CONCLUSION

The risk of CRC in long-standing UC and CD has resulted in the adoption of surveillance strategies with the goals of reducing morbidity and mortality associated with IBD-related CRC. Risk factors for the development of CRC in the setting of IBD include disease duration, anatomic extent of disease, age at time of diagnosis, severity of inflammation, family history of colon cancer, and concomitant PSC. Although guidelines currently exist, limitations of these guidelines, including sampling error at time of biopsy, interobserver disagreement in histologically grading dysplasia, and disagreement about the management of LGD indicate the need for continued research into the molecular pathogenesis of IBD-associated CRC, with the hope of identifying targets for prevention. Advances in endoscopic imaging techniques are already underway, and may potentially aid in dysplasia detection and improve overall surveillance outcomes. Management of dysplasia depends predominantly on the degree and focality of dysplasia, with the mainstay of management involving either proctocolectomy or continued colonoscopic surveillance. Lastly, continued research into additional chemopreventive agents may increase our arsenal in attempting to reduce the incidence of IBD-associated CRC.

## REFERENCES

1 **Svartz N**, Ernberg T. Cancer coli in cases of colitis ulcerosa. *Acta Med Scand* 1949; **135**: 444-447
2 **Rosenqvist H**, Ohrling H, Lagercrantz R, Edling N. Ulcerative colitis and carcinoma coli. *Lancet* 1950; **1**: 906-908
3 **Ekbom A**, Helmick C, Zack M, Adami HO. Increased risk of large-bowel cancer in Crohn's disease with colonic involvement. *Lancet* 1990; **336**: 357-359
4 **Jess T**, Winther KV, Munkholm P, Langholz E, Binder V.

Intestinal and extra-intestinal cancer in Crohn's disease: follow-up of a population-based cohort in Copenhagen County, Denmark. *Aliment Pharmacol Ther* 2004; **19**: 287-293
5 **Jess T**, Gamborg M, Matzen P, Munkholm P, Sørensen TI. Increased risk of intestinal cancer in Crohn's disease: a meta-analysis of population-based cohort studies. *Am J Gastroenterol* 2005; **100**: 2724-2729
6 **Pinczowski D**, Ekbom A, Baron J, Yuen J, Adami HO. Risk factors for colorectal cancer in patients with ulcerative colitis: a case-control study. *Gastroenterology* 1994; **107**: 117-120
7 **Eaden JA**, Abrams KR, Mayberry JF. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. *Gut* 2001; **48**: 526-535
8 **Bernstein CN**, Blanchard JF, Kliewer E, Wajda A. Cancer risk in patients with inflammatory bowel disease: a population-based study. *Cancer* 2001; **91**: 854-862
9 **Lakatos L**, Mester G, Erdelyi Z, David G, Pandur T, Balogh M, Fischer S, Vargha P, Lakatos PL. Risk factors for ulcerative colitis-associated colorectal cancer in a Hungarian cohort of patients with ulcerative colitis: results of a population-based study. *Inflamm Bowel Dis* 2006; **12**: 205-211
10 **Jess T**, Loftus EV Jr, Velayos FS, Harmsen WS, Zinsmeister AR, Smyrk TC, Schleck CD, Tremaine WJ, Melton LJ 3rd, Munkholm P, Sandborn WJ. Risk of intestinal cancer in inflammatory bowel disease: a population-based study from olmsted county, Minnesota. *Gastroenterology* 2006; **130**: 1039-1046
11 **Winther KV**, Jess T, Langholz E, Munkholm P, Binder V. Long-term risk of cancer in ulcerative colitis: a population-based cohort study from Copenhagen County. *Clin Gastroenterol Hepatol* 2004; **2**: 1088-1095
12 **Itzkowitz SH**, Yio X. Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. *Am J Physiol Gastrointest Liver Physiol* 2004; **287**: G7-G17
13 **Clevers H**. At the crossroads of inflammation and cancer. *Cell* 2004; **118**: 671-674
14 **Sato F**, Shibata D, Harpaz N, Xu Y, Yin J, Mori Y, Wang S, Olaru A, Deacu E, Selaru FM, Kimos MC, Hytiroglou P, Young J, Leggett B, Gazdar AF, Toyooka S, Abraham JM, Meltzer SJ. Aberrant methylation of the HPP1 gene in ulcerative colitis-associated colorectal carcinoma. *Cancer Res* 2002; **62**: 6820-6822
15 **Chang CL**, Marra G, Chauhan DP, Ha HT, Chang DK, Ricciardiello L, Randolph A, Carethers JM, Boland CR. Oxidative stress inactivates the human DNA mismatch repair system. *Am J Physiol Cell Physiol* 2002; **283**: C148-C154
16 **Redston MS**, Papadopoulos N, Caldas C, Kinzler KW, Kern SE. Common occurrence of APC and K-ras gene mutations in the spectrum of colitis-associated neoplasias. *Gastroenterology* 1995; **108**: 383-392
17 **Burmer GC**, Rabinovitch PS, Haggitt RC, Crispin DA, Brentnall TA, Kolli VR, Stevens AC, Rubin CE. Neoplastic progression in ulcerative colitis: histology, DNA content, and loss of a p53 allele. *Gastroenterology* 1992; **103**: 1602-1610
18 **Itzkowitz SH**, Harpaz N. Diagnosis and management of dysplasia in patients with inflammatory bowel diseases. *Gastroenterology* 2004; **126**: 1634-1648
19 **Ekbom A**, Helmick C, Zack M, Adami HO. Ulcerative colitis and colorectal cancer. A population-based study. *N Engl J Med* 1990; **323**: 1228-1233
20 **Greenstein AJ**, Sachar DB, Smith H, Pucillo A, Papatestas AE, Kreel I, Geller SA, Janowitz HD, Aufses AH Jr. Cancer in universal and left-sided ulcerative colitis: factors determining risk. *Gastroenterology* 1979; **77**: 290-294
21 **Rutter M**, Saunders B, Wilkinson K, Rumbles S, Schofield G, Kamm M, Williams C, Price A, Talbot I, Forbes A. Severity of inflammation is a risk factor for colorectal neoplasia in ulcerative colitis. *Gastroenterology* 2004; **126**: 451-459
22 **Rubin DT**, Huo D, Rothe JA, Hetzel JT, Sedrak M, Yadron N, Bunnag A, Hart J, Turner JR. Increased Inflammatory Activity Is An Independent Risk Factor for Dysplasia and Colorectal Cancer in Ulcerative Colitis: A Case-Control Analysis with Blinded Prospective Pathology Review. *Gastroenterology* 2006; **130**: A2
23 **Gupta RB**, Harpaz N, Itzkowitz S, Hossain S, Matula S, Kornbluth A, Bodian C, Ullman T. Histologic inflammation is a risk factor for progression to colorectal neoplasia in ulcerative colitis: a cohort study. *Gastroenterology* 2007; **133**: 1099-1105; quiz 1340-1341
24 **Velayos FS**, Loftus EV Jr, Jess T, Harmsen WS, Bida J, Zinsmeister AR, Tremaine WJ, Sandborn WJ. Predictive and protective factors associated with colorectal cancer in ulcerative colitis: A case-control study. *Gastroenterology* 2006; **130**: 1941-1949
25 **Rutter MD**, Saunders BP, Wilkinson KH, Rumbles S, Schofield G, Kamm MA, Williams CB, Price AB, Talbot IC, Forbes A. Cancer surveillance in longstanding ulcerative colitis: endoscopic appearances help predict cancer risk. *Gut* 2004; **53**: 1813-1816
26 **Haskell H**, Andrews CW Jr, Reddy SI, Dendrinos K, Farraye FA, Stucchi AF, Becker JM, Odze RD. Pathologic features and clinical significance of "backwash" ileitis in ulcerative colitis. *Am J Surg Pathol* 2005; **29**: 1472-1481
27 **Nuako KW**, Ahlquist DA, Mahoney DW, Schaid DJ, Siems DM, Lindor NM. Familial predisposition for colorectal cancer in chronic ulcerative colitis: a case-control study. *Gastroenterology* 1998; **115**: 1079-1083
28 **Askling J**, Dickman PW, Karlén P, Broström O, Lapidus A, Löfberg R, Ekbom A. Family history as a risk factor for colorectal cancer in inflammatory bowel disease. *Gastroenterology* 2001; **120**: 1356-1362
29 **Broomé U**, Lindberg G, Löfberg R. Primary sclerosing cholangitis in ulcerative colitis--a risk factor for the development of dysplasia and DNA aneuploidy? *Gastroenterology* 1992; **102**: 1877-1880
30 **Kornfeld D**, Ekbom A, Ihre T. Is there an excess risk for colorectal cancer in patients with ulcerative colitis and concomitant primary sclerosing cholangitis? A population based study. *Gut* 1997; **41**: 522-525
31 **Ullman T**, Croog V, Harpaz N, Sachar D, Itzkowitz S. Progression of flat low-grade dysplasia to advanced neoplasia in patients with ulcerative colitis. *Gastroenterology* 2003; **125**: 1311-1319
32 **Greenstein AJ**, Slater G, Heimann TM, Sachar DB, Aufses AH Jr. A comparison of multiple synchronous colorectal cancer in ulcerative colitis, familial polyposis coli, and de novo cancer. *Ann Surg* 1986; **203**: 123-128
33 **Harpaz N**, Talbot IC. Colorectal cancer in idiopathic inflammatory bowel disease. *Semin Diagn Pathol* 1996; **13**: 339-357
34 **Delaunoit T**, Limburg PJ, Goldberg RM, Lymp JF, Loftus EV Jr. Colorectal cancer prognosis among patients with inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2006; **4**: 335-342
35 **Riddell RH**, Goldman H, Ransohoff DF, Appelman HD, Fenoglio CM, Haggitt RC, Ahren C, Correa P, Hamilton SR, Morson BC. Dysplasia in inflammatory bowel disease: standardized classification with provisional clinical applications. *Hum Pathol* 1983; **14**: 931-968
36 **Blackstone MO**, Riddell RH, Rogers BH, Levin B. Dysplasia-associated lesion or mass (DALM) detected by colonoscopy in long-standing ulcerative colitis: an indication for colectomy. *Gastroenterology* 1981; **80**: 366-374
37 **Butt JH**, Konishi F, Morson BC, Lennard-Jones JE, Ritchie JK. Macroscopic lesions in dysplasia and carcinoma complicating ulcerative colitis. *Dig Dis Sci* 1983; **28**: 18-26
38 **Rubin PH**, Friedman S, Harpaz N, Goldstein E, Weiser J, Schiller J, Waye JD, Present DH. Colonoscopic polypectomy in chronic colitis: conservative management after endoscopic resection of dysplastic polyps. *Gastroenterology* 1999; **117**: 1295-1300
39 **Odze RD**, Goldblum J, Noffsinger A, Alsaigh N, Rybicki

LA, Fogt F. Interobserver variability in the diagnosis of ulcerative colitis-associated dysplasia by telepathology. *Mod Pathol* 2002; **15**: 379-386

40 **Eaden J**, Abrams K, McKay H, Denley H, Mayberry J. Inter-observer variation between general and specialist gastrointestinal pathologists when grading dysplasia in ulcerative colitis. *J Pathol* 2001; **194**: 152-157

41 **Itzkowitz SH**, Present DH. Consensus conference: Colorectal cancer screening and surveillance in inflammatory bowel disease. *Inflamm Bowel Dis* 2005; **11**: 314-321

42 **Karlén P**, Kornfeld D, Broström O, Löfberg R, Persson PG, Ekbom A. Is colonoscopic surveillance reducing colorectal cancer mortality in ulcerative colitis? A population based case control study. *Gut* 1998; **42**: 711-714

43 **Choi PM**, Nugent FW, Schoetz DJ Jr, Silverman ML, Haggitt RC. Colonoscopic surveillance reduces mortality from colorectal cancer in ulcerative colitis. *Gastroenterology* 1993; **105**: 418-424

44 **Rosenstock E**, Farmer RG, Petras R, Sivak MV Jr, Rankin GB, Sullivan BH. Surveillance for colonic carcinoma in ulcerative colitis. *Gastroenterology* 1985; **89**: 1342-1346

45 **Jones HW**, Grogono J, Hoare AM. Surveillance in ulcerative colitis: burdens and benefit. *Gut* 1988; **29**: 325-331

46 **Mpofu C**, Watson AJ, Rhodes JM. Strategies for detecting colon cancer and/or dysplasia in patients with inflammatory bowel disease. *Cochrane Database Syst Rev* 2004; CD000279

47 **Connell WR**, Lennard-Jones JE, Williams CB, Talbot IC, Price AB, Wilkinson KH. Factors affecting the outcome of endoscopic surveillance for cancer in ulcerative colitis. *Gastroenterology* 1994; **107**: 934-944

48 **Befrits R**, Ljung T, Jaramillo E, Rubio C. Low-grade dysplasia in extensive, long-standing inflammatory bowel disease: a follow-up study. *Dis Colon Rectum* 2002; **45**: 615-620

49 **Lim CH**, Dixon MF, Vail A, Forman D, Lynch DA, Axon AT. Ten year follow up of ulcerative colitis patients with and without low grade dysplasia. *Gut* 2003; **52**: 1127-1132

50 **Torres C**, Antonioli D, Odze RD. Polypoid dysplasia and adenomas in inflammatory bowel disease: a clinical, pathologic, and follow-up study of 89 polyps from 59 patients. *Am J Surg Pathol* 1998; **22**: 275-284

51 **Yamazaki Y**, Ribeiro MB, Sachar DB, Aufses AH Jr, Greenstein AJ. Malignant colorectal strictures in Crohn's disease. *Am J Gastroenterol* 1991; **86**: 882-885

52 **Velayos FS**, Terdiman JP, Walsh JM. Effect of 5-aminosalicylate use on colorectal cancer and dysplasia risk: a systematic review and metaanalysis of observational studies. *Am J Gastroenterol* 2005; **100**: 1345-1353

53 **Bernstein CN**, Blanchard JF, Metge C, Yogendran M. Does the use of 5-aminosalicylates in inflammatory bowel disease prevent the development of colorectal cancer? *Am J Gastroenterol* 2003; **98**: 2784-2788

54 **Tung BY**, Emond MJ, Haggitt RC, Bronner MP, Kimmey MB, Kowdley KV, Brentnall TA. Ursodiol use is associated with lower prevalence of colonic neoplasia in patients with ulcerative colitis and primary sclerosing cholangitis. *Ann Intern Med* 2001; **134**: 89-95

55 **Pardi DS**, Loftus EV Jr, Kremers WK, Keach J, Lindor KD. Ursodeoxycholic acid as a chemopreventive agent in patients with ulcerative colitis and primary sclerosing cholangitis. *Gastroenterology* 2003; **124**: 889-893

56 **Eaden J**, Abrams K, Ekbom A, Jackson E, Mayberry J. Colorectal cancer prevention in ulcerative colitis: a case-control study. *Aliment Pharmacol Ther* 2000; **14**: 145-153

57 **Bansal P**, Sonnenberg A. Risk factors of colorectal cancer in inflammatory bowel disease. *Am J Gastroenterol* 1996; **91**: 44-48

58 **Lashner BA**, Provencher KS, Seidner DL, Knesebeck A, Brzezinski A. The effect of folic acid supplementation on the risk for cancer or dysplasia in ulcerative colitis. *Gastroenterology* 1997; **112**: 29-32

59 **Matula S**, Croog V, Itzkowitz S, Harpaz N, Bodian C, Hossain S, Ullman T. Chemoprevention of colorectal neoplasia in ulcerative colitis: the effect of 6-mercaptopurine. *Clin Gastroenterol Hepatol* 2005; **3**: 1015-1021

60 **Jung M**, Kiesslich R. Chromoendoscopy and intravital staining techniques. *Baillieres Best Pract Res Clin Gastroenterol* 1999; **13**: 11-19

61 **Kiesslich R**, Fritsch J, Holtmann M, Koehler HH, Stolte M, Kanzler S, Nafe B, Jung M, Galle PR, Neurath MF. Methylene blue-aided chromoendoscopy for the detection of intraepithelial neoplasia and colon cancer in ulcerative colitis. *Gastroenterology* 2003; **124**: 880-888

62 **Hurlstone DP**, Sanders DS, Lobo AJ, McAlindon ME, Cross SS. Indigo carmine-assisted high-magnification chromoscopic colonoscopy for the detection and characterisation of intraepithelial neoplasia in ulcerative colitis: a prospective evaluation. *Endoscopy* 2005; **37**: 1186-1192

63 **Kiesslich R**, Neurath MF. Chromoendoscopy and other novel imaging techniques. *Gastroenterol Clin North Am* 2006; **35**: 605-619

**S- Editor** Tian L **L- Editor** Webster JR **E- Editor** Zheng XM

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.67

*World J Gastroenterol* 2009 January 7; 15(1): 67-80
***World Journal of Gastroenterology*** ISSN 1007-9327




**Dr. Peter V Draganov,** *Series Editor*

# Evaluation and management of patients with refractory ascites

Bahaa Eldeen Senousy, Peter V Draganov

Bahaa Eldeen Senousy, Ain Shams University school of Medicine, Abbassia Square 11566, Cairo, Egypt
Peter V Draganov, University of Florida, Gainesville, Florida 32610, United States
Author contributions: Draganov PV designed the concept and the format of the article; Senousy BE performed the literature search and wrote the first draft of the paper; Draganov PV contributed new references to the literature search and reviewed and edited the article.
Correspondence to: Peter V Draganov, MD, Department of Gastroenterology, Hepatology and Nutrition, University of Florida, 1600 SW Archer Rd, Room HD 602, PO Box 100214 Gainesville, Florida 32610,
United States. dragapv@medicine.ufl.edu
Telephone: +1-352-392-2877 Fax: +1-352-392-3618
Received: October 12, 2008 Revised: December 6, 2008
Accepted: December 13, 2008
Published online: January 7, 2009

## Abstract

Some patients with ascites due to liver cirrhosis become no longer responsive to diuretics. Once other causes of ascites such as portal vein thrombosis, malignancy or infection and non-compliance with medications and low sodium diet have been excluded, the diagnosis of refractory ascites can be made based on strict criteria. Patients with refractory ascites have very poor prognosis and therefore referral for consideration for liver transplantation should be initiated. Search for reversible components of the underlying liver pathology should be undertaken and targeted therapy, when available, should be considered. Currently, serial large volume paracentesis (LVP) and transjugular intrahepatic portasystemic stent-shunt (TIPS) are the two mainstay treatment options for refractory ascites. Other treatment options are available but not widely used either because they carry high morbidity and mortality (most surgical options) rates, or are new interventions that have shown promise but still need further evaluation. In this comprehensive review, we describe the evaluation and management of patients with refractory ascites from the prospective of the practicing physician.



**Key words:** Refractory ascites; Aquaretics; Albumin infusion; Transjugular intrahepatic portosystemic stent-shunt; Large volume paracentesis

**Peer reviewer:** Oliviero Riggio, Professor, Dipartimento di Medicina Clinica, Sapienza Università di Roma,Viale dell' Università 37, 00185 Roma, Italy

Senousy BE, Draganov PV. Evaluation and management of patients with refractory ascites. *World J Gastroenterol* 2009; 15(1): 67-80 Available from: URL: http://www.wjgnet.com/1007-9327/15/67.asp DOI: http://dx.doi.org/10.3748/wjg.15.67

## INTRODUCTION

Ascites means pathological fluid accumulation within the abdominal cavity. The word "ascites" itself is derived from the Greek word "askos," which means a bag or sack[1]. Cirrhosis accounts for over 75% of patients who present with ascites[2]. Ascites is the most common of the three major complications of cirrhosis; the other complications are hepatic encephalopathy and variceal hemorrhage[3]. Approximately 50%-60% of patients with compensated cirrhosis will develop ascites during 10 years of observation[3,4].

Patients with ascites can be divided into the following categories based on their response to treatment. (1) Less than 10% have natural sodium excretion (i.e. without diuretics) more than 78 meq/d. These patients, have relatively preserved liver functions and will respond to dietary salt restriction [88 meq (2000 mg) per day] alone[5]. (2) As liver function deteriorates, patients excrete less sodium in the urine and sodium restriction alone is no longer enough to create a negative sodium balance and control ascites[6]. Most patients will need diuretics combined with sodium-restricted diet[7]. This regimen is effective in about 90% of the patients[8]. Over time, up to 20% of patients that were initially diuretic-responsive will become diuretic-resistant[9]. (3) 5%-10% of patients never respond to this regimen and have refractory ascites[4,10].

Development of ascites is associated with a poor quality of life, increased risks of infections and renal failure, and a poor long-term outcome[11]. Furthermore, patients with refractory ascites have worse prognosis and shortened survival.

Cirrhotic patients who develop ascites have a probability of survival of 85% at 1 year and 56% at 5 years without liver transplantation[12]. In patients who

become resistant to diuretic therapy, the prognosis decreases to 50% survival at 2 years[13].

Patients with refractory ascites have lower sodium excretion compared to sensitive patients. It has been shown that patients with ascites and urinary sodium excretion below 10 meq/d had a mean survival rate of 5-6 mo compared to > 2 years in those with ascites and a higher rate of sodium excretion[14].

## DEFINITIONS

For the correct diagnosis of true refractory ascites, the patient's condition should fulfill the following criteria[2,15].

### *Diuretic-resistant ascites*

Failure of mobilization or the early recurrence of ascites which cannot be prevented because of a lack of response to sodium restriction and diuretic treatment is called diuretic-resistant ascites.

### *Diuretic-intractable ascites*

Failure of mobilization or the early recurrence of ascites which cannot be prevented because of the development of diuretic-induced complications that prevent the use of an effective diuretic dosage is called diuretic-intractable ascites.

### *Treatment duration*

Patients must be on intensive diuretic therapy (spironolactone 400 mg/d and furosemide 160 mg/d) for at least 1 wk and on a salt-restricted diet of less than 90 mmol/d.

### *Lack of response*

Mean weight loss of less than 0.8 kg over 4 d and urinary sodium output less than the sodium intake.

### *Early ascites recurrence*

There is an reappearance of grade 2 or 3 ascites (clinically detectable) within 4 wk of initial mobilization. However, it is important to notice that in patients with severe peripheral edema, reaccumulation of ascites within 2-3 d of paracentesis must not be considered as early ascites recurrence because it represents a shift of interstitial fluid to the intraperitoneal space[16].

### *Diuretic-induced complications*

Diuretic-induced hepatic encephalopathy is the development of encephalopathy in the absence of any other precipitating factor. Diuretic-induced renal impairment is indicated by an increase of serum creatinine by > 100% to a value of > 2 mg/dL in patients with ascites otherwise responding to treatment. Diuretic-induced hyponatremia is defined as a decrease of serum sodium by > 10 mEq/L to a serum sodium of < 125 mEq/L. Diuretic-induced hypo- or hyperkalemia is defined as a change in serum potassium to < 3 mEq/L or > 6 mEq/L despite appropriate measures.

In addition to this, we should exclude dietary non-compliance (patient taking excess sodium in diet) and exclude the use of nonsteroidal antiinflammatory drugs (NSAIDs), which can induce renal vasoconstriction and diminish diuretic responsiveness[17,18].

## EVALUATION OF PATIENT WITH REFRACTORY ASCITES

The aim of the work-up is to confirm the diagnosis and exclude other conditions that can be misdiagnosed as refractory ascites.

Exclude causes of ascites other than cirrhosis that are not responsive to diuretic therapy. This includes malignant ascites due to peritoneal carcinomatosis (but not due to massive hepatic metastasis)[19] and nephrogenic ascites, which develop in patients with end-stage renal disease[20]. This is important because about 5% of patients with ascites have more than one underlying etiology (mixed ascites)[21]; for example, a patient may have cirrhosis in addition to peritoneal carcinomatosis and be misdiagnosed as having true refractory ascites. The importance of this is that lines of therapy are different.

Patient should have ultrasound with portal vein Doppler and serum alpha fetoprotein level to exclude the presence of hepatocellular carcinoma or portal vein thrombosis[4], because these conditions are associated with lack of response to diuretics in patients with cirrhosis, while true refractory ascites represents actual progression of the liver disease[3] (discussed below).

Confirm compliance to dietary sodium restriction because patient may not be responding to diuretics simply because of the lack of dietary compliance. Therefore, the diagnosis of refractory ascites is not complete until it is proven that the patient has low urinary sodium excretion on the diuretic doses mentioned before[17]. This can be done through the following.

**Twenty-four-hour urinary sodium:** Patients who gain weight despite excreting more than 78 meq sodium per day are not compliant with the diet. The value of 78 meq sodium per day is derived from the difference between sodium intake (2 gm/d = 88 mEq) and non-urinary loss (10 mEq/d)[22]. The drawback of the 24-h urinary collections is that they are labor-intensive for patients and staff alike. Verbal and written instructions should be given to the patient in order to assure accurate collection. Completeness of collection can be assessed by measurement of urinary creatinine. Urinary creatinine excretion per day should be more than 15 mg of creatinine per kg of body weight for men and more than 10 mg/kg for women[23]. Samples with less creatinine indicate incomplete collection that may affect the results. However, this may not be very accurate because patients with advanced cirrhosis have muscle wasting and therefore lower creatinine excretion in urine even with complete collection[24,25].

**Sodium in spot urine specimen:** Measuring sodium in a spot urine specimen should be easier and more

convenient for the patient but lack of accuracy is the problem as excretion of sodium is not uniform throughout the day. Random urinary sodium concentrations are of value when they are 0 mmol/L (meaning low sodium excretion and lack of diuretic response) or greater than 100 mmol/L (means either adequate response to diuretics or diet non compliance) but are not helpful when they are intermediate[22].

**Random urinary Na/K ratio:** Random urinary Na/K ratio may be as helpful as 24-h urinary sodium collection, with accuracy rates of 86% according to one study and 90% according to another. A ratio of more than 1 is equivalent to 24 h sodium more than 78 mmol Na/d. This test is easier for the patient as it does not involve collection of 24-h urine.

**Furosemide-induced natriuresis:** Furosemide-induced natriuresis is another alternative where a single intravenous 80-mg dose of furosemide is given and urinary sodium is measured in the next 8 h. Patients with diuretic-resistant ascites have sodium excretion less than 50 mEq/8 h[26,27]. Another advantage of this test is that it allows more rapid identification of diuretic-resistant patients without the need to follow them up for weeks with increasing doses of diuretics.

## PATHOGENESIS OF ASCITES

Currently, the most widely accepted theory of ascites formation is the forward theory which is based on the peripheral arterial vasodilation hypothesis of renal dysfunction in cirrhosis[28]. According to this theory, the initial step is the development of sinusoidal portal hypertension[29,30]. This leads to systemic vasodilation and reduction in systemic vascular resistance, which is most evident in splanchnic blood vessels[31,32]. Portal hypertension causes vasodilation through increased release of local vasodilators such as nitric oxide (which seems to be the primary mediator[33]), glucagon[34], prostacyclins[35], vasoactive intestinal peptide, substance P and platelet activating factor[29]. Splanchnic vasodilation leads to a forward increase in filtration across splanchnic capillaries[36]. In patients with decompensated cirrhosis, the lymphatic system is not capable of returning back all the filtered fluid and causes accumulation of ascites fluid[37]. Besides this, systemic vasodilation causes systemic vascular underfilling, which stimulates the sodium-retaining neurohumoral mechanisms in order to refill the dilated vascular bed; these mechanisms include mainly the renin-angiotensin-aldosterone system, sympathetic nervous system, and antidiuretic hormone[38]. This leads to sodium retention, water retention (with dilutional hyonatremia) and renal vasoconstriction, which may later lead to hepatorenal syndrome[28]. This causes retention of more fluid, which is not effective in filling the systemic vascular bed because of the continuous leakage into the peritoneal cavity leading to more ascites formation[28]. These changes become more severe with the progression of liver disease, which is why the degree of sodium retention (measured as urinary sodium excretion)[14] and hyponatremia[39,40] correlate with worsening survival in cirrhotic patients.

Patients with more advanced cirrhosis have a marked degree of circulatory dysfunction and marked neurohumoral activation. This results in renal vasoconstriction and enhanced sodium reabsorption in the renal tubule and very low urinary excretion of sodium (even with high doses of diuretics), which is how refractory ascites develops[17]. Hepatorenal syndrome has a pathogenesis similar to that of ascites[41]; refractory ascites is considered a pre-hepatorenal state and actually refractory ascites is a usual manifestation of type 2-hepatorenal syndrome[4].

## TREATMENT OF REFRACTORY ASCITES

The ideal treatment of ascites should be effective in mobilization of ascites and prevention of recurrence, should improves patient's quality of life and survival, and should be acting directly on one or more steps in the pathogenesis of ascites and not just the mechanical removal of the fluid[42].

Currently, the main lines of treatment for refractory ascites are serial large volume paracentesis (LVP), transjugular intrahepatic portasystemic stent-shunt (TIPS), liver transplantation and peritoneovenous shunt[22]. We will also discuss promising new therapies that are currently being evaluated.

### *LVP*

LVP with administration of intravenous albumin represents the standard therapy for refractory ascites[43]. Several studies have shown its effectiveness and safety[44-46]. Beside rapid control of ascites, it may decrease the risk of variceal bleeding because it is associated with reductions in the hepatic venous pressure gradient and intravariceal pressure[47,48].

**Frequency of LVP:** Therapeutic paracentesis is a local therapy that does not modify the mechanisms that lead to ascites formation. Therefore ascites will always recur in patients with refractory ascites unless there is an improvement in liver disease, as in alcoholic liver disease when patients stop drinking, or after liver transplantation[49,50]. Two weeks are considered a reasonable interval between paracentesis sessions in patients with refractory ascites[22,51]. Less frequent sessions are needed in the patient with some sodium excretion and more frequent sessions are required in patients who are not compliant with dietary sodium restriction. The explanation requires knowing some details related to sodium balance in patients with ascites. The sodium concentration of ascitic fluid is approximately equivalent to that of plasma in these patients: 130 mmol/L. A 10-L paracentesis removes 1300 mmol (130 × 10). If the patient is adherent to the diet, he/she will consume 88 mmol sodium every day, and excrete 10 mmol/d in non-urinary loss and excrete nothing in the urine if there is no urinary sodium

excretion at all. Therefore, the net gain every day will be 78 mmol. Therefore, a 6-L paracentesis removes 10 d (780 mmol/78 mmol per day) of retained sodium, and a 10-L paracentesis removes approximately 17 d of retained sodium (1300 mmol/78 mmol per day = 16.7 d) in patients with no urinary sodium excretion[22].

Patients who are not compliant need education regarding their diet rather than more frequent LVP sessions. This is important because although the patients are no longer responding to diuretics, diet is still very important. One should not think about a more restricted diet as a solution for diuretic resistance, as it is not more effective and makes food less palatable therefore, malnutrition may result[52]. Fluid restriction is indicated in patients with ascites and serum sodium lower than 130 mEq/L[53].

At the time of LVP measurement of the white cell count with differential should be performed on the acidic fluid sample as a screening for spontaneous bacterial peritonitis even if the patient is asymptomatic, while if symptomatic, cultures should be added[50].

Most authors prefer total LVP than repeated LVP (removing 4-6 L daily until ascites completely disappears) because it is faster and can be done as an outpatient procedure; also, it is associated with lower incidences of complications that may be related to needle insertion and associated with no fluid leakage after paracentesis because no fluid stays in the abdominal cavity[38]. Another measure to reduce leakage is using the "Z" track where skin is penetrated perpendicularly, then the needle is advanced obliquely in subcutaneous tissue before the peritoneal cavity is punctured, so that the puncture site on the skin and the peritoneum are not overlying. Also asking the patient to recline for 2 h on the side opposite to the paracentesis site will prevent the leakage of ascitic fluid. If there is significant leakage that is not controlled with these measures, a suture or purse string may be inserted around the site of drainage[54].

**Complication associated with LVP:** It is considered a safe procedure associated with very low incidence of serious complications even in patients with coagulopathy[55,56]. The risk of developing a large hematoma is about 1% and the risk of hemoperitoneum or iatrogenic infection is only about 1 per 1000[57]. There is no evidence in clinical trials that transfusion of plasma or platelets before the procedure decreases the risk of bleeding[58]. However, one should avoid puncture of the visible dilated abdominal wall veins in order to avoid severe bleeding. Also, there is no coagulation profile cut-off value that paracentesis should be avoided beyond it. According to one study, patients tolerated the procedures with INR up to 8.7 and platelet counts as low as 19 000[59]. It may be that the only condition when the procedure should be avoided due to high bleeding risk is the presence of disseminated intravascular coagulation with clinically evident fibrinolysis[60].

One problem with repeated LVPs is ascitic fluid protein and complement depletion, which may predispose to ascitic fluid infections[61], in comparison with diuretic therapy, but this is of special concern in diuretic-sensitive patients while in refractory ascites, diuretics are no longer an option.

Another problem with large volume paracentesis is post-paracentesis circulatory dysfunction (PCD). Circulatory changes after LVP can be described as follows. (1) Immediately after paracentesis, there is an improvement in circulatory function in regard to increased cardiac output and suppression of the renin-angiotensin and sympathetic nervous systems. This effect is mostly due to mechanical factors that mainly increase venous return due to reduced intraabdominal pressure[62]. (2) After about 12 h, there are opposite hemodynamic changes, including a reduction in cardiac output to baseline values and marked activation of the renin-angiotensin and sympathetic nervous systems over the levels before paracentesis[63]. These changes are not spontaneously reversed as once plasma renin activity and plasma norepinephrine concentration increase, this elevation persists[38]. PCD has been defined as a 50% increase in plasma renin activity over baseline on the sixth day after treatment, up to a value greater than 4 ng/mL per hour[38,62,64]. Despite being asymptomatic, PCD adversely affects the clinical course of the disease with higher incidences of hyponatremia, and renal impairment. In patients who develop PCD, it is severity correlates inversely with patient survival[65]. Severity of the circulatory dysfunction correlates with the amount of fluid removed in paracentesis being most significant when it exceeds 5L[65].

A number of measures can be applied to prevent PCD.

**Albumin infusion:** Albumin infusion has been studied for the prevention of PCD[45,66]. Incidence of PCD following LVP reaches 80% when albumin is not used and albumin infusion reduces the incidence to 15%-20%[45]. However, some controversy still exists related to albumin infusion. The reasons behind this are: lack of direct survival advantage with albumin infusion[67]; albumin is very expensive and some studies state that albumin infusion inhibits synthesis of albumin[58] and stimulates albumin degradation inside the body[68,69]. Another reason for the controversy is that the circulatory changes that can follow LVP may not be related to a decreased intravascular volume due to rapid accumulation of ascitic fluid as was thought before[70], but it is actually due to accentuation of the arterial vasodilatation already present in these patients[38].

The current American Association for the Study of Liver Diseases (AASLD) guidelines state that post-paracentesis albumin infusion may not be necessary for a single paracentesis of less than 4-5 L. For LVP, an albumin infusion of 8-10 g per liter of fluid removed can be considered[22].

Albumin should be given once the session is completed[54]. Some authors recommend giving one half of the plasma expander immediately after the paracentesis and the other half 6 h later[65,71]. Others say this is unwarranted and converts an otherwise simple

outpatient procedure into an all-day clinic visit[67].

It has been suggested that reducing the flow rate of ascites extraction may help prevent PCD[72], however, this may need further evaluation before being applied in practice.

**Other alternatives to albumin for prevention of PCD:** Many trials were carried out to find less expensive alternatives to albumin therapy, however none is accepted currently to replace albumin. Some of the alternatives to albumin infusion include synthetic colloids, extracorporeal ultrafiltration and reinfusion, and vasoconstrictors.

**(Ⅰ) Synthetic colloids.** Studies comparing replacement of albumin with dextran 70 or polygeline showed no survival advantage[71] and PCD was much less common with albumin administration in patients where ⩾ 5 L of fluid were removed[65]. Saline also has been tried but without showing any survival advantage[73]. This is mostly related to the half-life of the colloid used, being highest with albumin (21 d), this may explain its effectiveness in prevention of PCD[74]. Some authors state that paracentesis of < 5 L can be followed by synthetic plasma expander and albumin is not required in this setting[54].

One important point is that hydroxyethyl starch can increase portal pressure as it fills Kupffer cells (lysosomal storage) and this may increase the risk of variceal bleeding[75].

**(Ⅱ) Extracorporeal ultrafiltration and reinfusion.** This procedure involves ultrafiltration of ascitic fluid and intraperitoneal or intravascular reperfusion[76]. Advantages compared to albumin are the reduced expenses and avoidance of depletion of complement lost with paracentesis[77]. The main problem reported is the development of disseminated intravascular coagulation (DIC) in some patients, and this may be why it is not approved for use now; however, one simplified method is suggested that limits the incidence of DIC[78]. One point is that only a few patients have been studied until now therefore, better evaluation with larger studies is needed.

**(Ⅲ) Vasoconstrictors.** Administration of vasoconstrictors may decrease the development of PCD and may prevent complications associated with a decrease in effective arterial blood volume as with the plasma volume expander albumin[64]. This may be due to the fact mentioned before, as the pathogenesis of PCD is accentuation of splanchnic vasodilation rather than depletion of intravascular volume.

**Terlipressin:** More than one study showed that terlipressin may be as effective as intravenous albumin and well tolerated in preventing paracentesis-induced circulatory dysfunction in patients with cirrhosis after therapeutic paracentesis[79-82]. One study recommended a dose of terlipressin (1 mg every 4 h for 48 h)[79], while another study suggested that a total dose of 3 mg terlipressin should be administered as an intravenous bolus of 1 mg terlipressin at the onset of paracentesis and then 8 and 16 h after the first bolus[80]. A problem with using terlipressin is that it requires hospital admission for a simple outpatient procedure, as it is given as intravenous injections for up to 48 h after the procedure.

**Midodrine:** One study carried out on 40 patients showed that midodrine may be as effective as albumin[83], while another one carried out on 24 patients showed that PCD developed in six patients of the midodrine group (60%) and in only four patients (31%) of the albumin group[64]. The dose given was 12.5 mg every 8 h post-paracentesis for 2 d. Being much cheaper than albumin and terlipressin and much easier to administer, midodrine may be worth more trials to assess its use instead of albumin.

**Noradrenaline:** Another study showed noradrenaline to be as effective as albumin in the prevention of PCD[84]. Noradrenaline was suggested as a less expensive alternative to albumin but no further studies were done to confirm this.

### *TIPS*

TIPS is a side-to-side portacaval shunt by which an intrahepatic communication between the portal and the hepatic vein is created[85]. It is a non-surgical procedure performed under local anesthesia by an interventional radiologist. A catheter is advanced through the jugular vein into a hepatic vein and into a main branch of the portal vein. There an expandable stent is introduced connecting hepatic and portal systems, which allows shunting of blood from the high-pressure portal circulation (splanchnic and sinusoidal beds) to the low-pressure systemic circulation (hepatic vein)[42].

The mechanism by which TIPS helps control ascites is decompression of the portal circulation and reduction in the portacaval gradient and the portal venous pressure[38]. As mentioned before, portal hypertension is essential in ascites formation so that cirrhotic patients with portal venous pressure less than 12 mm Hg do not develop ascites[29,30], and ascites in these patients disappears if portal venous pressure drops below 12 mm Hg[86,87]. Another mechanism is that the blood volume pooled in the dilated splanchnic vascular bed is transferred to the systemic circulation through the shunt, therefore, it corrects the systemic vascular underfilling and causes a decrease in the renin-angiotensin-aldosterone system and thereby improves renal sodium excretion[42,88].

Several studies showed that TIPS is highly effective in controlling ascites[54]. According to these studies, ascites was controlled in 27%-92%[89,90], with 75% of cases showing complete resolution[91]. It takes about 1 -3 mo for ascites to resolve after TIPS procedure[38]. One important point is that diuretic therapy will still be required in about 95% of patients. The explanation is that TIPS produces partial resolution of ascites pathogenesis, so portal pressure and renin and aldosterone levels, although they are markedly reduced after TIPS, they are not back to normal as in healthy subjects[38].

In patients with cirrhosis, TIPS may have some advantages beside ascites control. Improvement in

renal function is seen in these patients in the form of increased urine volume, increased sodium excretion[91,92] and even a reduction in serum creatinine level which is a delayed effect seen after 6 mo according to one study[93]. Another advantage is the improvement in the nutritional status (in the form of an increase in dry weight and total body nitrogen)[88,94] and improvements in quality of life[93]; however, these effects (nutrition and quality of life) may be simply due to improved eating when ascites is controlled[54].

**Complications associated with TIPS:** (1) Technical complications. Estimated technical success rate is reported in the range of 93%-100%[90,91]. Procedure-related mortality is very low (1%-2%) according to one study[95], and it was due to hemoperitoneum, hemobilia, hemolysis, and sepsis. The procedure-related complication rate is around 9%, with intraperitoneal hemorrhage and acute renal failure (mostly due to contrast media) being the most frequent[42]. Complications also include those of sedation and arrhythmia if the catheter enters the right atrium or right ventricle[96], and transient right bundle branch block, which may be significant in patients already with left bundle branch block as it may lead to complete heart block. The liver capsule is frequently punctured (reported frequency around 33%[97]), especially if the liver is shrunken but intraperitoneal bleed only occurs in 1%-2% of the cases[98]. (2) Hepatic encephalopathy occurs in about 30% of patients after TIPS[99,100]. Factors associated with the development of encephalopathy that can be used for patient selection are increasing age, advanced liver failure, and a history of encephalopathy before TIPS insertion[101,102]. Encephalopathy usually becomes clinically apparent 2-3 wk after TIPS insertion[99]. According to one study[100], encephalopathy starts to develop about 10 d after insertion, and then begins to decline (as measured by the portosystemic encephalopathy index) at 6 mo, however, it remained significantly higher than the baseline values. A possible explanation for this decline may be shunt stenosis with time. Treatment is medical in most of the cases and consists of controlling any precipitating factor, lactulose and non-absorbable antibiotics (neomycin or rifaximin)[100]. In case of medical therapy failure, the TIPS can be occluded[103] or the diameter of the shunt can be narrowed in some types with a "wasp waist" constrictor[104]. (3) Shunt occlusion. These problems occur in 22% to 50 % of patients[91,105,106]. This occurs due to growth of collagenous fibrils and endothelial cells (pseudointima) inside the stent[107]. It can diffuse all over the whole length (type 1) or localized to the hepatic venous end (type 2); however, both have the same management[108]. The incidence of this complication increases with time, according to one study, all patients surviving more than 2 years had shunt stenosis[108]. Shunt stenosis or occlusion presents as a recurrence of portal hypertension or variceal bleeding[109]. TIPS patency should be followed up after insertion, however, the best strategy for follow-up is not yet defined. Methods used to monitor patency include venography (which is the best test but not usually used because is invasive and caries a high cost), but Doppler sonography is the most frequently used (although it is less sensitive than venography)[110]. Helical CT angiography also can be used; one study showed a 92% correlation with venography[111]. One recommended approach for surveillance is duplex ultrasonography every 3 mo and venography annually. Venography can be done earlier if shunt obstruction is suspected clinically[110]. Treatment is redilation of the shunt done by interventional radiology[105]. New stents covered with polytetrafluoroethylene (Goretex) showed lower rates of occlusion and stenosis[112,113]. Using antiplatelet therapy was evaluated in the prevention of shunt stenosis[114] and it showed some efficacy, but it is not used in practice because of the increased risk of bleeding, as those patients already have bleeding tendencies and thrombocytopenia. (4) Haemolysis occurs in about 10% of patients and it is believed to be due to direct mechanical trauma to the red blood cells when they pass through the metallic stent[115]. This may be why spontaneous resolution is seen in most patients after 8-12 wk with covering of the metallic stent by pseudointima[116]. In most patients, the anemia is mild with less than 2 g/dL reduction in hemoglobin level starting 1-2 wk after placement. Blood smears may show schistocytes in patients who develop severe anemia[115]. (5) Infection. According to one study, infection occurred weeks to months after placement and presented with fever, continuous bacteremia and presence of vegetations or thrombi in the stent. It was treated with intravenous antibiotics[117]. (6) Portosystemic myelopathy (PSM, also called shunt myelopathy) is a rare syndrome that includes spastic paraparesis with intact sensation occurring in patients with surgical portosytemic shunts and also described after TIPS placement[118,119]. A possible explanation is accumulation of ammoniacal substances (that bypass the liver through the stent), leading to loss of motor neurons in the spinal cord. According to one study[118] carried out on 212 patients, four patients (1.89%) had this progressive spastic paresis starting to appear between 5 wk and 5 mo after stent placement. (7) Deterioration of cardiac function. TIPS increases the cardiac preload, and hence it may precipitate heart failure in those with pre-existing heart disease[120]. Echocardiography is usually done before the procedure to exclude patients with subtle heart failure; usually, patients with ejection fraction less than 60 are excluded.

### *TIPS versus paracentesis*

Several studies have compared TIPS to repeated LVP plus albumin, but the detailed discussion of these studies is beyond the scope of this article. However, the conclusion from these studies is: (1) TIPS controls ascites effectively and is associated with a lower rate of ascites recurrence[106,121-123]; (2) patients with ascites who undergo TIPS improve their nutritional status as mentioned before; (3) there is a higher incidence of side effects, mainly hepatic encephalopathy and shunt dysfunction in the

group treated with TIPS; and (4) there is no proven effect for TIPS on survival. In one study, TIPS had no effect on survival[123,124], while others have reported both reduced[106] as well as improved survival[106,121,122] compared with therapeutic paracentesis. For these reasons, we believe repeated LVP plus albumin should be considered the first-line therapy for refractory ascites, and TIPS should be used as a second line of management[15,125,126]. TIPS should be considered in appropriately selected patients who meet the following criteria.

Patients with very rapid recurrence of ascites (those who require paracentesis > 3 times/mo) and preserved liver function [bilirubin < 3 mg/dL, serum sodium level >130 mEq/L, Child-Pugh score < 12, model for end-stage liver disease (MELD) score < 18], aged < 70 years, without hepatic encephalopathy, central hepatocellular carcinoma, or cardiopulmonary disease[15,127].

## SURGICAL OPTIONS

Peritoneo-venous shunt is a surgically inserted shunt that drains ascitic fluid from the peritoneal cavity into the internal jugular vein. It has limited indications because there is no survival advantage in addition to frequent complications including bacteremia, small bowel obstruction and volume overload leading to variceal bleeding[10]. The use of the peritoneo-venous shunt is limited to patients with refractory ascites who are not candidate for TIPS or liver transplantation, and has a lot of abdominal scars that makes frequent paracentesis unsafe[22,67]. One study described percutaneous placement of a peritoneovenous shunt by interventional radiology which may carry less complications than surgery however further studies are needed to confirm this[128].

A more simple method of peritoneovenous drainage was described, the sapheno-peritoneal anastomosis[129-131]. Advantages over the ordinary peritoneovenous shunt are simpler and less expensive and use a biological shunt instead of a prosthetic one. Also, one study described the possibility of doing the procedure under local anesthesia[129], which is an advantage in cirrhotic patients. Patients had reduction in admissions and paracentesis, however, no survival advantage was noted.

Portosystemic shunt works, similar to TIPS, through decompression of the portal circulation; however, mortality is higher ranging from 12% to 39% and encephalopathy rates are more than 50%[132].

One study described a technique of peritoneal-urinary drainage of the fluid using a surgically implanted pump[133].

## MEASURES THAT MAY IMPROVE THE RESPONSE TO DIURETICS

Several medications have been suggested that attack certain step(s) in the pathogenesis of ascites.

### *Aquaretics*

Aquaretics are vasopressin receptor antagonists that act on the distal tubule of the kidney so as to increase the excretion of solute-free water[15]. They are already approved for management of hyponatremia due to syndrome of inappropriate anti-diuretic hormone secretion (SIADH), and are being evaluated for management of hyponatremia in cirrhosis and in refractory ascites to be combined with diuretics to improve response[134].

Vasopressin receptors are V1a, V1b and V2. The oral forms of aquaretics (e.g. lixivaptan and satavaptan) are selective for V2 receptors, which mediate the antidiuretic response of vasopressin. While, the intravenous forms (conivaptan) works on V2 and V1a receptors and V1a mediates the vasoconstrictor response of vasopressin[135]. Although conivaptan is the only approved one right now (used in SIADH), it cannot be used in ascites as it may cause variceal bleeding when it blocks the vasoconstrictor effect of the anti diuretic hormone[136].

One study carried out in 110 patients with cirrhosis and ascites receiving satavaptan or placebo in addition to diuretics[137]. Those receiving satavaptan had significant decrease in abdominal girth and more weight reduction without significant side effects. However, because of the short follow-up duration in this study (14 d), more studies are needed to evaluate the use of this drug in patients with ascites before they are approved for use in practice.

### *Vasoconstrictors*

Vasoconstrictors may theoretically improve the action of diuretics as they improve the systemic vasodilation, so as to reduce the antinaturetic factors described before[15]. They are already used in hepatorenal syndrome based on a similar mechanism of action[138].

**Terlipressin:** A potent vasoconstrictor approved for use in the acute control of variceal hemorrhage and hepatorenal syndrome. In one study in 15 patients with cirrhosis and ascites without hepatorenal syndrome[139], eight of them had refractory ascites and received terlipressin. This group had significant decreases in plasma norepinephrine and renin in addition to an increase in urinary sodium.

**Octerotide:** According to a case report[140], octreotide treatment improved renal function and diuretic response in two patients with refractory ascites. Octreotide administration has been associated with arterial splanchnic vasoconstriction, which is mediated by a reduction in glucagon secretion. In addition, octreotide inhibits the release of renin and aldosterone in both normal humans and cirrhotic patients, possibly through a direct effect on renin-producing cells and adrenals.

**Midodrine:** One study carried out in 39 cirrhotic patients[141] evaluated the effects of a 7-d treatment with midodrine. It showed a significant increase in mean arterial blood pressure and urine volume and decrease in plasma renin and aldosterone activity in those with ascites treated with midodrine.

One study evaluated the combination of midodrine

and octerotide[142], another one evaluated both drugs given with albumen[143].

### Clonidine

Several studies evaluated the response of diuretics in cirrhotic patients with ascites[144-147]. It is a centrally acting $\alpha$2-agonist, therefore, it decreases sympathetic over activity which increases renal sodium reabsorption and stimulates the renin-angiotensin-aldosterone system[148]. One study was carried out in 32 alcoholic cirrhotic patients with ascites to compare the effect of spironolactone, clonidine and the combination of both in control of ascites[147]. After 10 d of spironolactone and clonidine, patients had a significant decrease in plasma renin and aldosterone, decrease in body weight and increase in natriuresis without adverse effects.

### Chronic albumin infusion

Some studies showed better diuretic response when combined with albumin; this is noticed as decreased recurrence and shorter hospital admissions[149-151]. One study showed improved survival in patients receiving chronic albumin infusion[152]. Because of cost and lack of definite survival benefit, albumin infusion is not routinely recommended in patients with ascites. Currently, the accepted indications of albumin infusion in liver patients with ascites are with LVP to prevent PCD (discussed before), patients with spontaneous bacterial peritonitis[153,154] and those with hepatorenal syndrome[155,156].

### Splenic artery embolization

One case report described the successful control of refractory ascites with splenic artery embolization[157]. This was a 32-year-old female who developed refractory ascites due to portal vein thrombosis after liver transplantation due to Budd Chiari syndrome. With further evaluation, this can be an alternative for patients with refractory ascites who cannot tolerate TIPS or surgical shunts.

### Mannitol

In one study[158], a dose of 100 mL 20% mannitol was given as infusion followed by the usual dose of diuretics taken by the patient. Increase in urine volume and urinary sodium was noticed. Therefore, mannitol may be used in refractory ascites to improve response to diuretics.

The measures described above target mainly mobilization of ascites. In addition, as part of comprehensive strategy of managing refractory ascites, one can aim to prevent other complications of cirrhosis and also improve liver function by ether treating the underlying liver disease or liver transplantation. (1) Prevention of other complication of cirrhosis as patients with ascites due to liver cirrhosis are liable to other complications[7]. This includes portal hypertensive bleeding (prevention using either prophylactic banding or propranolol[159]), spontaneous bacterial peritonitis (using prophylactic antibiotics in patients with acute variceal bleeding or ascitic fluid protein less than 1 gm/dL[67]) and hepatorenal syndrome (using albumin infusion in patients with spontaneous bacterial peritonitis[154] and using pentoxyfillin in patients with severe alcoholic hepatitis[160]). (2) Correction of liver function through either liver transplantation or treatment of the underlying liver pathology: some causes of liver cirrhosis have a reversible element; this is most evident in patients with alcoholic liver disease in which stopping alcohol consumption can lead to improvement of portal hypertension and ascites control[161,162]. There is also some evidence of similar improvement in patients with cirrhosis due to hepatitis B (treated with antiviral therapy)[163] and patients with autoimmune hepatitis treated with steroids or azathioprine[164,165].

## LIVER TRANSPLANTATION

As mentioned above, patients with ascites have a poor long-term outcome and survival is shortened in those who become refractory to diuretic therapy. The 12-mo survival rate for patients with ascites refractory to medical therapy is only 25%[166]. The survival rate for liver transplantation is much higher[67]. Therefore, those who develop refractory ascites ideally should be on the transplantation list already.

After liver transplantation, portal hypertension reversed immediately and completely; however, ascites disappearance may take 3 to 6 mo[13]. The reason for this is not fully understood, but some studies showed that the systemic vasodilation and hyperdynamic circulation persist for months after transplantation[167,168].

Priority of receiving liver transplant is based upon the MELD score. Some authors suggested that it may not be accurate enough for all patients with ascites, particularly for those with persistent or refractory ascites, who may have a poor prognosis despite low MELD scores[7,15]. One possible explanation is that the MELD score includes serum bilirubin, serum creatinine, and the INR, and some patients with refractory ascites might have a near-normal serum creatinine (as a result of low endogenous production), despite a low glomerular filtration rate and this may affect the accuracy of MELD score in this setting[4].

Therefore, some studies suggested that addition of serum sodium to the MELD score may improve its accuracy[169-171], but one point is that serum sodium is a not as steady as other parameters of the MELD score; it can change rapidly with diuretics or fluid administration, so this change may not reflect an actual change in the prognosis[15]. Further evaluation is needed before modified MELD (with serum sodium added to it) replaces the current MELD score in allocation of liver transplantation.

## CONCLUSION

Refractory ascites is a relatively common condition in patients with liver cirrhosis. Wrong diagnosis may occur sometimes therefore, certain criteria should be fulfilled with exclusion of dietary non-compliance, which can

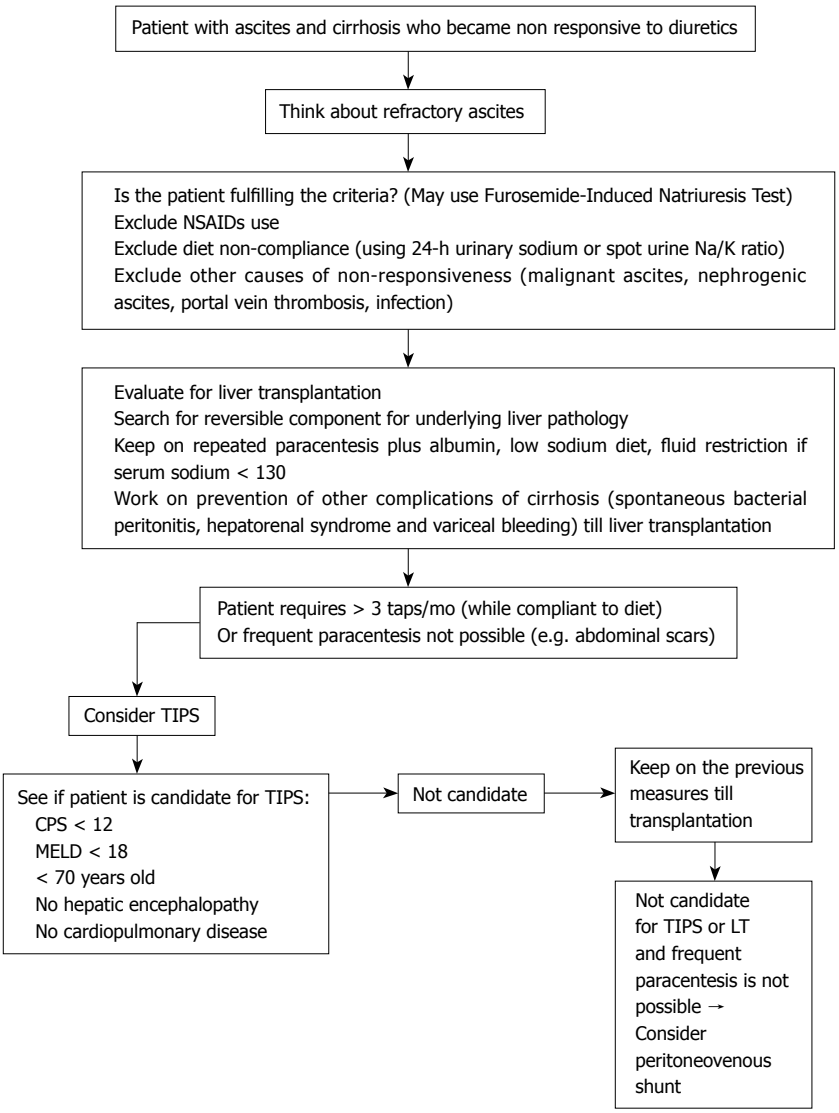


**Figure 1 Suggested approach to the patient with refractory ascites.** NSAIDs: Non-steroidal anti-inflammatory drugs; LT: Liver transplantation; CPS: Child-pugh score; MELD: Model for end-sage liver disease; TIPS: Transjugular intrahepatic portosystemic shunt.

be done through a variety of tests. Different treatment options are available, although definitive treatment is liver transplantation. An algorithmic approach to patients with refractory ascites is available (Figure 1). Other treatment options are not listed in the algorithm because they are still being evaluated and include mainly vasoconstrictor agents.

## REFERENCES


1 **Reynolds TB**. Ascites. *Clin Liver Dis* 2000; **4**: 151-168, vii
2 **Moore KP**, Wong F, Gines P, Bernardi M, Ochs A, Salerno F, Angeli P, Porayko M, Moreau R, Garcia-Tsao G, Jimenez W, Planas R, Arroyo V. The management of ascites in cirrhosis: report on the consensus conference of the International Ascites Club. *Hepatology* 2003; **38**: 258-266
3 **Gines P**, Quintero E, Arroyo V, Teres J, Bruguera M, Rimola A, Caballeria J, Rodes J, Rozman C. Compensated cirrhosis: natural history and prognostic factors. *Hepatology* 1987; **7**: 122-128
4 **Cardenas A**, Gines P. Management of refractory ascites. *Clin Gastroenterol Hepatol* 2005; **3**: 1187-1191
5 **Wongcharatrawee S**, Garcia-Tsao G. Clinical management of ascites and its complications. *Clin Liver Dis* 2001; **5**: 833-850
6 **Wensing G**, Lotterer E, Link I, Hahn EG, Fleig WE. Urinary sodium balance in patients with cirrhosis: relationship to quantitative parameters of liver function. *Hepatology* 1997; **26**: 1149-1155
7 **Gines P**, Cardenas A, Arroyo V, Rodes J. Management of cirrhosis and ascites. *N Engl J Med* 2004; **350**: 1646-1654
8 **Bernardi M**, Laffi G, Salvagnini M, Azzena G, Bonato S, Marra F, Trevisani F, Gasbarrini G, Naccarato R, Gentilini P. Efficacy and safety of the stepped care medical treatment of ascites in liver cirrhosis: a randomized controlled clinical trial comparing two diets with different sodium content. *Liver* 1993; **13**: 156-162
9 **Arroyo V**, Rodes J. A rational approach to the treatment of ascites. *Postgrad Med J* 1975; **51**: 558-562
10 **Stanley MM**, Ochi S, Lee KK, Nemchausky BA, Greenlee HB, Allen JI, Allen MJ, Baum RA, Gadacz TR, Camara DS. Peritoneovenous shunting as compared with medical treatment in patients with alcoholic cirrhosis and massive ascites. Veterans Administration Cooperative Study on Treatment of Alcoholic Cirrhosis with Ascites. *N Engl J Med* 1989; **321**: 1632-1638
11 **Guevara M**, Cardenas A, Uriz J, Gines P. Prognosis of Patients with Cirrhosis and Ascites. In: Gines P, Arroyo V, Rodes J, Schrier RW, eds. Ascites and renal dysfunction in liver disease: pathogenesis, diagnosis, and treatment. Malden, Mass: Blackwell Science, 2005: 260-271
12 **Planas R**, Montoliu S, Balleste B, Rivera M, Miquel M, Masnou H, Galeras JA, Gimenez MD, Santos J, Cirera I, Morillas RM, Coll S, Sola R. Natural history of patients hospitalized for management of cirrhotic ascites. *Clin Gastroenterol Hepatol* 2006; **4**: 1385-1394

13 **Wong F**, Blendis L. The pathophysiologic basis for the treatment of cirrhotic ascites. *Clin Liver Dis* 2001; **5**: 819-832
14 **Arroyo V**, Bosch J, Gaya-Beltran J, Kravetz D, Estrada L, Rivera F, Rodes J. Plasma renin activity and urinary sodium excretion as prognostic indicators in nonazotemic cirrhosis with ascites. *Ann Intern Med* 1981; **94**: 198-201
15 **Gines P**, Cardenas A. The management of ascites and hyponatremia in cirrhosis. *Semin Liver Dis* 2008; **28**: 43-58
16 **Arroyo V**, Gines P, Gerbes AL, Dudley FJ, Gentilini P, Laffi G, Reynolds TB, Ring-Larsen H, Scholmerich J. Definition and diagnostic criteria of refractory ascites and hepatorenal syndrome in cirrhosis. International Ascites Club. *Hepatology* 1996; **23**: 164-176
17 **Runyon BA**. Refractory ascites. *Semin Liver Dis* 1993; **13**: 343-351
18 **Wong F**, Massie D, Hsu P, Dudley F. Indomethacin-induced renal dysfunction in patients with well-compensated cirrhosis. *Gastroenterology* 1993; **104**: 869-876
19 **Pockros PJ**, Esrason KT, Nguyen C, Duque J, Woods S. Mobilization of malignant ascites with diuretics is dependent on ascitic fluid characteristics. *Gastroenterology* 1992; **103**: 1302-1306
20 **Han SH**, Reynolds TB, Fong TL. Nephrogenic ascites. Analysis of 16 cases and review of the literature. *Medicine* (Baltimore) 1998; **77**: 233-245
21 **Runyon BA**, Montano AA, Akriviadis EA, Antillon MR, Irving MA, McHutchison JG. The serum-ascites albumin gradient is superior to the exudate-transudate concept in the differential diagnosis of ascites. *Ann Intern Med* 1992; **117**: 215-220
22 **Runyon BA**. Management of adult patients with ascites due to cirrhosis. *Hepatology* 2004; **39**: 841-856
23 **Pirlich M**, Selberg O, Boker K, Schwarze M, Muller MJ. The creatinine approach to estimate skeletal muscle mass in patients with cirrhosis. *Hepatology* 1996; **24**: 1422-1427
24 **Caregaro L**, Menon F, Angeli P, Amodio P, Merkel C, Bortoluzzi A, Alberino F, Gatta A. Limitations of serum creatinine level and creatinine clearance as filtration markers in cirrhosis. *Arch Intern Med* 1994; **154**: 201-205
25 **Papadakis MA**, Arieff AI. Unpredictability of clinical evaluation of renal function in cirrhosis. Prospective study. *Am J Med* 1987; **82**: 945-952
26 **Spahr L**, Villeneuve JP, Tran HK, Pomier-Layrargues G. Furosemide-induced natriuresis as a test to identify cirrhotic patients with refractory ascites. *Hepatology* 2001; **33**: 28-31
27 **Cho HS**, Park GT, Kim YH, Shim SG, Kim JB, Lee OY, Choi HS, Hahm JS, Lee MH. [The significance of urine sodium measurement after furosemide administration in diuretics-unresponsive patients with liver cirrhosis] *Taehan Kan Hakhoe Chi* 2003; **9**: 324-331
28 **Cardenas A**, Bataller R, Arroyo V. Mechanisms of ascites formation. *Clin Liver Dis* 2000; **4**: 447-465
29 **Gines P**, Fernandez-Esparrach G, Arroyo V, Rodes J. Pathogenesis of ascites in cirrhosis. *Semin Liver Dis* 1997; **17**: 175-189
30 **Morali GA**, Sniderman KW, Deitel KM, Tobe S, Witt-Sullivan H, Simon M, Heathcote J, Blendis LM. Is sinusoidal portal hypertension a necessary factor for the development of hepatic ascites? *J Hepatol* 1992; **16**: 249-250
31 **Fernandez-Seara J**, Prieto J, Quiroga J, Zozaya JM, Cobos MA, Rodriguez-Eire JL, Garcia-Plaza A, Leal J. Systemic and regional hemodynamics in patients with liver cirrhosis and ascites with and without functional renal failure. *Gastroenterology* 1989; **97**: 1304-1312
32 **Schrier RW**, Arroyo V, Bernardi M, Epstein M, Henriksen JH, Rodes J. Peripheral arterial vasodilation hypothesis: a proposal for the initiation of renal sodium and water retention in cirrhosis. *Hepatology* 1988; **8**: 1151-1157
33 **Vallance P**, Moncada S. Hyperdynamic circulation in cirrhosis: a role for nitric oxide? *Lancet* 1991; **337**: 776-778
34 **Kravetz D**, Arderiu M, Bosch J, Fuster J, Visa J, Casamitjana R, Rodes J. Hyperglucagonemia and hyperkinetic circulation after portocaval shunt in the rat. *Am J Physiol* 1987; **252**: G257-G261
35 **Guarner C**, Soriano G, Such J, Teixido M, Ramis I, Bulbena O, Rosello J, Guarner F, Gelpi E, Balanzo J. Systemic prostacyclin in cirrhotic patients. Relationship with portal hypertension and changes after intestinal decontamination. *Gastroenterology* 1992; **102**: 303-309
36 **Korthuis RJ**, Kinden DA, Brimer GE, Slattery KA, Stogsdill P, Granger DN. Intestinal capillary filtration in acute and chronic portal hypertension. *Am J Physiol* 1988; **254**: G339-G345
37 **Dudley FJ**. Pathophysiology of ascites formation. *Gastroenterol Clin North Am* 1992; **21**: 215-235
38 **Arroyo V**, Navasa M. Ascites and Spontaneous Bacterial Peritonitis. In: Schiff ER, Sorrel MF, Maddrey WS. Schiff's diseases of the liver. 10th ed. Philadelphia: Lippincott Williams & Wilkins, 2007
39 **Rafael Valdivia L**, Ferrandiz Quiroz J. [Hyponatremia as a possible mortality factor in cirrhotic patients hospitalised in the Guillermo Almenara Irigoyen State Hospital, 2003-2005] *Rev Gastroenterol Peru* 2007; **27**: 37-46
40 **Biggins SW**, Rodriguez HJ, Bacchetti P, Bass NM, Roberts JP, Terrault NA. Serum sodium predicts mortality in patients listed for liver transplantation. *Hepatology* 2005; **41**: 32-39
41 **Bataller R**, Gines P, Guevara M, Arroyo V. Hepatorenal syndrome. *Semin Liver Dis* 1997; **17**: 233-247
42 **Garcia-Tsao G**. Transjugular Intrahepatic Portosystemic Shunt (TIPS) for the Management of Refractory Ascites in Cirrhosis. In: Gines P, Arroyo V, Rodes J, Schrier RW, eds. Ascites and renal dysfunction in liver disease: pathogenesis, diagnosis, and treatment. Malden, Mass: Blackwell Science, 2005: 251-260
43 **Cardenas A**, Gines P. A Practical Approach to Treatment of Patients with Cirrhosis and Ascites. In: Gines P, Arroyo V, Rodes J, Schrier RW, eds. Ascites and renal dysfunction in liver disease: pathogenesis, diagnosis, and treatment. Malden, Mass: Blackwell Science, 2005: 286-293
44 **Gines P**, Arroyo V, Quintero E, Planas R, Bory F, Cabrera J, Rimola A, Viver J, Camps J, Jimenez W. Comparison of paracentesis and diuretics in the treatment of cirrhotics with tense ascites. Results of a randomized study. *Gastroenterology* 1987; **93**: 234-241
45 **Gines P**, Tito L, Arroyo V, Planas R, Panes J, Viver J, Torres M, Humbert P, Rimola A, Llach J. Randomized comparative study of therapeutic paracentesis with and without intravenous albumin in cirrhosis. *Gastroenterology* 1988; **94**: 1493-502
46 **Salerno F**, Badalamenti S, Incerti P, Tempini S, Restelli B, Bruno S, Bellati G, Roffi L. Repeated paracentesis and i.v. albumin infusion to treat 'tense' ascites in cirrhotic patients. A safe alternative therapy. *J Hepatol* 1987; **5**: 102-108
47 **Kravetz D**, Romero G, Argonz J, Guevara M, Suarez A, Abecasis R, Bildozola M, Valero J, Terg R. Total volume paracentesis decreases variceal pressure, size, and variceal wall tension in cirrhotic patients. *Hepatology* 1997; **25**: 59-62
48 **Nevens F**, Bustami R, Scheys I, Lesaffre E, Fevery J. Variceal pressure is a factor predicting the risk of a first variceal bleeding: a prospective cohort study in cirrhotic patients. *Hepatology* 1998; **27**: 15-19
49 **Fernandez-Esparrach G**, Guevara M, Sort P, Pardo A, Jimenez W, Gines P, Planas R, Lebrec D, Geuvel A, Elewaut A, Adler M, Arroyo V. Diuretic requirements after therapeutic paracentesis in non-azotemic patients with cirrhosis. A randomized double-blind trial of spironolactone versus placebo. *J Hepatol* 1997; **26**: 614-620
50 **Morillas RM**, Santos J, Montoliu S, Planas R. Paracentesis for Cirrhotic Ascites. In: Gines P, Arroyo V, Rodes J, Schrier RW, eds. Ascites and renal dysfunction in liver disease: pathogenesis, diagnosis, and treatment. Malden, Mass: Blackwell Science, 2005: 241-250
51 **Runyon BA**. Care of patients with ascites. *N Engl J Med*

1994; **330**: 337-342
52 **Soulsby CT**, Morgan MY. Dietary management of hepatic encephalopathy in cirrhotic patients: survey of current practice in United Kingdom. *BMJ* 1999; **318**: 1391
53 **Gines P**, Berl T, Bernardi M, Bichet DG, Hamon G, Jimenez W, Liard JF, Martin PY, Schrier RW. Hyponatremia in cirrhosis: from pathogenesis to treatment. *Hepatology* 1998; **28**: 851-864
54 **Moore KP**, Aithal GP. Guidelines on the management of ascites in cirrhosis. *Gut* 2006; **55** Suppl 6: vi1-vi12
55 **Grabau CM**, Crago SF, Hoff LK, Simon JA, Melton CA, Ott BJ, Kamath PS. Performance standards for therapeutic abdominal paracentesis. *Hepatology* 2004; **40**: 484-488
56 **McVay PA**, Toy PT. Lack of increased bleeding after paracentesis and thoracentesis in patients with mild coagulation abnormalities. *Transfusion* 1991; **31**: 164-171
57 **Runyon BA**. Paracentesis of ascitic fluid. A safe procedure. *Arch Intern Med* 1986; **146**: 2259-2261
58 **Runyon BA**. Management of adult patients with ascites caused by cirrhosis. *Hepatology* 1998; **27**: 264-272
59 **Pache I**, Bilodeau M. Severe haemorrhage following abdominal paracentesis for ascites in patients with liver disease. *Aliment Pharmacol Ther* 2005; **21**: 525-529
60 **Hu KQ**, Yu AS, Tiyyagura L, Redeker AG, Reynolds TB. Hyperfibrinolytic activity in hospitalized cirrhotic patients in a referral liver unit. *Am J Gastroenterol* 2001; **96**: 1581-1586
61 **Runyon BA**, Antillon MR, Montano AA. Effect of diuresis versus therapeutic paracentesis on ascitic fluid opsonic activity and serum complement. *Gastroenterology* 1989; **97**: 158-162
62 **Pozzi M**, Redaelli E, Ratti L, Poli G, Guidi C, Milanese M, Calchera I, Mancia G. Time-course of diastolic dysfunction in different stages of chronic HCV related liver diseases. *Minerva Gastroenterol Dietol* 2005; **51**: 179-186
63 **Ruiz-del-Arbol L**, Monescillo A, Jimenez W, Garcia-Plaza A, Arroyo V, Rodes J. Paracentesis-induced circulatory dysfunction: mechanism and effect on hepatic hemodynamics in cirrhosis. *Gastroenterology* 1997; **113**: 579-586
64 **Appenrodt B**, Wolf A, Grunhage F, Trebicka J, Schepke M, Rabe C, Lammert F, Sauerbruch T, Heller J. Prevention of paracentesis-induced circulatory dysfunction: midodrine vs albumin. A randomized pilot study. *Liver Int* 2008; **28**: 1019-1025
65 **Gines A**, Fernandez-Esparrach G, Monescillo A, Vila C, Domenech E, Abecasis R, Angeli P, Ruiz-Del-Arbol L, Planas R, Sola R, Gines P, Terg R, Inglada L, Vaque P, Salerno F, Vargas V, Clemente G, Quer JC, Jimenez W, Arroyo V, Rodes J. Randomized trial comparing albumin, dextran 70, and polygeline in cirrhotic patients with ascites treated by paracentesis. *Gastroenterology* 1996; **111**: 1002-1010
66 **Moreau R**, Valla DC, Durand-Zaleski I, Bronowicki JP, Durand F, Chaput JC, Dadamessi I, Silvain C, Bonny C, Oberti F, Gournay J, Lebrec D, Grouin JM, Guemas E, Golly D, Padrazzi B, Tellier Z. Comparison of outcome in patients with cirrhosis and ascites following treatment with albumin or a synthetic colloid: a randomised controlled pilot trail. *Liver Int* 2006; **26**: 46-54
67 **Runyon BA**. Ascites and spontaneous bacterial peritonitis. In: Feldman M, Friedman L, Brandt LJ, eds. Sleisenger and Fordtran's Gastrointestinal & Liver Disease: pathophysiology, diagnosis, management. 8th ed. Philadephia: Saunders, 2006: 1935-1964
68 **Wilkinson P**, Sherlock S. The effect of repeated albumin infusions in patients with cirrhosis. *Lancet* 1962; **2**: 1125-1129
69 **Rothschild MA**, Oratz M, Evans C, Schreiber SS. Alterations in Albumin Metabolism after Serum and Albumin Infusions. *J Clin Invest* 1964; **43**: 1874-1880
70 **Salo J**, Gines A, Gines P, Piera C, Jimenez W, Guevara M, Fernandez-Esparrach G, Sort P, Bataller R, Arroyo V, Rodes J. Effect of therapeutic paracentesis on plasma volume and transvascular escape rate of albumin in patients with cirrhosis. *J Hepatol* 1997; **27**: 645-653
71 **Planas R**, Gines P, Arroyo V, Llach J, Panes J, Vargas V, Salmeron JM, Gines A, Toledo C, Rimola A. Dextran-70 versus albumin as plasma expanders in cirrhotic patients with tense ascites treated with total paracentesis. Results of a randomized study. *Gastroenterology* 1990; **99**: 1736-1744
72 **Coll S**, Vila MC, Molina L, Gimenez MD, Guarner C, Sola R. Mechanisms of early decrease in systemic vascular resistance after total paracentesis: influence of flow rate of ascites extraction. *Eur J Gastroenterol Hepatol* 2004; **16**: 347-353
73 **Cabrera J**, Inglada L, Quintero E, Jimenez W, Losada A, Mayor J, Guerra C. Large-volume paracentesis and intravenous saline: effects on the renin-angiotensin system. *Hepatology* 1991; **14**: 1025-1028
74 **Sola-Vera J**, Such J. Understanding the mechanisms of paracentesis-induced crculatory dysfunction. *Eur J Gastroenterol Hepatol* 2004; **16**: 295-298
75 **Christidis C**, Mal F, Ramos J, Senejoux A, Callard P, Navarro R, Trinchet JC, Larrey D, Beaugrand M, Guettier C. Worsening of hepatic dysfunction as a consequence of repeated hydroxyethylstarch infusions. *J Hepatol* 2001; **35**: 726-732
76 **Cadranel JF**, Gargot D, Grippon P, Lunel F, Bernard B, Valla D, Opolon P. Spontaneous dialytic ultrafiltration with intraperitoneal reinfusion of the concentrate versus large paracentesis in cirrhotic patients with intractable ascites: a randomized study. *Int J Artif Organs* 1992; **15**: 432-435
77 **Bernardi M**, Rimondi A, Gasbarrini A, Trevisani F, Caraceni P, Legnani C, Palareti G, Gasbarrini G. Ascites apheresis, concentration and reinfusion for the treatment of massive or refractory ascites in cirrhosis. *J Hepatol* 1994; **20**: 289-295
78 **Albalate M**, Lopez Garcia MD, Vazquez A, De Sequera P, Marriott E, Tan D, Ortiz A, Casado S, Carreno V, Caramelo C. Concentrated ascitic fluid reinfusion in cirrhotic patients: a simplified method. *Am J Kidney Dis* 1997; **29**: 392-398
79 **Lata J**, Marecek Z, Fejfar T, Zdenek P, Bruha R, Safka V, Hulek P, Hejda V, Dolina J, Stehlik J, Tozzi I. The efficacy of terlipressin in comparison with albumin in the prevention of circulatory changes after the paracentesis of tense ascites--a randomized multicentric study. *Hepatogastroenterology* 2007; **54**: 1930-1933
80 **Moreau R**, Asselah T, Condat B, de Kerguenec C, Pessione F, Bernard B, Poynard T, Binn M, Grange JD, Valla D, Lebrec D. Comparison of the effect of terlipressin and albumin on arterial blood volume in patients with cirrhosis and tense ascites treated by paracentesis: a randomised pilot study. *Gut* 2002; **50**: 90-94
81 **Singh Ranger G**. Terlipressin and arterial blood volume after paracentesis for tense ascites in cirrhosis. *Gut* 2002; **51**: 755
82 **Singh V**, Kumar R, Nain CK, Singh B, Sharma AK. Terlipressin versus albumin in paracentesis-induced circulatory dysfunction in cirrhosis: a randomized study. *J Gastroenterol Hepatol* 2006; **21**: 303-307
83 **Singh V**, Dheerendra PC, Singh B, Nain CK, Chawla D, Sharma N, Bhalla A, Mahi SK. Midodrine versus albumin in the prevention of paracentesis-induced circulatory dysfunction in cirrhotics: a randomized pilot study. *Am J Gastroenterol* 2008; **103**: 1399-1405
84 **Singh V**, Kumar B, Nain CK, Singh B, Sharma N, Bhalla A, Sharma AK. Noradrenaline and albumin in paracentesis-induced circulatory dysfunction in cirrhosis: a randomized pilot study. *J Intern Med* 2006; **260**: 62-68
85 **Rosch J**, Uchida BT, Putnam JS, Buschman RW, Law RD, Hershey AL. Experimental intrahepatic portacaval anastomosis: use of expandable Gianturco stents. *Radiology* 1987; **162**: 481-485
86 **Casado M**, Bosch J, Garcia-Pagan JC, Bru C, Banares R, Bandi JC, Escorsell A, Rodriguez-Laiz JM, Gilabert R, Feu F, Schorlemer C, Echenagusia A, Rodes J. Clinical events after transjugular intrahepatic portosystemic shunt: correlation with hemodynamic findings. *Gastroenterology* 1998; **114**:

1296-1303
87 **Reichle FA**, Owen OE. Hemodynamic patterns in human hepatic cirrhosis: a prospective randomized study of the hemodynamic sequelae of distal splenorenal (Warren) and mesocaval shunts. *Ann Surg* 1979; **190**: 523-534
88 **Rossle M**, Siegerstetter V, Huber M, Ochs A. The first decade of the transjugular intrahepatic portosystemic shunt (TIPS): state of the art. *Liver* 1998; **18**: 73-89
89 **Jalan R**, Lui HF, Redhead DN, Hayes PC. TIPSS 10 years on. *Gut* 2000; **46**: 578-581
90 **Forrest EH**, Stanley AJ, Redhead DN, McGilchrist AJ, Hayes PC. Clinical response after transjugular intrahepatic portosystemic stent shunt insertion for refractory ascites in cirrhosis. *Aliment Pharmacol Ther* 1996; **10**: 801-806
91 **Ochs A**, Rossle M, Haag K, Hauenstein KH, Deibert P, Siegerstetter V, Huonker M, Langer M, Blum HE. The transjugular intrahepatic portosystemic stent-shunt procedure for refractory ascites. *N Engl J Med* 1995; **332**: 1192-1197
92 **Wong W**, Liu P, Blendis L, Wong F. Long-term renal sodium handling in patients with cirrhosis treated with transjugular intrahepatic portosystemic shunts for refractory ascites. *Am J Med* 1999; **106**: 315-322
93 **Nazarian GK**, Ferral H, Bjarnason H, Castaneda-Zuniga WR, Rank JM, Bernadas CA, Hunter DW. Effect of transjugular intrahepatic portosystemic shunt on quality of life. *AJR Am J Roentgenol* 1996; **167**: 963-969
94 **Allard JP**, Chau J, Sandokji K, Blendis LM, Wong F. Effects of ascites resolution after successful TIPS on nutrition in cirrhotic patients with refractory ascites. *Am J Gastroenterol* 2001; **96**: 2442-2447
95 **Boyer TD**. Transjugular intrahepatic portosystemic shunt: current status. *Gastroenterology* 2003; **124**: 1700-1710
96 **Pidlich J**, Peck-Radosavljevic M, Kranz A, Wildling R, Winkelbauer FW, Lammer J, Mayer C, Muller C, Stix G, Gangl A, Schmidinger H. Transjugular intrahepatic portosystemic shunt and cardiac arrhythmias. *J Clin Gastroenterol* 1998; **26**: 39-43
97 **Freedman AM**, Sanyal AJ, Tisnado J, Cole PE, Shiffman ML, Luketic VA, Purdum PP, Darcy MD, Posner MP. Complications of transjugular intrahepatic portosystemic shunt: a comprehensive review. *Radiographics* 1993; **13**: 1185-1210
98 **LaBerge JM**, Ring EJ, Gordon RL, Lake JR, Doherty MM, Somberg KA, Roberts JP, Ascher NL. Creation of transjugular intrahepatic portosystemic shunts with the wallstent endoprosthesis: results in 100 patients. *Radiology* 1993; **187**: 413-420
99 **Riggio O**, Merlli M, Pedretti G, Servi R, Meddi P, Lionetti R, Rossi P, Bezzi M, Salvatori F, Ugolotti U, Fiaccadori F, Capocaccia L. Hepatic encephalopathy after transjugular intrahepatic portosystemic shunt. Incidence and risk factors. *Dig Dis Sci* 1996; **41**: 578-584
100 **Sanyal AJ**, Freedman AM, Shiffman ML, Purdum PP 3rd, Luketic VA, Cheatham AK. Portosystemic encephalopathy after transjugular intrahepatic portosystemic shunt: results of a prospective controlled study. *Hepatology* 1994; **20**: 46-55
101 **Rossle M**, Piotraschke J. Transjugular intrahepatic portosystemic shunt and hepatic encephalopathy. *Dig Dis* 1996; **14** Suppl 1: 12-19
102 **Somberg KA**, Riegler JL, LaBerge JM, Doherty-Simor MM, Bachetti P, Roberts JP, Lake JR. Hepatic encephalopathy after transjugular intrahepatic portosystemic shunts: incidence and risk factors. *Am J Gastroenterol* 1995; **90**: 549-555
103 **Kerlan RK Jr**, LaBerge JM, Baker EL, Wack JP, Marx M, Somberg KA, Gordon RL, Ring EJ. Successful reversal of hepatic encephalopathy with intentional occlusion of transjugular intrahepatic portosystemic shunts. *J Vasc Interv Radiol* 1995; **6**: 917-921
104 **Hauenstein KH**, Haag K, Ochs A, Langer M, Rossle M. The reducing stent: treatment for transjugular intrahepatic portosystemic shunt-induced refractory hepatic encephalopathy and liver failure. *Radiology* 1995; **194**: 175-179
105 **Martinet JP**, Fenyves D, Legault L, Roy L, Dufresne MP, Spahr L, Lafortune M, Pomier-Layrargues G. Treatment of refractory ascites using transjugular intrahepatic portosystemic shunt (TIPS): a caution. *Dig Dis Sci* 1997; **42**: 161-166
106 **Lebrec D**, Giuily N, Hadengue A, Vilgrain V, Moreau R, Poynard T, Gadano A, Lassen C, Benhamou JP, Erlinger S. Transjugular intrahepatic portosystemic shunts: comparison with paracentesis in patients with cirrhosis and refractory ascites: a randomized trial. French Group of Clinicians and a Group of Biologists. *J Hepatol* 1996; **25**: 135-144
107 **LaBerge JM**, Ferrell LD, Ring EJ, Gordon RL. Histopathologic study of stenotic and occluded transjugular intrahepatic portosystemic shunts. *J Vasc Interv Radiol* 1993; **4**: 779-786
108 **Sanyal AJ**, Freedman AM, Luketic VA, Purdum PP 3rd, Shiffman ML, DeMeo J, Cole PE, Tisnado J. The natural history of portal hypertension after transjugular intrahepatic portosystemic shunts. *Gastroenterology* 1997; **112**: 889-898
109 **LaBerge JM**, Somberg KA, Lake JR, Gordon RL, Kerlan RK Jr, Ascher NL, Roberts JP, Simor MM, Doherty CA, Hahn J. Two-year outcome following transjugular intrahepatic portosystemic shunt for variceal bleeding: results in 90 patients. *Gastroenterology* 1995; **108**: 1143-1151
110 **Rosado B**, Kamath PS. Transjugular intrahepatic portosystemic shunts: an update. *Liver Transpl* 2003; **9**: 207-217
111 **Chopra S**, Dodd GD 3rd, Chintapalli KN, Rhim H, Encarnacion CE, Palmaz JC, Esola CC, Ghiatas AA. Transjugular intrahepatic portosystemic shunt: accuracy of helical CT angiography in the detection of shunt abnormalities. *Radiology* 2000; **215**: 115-122
112 **Angermayr B**, Cejna M, Koenig F, Karnel F, Hackl F, Gangl A, Peck-Radosavljevic M. Survival in patients undergoing transjugular intrahepatic portosystemic shunt: ePTFE-covered stentgrafts versus bare stents. *Hepatology* 2003; **38**: 1043-1050
113 **Bureau C**, Garcia-Pagan JC, Otal P, Pomier-Layrargues G, Chabbert V, Cortez C, Perreault P, Peron JM, Abraldes JG, Bouchard L, Bilbao JI, Bosch J, Rousseau H, Vinel JP. Improved clinical outcome using polytetrafluoroethylene-coated stents for TIPS: results of a randomized study. *Gastroenterology* 2004; **126**: 469-475
114 **Siegerstetter V**, Huber M, Ochs A, Blum HE, Rossle M. Platelet aggregation and platelet-derived growth factor inhibition for prevention of insufficiency of the transjugular intrahepatic portosystemic shunt: a randomized study comparing trapidil plus ticlopidine with heparin treatment. *Hepatology* 1999; **29**: 33-38
115 **Sanyal AJ**, Freedman AM, Purdum PP, Shiffman ML, Luketic VA. The hematologic consequences of transjugular intrahepatic portosystemic shunts. *Hepatology* 1996; **23**: 32-39
116 **Conn HO**. Hemolysis after transjugular intrahepatic portosystemic shunting: the naked stent syndrome. *Hepatology* 1996; **23**: 177-181
117 **Sanyal AJ**, Reddy KR. Vegetative infection of transjugular intrahepatic portosystemic shunts. *Gastroenterology* 1998; **115**: 110-115
118 **Wang MQ**, Dake MD, Cui ZP, Wang ZQ, Gao YA. Portal-systemic myelopathy after transjugular intrahepatic portosystemic shunt creation: report of four cases. *J Vasc Interv Radiol* 2001; **12**: 879-881
119 **Conn HO**, Rossle M, Levy L, Glocker FX. Portosystemic myelopathy: spastic paraparesis after portosystemic shunting. *Scand J Gastroenterol* 2006; **41**: 619-625
120 **Huonker M**, Schumacher YO, Ochs A, Sorichter S, Keul J, Rossle M. Cardiac function and haemodynamics in alcoholic cirrhosis and effects of the transjugular intrahepatic portosystemic stent shunt. *Gut* 1999; **44**: 743-748

121 **Rossle M**, Ochs A, Gulberg V, Siegerstetter V, Holl J, Deibert P, Olschewski M, Reiser M, Gerbes AL. A comparison of paracentesis and transjugular intrahepatic portosystemic shunting in patients with ascites. *N Engl J Med* 2000; **342**: 1701-1707

122 **Salerno F**, Merli M, Riggio O, Cazzaniga M, Valeriano V, Pozzi M, Nicolini A, Salvatori F. Randomized controlled study of TIPS versus paracentesis plus albumin in cirrhosis with severe ascites. *Hepatology* 2004; **40**: 629-635

123 **Gines P**, Uriz J, Calahorra B, Garcia-Tsao G, Kamath PS, Del Arbol LR, Planas R, Bosch J, Arroyo V, Rodes J. Transjugular intrahepatic portosystemic shunting versus paracentesis plus albumin for refractory ascites in cirrhosis. *Gastroenterology* 2002; **123**: 1839-1847

124 **Sanyal AJ**, Genning C, Reddy KR, Wong F, Kowdley KV, Benner K, McCashland T. The North American Study for the Treatment of Refractory Ascites. *Gastroenterology* 2003; **124**: 634-641

125 **Albillos A**, Banares R, Gonzalez M, Catalina MV, Molinero LM. A meta-analysis of transjugular intrahepatic portosystemic shunt versus paracentesis for refractory ascites. *J Hepatol* 2005; **43**: 990-996

126 **Deltenre P**, Mathurin P, Dharancy S, Moreau R, Bulois P, Henrion J, Pruvot FR, Ernst O, Paris JC, Lebrec D. Transjugular intrahepatic portosystemic shunt in refractory ascites: a meta-analysis. *Liver Int* 2005; **25**: 349-356

127 **Salerno F**, Camma C, Enea M, Rossle M, Wong F. Transjugular intrahepatic portosystemic shunt for refractory ascites: a meta-analysis of individual patient data. *Gastroenterology* 2007; **133**: 825-834

128 **Park JS**, Won JY, Park SI, Park SJ, Lee DY. Percutaneous peritoneovenous shunt creation for the treatment of benign and malignant refractory ascites. *J Vasc Interv Radiol* 2001; **12**: 1445-1448

129 **Lasheen AE**, Elzeftawy A, Ibrahim S, Attia M, Emam M. Implantation of a skin graft tube to create a saphenoperitoneal shunt for refractory ascites. *Surg Today* 2007; **37**: 622-625

130 **Deen KI**, de Silva AP, Jayakody M, de Silva HJ. Saphenoperitoneal anastomosis for resistant ascites in patients with cirrhosis. *Am J Surg* 2001; **181**: 145-148

131 **Vadeyar HJ**, Doran JD, Charnley R, Ryder SD. Saphenoperitoneal shunts for patients with intractable ascites associated with chronic liver disease. *Br J Surg* 1999; **86**: 882-885

132 **Zervos EE**, Rosemurgy AS. Management of medically refractory ascites. *Am J Surg* 2001; **181**: 256-264

133 **Rozenblit GN**, Del Guercio LR, Rundback JH, Poplausky MR, Lebovics E. Peritoneal-urinary drainage for treatment of refractory ascites: a pilot study. *J Vasc Interv Radiol* 1998; **9**: 998-1005

134 **Ali F**, Guglin M, Vaitkevicius P, Ghali JK. Therapeutic potential of vasopressin receptor antagonists. *Drugs* 2007; **67**: 847-858

135 **Pham PC**, Pham PM, Pham PT. Vasopressin excess and hyponatremia. *Am J Kidney Dis* 2006; **47**: 727-737

136 **Greenberg A**, Verbalis JG. Vasopressin receptor antagonists. *Kidney Int* 2006; **69**: 2124-2130

137 **Gines P**, Wong F, Watson H, Milutinovic S, del Arbol LR, Olteanu D. Effects of satavaptan, a selective vasopressin V(2) receptor antagonist, on ascites and serum sodium in cirrhosis with hyponatremia: a randomized trial. *Hepatology* 2008; **48**: 204-213

138 **Angeli P**, Volpin R, Gerunda G, Craighero R, Roner P, Merenda R, Amodio P, Sticca A, Caregaro L, Maffei-Faccioli A, Gatta A. Reversal of type 1 hepatorenal syndrome with the administration of midodrine and octreotide. *Hepatology* 1999; **29**: 1690-1697

139 **Krag A**, Moller S, Henriksen JH, Holstein-Rathlou NH, Larsen FS, Bendtsen F. Terlipressin improves renal function in patients with cirrhosis and ascites without hepatorenal syndrome. *Hepatology* 2007; **46**: 1863-1871

140 **Kalambokis G**, Fotopoulos A, Economou M, Tsianos EV. Octreotide in the treatment of refractory ascites of cirrhosis. *Scand J Gastroenterol* 2006; **41**: 118-121

141 **Kalambokis G**, Fotopoulos A, Economou M, Pappas K, Tsianos EV. Effects of a 7-day treatment with midodrine in non-azotemic cirrhotic patients with and without ascites. *J Hepatol* 2007; **46**: 213-221

142 **Kalambokis G**, Economou M, Fotopoulos A, Al Bokharhii J, Pappas C, Katsaraki A, Tsianos EV. The effects of chronic treatment with octreotide versus octreotide plus midodrine on systemic hemodynamics and renal hemodynamics and function in nonazotemic cirrhotic patients with ascites. *Am J Gastroenterol* 2005; **100**: 879-885

143 **Tandon P**, Tsuyuki RT, Mitchell L, Hoskinson M, Ma MM, Wong WW, Mason AL, Gutfreund K, Bain VG. The effect of 1 month of therapy with midodrine, octreotide-LAR and albumin in refractory ascites: a pilot study. *Liver Int* 2008

144 **Lenaerts A**, Van Cauter J, Moukaiber H, Meunier JC, Ligny G. [Treatment of refractory ascites with clonidine and spironolactone] *Gastroenterol Clin Biol* 1997; **21**: 524-525

145 **Lenaerts A**, Codden T, Meunier JC, Henry JP, Ligny G. Effects of clonidine on diuretic response in ascitic patients with cirrhosis and activation of sympathetic nervous system. *Hepatology* 2006; **44**: 844-849

146 **Rai RR**, Jain P. Combination of clonidine and diuretic in cirrhotic ascites with activated sympathetic nervous system: Would it be a magical bullet? *Hepatology* 2008; **47**: 360

147 **Lenaerts A**, Codden T, Van Cauter J, Meunier JC, Henry JP, Ligny G. Interest of the association clonidine-spironolactone in cirrhotic patients with ascites and activation of sympathetic nervous system. *Acta Gastroenterol Belg* 2002; **65**: 1-5

148 **Henriksen JH**, Ring-Larsen H. Hepatorenal disorders: role of the sympathetic nervous system. *Semin Liver Dis* 1994; **14**: 35-43

149 **Laffi G**, Gentilini P, Romanelli RG, La Villa G. Is the use of albumin of value in the treatment of ascites in cirrhosis? The case in favour. *Dig Liver Dis* 2003; **35**: 660-663

150 **Gentilini P**, Casini-Raggi V, Di Fiore G, Romanelli RG, Buzzelli G, Pinzani M, La Villa G, Laffi G. Albumin improves the response to diuretics in patients with cirrhosis and ascites: results of a randomized, controlled trial. *J Hepatol* 1999; **30**: 639-645

151 **Trotter J**, Pieramici E, Everson GT. Chronic albumin infusions to achieve diuresis in patients with ascites who are not candidates for transjugular intrahepatic portosystemic shunt (TIPS). *Dig Dis Sci* 2005; **50**: 1356-1360

152 **Romanelli RG**, La Villa G, Barletta G, Vizzutti F, Lanini F, Arena U, Boddi V, Tarquini R, Pantaleo P, Gentilini P, Laffi G. Long-term albumin infusion improves survival in patients with cirrhosis and ascites: an unblinded randomized trial. *World J Gastroenterol* 2006; **12**: 1403-1407

153 **Fernandez J**, Monteagudo J, Bargallo X, Jimenez W, Bosch J, Arroyo V, Navasa M. A randomized unblinded pilot study comparing albumin versus hydroxyethyl starch in spontaneous bacterial peritonitis. *Hepatology* 2005; **42**: 627-634

154 **Sort P**, Navasa M, Arroyo V, Aldeguer X, Planas R, Ruiz-del-Arbol L, Castells L, Vargas V, Soriano G, Guevara M, Gines P, Rodes J. Effect of intravenous albumin on renal impairment and mortality in patients with cirrhosis and spontaneous bacterial peritonitis. *N Engl J Med* 1999; **341**: 403-409

155 **Gines P**, Guevara M, De Las Heras D, Arroyo V. Review article: albumin for circulatory support in patients with cirrhosis. *Aliment Pharmacol Ther* 2002; **16** Suppl 5: 24-31

156 **Gines P**, Torre A, Terra C, Guevara M. Review article: pharmacological treatment of hepatorenal syndrome. *Aliment Pharmacol Ther* 2004; **20** Suppl 3: 57-62; discussion 63-64

157 **Chang CY**, Singal AK, Ganeshan SV, Schiano TD, Lookstein R, Emre S. Use of splenic artery embolization to relieve tense ascites following liver transplantation in a patient

with paroxysmal nocturnal hemoglobinuria. *Liver Transpl* 2007; **13**: 1532-1537

158 **Pamuk ON**, Sonsuz A. The effect of mannitol infusion on the response to diuretic therapy in cirrhotic patients with ascites. *J Clin Gastroenterol* 2002; **35**: 403-405

159 **Garcia-Tsao G**, Sanyal AJ, Grace ND, Carey W. Prevention and management of gastroesophageal varices and variceal hemorrhage in cirrhosis. *Hepatology* 2007; **46**: 922-938

160 **Akriviadis E**, Botla R, Briggs W, Han S, Reynolds T, Shakil O. Pentoxifylline improves short-term survival in severe acute alcoholic hepatitis: a double-blind, placebo-controlled trial. *Gastroenterology* 2000; **119**: 1637-1648

161 **Runyon BA**. Historical aspects of treatment of patients with cirrhosis and ascites. *Semin Liver Dis* 1997; **17**: 163-173

162 **Veldt BJ**, Laine F, Guillygomarc'h A, Lauvin L, Boudjema K, Messner M, Brissot P, Deugnier Y, Moirand R. Indication of liver transplantation in severe alcoholic liver cirrhosis: quantitative evaluation and optimal timing. *J Hepatol* 2002; **36**: 93-98

163 **Malekzadeh R**, Mohamadnejad M, Rakhshani N, Nasseri-Moghaddam S, Merat S, Tavangar SM, Sohrabpour AA. Reversibility of cirrhosis in chronic hepatitis B. *Clin Gastroenterol Hepatol* 2004; **2**: 344-347

164 **Malekzadeh R**, Mohamadnejad M, Nasseri-Moghaddam S, Rakhshani N, Tavangar SM, Sohrabpour AA, Tahaghoghi S. Reversibility of cirrhosis in autoimmune hepatitis. *Am J Med* 2004; **117**: 125-129

165 **Dufour JF**, DeLellis R, Kaplan MM. Reversibility of hepatic fibrosis in autoimmune hepatitis. *Ann Intern Med* 1997; **127**: 981-985

166 **Bories P**, Garcia Compean D, Michel H, Bourel M, Capron JP, Gauthier A, Lafon J, Levy VG, Pascal JP, Quinton A. The treatment of refractory ascites by the LeVeen shunt. A multi-centre controlled trial (57 patients). *J Hepatol* 1986; **3**: 212-218

167 **Henderson JM**, Mackay GJ, Hooks M, Chezmar JL, Galloway JR, Dodson TF, Kutner MH. High cardiac output of advanced liver disease persists after orthotopic liver transplantation. *Hepatology* 1992; **15**: 258-262

168 **Hadengue A**, Lebrec D, Moreau R, Sogni P, Durand F, Gaudin C, Bernuau J, Belghiti J, Gayet B, Erlinger S. Persistence of systemic and splanchnic hyperkinetic circulation in liver transplant patients. *Hepatology* 1993; **17**: 175-178

169 **Ruf AE**, Kremers WK, Chavez LL, Descalzi VI, Podesta LG, Villamil FG. Addition of serum sodium into the MELD score predicts waiting list mortality better than MELD alone. *Liver Transpl* 2005; **11**: 336-343

170 **Biggins SW**, Kim WR, Terrault NA, Saab S, Balan V, Schiano T, Benson J, Therneau T, Kremers W, Wiesner R, Kamath P, Klintmalm G. Evidence-based incorporation of serum sodium concentration into MELD. *Gastroenterology* 2006; **130**: 1652-1660

171 **Luca A**, Angermayr B, Bertolini G, Koenig F, Vizzini G, Ploner M, Peck-Radosavljevic M, Gridelli B, Bosch J. An integrated MELD model including serum sodium and age improves the prediction of early mortality in patients with cirrhosis. *Liver Transpl* 2007; **13**: 1174-1180

**S- Editor** Tian L **L- Editor** Alpini GD **E- Editor** Lin YP

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.81

*World J Gastroenterol* 2009 January 7; 15(1): 81-85
***World Journal of Gastroenterology*** ISSN 1007-9327




**Dr. Peter V Draganov,** *Series Editor*

# Recent advances and remaining gaps in our knowledge of associations between gut microbiota and human health

Volker Mai, Peter V Draganov

Volker Mai, Peter V Draganov, Department of Gastroenterology, Hepatology and Nutrition, University of Florida, 1600 SW Archer Rd, Room HD 602, PO Box 100214 Gainesville, Florida 32610, United States
Author contributions: Mai V and Draganov PV have contributed equally.
Correspondence to: Peter V Draganov, MD, Department of Gastroenterology, Hepatology and Nutrition, University of Florida, 1600 SW Archer Rd, Room HD 602, PO Box 100214 Gainesville, Florida 32610,
United States. dragapv@medicine.ufl.edu
Telephone: +1-352-392-2878 Fax: +1-352-392-3618
Received: October 11, 2008 Revised: December 3, 2008
Accepted: December 10, 2008
Published online: January 7, 2009

## Abstract

The complex gut microbial flora harbored by individuals (microbiota) has long been proposed to contribute to intestinal health as well as disease. Pre- and probiotic products aimed at improving health by modifying microbiota composition have already become widely available and acceptance of these products appears to be on the rise. However, although required for the development of effective microbiota based interventions, our basic understanding of microbiota variation on a population level and its dynamics within individuals is still rudimentary. Powerful new parallel sequence technologies combined with other efficient molecular microbiota analysis methods now allow for comprehensive analysis of microbiota composition in large human populations. Recent findings in the field strongly suggest that microbiota contributes to the development of obesity, atopic diseases, inflammatory bowel diseases and intestinal cancers. Through the ongoing National Institutes of Health Roadmap 'Human Microbiome Project' and similar projects in other parts of the world, a large coordinated effort is currently underway to study how microbiota can impact human health. Translating findings from these studies into effective interventions that can improve health, possibly personalized based on an individuals existing microbiota, will be the task for the next decade(s).



**Key words:** Microbiota; Intestinal microbial flora; Probiotic; Gut; Intestine

**Peer reviewers:** Lynne V McFarland, Puget Sound VA, HSR&D, 1100 Olive Street, Suite #1400, Seattle, Washington, WA 98101, United States; Yi Liu, MD, PhD, Department of Pathology, Academic Medical Center, University of Amsterdam, Meiberdreef 9, Postbus 22660, Amsterdam 1100 DD, The Netherlands

Mai V, Draganov PV. Recent advances and remaining gaps in our knowledge of associations between gut microbiota and human health. *World J Gastroenterol* 2009; 15(1): 81-85 Available from: URL: http://www.wjgnet.com/1007-9327/15/81.asp DOI: http://dx.doi.org/10.3748/wjg.15.81

## INTRODUCTION

In recent years, the commensal microbiota, including that of the small and large intestines, has received renewed research interest for potential associations with human health. The commensal microbiota consists of a diverse population of prokaryotic (eubacteria and archaea) as well as eukaryotic microbes that live synergistically within their human host. As early as the beginning of the 20th century, Metchnikoff proposed that putrefactive bacteria contribute to various disease processes and that modification of microbiota composition through consumption of viable microbes might help to improve health and longevity[1]. A variety of potential associations between gut microbiota composition/activities and health or disease have undergone scientific scrutiny. Evidence is mounting in support of an association between microbiota and diseases associated with failures in appropriate immune responses leading to excessive inflammation, such as atopic disease, inflammatory bowel disease and intestinal cancers[2,3,4]. During the next decade, findings from comprehensive microbiota studies currently underway can be expected to revolutionize the way we think about our microbial "friends and foes".

## CONSUMPTION OF LIVE BACTERIA TO PROMOTE HEALTH

A wide variety of often milk-based fermented foods containing viable beneficial microbes, mostly lactic-acid bacteria and bifidobacteria, but also other bacteria

and fungi, have traditionally been part of the diet in many cultures. Various cultures share the belief that home-made products, such as yoghurt, curd, kefir, chal, kombucha *etc*, can help to maintain good health. Microbial cultures used for preparing these fermented products have sometimes been propagated for generations. They range from simple cultures that contain a few lactic-acid bacterial strains to complex consortia containing various bacteria and yeasts (kefir grains). More recently, commercial products claiming to contain beneficial bacteria that can establish residency in the gut (probiotics), fermentable substrates that enrich for beneficial bacteria (prebiotics), or mixtures of both (synbiotics), continue to expand their market share. Such products have been quite popular in Europe and Asia for a while but they are now also becoming more common in other parts of the world including the US. Although it is clear that there is significant potential for such products to help improve or maintain health, the research validating many of the current health claims is sparse. We discuss below some of the recent findings and future opportunities to advance this promising approach.

## THE HUMAN/MICROBIOTA 'SUPERORGANISM'

It is well established that commensal microbial cells living in intimate contact with their human host far exceed the number of human cells. Bacteria belonging to a few phyla, particularly Firmicutes and Bacteroidetes, appear to dominate in most healthy individuals[5-7]. Estimates for the total number of bacterial species compromising the collective gut microbiome have recently been extended up to 40 000[8], but, due to the large amount of emerging sequence data, the bacterial species concept likely will soon undergo revision. Most gut microbiota research to date has focused on exploring the eubacterial community, but archaea (prokaryotes resembling bacteria but different in certain aspects of their chemical structure, such as the composition of their cell walls), viruses, fungi and other microbes can frequently be detected in intestinal contents[9-12].

The combined microbial gene pool, studied by metagenomic approaches, exceeds the complexity of the human genome, extending the metabolic abilities of the human/microbiota "supra- or superorganism"[3,13,14], which is the combined host/microbe consortium. Through its immense metabolic capabilities, the gut microbiota contributes to human physiology by transforming complex nutrients, such as dietary fiber or intestinal mucins that otherwise would be lost to the human host, into simple sugars, short-chain fatty acids and other nutrients that can be absorbed[5,15]. Furthermore, the microbiota produces some essential vitamins including vitamin K, vitamin B12 and folic acid, contributes to intestinal bile acid metabolism and recirculation, transforms potential carcinogens such as N-nitroso compounds (NOCs) and heterocyclic amines (HCAs) and activates bioactive compounds including phytoestrogens[16-18]. Differences in environmental factors, including diet, as well as hosts genetics are thought to contribute to microbiota diversity[18,19]. However, as genetically similar mice obtained from a dedicated breeding colony and fed the same amounts of the same defined diet develop striking differences in microbiota profiles[20], factors beyond our current comprehension or even random chance might contribute.

## DISEASES ASSOCIATED WITH GUT MICROBIOTA DISTORTIONS

Distortions in any one of the microbiota functions or signaling pathways could potentially contribute to a wide range of diseases, including cardiovascular diseases (IBD) (bile-acid-associated regulation of serum cholesterol levels, chronic inflammation), diabetes (carbohydrate uptake and glycemic control), inflammatory diseases including atopic diseases, inflammatory bowel disease (inappropriate immune stimulation) and neoplastic diseases (carcinogen activation, chronic inflammation related hyperproliferation). Eloquent studies suggesting microbiota associations with obesity have recently received significant publicity[21-25] but other studies have refuted the existence of such an association[26]. Undoubtedly, the gut microbiota can contribute to differences in energy gain from fiber fermentation. The resulting small amounts of additional energy, if absorbed by the host, can over time, contribute to weight gain, and signaling from gut bacteria might contribute to fat storage. However, from a public health perspective, we might want to avoid shifting the focus away from a more direct path to avoid obesity: balance energy intake and output.

Changes in gut microbiota composition by probiotic supplementation of infant diets have been shown to reduce atopic disease[2,27]. Associations between the microbiota development in infants and health later in life have long been proposed[28,29]. Utilizing microarray technology to monitor microbiota, Palmer *et al*[30] recently reported changes in the microbiota composition in 14 infants during the first year of life, pointing to considerable temporal variation and distinct features in each infant.

IBD has been linked to microbiota composition in a variety of studies[3,7,31-36], and successful interventions using a pre- and/or probiotic approach have been reported. In addition to reports of differences in microbiota composition analyzed in fecal samples, the kinds and amounts of mucosa-adherent bacteria also seem to differ between cases with IBD and healthy controls[7,37-39].

Colorectal cancer (CRC) risk also has been proposed to be associated with microbiota composition through various mechanisms[4,40]. Pre- and or probiotics have reduced carcinogenesis in some but not all animal studies[41,42]. Dietary prevention of intestinal carcino-

genesis in $APC^{Min}$ mice (mice that develop large numbers of intestinal tumors due to mutation in the adenomatous polyposis coli gene) was associated with correlated differences in overall microbiota profiles as well as with the presence of specific bacterial signatures[20]. Increases in the amounts of intraepithelial *Escherichia coli* (*E. coli*) in CRC patients have been suggested[43].

Interest has recently also been directed towards establishing a potential association between microbiota composition and both type 1 as well as type 2 diabetes mellitus. Brugman *et al*[44] showed that antibiotics affected type 1 diabetes mellitus incidence but, more importantly, that microbiota differed before the onset of disease in diabetes-prone rats that developed type 2 diabetes. Similar data have recently been reported in immune system-associated studies in non-obese diabetic (NOD)-mice[45]. Antibiotic-induced microbiota changes have also been shown to affect type 2 diabetes, but systemic effects likely contributed to this observation[46].

Current studies of associations between microbiota composition and disease suffer from a lack of understanding regarding the normal range of microbiota diversity on a population level. Furthermore, the presence of particular microbes or microbiota pattern has been studied almost exclusively in observational studies, in which differences in microbiota were evaluated between subjects suffering from the respective disease and normal controls. This study design does not allow us to distinguish if differences in microbiota composition are causing the disease or if they are simply a result of the changed gut environment in diseased subjects. Prospective studies evaluating microbiota composition in individuals before they develop disease will be required to attribute causality to potential associations between microbiota and disease. Because such microbiota studies would be expensive and time consuming, they should be designed as ancillary projects as part of larger cohort studies.

## NEW OPPORTUNITIES TO STUDY GUT MICROBIOTA AND HEALTH

Powerful molecular microbiota analyses methods, including 16S rRNA sequencing through a massively parallel barcoded pyrosequencing approach, facilitate for the first time our ability to analyze microbiota in depth and in an efficient manner. Studies of gut microbiota interactions with metabolic phenotypes (so-called functional metagenomics) are now possible through the use of proton nuclear magnetic resonance ($^1$H NMR)-based profiling of fecal, urine or other extracts. Early results in this area that tried to correlated microbiota and probiotic supplementation-induced changes in its composition are promising[47,48].

Last year, the National Institutes of Health announced its roadmap Human Microbiome Project (HMP) with funding in excess of one hundred million US dollars, allocated to improve our understanding of associations between human health and microbiota at five major sites: nasal and oral cavities, gastrointestinal and urogenital tracts and skin[49] (http://nihroadmap.nih.gov/hmp/). Efforts to sequence the genomes of hundreds of human-associated microbes are currently underway and multiple projects that will explore potential associations with human health are currently being funded. European and Asian countries are undertaking similar endeavors and international efforts have been made to coordinate projects. It can be expected that the studies to determine the composition, activities and dynamics of the human microbiota and its overall genomic content, the human microbiome, will expand our ability to utilize microbiota for maintaining/improving health.

## REMAINING GAPS AND CONCLUSIONS

Studies of microbiota composition have so far been limited to fairly small populations. We are clearly lacking an understanding of microbiota diversity on a population level and across various cultural and ethnic groups. Few studies have extensively investigated microbiota dynamics in adults; the causes for variations over time have not been well explored. The many interventions aimed at improving health parameters through microbiota modifications with pre-and probiotic supplements have often been short-term. Thus, effects of microbiota changes on long-term health are unknown. Furthermore, the types and concentration of pre- and probiotic supplements significantly vary from study to study, making firm conclusions difficult to draw.

To improve statistical power for defining disease-specific microbiota pattern, it is frequently necessary to combine results from various individuals into disease and control groups. However, it is crucial to recognize that inter-individual variations in microbiota composition may be so large and its statistical distribution so far from normal that combining individuals might not be appropriate. The true extent of microbiota variation will only be known after we have studied a sufficient number of individuals. Massive parallel sequencing technologies and the necessary bioinformatics tools to handle the resulting large datasets have been and continue to be adapted for human microbiota analysis[50,51].

To date, little effort has been made to standardize the microbiota analysis methodology used in human studies. Furthermore, the extent of the bias introduced by different sample collection, storage and analysis methods has only been superficially investigated. This makes it almost impossible to directly compare findings from different groups, limiting our ability to generalize findings.

Successfully correlating microbiota composition with disease risk, rather than correlating it with disease status only, will likely require large prospective epidemiological studies sufficiently powered to detect disease predicting microbiota differences, even with the predicted large inter- and intra-individual variation. Such findings could lead to future microbiota based preventions, which may have to be individualized based on the subjects' existing microbiota. It is also important to establish microbiota

changes that are caused by, but are not causally associated with, disease progression. Such knowledge might facilitate the development of efficient microbiota-based screening tests (IBD, CHC *etc*). We have all the reasons to be optimistic that, based on new findings, expected through the current large multi-national efforts to better understand microbiota, we will finally be able to 'domesticate' our own complex gut microbiota as a means for improving health.

## REFERENCES


1 **Metchnikoff E**. In The prolongation of life. London: William Heinemann, 1907
2 **Isolauri E**, Kalliomäki M, Laitinen K, Salminen S. Modulation of the maturing gut barrier and microbiota: a novel target in allergic disease. *Curr Pharm Des* 2008; **14**: 1368-1375
3 **Peterson DA**, Frank DN, Pace NR, Gordon JI. Metagenomic approaches for defining the pathogenesis of inflammatory bowel diseases. *Cell Host Microbe* 2008; **3**: 417-427
4 **Huycke MM**, Gaskins HR. Commensal bacteria, redox stress, and colorectal cancer: mechanisms and models. *Exp Biol Med* (Maywood) 2004; **229**: 586-597
5 **Zoetendal EG**, Vaughan EE, de Vos WM. A microbial world within us. *Mol Microbiol* 2006; **59**: 1639-1650
6 **Eckburg PB**, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA. Diversity of the human intestinal microbial flora. *Science* 2005; **308**: 1635-1638
7 **Frank DN**, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci USA* 2007; **104**: 13780-13785
8 **Frank DN**, Pace NR. Gastrointestinal microbiology enters the metagenomics era. *Curr Opin Gastroenterol* 2008; **24**: 4-10
9 **Scanlan PD**, Shanahan F, Marchesi JR. Human methanogen diversity and incidence in healthy and diseased colonic groups using mcrA gene analysis. *BMC Microbiol* 2008; **8**: 79
10 **Zhang T**, Breitbart M, Lee WH, Run JQ, Wei CL, Soh SW, Hibberd ML, Liu ET, Rohwer F, Ruan Y. RNA viral community in human feces: prevalence of plant pathogenic viruses. *PLoS Biol* 2006; **4**: e3
11 **Finkbeiner SR**, Allred AF, Tarr PI, Klein EJ, Kirkwood CD, Wang D. Metagenomic analysis of human diarrhea: viral detection and discovery. *PLoS Pathog* 2008; **4**: e1000011
12 **Scanlan PD**, Marchesi JR. Micro-eukaryotic diversity of the human distal gut microbiota: qualitative assessment using culture-dependent and -independent analysis of faeces. *ISME J* 2008; **2**: 1183-1193
13 **Xu J**, Gordon JI. Inaugural Article: Honor thy symbionts. *Proc Natl Acad Sci USA* 2003; **100**: 10452-10459
14 **Gill SR**, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JI, Relman DA, Fraser-Liggett CM, Nelson KE. Metagenomic analysis of the human distal gut microbiome. *Science* 2006; **312**: 1355-1359
15 **Dethlefsen L**, Eckburg PB, Bik EM, Relman DA. Assembly of the human intestinal microbiota. *Trends Ecol Evol* 2006; **21**: 517-523
16 **Jones BV**, Begley M, Hill C, Gahan CG, Marchesi JR. Functional and comparative metagenomic analysis of bile salt hydrolase activity in the human gut microbiome. *Proc Natl Acad Sci USA* 2008; **105**: 13580-13585
17 **Lunn JC**, Kuhnle G, Mai V, Frankenfeld C, Shuker DE, Glen RC, Goodman JM, Pollock JR, Bingham SA. The effect of haem in red and processed meat on the endogenous formation of N-nitroso compounds in the upper gastrointestinal tract. *Carcinogenesis* 2007; **28**: 685-690
18 **Mai V**. Dietary modification of the intestinal microbiota. *Nutr Rev* 2004; **62**: 235-242
19 **Khachatryan ZA**, Ktsoyan ZA, Manukyan GP, Kelly D, Ghazaryan KA, Aminov RI. Predominant role of host genetics in controlling the composition of gut microbiota. *PLoS ONE* 2008; **3**: e3064
20 **Mai V**, Colbert LH, Perkins SN, Schatzkin A, Hursting SD. Intestinal microbiota: a potential diet-responsive prevention target in ApcMin mice. *Mol Carcinog* 2007; **46**: 42-48
21 **Bäckhed F**, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proc Natl Acad Sci USA* 2007; **104**: 979-984
22 **Bäckhed F**, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, Gordon JI. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci USA* 2004; **101**: 15718-15723
23 **Ley RE**, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. *Proc Natl Acad Sci USA* 2005; **102**: 11070-11075
24 **Ley RE**, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature* 2006; **444**: 1022-1023
25 **Turnbaugh PJ**, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006; **444**: 1027-1031
26 **Duncan SH**, Lobley GE, Holtrop G, Ince J, Johnstone AM, Louis P, Flint HJ. Human colonic microbiota associated with diet, obesity and weight loss. *Int J Obes* (Lond) 2008; **32**: 1720-1724
27 **Kalliomäki M**, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* 2001; **357**: 1076-1079
28 **Harmsen HJ**, Wildeboer-Veloo AC, Raangs GC, Wagendorp AA, Klijn N, Bindels JG, Welling GW. Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. *J Pediatr Gastroenterol Nutr* 2000; **30**: 61-67
29 **Guarner F**, Malagelada JR. Gut flora in health and disease. *Lancet* 2003; **361**: 512-519
30 **Palmer C**, Bik EM, Digiulio DB, Relman DA, Brown PO. Development of the Human Infant Intestinal Microbiota. *PLoS Biol* 2007; **5**: e177
31 **Farrell RJ**, LaMont JT. Microbial factors in inflammatory bowel disease. *Gastroenterol Clin North Am* 2002; **31**: 41-62
32 **Kleessen B**, Kroesen AJ, Buhr HJ, Blaut M. Mucosal and iUse of uninitialized value in scalar chomp at E:\wwwroot\cgi-bin\submit.pl line 49. nvading bacteria in patients with inflammatory bowel disease compared with controls. *Scand J Gastroenterol* 2002; **37**: 1034-1041
33 **Sokol H**, Seksik P, Rigottier-Gois L, Lay C, Lepage P, Podglajen I, Marteau P, Doré J. Specificities of the fecal microbiota in inflammatory bowel disease. *Inflamm Bowel Dis* 2006; **12**: 106-111
34 **Bengmark S**. Pre-, pro- and synbiotics. *Curr Opin Clin Nutr Metab Care* 2001; **4**: 571-579
35 **Marteau P**, Seksik P, Shanahan F. Manipulation of the bacterial flora in inflammatory bowel disease. *Best Pract Res Clin Gastroenterol* 2003; **17**: 47-61
36 **Teitelbaum JE**, Walker WA. Nutritional impact of pre- and probiotics as protective gastrointestinal organisms. *Annu Rev Nutr* 2002; **22**: 107-138
37 **Swidsinski A**, Loening-Baucke V, Vaneechoutte M, Doerffel Y. Active Crohn's disease and ulcerative colitis can be specifically diagnosed and monitored based on the biostructure of the fecal flora. *Inflamm Bowel Dis* 2008; **14**: 147-161
38 **Swidsinski A**, Weber J, Loening-Baucke V, Hale LP, Lochs H. Spatial organization and composition of the mucosal flora in patients with inflammatory bowel disease. *J Clin Microbiol* 2005; **43**: 3380-3389

39 **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

40 **Mai V**, Morris JG Jr. Colonic bacterial flora: changing understandings in the molecular age. *J Nutr* 2004; **134**: 459-464

41 **Brady LJ**, Gallaher DD, Busta FF. The role of probiotic cultures in the prevention of colon cancer. *J Nutr* 2000; **130**: 410S-414S

42 **Saikali J**, Picard C, Freitas M, Holt P. Fermented milks, probiotic cultures, and colon cancer. *Nutr Cancer* 2004; **49**: 14-24

43 **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

44 **Brugman S**, Klatter FA, Visser JT, Wildeboer-Veloo AC, Harmsen HJ, Rozing J, Bos NA. Antibiotic treatment partially protects against type 1 diabetes in the Bio-Breeding diabetes-prone rat. Is the gut flora involved in the development of type 1 diabetes? *Diabetologia* 2006; **49**: 2105-2108

45 **Wen L**, Ley RE, Volchkov PY, Stranges PB, Avanesyan L, Stonebraker AC, Hu C, Wong FS, Szot GL, Bluestone JA, Gordon JI, Chervonsky AV. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. *Nature* 2008; **455**: 1109-1113

46 **Membrez M**, Blancher F, Jaquet M, Bibiloni R, Cani PD, Burcelin RG, Corthesy I, Macé K, Chou CJ. Gut microbiota modulation with norfloxacin and ampicillin enhances glucose tolerance in mice. *FASEB J* 2008; **22**: 2416-2426

47 **Li M**, Wang B, Zhang M, Rantalainen M, Wang S, Zhou H, Zhang Y, Shen J, Pang X, Zhang M, Wei H, Chen Y, Lu H, Zuo J, Su M, Qiu Y, Jia W, Xiao C, Smith LM, Yang S, Holmes E, Tang H, Zhao G, Nicholson JK, Li L, Zhao L. Symbiotic gut microbes modulate human metabolic phenotypes. *Proc Natl Acad Sci USA* 2008; **105**: 2117-2122

48 **Martin FP**, Wang Y, Sprenger N, Yap IK, Lundstedt T, Lek P, Rezzi S, Ramadan Z, van Bladeren P, Fay LB, Kochhar S, Lindon JC, Holmes E, Nicholson JK. Probiotic modulation of symbiotic gut microbial-host metabolic interactions in a humanized microbiome mouse model. *Mol Syst Biol* 2008; **4**: 157

49 **Turnbaugh PJ**, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. *Nature* 2007; **449**: 804-810

50 **Andersson AF**, Lindberg M, Jakobsson H, Bäckhed F, Nyrén P, Engstrand L. Comparative analysis of human gut microbiota by barcoded pyrosequencing. *PLoS ONE* 2008; **3**: e2836

51 **Hamady M**, Walker JJ, Harris JK, Gold NJ, Knight R. Error-correcting barcoded primers for pyrosequencing hundreds of samples in multiplex. *Nat Methods* 2008; **5**: 235-237


**S- Editor** Tian L **L- Editor** Negro F **E- Editor** Zheng XM

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.86

*World J Gastroenterol* 2009 January 7; 15(1): 86-94
***World Journal of Gastroenterology*** ISSN 1007-9327


*ORIGINAL ARTICLES*

# Prevalence of clonorchiasis in patients with gastrointestinal disease: A Korean nationwide multicenter survey

Ho Gak Kim, Jimin Han, Myung-Hwan Kim, Kyu Hyun Cho, Im Hee Shin, Gwang Ha Kim, Jae Seon Kim, Jin Bong Kim, Tae Nyeun Kim, Tae Hyeon Kim, Tae Hyo Kim, Jae Woo Kim, Ji Kon Ryu, Young-Soo Moon, Jong Ho Moon, Sung Jae Park, Chan Guk Park, Sung-Jo Bang, Chang Heon Yang, Kyo-Sang Yoo, Byung Moo Yoo, Kyu Taek Lee, Dong Ki Lee, Byung Seok Lee, Sang Soo Lee, Seung Ok Lee, Woo Jin Lee, Chang Min Cho, Young-Eun Joo, Gab Jin Cheon, Young Woo Choi, Jae Bok Chung, Yong Bum Yoon

Ho Gak Kim, Jimin Han, Kyu Hyun Cho, Department of Internal Medicine, Catholic University of Daegu School of Medicine, Daegu 705-718, South Korea
Myung-Hwan Kim, Sang Soo Lee, Department of Internal Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul 138-736, South Korea
Im Hee Shin, Department of Medical Statistics, Catholic University of Daegu School of Medicine, Daegu 705-718, South Korea
Gwang Ha Kim, Department of Internal Medicine, Pusan National University School of Medicine, Busan 602-739, South Korea
Jae Seon Kim, Department of Internal Medicine, Korea University College of Medicine, Seoul 136-705, South Korea
Jin Bong Kim, Department of Internal Medicine, Hallym University College of Medicine, Chuncheon 200-704, South Korea
Tae Nyeun Kim, Department of Internal Medicine, Yeungnam University College of Medicine, Daegu 705-717, South Korea
Tae Hyeon Kim, Department of Internal Medicine, Wonkwang University College of Medicine, Iksan, Jeonbuk 570-711, South Korea
Tae Hyo Kim, Department of Internal Medicine, Gyeongsang National University School of Medicine, Jinju, Gyeongnam 660-702, South Korea
Jae Woo Kim, Department of Internal Medicine, Yonsei University Wonju College of Medicine, Wonju, Gangwon 220-701, South Korea
Ji Kon Ryu, Yong Bum Yoon, Department of Internal Medicine, Seoul National University College of Medicine, Seoul 110-799, South Korea
Young-Soo Moon, Department of Internal Medicine, Inje University College of Medicine, Seoul 139-713, South Korea
Jong Ho Moon, Department of Internal Medicine, Soonchunhyang University College of Medicine, Bucheon, Gyunggi 420-767, South Korea
Sung Jae Park, Department of Internal Medicine, Inje University College of Medicine, Busan 614-735, South Korea
Chan Guk Park, Department of Internal Medicine, Chosun University College of Medicine, Gwangju, Jeonbuk 501-717, South Korea
Sung-Jo Bang, Department of Internal Medicine, Ulsan University Hospital University of Ulsan College of Medicine, Ulsan 681-714, South Korea
Chang Heon Yang, Department of Internal Medicine, Dongguk University College of Medicine, Gyeongju, Gyeongbuk 791-707, South Korea
Kyo-Sang Yoo, Department of Internal Medicine, Hallym University College of Medicine, Anyang, Gyeonggi 431-070, South Korea
Byung Moo Yoo, Department of Internal Medicine, Ajou University School of Medicine, Suwon, Gyeonggi 443-721, South Korea
Kyu Taek Lee, Department of Internal Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul 135-710, South Korea
Dong Ki Lee, Jae Bok Chung, Department of Internal Medicine, Yonsei University College of Medicine, Seoul 120-749, South Korea
Byung Seok Lee, Department of Internal Medicine, Chungnam National University College of Medicine, Daejeon 301-747, South Korea
Seung Ok Lee, Department of Internal Medicine, Chonbuk National University Medical School, Jeonju 561-712, South Korea
Woo Jin Lee, Department of Internal Medicine, National Cancer Center, Ilsan, Gyeonggi 410-769, South Korea
Chang Min Cho, Department of Internal Medicine, Kyungpook National University School of Medicine, Daegu 700-721, South Korea
Young-Eun Joo, Department of Internal Medicine, Chonnam National University Medical School, Gwangju 501-746, South Korea
Gab Jin Cheon, Department of Internal Medicine, Kangneung Asan Hospital, Gangneung, Gangwon 210-711, South Korea
Young Woo Choi, Department of Internal Medicine, Konyang University College of Medicine, Daejeon 302-718, South Korea
**Author contributions:** Kim HG and Han J contributed equally to this work; Kim HG and Kim MH designed research; Kim HG, Han J, Kim MH, Cho KH, Kim GH, Kim JS, Kim JB, Kim TN, Kim TH, Kim TH, Kim JW, Ryu JK, Moon YS, Moon JH, Park SJ, Park CG, Bang SJ, Yang CH, Yoo KS, Yoo BM, Lee KT, Lee DK, Lee BS, Lee SS, Lee SO, Lee WJ, Cho CM, Joo YE, Cheon GJ, Choi YW, Chung JB, and Yoon YB performed research; Kim HG, Han J, Choi KH, and Shin IH analyzed data; and Kim HG, Han J, and Choi KH wrote the paper.
**Supported by** The Korean Society of Gastroenterology Research Fund, No. 2005-1
**Correspondence to: Ho Gak Kim,** Department of Internal Medicine, Catholic University of Daegu School of Medicine, 3056-6 Daemyung 4-dong, Nam-gu, Daegu 705-718, South Korea. hgkim@cu.ac.kr
**Telephone:** +82-53-6504041 **Fax:** +82-53-6243281
**Received:** September 1, 2008 **Revised:** October 10, 2008
**Accepted:** October 17, 2008
**Published online:** January 7, 2009

## Abstract

**AIM:** To investigate prevalence of *Clonorchis sinensis* in patients with gastrointestinal symptoms, and the relation of the infection to hepatobiliary diseases in 26 hospitals in Korea.

**METHODS:** Consecutive patients who had been admitted to the Division of Gastroenterology with gastrointestinal symptoms were enrolled from March to April 2005. Of those who had been diagnosed with clonorchiasis, epidemiology and correlation between infection and hepatobiliary diseases were surveyed by questionnaire.

**RESULTS:** Of 3080 patients with gastrointestinal diseases, 396 (12.9%) had clonorchiasis and 1140 patients (37.2%) had a history of eating raw freshwater fish. Of those with a history of raw freshwater fish ingestion, 238 (20.9%) patients had clonorchiasis. Cholangiocarcinoma was more prevalent in *C. sinensis*-infected patients than non-infected patients [34/396 (8.6%) *vs* 145/2684 (5.4%), $P = 0.015$]. Cholangiocarcinoma and clonorchiasis showed statistically significant positive cross-relation ($P = 0.008$). Choledocholithiasis, cholecystolithiasis, cholangitis, hepatocellular carcinoma, and biliary pancreatitis did not correlate with clonorchiasis.

**CONCLUSION:** Infection rate of clonorchiasis was still high in patients with gastrointestinal diseases in Korea, and has not decreased very much during the last two decades. Cholangiocarcinoma was related to clonorchiasis, which suggested an etiological role for the parasite.



**Key words:** *Clonorchis sinensis*; Epidemiology; Cholangiocarcinoma; Korea; Multicenter study; Clonorchiasis

**Peer reviewer:** Heitor Rosa, Professor, Department of Gastroenterology and Hepatology, Federal University School of Medicine, Rua 126 n.21, Goiania GO 74093-080, Brazil

Kim HG, Han J, Kim MH, Cho KH, Shin IH, Kim GH, Kim JS, Kim JB, Kim TN, Kim TH, Kim TH, Kim JW, Ryu JK, Moon YS, Moon JH, Park SJ, Park CG, Bang SJ, Yang CH, Yoo KS, Yoo BM, Lee KT, Lee DK, Lee BS, Lee SS, Lee SO, Lee WJ, Cho CM, Joo YE, Cheon GJ, Choi YW, Chung JB, Yoon YB. Prevalence of clonorchiasis in patients with gastrointestinal disease: A Korean nationwide multicenter survey. *World J Gastroenterol* 2009; 15(1): 86-94 Available from: URL: http://www.wjgnet.com/1007-9327/15/86.asp DOI: http://dx.doi.org/10.3748/wjg.15.86

## INTRODUCTION

Clonorchiasis is a parasitic infection caused by *C. sinensis,* and is one of the most prevalent endemic diseases in eastern Asia[1-3]. According to a report by World Health Organization (WHO) and International Agency for Research on Cancer in 1994, about 7 million people in the world were infected with *C. sinensis*[4]. In Korea, the stool egg-positive rate for *C. sinensis* has decreased dramatically from 4.6% in 1971 to 2.7% in 1986, after the introductioin of praziquantel, but the rate remained at 1.4% in 1997[5-8]. The high prevalence rate of clonorchiasis in Korea results from a long tradition of consuming raw freshwater fish and/or shellfish[9].

Infection rate in Korea differs from one major river basin to another. According to a national survey conducted in 1981, the stool egg-positive rate for *C. sinensis* in people of southern river basins (Nakdong, Yeongsan, and Seomjin Rivers) was 17%-40%, while that in people of middle river basins (Han, Geum, and Dangjim-Mankyong Rivers) was lower at 8%-12%[10]. However, there have been no data on infection rate among people in other river basins in middle and eastern areas (North Han, Bulyeong-Wangpi, and Namdae-Yeonggok-Osip Rivers).

Fecal examination for eggs has bee used in population-based studies for diagnosis of clonorchiasis[11]. However, this method has low sensitivity, which results in lower prevalence rates. Other methods for diagnosis include intradermal test using diluted antigens of *C. sinensis*[12], ELISA for circulating antibody against the parasite[13], radiological studies of the liver[14], and bile examination for eggs, metacercariae and cercariae. Of these, intradermal test is the easiest to perform, but has low specificity because of cross-reactivity with other parasites such as *Paragonimus westermani*[12]. Diffuse dilatation of the intrahepatic bile ducts detected by abdominal ultrasonography (US), computed tomography (CT), or cholangiography can easily establish clonorchiasis. In addition, detection of eggs in bile collected by endoscopic or percutaneous biliary drainage can lead to a definite diagnosis.

Adult worms of *C. sinensis* migrate from the common bile duct to peripheral intrahepatic bile duct, and remain there for 20-30 years causing chronic persistent infection[15]. In humans, clinical manifestations of light parasite loads are often asymptomatic. On the other hand, chronic infection with heavy parasite loads has been associated with various hepatobiliary diseases, such as biliary obstruction, recurrent pyogenic cholangitis[16], hepatolithiasis[17,18], and cholangiocarcinoma[19-21]. According to several experimental and clinical studies, clonorchiasis has been associated with carcinogenesis in the bile duct mucosa[4,19,22]. Adult worms, eggs, or mucoid material after infection can also be the nidus of hepatolithiasis[16,23,24]. Although there have been several studies on association between clonorchiasis and several hepatobiliary diseases, there has been no recent nationwide multicenter study in endemic areas and no investigation on prevalence and infection rates after raw freshwater fish and/or shellfish ingestion.

Therefore, we conducted a prospective nationwide multicenter study to investigate infection rate of *C. sinensis* in patients with gastrointestinal symptoms, and

the relation of *C. sinensis* infection with hepatobiliary diseases in 26 secondary and tertiary hospitals in Korea.

## MATERIALS AND METHODS

### Subjects

This prospective study was conducted in 26 secondary and tertiary hospitals in South Korea from March to April 2005. Subjects included consecutive patients with gastrointestinal symptoms who were admitted to the Department of Internal Medicine during the study period. Gastrointestinal symptoms were defined as the presence of any of the following: nausea, vomiting, diarrhea, constipation, abdominal pain, heartburn, dyspepsia, jaundice, indigestion, and fecal incontinence. Patients were excluded if they had been admitted with non-gastrointestinal symptoms, admitted more than twice during the study period, unable to give a thorough history, < 14 years old, and declined to participate in this study.

The institutional review board of each participating hospital approved this study. Informed consent for participation in this study was obtained from each patient included in the study.

### Questionnaires

Upon admission, attending physicians filled out a structured questionnaire for each subject after a medical interview. The questionnaire included the following information: rivers nearest to the birthplace or place of current residence in order of decreasing duration, history of eating raw freshwater fish and/or shellfish, including the time, place and species of the fish and/or shellfish consumed, past history of clonorchiasis and treatment including type and duration, and past history of hepatobiliary diseases. Rivers nearest to the birthplace or place of current residence included 10 major rivers in South Korea: Nakdong, South Han, North Han, Geum, Yeongsan, Seomjin, Mangyong-Dongjin, Hyeongsan, Bulyeong-Wangpi, and Namdae-Yeongok-Osip.

The questionnaire also included close-ended questions such as: (1) did you (the patient him/herself) know that clonorchiasis can be acquired by ingesting raw freshwater fish? (2) Did you know that clonorchiasis can also be acquired by eating freshwater shellfish? (3) Did you know that clonorchiasis can be transmitted *via* kitchen knives and/or towels? (4) Did you know clonorchiasis can be transmitted by unwashed hands of raw freshwater fish handlers? (5) Did you know clonorchiasis can be prevented by eating fully cooked freshwater fish?

### Diagnostic methods for C. sinensis infection

After admission, all the patients underwent laboratory tests, which included complete blood count with differential count, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase, gamma-glutamyl transpeptidase (GGT), and total bilirubin. When transabdominal US or abdominal CT was done after admission or within 6 mo of admission, presence of dilatation of the intrahepatic bile ducts and/or extrahepatic bile duct was recorded.

Diagnosis of clonorchiasis was based on the presence of one or more of the following findings: (1) detection of *C. sinensis* eggs, metacercariae, or adult worms in stools collected during admission; (2) presence of induration with area of 60 $mm^2$ or greater on the forearm after skin test with an intradermal injection of diluted crude antigen of *C. sinensis*; (3) positive for serum antibodies to *C. sinensis* using ELISA; (4) detection of *C. sinensis* eggs, metacercariae, or adult worms in bile collected during percutaneous transhepatic biliary drainage or endoscopic nasobiliary drainage; (5) finding of diffuse dilatation of intrahepatic bile ducts in transabdominal US, abdominal CT, or cholangiography; and (6) detection of *C. sinensis* infection in stools or bile examination, and/or presence of positive intradermal test described in medical records. To investigate the possible association between clonorchiasis and hepatobiliary diseases, medical records of each patient were reviewed for diagnosis of the following diseases: cholangitis, choledocholithiasis, gallbladder stones, hepatocellular carcinoma, cholangiocarcinoma, gallbladder cancer, biliary pancreatitis, and alcoholic pancreatitis.

### Statistical analysis

Overall infection rate of *C. sinensis* and that according to the river basins were calculated. Infection rates between raw freshwater fish and/or shellfish eaters and non-eaters were also compared. Level of knowledge on transmission and prevention of clonorchiasis was assessed. By using the $\chi^2$ test and independent *t* test, the differences between infected and non-infected patients were assessed with regard to the presence of peripheral eosinophilia and abnormal liver function tests. Sensitivities of various diagnostic methods used in this study were evaluated.

Association with hepatobiliary diseases was evaluated for infected and non-infected patients by using the $\chi^2$ test. Association between clonorchiasis and cholangiocarcinoma among raw freshwater fish and/or shellfish eaters from high prevalence river basins (Nakdong, Yeongsan, Seomjin, and Hyeongsan Rivers) and lower prevalence river basins (the remainder) was assessed using the *t* test and odds ratio. After correction for regions, Cochran's Mantel-Haenszel $\chi^2$ test was used to evaluate the association between clonorchiasis and cholangiocarcinoma. SPSS (version 12.0 for Windows; Chicago, IL, USA) was used for statistical analysis and $P < 0.05$ was considered statistically significant.

## RESULTS

### Patient characteristics

Subjects included 3080 patients from 26 hospitals. Number of patients according to rivers nearest to the birthplace or place of current residence was 947 (31.5%) in Nakdong, 774 (25.7%) in South Han, 270 (9.0%) in North Han, 303 (10.1%) in Geum, 137 (4.6%)

**Table 1 Distribution of patients according to rivers nearest to the birthplace or place of current residence *n* (%)**

| Rivers | Number of patients |
|---|---|
| Nakdong | 947 (31.5) |
| South Han | 774 (25.7) |
| North Han | 270 (9.0) |
| Geum | 303 (10.1) |
| Yeongsan | 137 (4.6) |
| Seomjin | 97 (3.2) |
| Mangyong-Dongjin | 266 (8.8) |
| Hyeongsan | 145 (4.8) |
| Bulyeong-Wangpi | 6 (0.2) |
| Namdae-Yongok-Osip | 64 (2.1) |
| Not answered | 71 |
| Total | 3080 |

in Yeongsan, 97 (3.2%) in Seomjin, 266 (8.8%) in Mangyong-Dongjin, 154 (4.8%) in Hyeongsan, six (0.2%) in Bulyeong-Wangpi, 64 (2.1%) in Namdae-Yeongok-Osip Rivers and 71 unanswered (Table 1). There were 1953 male and 1127 female patients. Male to female ratio was 1.7:1. Mean age of the patients was 58.2 years old (range, 14-98).

### *Past history of raw freshwater fish and/or shellfish ingestion and C. sinensis infection*

The number of patients with past history of raw freshwater fish and/or shellfish ingestion was 1140 (37.3%) out of 3055 of those who answered the questionnaire, while there were 191 (62.7%) patients with no past history of ingestion, and there were 25 unanswered questionnaires. Of those with a past history of ingestion, 156 out of 1140 patients (13.9%) ingested only once, 318 patients (28.3%) two to four times, and 648 patients (57.8%) more than five times. Initial time of raw freshwater fish and/or shellfish ingestion was within 10 years in 278 patients (25.5%), 11-20 years ago in 207 (19.0%), 21–30 years ago in 179 (16.4%), > 30 years ago in 427 (39.1%), and 49 questionnaires were unanswered (Table 2).

In 1140 patients with a past history of ingestion, river basins nearest to the place of ingestion were Nakdong for 394 patients (35.2%), South Han for 156 (13.9%), North Han for 207 (18.5%), Geum for 84 (7.5%), Yeongsan for 47 (4.2%), Seomjin for 65 (5.8%), Mangyong-Dongjin for 78 (7.0%), Hyeongsan for 54 (4.8%), Bulyeong-Wangpi for three (0.3%), Namdae-Yeongok-Osip Rivers for 32 (2.9%), and 20 questionnaires were unanswered (Table 3).

Only 150 (5.0%) patients had been diagnosed with clonorchiasis in the past. The number of patients without a past history of diagnosis or treatment was 2880 (95.0%) and 50 questionnaires were unanswered. Of those 150 patients with a past history of clonorchiasis, 120 (81.6%) had received eradication therapy, 14 (9.5%) did not receive any therapy, 13 (8.8%) had undergone treatment without definite diagnosis, and three did not answer. There were 657 patients (21.3%) with a past history of hepato-biliary diseases. Of these patients, 395 (60.4%) had bile duct stones, 118 (18.1%) cholangitis, 64 (9.6%) cholangiocarcinoma, 116 (17.6%) jaundice of uncertain cause, and 112 (16.5%) pancreatitis.

**Table 2 Frequency and time of raw freshwater fish or snail ingestion among the patients with positive history *n* (%)**

| Characteristics | Number of patients |
|---|---|
| History of ingestion | |
| Present | 1140 (37.3) |
| Frequency | |
| Once | 156 (13.9) |
| 2-4 times | 318 (28.3) |
| ⩾ 5 times | 648 (57.8) |
| Unknown or not answered | 18 |
| Time of first ingestion | |
| 0-10 yr ago | 278 (25.5) |
| 11-20 yr ago | 107 (19.0) |
| 21-30 yr ago | 179 (16.4) |
| ⩾ 30 yr ago | 427 (39.1) |
| Unknown or not answered | 49 |
| None | 1915 (62.7) |
| Not answered | 25 |
| Total | 3080 |

**Table 3 Distribution and infection rate of the patients with positive history of raw freshwater fish or snail ingestion according to river basins nearest to place of residence**

| River basins | Patients, *n* (%) | Infected patients (*n*) | Infection rate (%) |
|---|---|---|---|
| Nakdong | 394 (35.2) | 132 | 33.5 |
| South Han | 156 (13.9) | 12 | 7.7 |
| North Han | 207 (18.5) | 15 | 7.2 |
| Geum | 84 (7.5) | 7 | 8.3 |
| Yeongsan | 47 (4.2) | 19 | 40.4 |
| Seomjin | 65 (5.8) | 14 | 21.5 |
| Mangyong-Dongjin | 78 (7.0) | 10 | 1.8 |
| Hyeongsan | 54 (4.8) | 24 | 44.4 |
| Bulyeong-Wangpi | 3 (0.3) | 0 | 0 |
| Namdae-Yongok-Osip | 32 (2.9) | 4 | 12.5 |
| Not answered | 20 | 3 | |
| Total | 1140 | 238 | 20.9 |

### *Questionnaire on route of C. sinensis infection*

Of 3049 patients who answered the questionnaire, 2464 (80.8%) knew that clonorchiasis can be acquired by ingesting raw freshwater fish and 1629 (53.3%) knew that clonorchiasis can also be acquired by eating raw freshwater shellfish. Also, 1141 (47.2%) knew that clonorchiasis can be transmitted *via* kitchen knives and/or towels and 1192 (39.1%) acknowledged that clonorchiasis can be transmitted by unwashed hands of raw freshwater fish handlers. In addition, 2371 patients (77.8%) knew that clonorchiasis can be prevented by eating fully cooked freshwater fish (Table 4).

### *Presence of C. sinensis infection*

**Diagnosis of infection:** Of 3080 patients admitted to the Department of Internal Medicine during the study period, 396 (12.9%) had been diagnosed with clonorchiasis. Stool examination was positive for *C. sinensis* eggs, metacercariae, or adult worms in 55 patients. Intradermal test was positive in 225 patients and serum antibodies to *C. sinensis* using an ELISA

**Table 4 Answers to questionnaires regarding knowledge on route of *C. sinensis* infection**

| Questions | patients with "Yes" *n* (%) | Patients with "No" *n* (%) | Number of not answered (*n*) |
|---|---|---|---|
| Did you (the patient him/herself) know that clonorchiasis can be acquired by ingesting raw freshwater fish? | 2464 (80.8) | 585 (19.2) | 31 |
| Did you know that clonorchiasis can also be acquired by eating freshwater shellfish? | 1626 (53.3) | 1423 (46.7) | 31 |
| Did you know that clonorchiasis can be transmitted via kitchen knifes and/or towels? | 1441 (47.3) | 1608 (52.7) | 31 |
| Did you know clonorchiasis can be transmitted by unwashed hands of raw freshwater fish handlers? | 1192 (39.1) | 1855 (60.9) | 33 |
| Did you know that clonorchiasis can be prevented by eating fully cooked freshwater fish? | 2371 (77.8) | 676 (22.2) | 33 |

**Table 5 Sensitivities of various diagnostic modalities for detection of clonorchiasis**

| Diagnostic modalities | Infected persons, who were tested (*n*) | Positive results (*n*) | Sensitivity (%) |
|---|---|---|---|
| Fecal exam for eggs | 321 | 55 | 17.1 |
| ELISA for circulating antibody | 362 | 157 | 43.40 |
| Intradermal test | 302 | 225 | 74.50 |
| Examination of collected bile | 134 | 14 | 10.40 |
| Radiologic findings | 295 | 34 | 11.50 |

**Table 6 Distribution of patients with clonorchiasis according to age group *n* (%)**

| Age group (yr) | Number of patients (*n*) | Number of patients with clonorchiasis |
|---|---|---|
| 10-19 | 26 | 0 (0) |
| 20-29 | 107 | 7 (6.5) |
| 30-39 | 222 | 25 (11.3) |
| 40-49 | 463 | 79 (17.1) |
| 50-59 | 695 | 99 (14.2) |
| 60-69 | 831 | 113 (13.6) |
| 70-79 | 575 | 61 (10.6) |
| ⩾ 80 | 161 | 12 (7.5) |
| Total | 3080 | 396 |

were positive in 157 patients. In 14 patients, *C. sinensis* eggs, metacercariae, or adult worms were detected in bile collected during percutaneous transhepatic biliary drainage or endoscopic nasobiliary drainage. Diffuse dilatation of intrahepatic bile ducts in transabdominal US, abdominal CT, or cholangiography was found in 34 patients. *C. sinensis* infection in stools or bile examination and/or presence of positive intradermal test was described in the medical records of 150 patients.

Sensitivities of the diagnostic tests were highest for intradermal test (74.5%) and second highest for serum antibodies to *C. sinensis* using an ELISA (43.4%) (Table 5).

Among patients with clonorchiasis, there was no patient younger than 19 year old. There were seven patients (6.5%) out of 107 in their twenties, 25 (11.3%) out of 222 in their thirties, 79 (17.1%) out 463 in their forties, 99 (14.2%) out of 695 in their fifties, 113 (13.6%) out of 831 in their sixties, and 61 (10.6%) out of 575 in their seventies. There were 12 (7.5%) out of 161 patients older than 80 years (Table 6).

**Table 7 Presence of clonorchiasis according to history of raw freshwater fish ingestion *n* (%)**

| | Number of patients with clonorchiasis |
|---|---|
| Total number of patients (*n* = 3080) | 396 (12.9) |
| Patients with positive history of raw freshwater fish ingestion (*n* = 1140) | 238 (20.9) |
| Patients without raw freshwater fish ingestion (*n* = 1940) | 158 (8.1) |

**Distribution of infected patients according to river basins from where ingested raw freshwater fishes originated:** Of 1140 patients with a history of raw freshwater fish and/or shellfish ingestion, 238 (20.9%) had been diagnosed with clonorchiasis. Also, there was evidence of clonorchiasis in 157 out of 1940 patients (6.5%) who had no history of ingestion or had not answered the questionnaire (Table 7).

Of 1120 patients who answered, the river basin nearest to the place of raw freshwater fish or shellfish ingestion was Nakdong for 132 patients, South Han for 12 patients, North Han for 15 patients, Geum for seven patients, Yeongsan for 19 patients, Seomjin for 14 patients, Mangyong-Dongjin for 10 patients, Hyeongsan for 24 patients, Bulyeong-Wangpi for none, and Namdae-Yeongok-Osip Rivers for four patients (Table 4). The river basin with highest infection rate was Hyeongsan (44.4%). Other river basins in decreasing order of infection rate were Yeongsan (40.4%) and Nakdong (33.5%).

**Laboratory findings:** Eosinophilia in the peripheral blood (> 400/mm$^3$) was found in 65 of 389 patients with clonorchiasis (16.7%), while it was found in 250 of 2617 patients (9.6%) without clonorchiasis ($P$ = 0.000) (Table 8). Serum alkaline phosphatase was 304.8 ± 418.35 U/L in 382 patients with clonorchiasis, but 234.4 ± 350.81 U/L in 2611 patients without clonorchiasis ($P$ = 0.002). However, levels of AST, ALT, GGT, and total bilirubin were not significantly different between

**Table 8 Comparison of laboratory findings between patients with clonorchiasis and without clonorchiasis**

| Laboratory findings | With clonorchiasis ($n$ = 396) | | Without clonorchiasis ($n$ = 2684) | | $P$ |
|---|---|---|---|---|---|
| | Number of patients | mean ± SD | Number of patients | mean ± SD | |
| Eosinophilia[1] | 65 | NA | 250 | NA | 0 |
| AST (U/L) | 393 | 104.5 ± 269.56 | 2671 | 110.7 ± 263.59 | 0.665 |
| ALT (U/L) | 393 | 113.5 ± 254.01 | 2669 | 107.7 ± 258.17 | 0.68 |
| Alkaline phosphatase (U/L) | 382 | 304.8 ± 418.35 | 2611 | 234.4 ± 350.81 | 0.002 |
| γ-glutamyl transpeptidase (U/L) | 362 | 200.0 ± 261.19 | 2233 | 187.0 ± 440.01 | 0.585 |
| Total bilirubin (mg/dL) | 392 | 3.07 ± 17.246 | 2668 | 3.28 ± 5.401 | 0.81 |

[1]> 400/mm$^3$ in peripheral blood; NA: Not available.

**Table 9 Association between hepatobiliary diseases and presence of clonorchiasis $n$ (%)**

| Hepatobiliary diseases | Patients with clonorchiasis ($n$ = 396) | Patients without clonorchiasis ($n$ = 2684) | $P$ |
|---|---|---|---|
| Cholangitis | 32 (8.0) | 242 (9.0) | NS |
| Bile duct stones | 92 (23.2) | 716 (26.7) | NS |
| Gallstone | 45 (11.4) | 340 (12.7) | NS |
| Intrahepatic bile duct stones | 13 (3.3) | 107 (4.0) | NS |
| Extrahepatic bile duct stones | 34 (8.6) | 269 (10.0) | NS |
| Hepatitis | 100 (25.3) | 650 (24.2) | NS |
| Hepatitis B virus | 58 (14.6) | 336 (12.5) | NS |
| Hepatitis C virus | 5 (1.3) | 66 (2.5) | NS |
| Alcoholic | 25 (6.3) | 159 (5.9) | NS |
| Toxic | 5 (1.3) | 40 (1.5) | NS |
| Autoimmune | 1 (0.3) | 5 (0.2) | NS |
| Other causes | 6 (1.5) | 44 (1.6) | NS |
| Hepatocellular carcinoma | 51 (12.9) | 391 (14.6) | NS |
| Cholangiocarcinoma | 34 (8.6) | 145 (5.4) | 0.015 |
| Gallbladder cancer | 9 (2.3) | 75 (2.8) | NS |
| Biliary pancreatitis | 6 (1.5) | 71 (2.6) | NS |

NS: Not significant.

the two groups (Table 8).

**Association between clonorchiasis and hepatobiliary diseases:** When prevalence of various hepatobiliary diseases was evaluated between patients with and without clonorchiasis, a statistically significant difference was found only for cholangiocarcinoma [34 (8.6%) *vs* 145 (5.4%), $P$ = 0.015] (Figure 1A). There was no significant difference regarding cholangitis, bile duct stones, hepatitis, hepatocellular carcinoma, gallstone pancreatitis, and gallbladder cancer between patients with and without clonorchiasis (Table 9).

Patients were divided into two groups according to regions of the river basins nearest to the place of residence, and analyzed for association between presence of clonorchiasis and cholangiocarcinoma. Rivers of the southern region of the Korean Peninsula included Nakdong, Yeongsan, Seomjin and Hyeongsan Rivers and those of the middle region included South Han, North Han, Geum, Bulyeong-Wangpi, Namdae-Yeongok-Osip, and Mangyong-Dongjin Rivers (Figure 2). Clonorchiasis was present in 189 of 560 patients (33.8%) from the southern region, but in only 48 of 560 patients (8.6%) from the middle region ($P$ = 0.000). While cholangiocarcinoma was found in 39 of 560 patients (7.0%) from the southern region, it was found in 19 of 560 patients (3.4%) from the middle region ($P$ = 0.007) (Figure 1B).

In both southern and middle region groups, there was a significant association between presence of clonorchiasis and cholangiocarcinoma [$P$ = 0.005, odds ratio: 4.136 (95% CI, 1.422-12.030] and $P$ = 0.040, odds ratio: 1.961 (95% CI, 1.020-3.773), respectively]. Even after correction for regional influence, there was a significant association between presence of clonorchiasis and cholangiocarcinoma [$P$ = 0.003, common odds ratio: 2.289 (95% CI, 1.297-4.038)] (Table 10, Figure 1B).

Twenty-four of 34 patients with clonorchiasis and cholangiocarcinoma had a history of raw freshwater fish and/or shellfish ingestion. Fifteen of these patients (62.5%) had ingested raw freshwater fish and/or shellfish 30 years ago, seven patients did so within last 10 years, and the other two patients ingested raw freshwater fish and/or shellfish between 21 and 30 years ago.

## DISCUSSION

In this prospective study of Korean patients who had been admitted with gastrointestinal symptoms, 37.2% had a history of ingesting raw freshwater fish and/or shellfish more than once. Many Koreans still enjoy raw freshwater fish and/or shellfish and most do so more than once in his/her lifetime.

More than 80% of the patients knew that ingesting raw freshwater fish can result in clonorchiasis and 78% answered that eating fully cooked freshwater fish can prevent clonorchiasis. However, only 50% knew that eating raw freshwater shellfish could result in clonorchiasis. About 40%-50% of the patients also knew that clonorchiasis can be transmitted through kitchen knives, towels, kitchen boards, and/or unwashed hands of the cook or handler. Many people knew the transmission route of clonorchiasis, but still enjoyed eating raw freshwater fish and/or shellfish. Perhaps, these people believed that clonorchiasis can be easily treated with oral medication and clonorchiasis will not result in serious hepatobiliary diseases.

Since the objective of this study was to evaluate the association between clonorchiasis and various hepatobiliary diseases, rather than to evaluate epidemiology of clonorchiasis in the Korean population,

**Table 10 Association between prevalence of clonorchiasis and cholangiocarcinoma according to regions of the rivers ($n$ = 560)**

| | Rivers in southern region[1] | | | Rivers in middle region[2] | | |
|---|---|---|---|---|---|---|
| | With cholangiocarcinoma | Without cholangiocarcinoma | $\chi^2$ ($P$) | With cholangiocarcinoma | Without cholangiocarcinoma | $\chi^2$ ($P$) |
| With clonorchiasis, $n$ (%) | 5 (10.4) | 43 (89.6) | 7.902 (0.005) | 19 (10.1) | 170 (89.9) | 4.2 (0.04) |
| Without clonorchiasis, $n$ (%) | 14 (2.7) | 498 (97.3) | | 20 (5.4) | 351 (94.6) | |
| Odd ratio | 4.136 (95% CI 1.422-12.030) | | | 1.961 (95% CI 1.020-3.773) | | |
| Common odds ratio[3] | 2.289 (95% CI 1.297-4.038) | | | | | |

[1]Nakdong, Yeongsan, Seomjin, Hyeongsan Rivers; [2]South Han, North Han, Geum, Bulyeong-Wangpi, Namdae-Yeongok-Osip, Mangyong-Dongjin Rivers; [3]$P$ = 0.003, result of Cochran's Mantel-Haenzel test adjusted area.

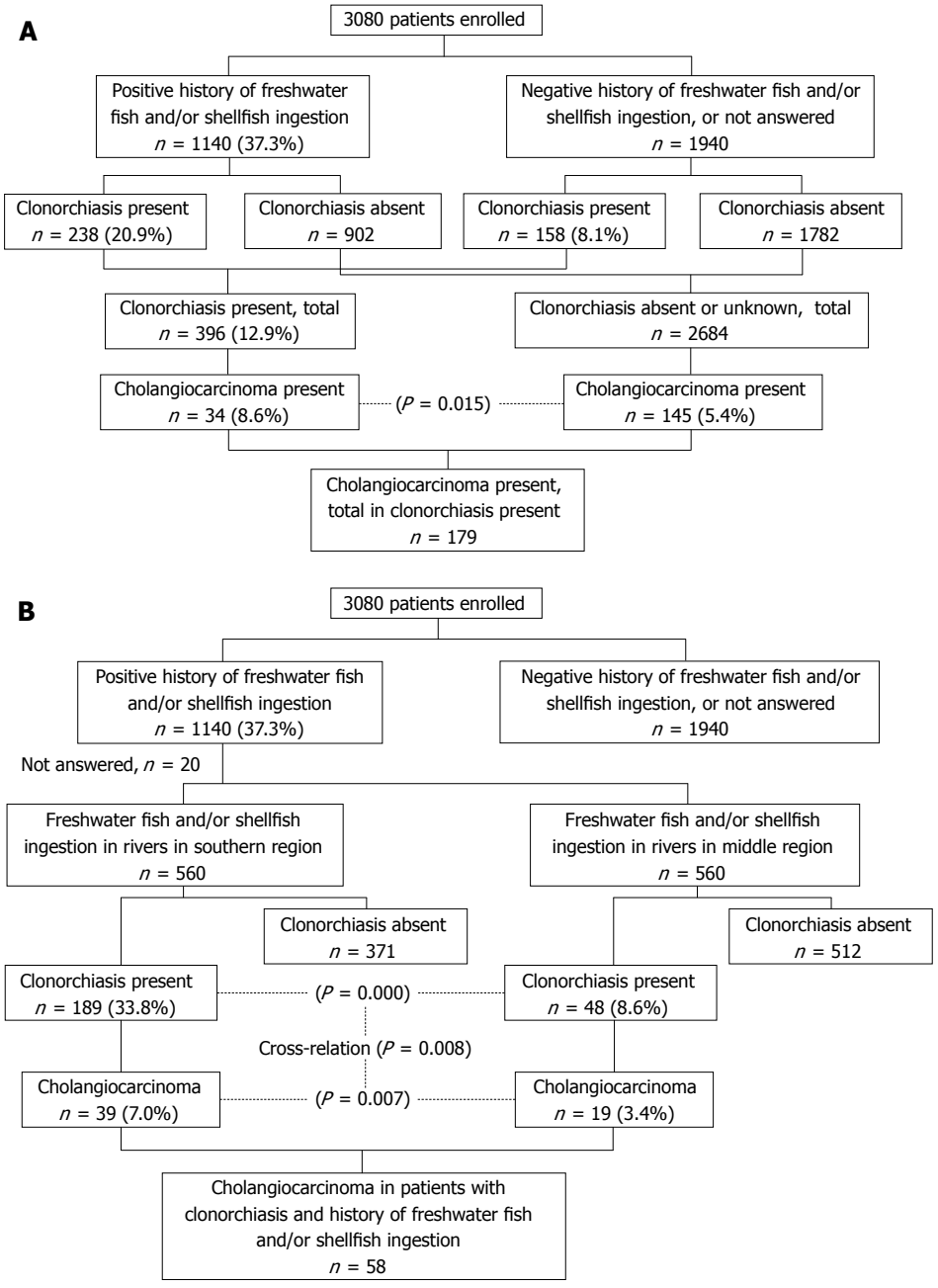


**Figure 1 Flowchart for the comparison of the frequency of cholangiocarcinoma.** A: According to the presence or absence of clonorchiasis; B: According to the place of residence.

diagnosis of clonorchiasis was based not only on positive laboratory findings, but also on presence of peripheral intrahepatic bile duct dilatation and clonorchiasis documented in medical records. Of 3080 patients admitted with gastrointestinal symptoms, 12.9% had been diagnosed with clonorchiasis. It is apparent that clonorchiasis is still prevalent in the Korean population. In the past, epidemiological studies of clonorchiasis in Korea have been based on stool examination and/or intradermal tests[11,12]. One epidemiological study in 1969

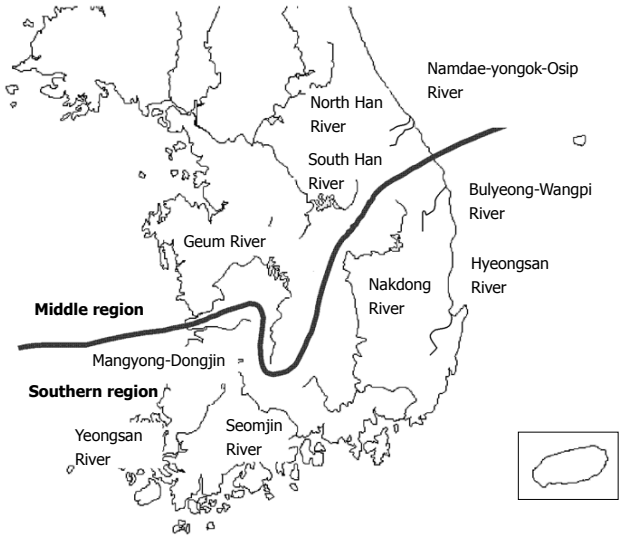


**Figure 2 Rivers in South Korea.** The basins of rives are divided into middle and southern regions.

utilizing cellophane thick smear revealed that 11.6% of 3880 subjects were infected with *C. sinensis*[25]. Another study in 1973 using an intradermal test showed an infection rate of 21.1%[5]. In 1981, stool examination of 13 000 Koreans from seven river basins demonstrated a clonorchiasis infection rate of 21.5%. From national surveys of clonorchiasis done every 5 years since 1971, the Ministry of Health and Welfare have reported an infection rate of 1.8%-4.6% in the Korean population[2]. Although the present study enrolled patients admitted with gastrointestinal symptoms, clonorchiasis seemed still prevalent with an infection rate of 12.9%. About one out of five patients who had ingested raw freshwater fish had clonorchiasis. Also, 6.5% of those without a history of raw freshwater fish ingestion had clonorchiasis, which implied that there might be routes of infection other than ingestion of raw freshwater fish or shellfish.

In a previous study, people from river basins in southern region of the Korean Peninsula showed higher infection rates when compared to those from river basins in the middle region (17.3%-40.2% *vs* 8.0%-17.3%, respectively)[10]. The results of the present study were similar to those of the previous study. While 33.8% of patients from river basins in southern region were infected with clonorchiasis, only 8.3% of those from the middle region were infected.

Among various methods used to diagnose clonorchiasis in the present study, intradermal tests showed the highest sensitivity of 74.5%, followed by detection of serum antibodies using ELISA, with a sensitivity of 46.4%. These two methods are limited by cross-reactivity and low specificity. Stool examination and bile cytology for adult worms and/or eggs have high specificity, but low sensitivity of 10%-12%. Radiological findings of intrahepatic bile duct dilatation also showed low sensitivity of 11.5%. In order to increase sensitivity, more than two diagnostic studies are needed.

Eosinophilia was found in 16.7% of patients with infection, while it was found in 9.6% of patients without clonorchiasis. Mean level of serum alkaline phosphatase was 304 U/L in patients with infection and 234.4 U/L in those without clonorchiasis. When laboratory tests during admission show eosinophilia with elevated alkaline phosphatase, clonorchiasis should be considered. There was no significant difference regarding other laboratory tests such as AST, ALT, GGT, and total bilirubin. Therefore, clonorchiasis cannot be excluded by liver function test only.

Adult worms of *C. sinensis* attach themselves with suckers to the walls of peripheral intrahepatic bile ducts. Long-term infection with *C. sinensis* is associated with various hepatobiliary diseases. It has been reported that cholangiocarcinoma has originated from papillary or adenomatous hyperplasia of the bile ducts infected with *C. sinensis*[26]. In a recent case-control study of Korean patients, peripheral intrahepatic bile dilatation or positive serum antibodies has been a risk factor for cholangiocarcinoma[27]. In the present study, there was no association of cholangitis and bile duct stones with clonorchiasis. Even after dividing bile duct stones into intrahepatic, extrahepatic, and gallbladder stones, there was no association between bile duct stones and clonorchiasis. In the present study, in patients admitted with gastrointestinal symptoms, cholangitis and bile ducts stones were present in 9% and 26.7% of patients without clonorchiasis. This may explain the absence of association of these diseases with clonorchiasis. Neither gallstone pancreatitis nor hepatitis of various causes was associated with clonorchiasis. Also, hepatocellular carcinoma and gallbladder cancer showed no association with clonorchiasis. Similar to results of other studies[26,27], cholangiocarcinoma was associated with the presence of clonorchiasis.

River basins of the southern region showed a higher infection rate of *C. sinensis* than those of the middle region (33.8% *vs* 8.6%, $P = 0.000$). Also, river basins of the southern region showed a higher prevalence rate of cholangiocarcinoma compared to those of the middle region (7.0% *vs* 3.4%, $P = 0.007$). The odds ratio of patients with clonorchiasis for cholangiocarcinoma was 4.136 (95% CI, 1.422-12.030) in the southern region and 1.961 (95% CI, 1.020-3.773) in the middle region. Even after correction for regional influence, the odds ratio was 2.289. According to these data, there was a strong correlation between clonorchiasis and cholangiocarcinoma. These findings were similar to the results of a previous study on the Korean population[28].

Initial ingestion of raw freshwater fish or shellfish dated back to 20 years ago in 70.8% of 24 patients with clonorchiasis and cholangiocarcinoma. Long-term infestation with *C. sinensis* is associated with development of cholangiocarcinoma, therefore, clonorchiasis should be treated as soon as possible when suspected. In this prospective multicenter nationwide study, prevalence of clonorchiasis and the association between clonorchiasis and hepatobiliary diseases in the Korean population were evaluated. Unlike other intestinal nematode infections, clonorchiasis is still prevalent. This seems to result from the habit of raw freshwater fish and/or

shellfish ingestion. Since prevalence of clonorchiasis in river basins of the southern region was higher than other parts, there is an urgent need for public education to prevent further raw freshwater fish or shellfish ingestion. Also, the presence of clonorchiasis was associated with cholangiocarcinoma and is a risk factor for cholangiocarcinoma.

## COMMENTS

### Background

Clonorchiasis is an infection caused by the parasite *C. sinensis*, and has been one of the most important endemic diseases in eastern Asia. Clonorchiasis can cause a variety of gastrointestinal diseases such as bile duct obstruction, acute cholangitis, hepatolithiasis, and cholangiocarcinoma.

### Research frontiers

Important areas in the research field related to this article are development of more rapid and convenient diagnostic modalities and antihelminthic vaccines, and investigation of mechanisms by which *C. sinensis* causes cellular injury.

### Innovations and breakthroughs

Despite public efforts and education, clonorchiasis is still present in the Korean population, and more so in certain regions of the country. Also, clonorchiasis is associated with cholangiocarcinoma and is a risk factor for cholangiocarcinoma.

### Applications

To prevent clonorchiasis and cholangiocarcinoma associated with this parasite infection, there is a need for public education to prevent further raw freshwater fish or shellfish ingestion.

### Terminology

Acute cholangitis is inflammation of bile duct that can cause fever, abdominal pain and abnormal blood test results. Hepatolithiasis refers to stone formation inside the liver. Cholangiocarcinoma is a certain type of liver cancer that originates from bile duct.

### Peer review

This study showed an impressive epidemiology and way of infection of clornochiasis in riverside populations of Korea. The authors collected data from 26 hospitals in only 1 mo. This is an important contribution to the etiology of cholangiocarcionoma in this part of the world. Also, the results of this study can serve as a basis for public health initiatives for prevention and mass treatment of clonorchiasis.

## REFERENCES


1 **Furuta GT**, Liacouras CA, Collins MH, Gupta SK, Justinich C, Putnam PE, Bonis P, Hassall E, Straumann A, Rothenberg ME. Eosinophilic esophagitis in children and adults: a systematic review and consensus recommendations for diagnosis and treatment. *Gastroenterology* 2007; **133**: 1342-1363

2 **Rothenberg ME**. Eosinophilic gastrointestinal disorders (EGID). *J Allergy Clin Immunol* 2004; **113**: 11-28; quiz 29

3 **Ona FV**, Dytoc JN. Clonorchis-associated cholangiocarcinoma: a report of two cases with unusual manifestations. *Gastroenterology* 1991; **101**: 831-839

4 Infection with liver flukes (Opisthorchis viverrini, Opisthorchis felineus and Clonorchis sinensis). *IARC Monogr Eval Carcinog Risks Hum* 1994; **61**: 121-175

5 **Rim HJ**. Clonorchiasis in Korea. *Kisaengchunghak Chapchi* 1990; **28** Suppl: 63-78

6 **Ministry of Health and Welfare and Korean Association of Health**. Prevalence of intestinal parasitic infections in Korea. The sixth report; 1997; Seoul, Korea

7 **Kim SS**, Han MH, Park SG, Lim HS, Hong ST. [A survey on the epidemiological factors of clonorchiasis in the Pohang industrial belt along the Hyungsan river, Kyongsangbuk-do] *Kisaengchunghak Chapchi* 1990; **28**: 213-219

8 **Min DY**, Ahn MH, Kim KM, Kim CW. [Intestinal parasite survey in Seoul by stool examination at Hanyang University Hospital] *Kisaengchunghak Chapchi* 1986; **24**: 209-212

9 **Choi BI**, Park JH, Kim YI, Yu ES, Kim SH, Kim WH, Kim CY, Han MC. Peripheral cholangiocarcinoma and clonorchiasis: CT findings. *Radiology* 1988; **169**: 149-153

10 **Seo BS**, Lee SH, Cho SY, Chai JY, Hong ST, Han IS, Sohn JS, Cho BH, Ahn SR, Lee SK, Chung SC, Kang KS, Shim HS, Hwang IS. An Epidemiologic Study On Clonorchiasis And Metagonimiasis In Riverside Areas In Korea. *Kisaengchunghak Chapchi* 1981; **19**: 137-150

11 **Choi MH**, Ge T, Yuan S, Hong ST. Correlation of egg counts of Clonorchis sinensis by three methods of fecal examination. *Korean J Parasitol* 2005; **43**: 115-117

12 **Shin BM**, Choi KI. Diagnostic significance of intradermal test compared with radiologic findings for clonorchiasis. *Korean J Clin Pathol* 2000; **20**: 81-86

13 **Kim SI**. A Clonorchis sinensis-specific antigen that detects active human clonorchiasis. *Korean J Parasitol* 1998; **36**: 37-45

14 **Choi BI**, Han JK, Hong ST, Lee KH. Clonorchiasis and cholangiocarcinoma: etiologic relationship and imaging diagnosis. *Clin Microbiol Rev* 2004; **17**: 540-552, table of contents

15 **Hou PC**. The pathology of Clonorchis sinensis infestation of the liver. *J Pathol Bacteriol* 1955; **70**: 53-64

16 **Lim JH**. Oriental cholangiohepatitis: pathologic, clinical, and radiologic features. *AJR Am J Roentgenol* 1991; **157**: 1-8

17 **Huang MH**, Chen CH, Yen CM, Yang JC, Yang CC, Yeh YH, Chou DA, Yueh SK, Yang YY, Nien CK. Relation of hepatolithiasis to helminthic infestation. *J Gastroenterol Hepatol* 2005; **20**: 141-146

18 **Kubo S**, Kinoshita H, Hirohashi K, Hamba H. Hepatolithiasis associated with cholangiocarcinoma. *World J Surg* 1995; **19**: 637-641

19 **Hou PC**. The relationship between primary carcinoma of the liver and infestation with Clonorchis sinensis. *J Pathol Bacteriol* 1956; **72**: 239-246

20 **Belamaric J**. Intrahepatic bile duct carcinoma and C. sinensis infection in Hong Kong. *Cancer* 1973; **31**: 468-473

21 **Chung CS**, Lee SK. An epidemiological study of primary liver carcinomas in pusan area with special reference to clonorchiasis. *Korean J Pathol* 1976; **10** :33-64

22 **Choi BI**, Han JK. Other parasitic diseases. In: Okuda K, Mitchell DG, Itai Y, Ariyama J, eds. Hepatobiliary disease pathophysiology and imaging. London: Blackwell Science, 2001: 579-581

23 **Callea F**, Sergi C, Fabbretti G, Brisigotti M, Cozzutto C, Medicina D. Precancerous lesions of the biliary tree. *J Surg Oncol Suppl* 1993; **3**: 131-133

24 **Teoh TB**. A study of gall-stones and included worms in recurrent pyogenic cholangitis. *J Pathol Bacteriol* 1963; **86**: 123-129

25 **Seo BS**, Rim HJ, Loh IK, Lee SH, Cho SY, Park SC, Bae JW, Kim JH, Lee JS, Koo BY, Kim KS. [Study On The Status Of Helminthic Infections In Koreans] *Kisaengchunghak Chapchi* 1969; **7**: 53-70

26 **Lee JH**, Yang HM, Bak UB, Rim HJ. Promoting role of Clonorchis sinensis infection on induction of cholangiocarcinoma during two-step carcinogenesis. *Korean J Parasitol* 1994; **32**: 13-18

27 **Choi D**, Lim JH, Lee KT, Lee JK, Choi SH, Heo JS, Jang KT, Lee NY, Kim S, Hong ST. Cholangiocarcinoma and Clonorchis sinensis infection: a case-control study in Korea. *J Hepatol* 2006; **44**: 1066-1073

28 **Lim MK**, Ju YH, Franceschi S, Oh JK, Kong HJ, Hwang SS, Park SK, Cho SI, Sohn WM, Kim DI, Yoo KY, Hong ST, Shin HR. Clonorchis sinensis infection and increasing risk of cholangiocarcinoma in the Republic of Korea. *Am J Trop Med Hyg* 2006; **75**: 93-96


**S- Editor** Li DL **L- Editor** Kerr C **E- Editor** Zheng XM

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.95

*World J Gastroenterol* 2009 January 7; 15(1): 95-101
***World Journal of Gastroenterology*** ISSN 1007-9327


*ORIGINAL ARTICLES*

# Treatment of chronic proliferative cholangitis with c-myc shRNA

Fu-Yu Li, Nan-Sheng Cheng, Jing-Qiu Cheng, Hui Mao, Li-Sheng Jiang, Ning Li, Sheng He

Fu-Yu Li, Nan-Sheng Cheng, Li-Sheng Jiang, Ning Li, Sheng He, Department of Hepatobiliary Surgery, West China Hospital of Sichuan University, Chengdu 610041, Sichuan Province, China
Jing-Qiu Cheng, Hui Mao, Key Laboratory of Transplantation Engineering and Immunology, West China Hospital of Sichuan University, Chengdu 610041, Sichuan Province, China
Author contributions: Li FY and He S designed the research; Cheng NS and Jiang LS established the animal model and immunohistochemistry; Li N performed the histochemistry and biochemistry; Cheng JQ and Mao H performed the real-time PCR and Western blot.
Correspondence to: Fu-Yu Li, Department of Hepatobiliary Surgery, West China Hospital of Sichuan University, Chengdu 610041, Sichuan Province, China. lfy_74@vip.163.com
Telephone: +86-28-85422465 Fax: +86-28-85164029
Received: April 19, 2008 Revised: July 14, 2008
Accepted: July 21, 2008
Published online: January 7, 2009

## Abstract

**AIM:** To investigate the feasibility and effectiveness of c-myc shRNA in inhibiting the hyperplastic behavior and lithogenic potentiality of chronic proliferative cholangitis (CPC), in order to prevent stone recurrence and biliary restenosis.

**METHODS:** An animal model of CPC was established by giving intralumenally 0.5 mL of c-myc shRNA. Then, the effects of c-myc shRNA on hyperplastic behavior and lithogenic potentiality of CPC were evaluated by histological observation, immunohistochemistry, real-time PCR and Western blotting for c-myc, proliferating cell nuclear antigen (PCNA), procollagen Ⅲ, mucin 5AC, enzymatic histochemistry for β-glucuronidase, and biochemistry for hydroxyproline in the diseased bile duct.

**RESULTS:** Treatment with c-myc shRNA efficiently suppressed the hyperplasia of biliary epithelium, submucosal gland, and collagen fiber by inhibiting mRNA and protein expression of c-myc. More importantly, it decreased the lithogenic potentiality of CPC by inhibiting the expression of mucin 5AC and the secretion of endogenous β-glucuronidase. Further investigation indicated that c-myc shRNA-3 had a better inhibitory effect on CPC.

**CONCLUSION:** Treatment with c-myc shRNA-3 can control CPC and reduce the lithogenic potentiality of CPC.



**Key words:** Chronic proliferative cholangitis; Hepatolithiasis; Recurrence; C-myc; Prevention

**Peer reviewer:** Tom H Karlsen, MD, Institute of Immunology, Rikshospitalet University Hospital, N-0027 Oslo, Norway

Li FY, Cheng NS, Cheng JQ, Mao H, Jiang LS, Li N, He S. Treatment of chronic proliferative cholangitis with c-myc shRNA. *World J Gastroenterol* 2009; 15(1): 95-101 Available from: URL: http://www.wjgnet.com/1007-9327/15/95.asp DOI: http://dx.doi.org/10.3748/wjg.15.95

## INTRODUCTION

Hepatolithiasis, a commonly encountered disease in the Asian-Pacific region, is more refractory to surgical treatment than most other benign diseases of the biliary tract[1,2]. Its therapeutic challenges include the difficulty in completely correcting the biliary stenosis and a high rate of stone recurrence, leading to a re-operative rate of 37.1%-74.4%[1-3]. Unfortunately, how to prevent the stone recurrence and biliary restenosis is still a problem to be solved in hepatobiliary surgery. Since hepatectomy cannot eliminate the possibility of stone recurrence, 16% of postoperative patients may develop new stones at other sites[3-5]. Therefore, surgery itself cannot achieve its long-term therapeutic effectiveness on hepatolithiasis[2-7]. In recent years, with a deeper understanding of the pathological changes in hepatolithiasis, the high stone recurrence rate and biliary restenosis rate in hepatolithiasis patents have been found to be related to the residual chronic proliferative cholangitis (CPC) after operation[8-11].

In the past, we paid too much attention to the improvement of surgical skills for treatment of hepatolithiasis, but failed to sufficiently recognize the connection of CPC to the formation of intraheptic calculi, and to pay enough attention to the treatment of residual CPC after removal of stones[3,12-14]. Thus, even though the stone is removed completely and the biliary tract stenosis is corrected, the residual CPC induced by the stone would still exist persistently and

extensively, which would facilitate formation of new stones by producing mucoprotein or by changing the lithogenic pathology and biliary stricture, which leads to cholestasis. Therefore, treatment of CPC after operation might increase its curative effect on hepatolithiasis. Unfortunately, there is no definitely effective therapy for CPC at present[15-17]. Since hepatolithiasis is a chronic proliferative disease, we designed this study to investigate the preliminary effectiveness of c-myc shRNA on hyperplastic behavior and lithogenic potentiality of CPC, expecting to prevent stone recurrence or biliary restenosis by controlling or eradicating CPC[18,19].

## MATERIALS AND METHODS

### Study design and surgical procedure

A total of 56 Sprague-Dawley rats weighing 220-250 g were randomly divided into six groups. (1) CPC group ($n$ = 10) in which a 5-0 nylon thread was inserted into the common bile duct through the duodenal papilla[20]. (2) Four c-myc shRNA treatment groups ($n$ = 10), in which a nylon thread was used as a guidwire and a 20 G veinous retaining needle was introduced into the common bile duct. Then, a total of $3 \times 10^9$ plaque-forming units (pfu) of four kinds of c-myc shRNA (shRNA-1, shRNA-2, shRNA-3, and a negative control sequence provided by Genesil Biotechnology Co. Ltd, Wuhan, China) in a total volume of 0.5 mL mediated by liposome 2000 (Invitrogen, USA) were respectively infused. (3) Sham operation (SO) group ($n$ = 6) in which the common bile duct was dissected only. Transfection efficiency was detected after 48 h. One week later, all the rats were sacrificed with their common bile ducts removed, and fixed in liquid nitrogen and 10% formaldehyde for further tests.

### Immunohistochemistry or immunofluorescence staining of c-myc and mucin 5AC

The avidin-biotin-peroxidase complex method was used to detect the expression of c-myc. Briefly, tissue sections were incubated overnight at 4℃ with primary antibody (Zymed Co, USA), followed by incubation with biotinylated second antibody for 1 h at 37℃. Expression of mucin 5AC was detected with immunofluorescence staining. Briefly, cryostat slides were incubated with primary antibodies (Santa Cruz Biotechnology, USA) at 4℃ overnight. After incubation with secondary antibody labeled with fluorescein at 37℃ for 1 h, the expression of mucin 5AC was observed under a fluorescent microscope at once.

### Detection of c-myc and mucin 5AC by real time-PCR

Total RNA was extracted from the bile duct wall using Trizol (Gibco, USA). Reverse transcription was performed according to the manufacturer's instructions (Gibco, USA). Real-time analysis was performed on the cycle (Bio-Rad, Germany) using SYBR Green (TaKaRa, Dalian, China). Levels of target gene expression in the tested samples were normalized to the corresponding GAPDH mRNA transcript.

### Detection of c-myc, PCNA and procollagen Ⅲ by Western blotting

After protein concentration was determined, 100 μg protein was loaded in each lane and subjected to 8% SDS-PAGE gel electrophoresis, then transferred to nitrocellulose membrane for immunoblotting. The blots were probed with antibodies against c-myc, proliferating cell nuclear antigen (PCNA) and procollagen Ⅲ (dilution 1:1000, Zymed Co, USA) overnight at 4℃. After washed with TBST, the membrane was incubated for 2 h with HRP-conjugated rabbit anti-rat secondary antibody (dilution 1:3000). Immunoreactive bands were visualized with enhanced chemiluminescence and captured on a X-ray film.

### Enzymatic histochemical staining of endogenous β-glucuronidase in bile duct wall

The method of Ballantyne was used to perform enzymatic histochemical staining using nephthol-AS-SI-β-D-glucuronide (β-G; Sigma) as the substrate[21]. Briefly, cryostat sections were incubated at 37℃ for 1 h in a pH-4.95 solution containing the β-G substrate and hepatocyte nuclei were counterstained with methyl green for 3 min. Positive expression of endogenous β-G was observed as a rose-red signal in cytoplasm.

### Assessment of hydroxyproline content (mg/g of bile duct)

Connective tissue in the blie duct was estimated by quantifying hydroxyproline, an amino acid found primarily in collagen, the principal component of extracellular matrix. The hydroxyproline content was detected as previously described[22].

### Statistical analysis

All the data were presented as mean ± SD and analyzed using the SPSS10.0 software. Statistical analysis was conducted using the non-parametric ANOVA to evaluate the variance among more than two groups. $P < 0.05$ was considered statistically significant.

## RESULTS

### Histopathological examination

Biliary epithelium mucosa in the CPC group was histologically characterized by papillary hyperplasia projection, which led to obstruction of the bile duct lumen. Analogical histological changes were observed in the negative control group. In contrast, proliferative degrees of biliary epithelium, submucosal gland, and collagen fiber in the three c-myc shRNA treatment groups were obviously lower than those in the CPC group, especially in the c-myc shRNA-3 group. More specifically, the degree of fibrous thickening in the diseased bile duct wall was obviously relieved after treatment with c-myc shRNA (Figure 1A-C).

### Determination of c-myc and PCNA by immunohistochemistry, RT-PCR and Western blotting

To determine the anti-proliferative effect of c-myc

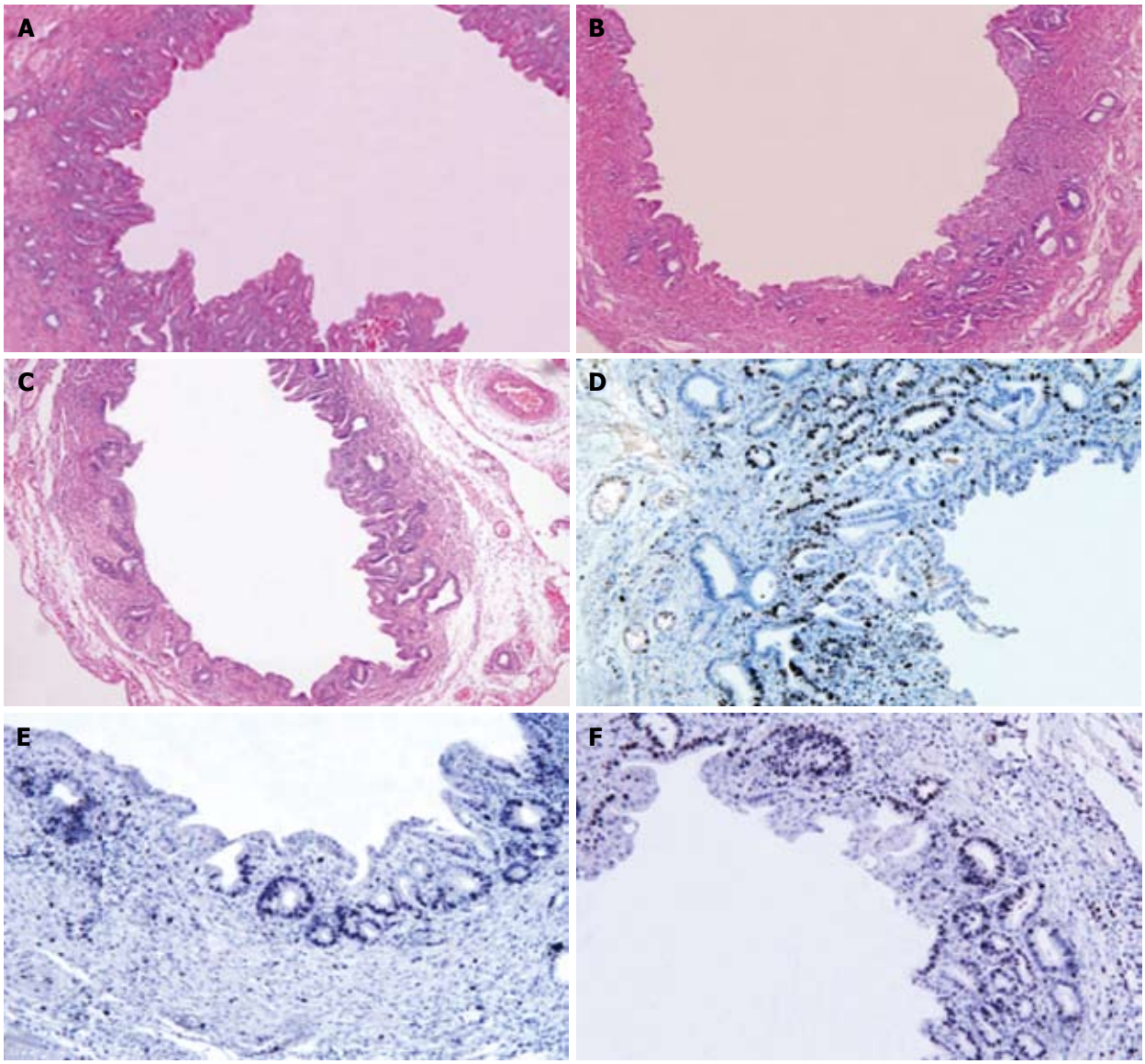


**Figure 1 HE staining and immunohistochemistry for c-myc in CPC group (A, D), c-myc shRNA-3 treatment group (B, E), and c-myc shRNA-2 treatment group (C, F).** Briefly, treatment with c-myc shRNA, especially with c-myc shRNA-3, can efficaciously inhibit hyperplasia of biliary epithelium, submucosal gland, collagen fiber, and down-regulate c-myc expression (A-C, × 50; D-F, × 100).

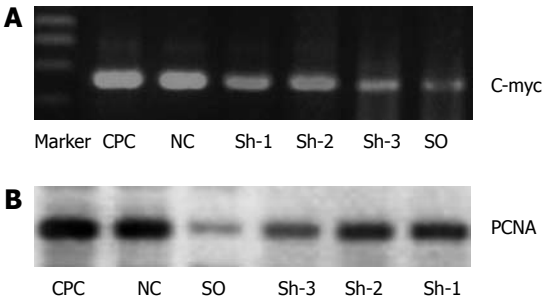


**Figure 2 Real-time PCR (A) and Western blot (B) analysis of c-myc and PCNA expression in biliary duct wall.**

shRNA, we compared the expression of c-myc, PCNA mRNA and protein in the diseased bile duct wall, revealing a remarkable decrease of c-myc, PCNA mRNA and protein expression in the c-myc shRNA treatment groups ($P < 0.0001$ and $P = 0.001$, respectively), c-myc shRNA-3 treatment group, but still significantly higher than that in the SO group ($0.97 \pm 0.28$ *vs* $0.22 \pm 0.09$, $P < 0.0001$; $0.82 \pm 0.22$ *vs* $0.45 \pm 0.08$, $P = 0.011$). The mRNA and protein levels of c-myc, and PCNA did not differ significantly between the c-myc shRNA-1 and the c-myc shRNA-2 treatment groups. Also, the difference between the CPC group and the negative control group was not statistically significant (Table 1, Figures 1D-F and 2).

### *Detection of mucin 5AC expression by RT-PCR and immunohistochemistry*

To probe the influence of c-myc shRNA on lithogenic potentiality of CPC, RT-PCR and Immunohistochemistry analysis of mucin 5AC were performed, showing a significant decrease of mucin 5AC mRNA and protein expression in the c-myc shRNA treatment groups, which was even more prominent in the c-myc shRNA-3 treatment group ($0.42 \pm 0.16$), when compared with the

**Table 1 HYP content and expression level of c-myc, PCNA, mucin5AC, procollagen Ⅲ**

| | CPC | shRNA-1 | shRNA-2 | shRNA-3 | NC | SO |
|---|---|---|---|---|---|---|
| c-myc/GAPDH | 6.21 ± 1.97 | 2.37 ± 0.77 | 2.93 ± 0.84 | 0.97 ± 0.28 | 6.57 ± 2.11 | 0.22 ± 0.09 |
| *P* | < 0.0001 | 0.001 | < 0.0001 | | < 0.0001 | < 0.0001 |
| PCNA/β-actin | 3.20 ± 0.81 | 1.52 ± 0.28 | 1.83 ± 0.42 | 0.82 ± 0.22 | 2.71 ± 0.63 | 0.45 ± 0.08 |
| *P* | < 0.0001 | 0.013 | 0.005 | | 0 | 0.011 |
| Mucin5AC/GAPDH | 1.87 ± 0.47 | 0.96 ± 0.28 | 1.05 ± 0.30 | 0.42 ± 0.16 | 1.69 ± 0.41 | 0.12 ± 0.04 |
| *P* | < 0.0001 | 0.004 | 0.002 | | < 0.0001 | < 0.0001 |
| Procol-Ⅲ/β-actin | 4.79 ± 1.27 | 2.83 ± 0.85 | 2.39 ± 0.58 | 1.23 ± 0.35 | 5.40 ± 1.76 | 0.52 ± 0.13 |
| *P* | 0 | 0.001 | 0.006 | | < 0.0001 | 0.001 |
| HYP content | 1.29 ± 0.32 | 0.78 ± 0.16 | 0.83 ± 0.18 | 0.55 ± 0.13 | 1.41 ± 0.36 | 0.39 ± 0.08 |
| *P* | 0.003 | 0.041 | 0.028 | | 0.001 | 0.038 |

*P* value was compared with c-myc shRNA-3 treatment group.

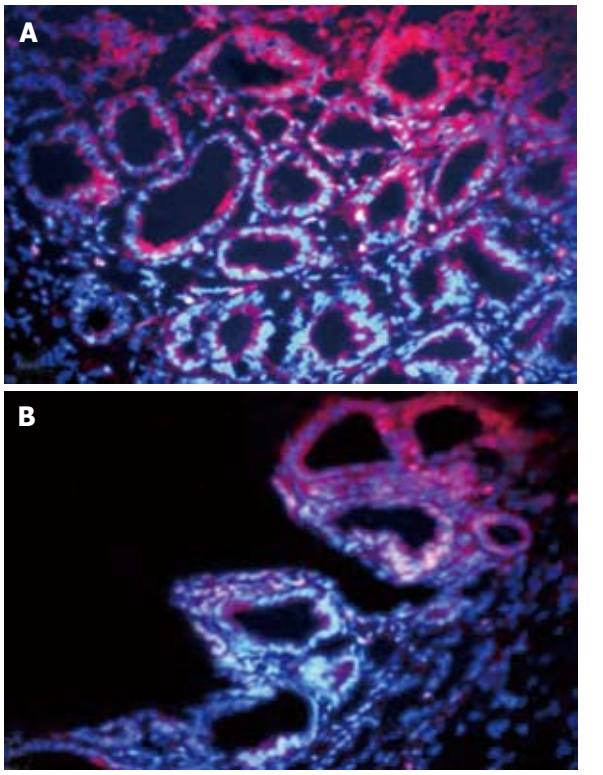


**Figure 3 Immunofluorescence (A, B) and RT-PCR (C) analysis of mucin 5AC in bile duct wall.** CPC group (A), c-myc shRNA-3 treatment group (B). Briefly, treatment with c-myc shRNA-3 can result in a more prominent down-regulation of mucin 5AC expression (A and B, × 400).

c-myc shRNA-1 and c-myc shRNA-2 treatment groups (0.96 ± 0.28, 1.05 ± 0.30; $P = 0.004$ and $P = 0.002$, Table 1, Figure 3).

### *Enzymatic histochemical staining of endogenous β-G*

To further explore the influence of c-myc shRNA on pigment stone formation, we performed enzymatic histochemical staining of endogenous β-G, which showed a significant increase of endogenous β-G expression in the CPC group. However, β-G expression was significantly decreased in the c-myc shRNA-1, shRNA-2, and shRNA-3 treatment groups, but the difference was not significant in the three groups (Figure 4).

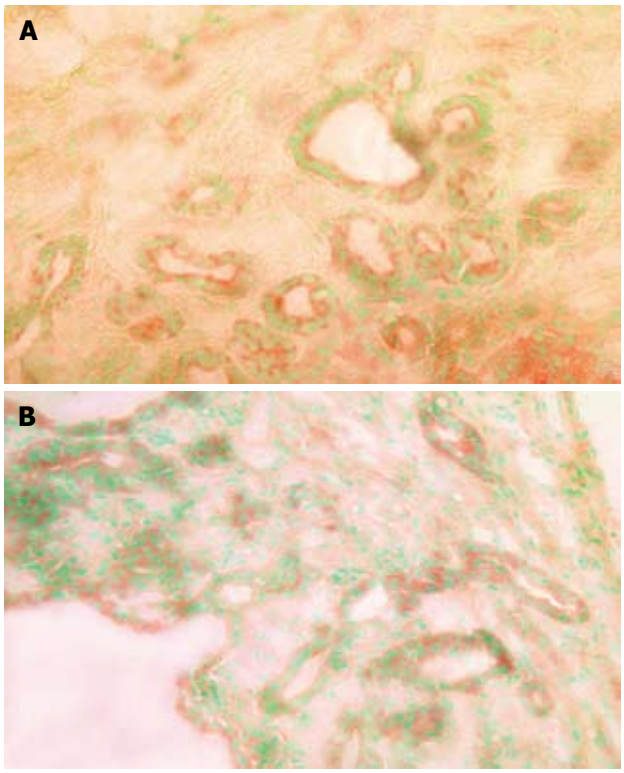


**Figure 4 Enzymatic histochemistry staining of endogenous β-G (cryostat section, × 400) in CPC group (A) and c-myc shRNA-3 treatment group (B).** A notable reduction of endogenous β-G was observed in the bile duct wall following c-myc shRNA treatment.

### *Western blot analysis of procollagen Ⅲ in biliary duct wall*

To explore the influence of c-myc shRNA on collagen fiber proliferation, we examined the procollagen Ⅲ protein expression in the diseased bile duct. The bile duct in the CPC group displayed a very high level of procollagen Ⅲ protein expression. However, the expression of procollagen Ⅲ protein was significantly decreased after treatment with c-myc shRNA, which was even more prominent in the c-myc shRNA-3 treatment group (1.23

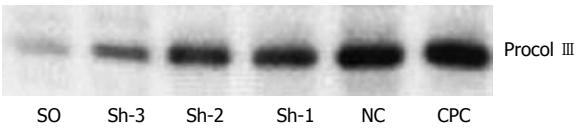


**Figure 5 Western blot analysis of procollagen Ⅲ expression in biliary duct wall.**

± 0.35 *vs* 2.83 ± 0.85 for the shRNA-1 treatment group, 2.39 ± 0.58 for the shRNA-2 treatment group, $P = 0.001$ and $P = 0.006$), although it was signifcantly higher than that in the SO group (Table 1, Figure 5).

### *Assessment of hydroxyproline (HYP) content (mg/g of bile duct)*

Subsequently, quantitative determination of connective tissue in the diseased bile duct was performed, which showed that the HYP content in the CPC and negative control groups was approximately increased up to three-fold, when compared with the SO group (1.29 ± 0.32 and 1.41 ± 0.36 *vs* 0.39 ± 0.08). However, after treatment with c-myc shRNA, the HYP content was significantly decreased, especially in the c-myc shRNA-3 treatment group (0.55 ± 0.13 *vs* 0.78 ± 0.16 for the shRNA-1 treatment group, 0.83 ± 0.18 for the shRNA-2 treatment group, $P = 0.041$ and 0.028, respectively; Table 1).

## DISCUSSION

Since hepatolithiasis is a common disease in Asia and its etiology remains obscure, it is quite difficult to treat and prevent this disease from its lithogenesis. According to its pathology, 75%-100% of hepatolithiasis patients in Asia are characterized by CPC. Increased attention has been paid in recent years to the cause-and-effect relationship of CPC and formation of stones[10,15-17]. Firstly, the stone can not only bring an inflammatory full-thickness penetrating damage to the local biliary duct mucosa in the obstructed part, but also lead to a down-stream mucosal injury in the distant bile duct as the stone and inflamed bile move downwards. Therefore, damage to the related biliary ducts caused by the stone is extensive, and not merely localized to the stone resident part. This is why stone-induced CPC exists widely after removal of the stone[11-16]. On the other hand, recurrent attacks of CPC would in turn facilitate formation of new stones by causing such lithogenic pathology changes as biliary stricture and biliary infection. Furthermore, recurrent CPC can be directly involved in formation of stones by producing mucoglycoproteins secreted by the proliferated submucosal gland. As we know, mucoglycoprotein is not only a contributing factor for intrahepatic stones but also a protein that can directly participate in formation of stone nucleation or reticular lithogenesis framework[23-26]. As mentioned above, a vicious cycle of CPC, biliary stricture and stone may develop. It is thereby reasonable that treatment of heaptolithiasis should be directed not only at removal of the stone and correction of the biliary stricture, but also at control of postoperative CPC, a key factor for this vicious cycle[12-16,19,20].

Recently, proto-oncogene c-myc has become an attractive target for anti-proliferative treatment of hypernomic proliferation diseases, because it is at the center of a transcription factor network that regulates cellular proliferation, replication, growth, differentiation, and apoptosis[27-30]. Specific blockage of the c-myc gene expression can partially inhibit cellular proliferation, thus efficiently preventing vascular restenosis after angioplasty by inhibiting endangium cell proliferation[27,28]. In this study, we also investigated the anti-proliferative effectiveness of c-myc shRNA on hypernomic proliferation behavior of CPC[17,18,29]. As expected, HE staining, immunohistochemistry, RT-PCR and Western blotting showed that treatment with c-myc shRNA could efficiently inhibit hyperplasia of the biliary epithelium, submucosal gland and collagen fiber by specifically blocking mRNA and protein expression of the proliferation-related gene c-myc and PCNA. The levels of c-myc, and PCNA mRNA and protein expression were much lower in the c-myc shRNA-3 treatment group than in the c-myc shRNA-1 and shRNA-2 treatment groups, which indicates that c-myc shRNA-3 may have a better anti-proliferative effect on CPC[9,15,27-30].

To analyze the effect of this gene therapy on the lithogenic potentiality of CPC, we compared the expression of mucin5AC and endogenous β-G in the diseased bile duct. Among the nine mucoglycoproteins identified so far, up-regulation of mucin5AC expression is considered to be closely related to the formation of stones[25,31], which is consistent with our findings. In the present study, the expression of mucin5AC mRNA and protein was significantly increased in the CPC group. However, the expression of mucin5AC was obviously decreased after treatment with c-myc shRNA, especially after treatment with c-myc shRNA-3, which suggests that c-myc shRNA can effectively inhibit the inactivation of such mucin genes as mucin5AC and secretion of muglycoprotein. It is noteworthy that reduced muglycoprotein helps decrease bile viscidity and aggregation or sedimentation of lithogenic ingredients in the bile, which might be significant in preventing stone recurrence[24,26,32]. The expression of endogenous β-G in the diseased bile duct was also obviously decreased after treatment with c-myc shRNA, which may be explained by the inhibitory effect of c-myc shRNA on the proliferation of biliary epithelium and submucosal gland. The inhibitory effect of c-myc shRNA on endogenous β-G would, to some degree, be helpful in preventing postoperative biliary stone recurrence[21,33].

Considering the potential anti-proliferative effect of c-myc shRNA on collagen fiber proliferation, c-myc shRNA treatment may prevent biliary tract restenosis secondary to CPC[8,9]. In our study, HE staining showed that collagen fiber proliferation was significantly lower in the diseased bile duct after treatment with c-myc shRNA than CPC, which suggests that the incidence of biliary tract stricture secondary to CPC can be reduced. Further comparison displayed that treatment with c-myc shRNA-3 demonstrated a better inhibitory effect on procollagen Ⅲ protein and HYP content than treatment

with shRNA-2 and shRNA-1, which indicates that c-myc shRNA-3 has a bright future in preventing bile duct fibrosis and biliary stricture[20,28,29,34].

In conclusion, anti-proliferative treatment with c-myc shRNA is likely to open a new feasible approach to the treatment of postoperative residual CPC. Furthermore, the inhibitory effects of c-myc shRNA on the lithogenic potentiality of CPC can assist in reducing postoperative recurrence of intrahepatic calculi. More importantly, this novel treatment would lay an experimental foundation of development of drugs for preventing stone recurrence after choledochoscopic lithotomy, at least in part, and reducing the incidence of reoperation and choledochoscopic lithotomy[2,13-18,20,30]. However, further study is needed on its long-term effect, related complications, and more efficient gene expression vectors before its clinical application[13,33,35].

## COMMENTS

### Background

In recent years, with a deeper understanding of the pathological changes in hepatolithiasis, the high stone recurrence rate has gradually been recognized, and is currently considered due to the postoperative chronic proliferative cholangitis (CPC). In this study, we investigated the inhibitory effect of c-myc shRNA on hyperplastic behavior and lithogenic potentiality of CPC.

### Research frontiers

Multiple factors for lithogenesis of intrahepatic stones have brought enormous difficulties to its prevention and treatment and 75%-100% of hepatolithiasis patients in the Asian-Pacific regions are pathologically characterized by CPC, a key factor for preventing calculus recurrence. Treatment of CPC after operation might assist in increasing the curative effect on hepatolithiasis.

### Innovations and breakthroughs

The high recurrence rate of intrahepatic stones is still a problem to be solved in hepatobiliary surgery. Since there is no effective medication for preventing stone recurrence after choledochoscopic lithotomy and for testing its pathology, stone recurrence and reoperation cannot be avoided. Our preliminary results showed that c-myc shRNA can inhibit hyperplastic behavior and lithogenic potentiality of CPC, thus laying an experimental foundation of development of drugs for preventing stone recurrence.

### Applications

Intralumenal administration of c-myc shRNA is a promising therapeutic approach to CPC, and might assist in reducing the lithogenic potentiality of CPC.

### Peer review

The authors of this paper investigated the efficacy of c-myc shRNA in ameliorating histological and molecular manifestations in an animal model of hepatolithiasis. The study was well designed and its findings are interesting and informative.

## REFERENCES


1 **Li SQ**, Liang LJ, Peng BG, Lai JM, Lu MD, Li DM. Hepaticojejunostomy for hepatolithiasis: a critical appraisal. *World J Gastroenterol* 2006; **12**: 4170-4174

2 **Chijiiwa K**, Yamashita H, Yoshida J, Kuroki S, Tanaka M. Current management and long-term prognosis of hepatolithiasis. *Arch Surg* 1995; **130**: 194-197

3 **Chen DW**, Tung-Ping Poon R, Liu CL, Fan ST, Wong J. Immediate and long-term outcomes of hepatectomy for hepatolithiasis. *Surgery* 2004; **135**: 386-393

4 **Kim BW**, Wang HJ, Kim WH, Kim MW. Favorable outcomes of hilar duct oriented hepatic resection for high grade Tsunoda type hepatolithiasis. *World J Gastroenterol* 2006; **12**: 431-436

5 **Hwang JH**, Yoon YB, Kim YT, Cheon JH, Jeong JB. Risk factors for recurrent cholangitis after initial hepatolithiasis treatment. *J Clin Gastroenterol* 2004; **38**: 364-367

6 **Buscaino GA**, Striano S. [Neurochemistry of convulsiveness. Some neurobiological mechanisms of experimental and human epilepsy] *Acta Neurol* (Napoli) 1976; **31**: 413-423

7 **Freitas ML**, Bell RL, Duffy AJ. Choledocholithiasis: evolving standards for diagnosis and management. *World J Gastroenterol* 2006; **12:** 3162-3167

8 **Fujii H**, Yang Y, Matsumoto Y, Suda K. Current problems with intrahepatic bile duct stones in Japan--congenital biliary malformations as a cause. *Hepatogastroenterology* 1997; **44**: 328-341

9 **Geng ZM**, Yao YM, Liu QG, Niu XJ, Liu XG. Mechanism of benign biliary stricture: a morphological and immunohistochemical study. *World J Gastroenterol* 2005; **11**: 293-295

10 **De Palma GD**, Masone S, Rega M, Simeoli I, Salvatori F, Siciliano S, Maione F, Girardi V, Celiento M, Persico G. Endoscopic approach to malignant strictures at the hepatic hilum. *World J Gastroenterol* 2007; **13**: 4042-4045

11 **Terada T**, Nakanuma Y. Pathologic observations of intrahepatic peribiliary glands in 1,000 consecutive autopsy livers: IV. Hyperplasia of intramural and extramural glands. *Hum Pathol* 1992; **23**: 483-490

12 **Li FY**, Cheng JQ, Li N, He S, Zhang MM, Dong JH, Jiang LS, Cheng NS. Effectiveness of chemical biliary duct embolization for chemical hepatectomy. *J Gastroenterol Hepatol* 2006; **21**: 880-886

13 **Li F**, Cheng J, He S, Li N, Zhang M, Dong J, Jiang L, Cheng N, Xiong X. The practical value of applying chemical biliary duct embolization to chemical hepatectomy for treatment of hepatolithiasis. *J Surg Res* 2005; **127**: 131-138

14 **Li FY**, Cheng JQ, Mao H, Li N, Jiang LS, Cheng NS, Wu XW. Treatment of hepatolithiasis by endoscopic chemoembolization of the left hepatic duct. *Endoscopy* 2006; **38**: 845-847

15 **Terada T**, Nakanuma Y. Innervation of intrahepatic bile ducts and peribiliary glands in normal human livers, extrahepatic biliary obstruction and hepatolithiasis. An immunohistochemical study. *J Hepatol* 1989; **9**: 141-148

16 **Lu S**, Yan L, Rao L, Xia T, Gou J, Zhang S, Lei S. Down stream involvement of the bile duct in hepatolithiasis. *Chin Med J* (Engl) 2002; **115**: 62-64

17 **Nakanuma Y**, Yamaguchi K, Ohta G, Terada T. Pathologic features of hepatolithiasis in Japan. *Hum Pathol* 1988; **19**: 1181-1186

18 **Ehsan A**, Mann MJ. Antisense and gene therapy to prevent restenosis. *Vasc Med* 2000; **5**: 103-114

19 **Yamamoto K**. Intrahepatic periductal glands and their significance in primary intrahepatic lithiasis. *Jpn J Surg* 1982; **12**: 163-170

20 **Park SM**, Choi JW, Kim ST, Cho MC, Sung RH, Jang LC, Park JW, Lee SP, Park YH. Suppression of proliferative cholangitis in a rat model by local delivery of paclitaxel. *J Hepatobiliary Pancreat Surg* 2003; **10**: 176-182

21 **Ballantyne B**, Wood WG. Biochemical and histochemical observations on Beta-glucuronidase in the mammalian gallbladder. *Am J Dig Dis* 1968; **13**: 551-557

22 **Jamall IS**, Finelli VN, Que Hee SS. A simple method to determine nanogram levels of 4-hydroxyproline in biological tissues. *Anal Biochem* 1981; **112**: 70-75

23 **Weber A**, Huber W, Kamereck K, Winkle P, Voland P, Weidenbach H, Schmid RM, Prinz C. In vitro activity of moxifloxacin and piperacillin/sulbactam against pathogens of acute cholangitis. *World J Gastroenterol* 2008; **14**: 3174-3178

24 **Yang HM**, Wu J, Li JY, Gu L, Zhou MF. Role of nucleation of bile liquid crystal in gallstone formation. *World J Gastroenterol* 2003; **9**: 1791-1794

25 **Yamasaki T**, Nakayama F, Tamura S, Endo M. Characterization of mucin in the hepatic bile of patients with intrahepatic pigment stones. *J Gastroenterol Hepatol* 1992; **7**: 36-41

26 **Li N**, Xiao LJ, Chen SW, Li L, Xiao BL, Chen WB, Gao XK, Gu SJ. [Mucus histochemical study of bilirubin cholangiolithiasis in rabbit model] *Huaxi Yike Daxue Xuebao* 1989; **20**: 417-420
27 **Hoffman B**, Liebermann DA, Selvakumaran M, Nguyen HQ. Role of c-myc in myeloid differentiation, growth arrest and apoptosis. *Curr Top Microbiol Immunol* 1996; **211**: 17-27
28 **Biro S**, Fu YM, Yu ZX, Epstein SE. Inhibitory effects of antisense oligodeoxynucleotides targeting c-myc mRNA on smooth muscle cell proliferation and migration. *Proc Natl Acad Sci USA* 1993; **90**: 654-658
29 **Ehsan A**, Mann MJ. Antisense and gene therapy to prevent restenosis. *Vasc Med* 2000; **5**: 103-114
30 **Edelman ER**, Simons M, Sirois MG, Rosenberg RD. c-myc in vasculoproliferative disease. *Circ Res* 1995; **76**: 176-182
31 **Zen Y**, Harada K, Sasaki M, Tsuneyama K, Katayanagi K, Yamamoto Y, Nakanuma Y. Lipopolysaccharide induces overexpression of MUC2 and MUC5AC in cultured biliary epithelial cells: possible key phenomenon of hepatolithiasis. *Am J Pathol* 2002; **161**: 1475-1484
32 **Lee KT**, Liu TS. Altered mucin gene expression in stone-containing intrahepatic bile ducts and cholangiocarcinomas. *Dig Dis Sci* 2001; **46**: 2166-2172
33 **Chen CY**, Shiesh SC, Tsao HC, Lin XZ. Human biliary beta-glucuronidase activity before and after relief of bile duct obstruction: is it the major role in the formation of pigment gallstones? *J Gastroenterol Hepatol* 2000; **15**: 1071-1075
34 **Mitra AK**, Agrawal DK. Gene therapy of fibroproliferative vasculopathies: current ideas in molecular mechanisms and biomedical technology. *Pharmacogenomics* 2006; **7**: 1185-1198
35 **Bennett MR**, Schwartz SM. Antisense therapy for angioplasty restenosis. Some critical considerations. *Circulation* 1995; **92**: 1981-1993

**S- Editor** Zhong XY **L- Editor** Wang XL **E- Editor** Yin DH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.102

*World J Gastroenterol* 2009 January 7; 15(1): 102-105
***World Journal of Gastroenterology*** ISSN 1007-9327


*BRIEF ARTICLES*

# Seroepidemiology of hepatitis A virus in Kuwait

Jameela Alkhalidi, Bader Alenezi, Seham Al-mufti, Entisar Hussain, Haifa Askar, Nyingi Kemmer, Guy W Neff

Jameela Alkhalidi, Bader Alenezi, Seham Al-mufti, Entisar Hussain, Haifa Askar, Thuniyan Al-Ghanim, GI Center & University of Kuwait, PO Box 4077, Safat 13041, Kuwait
Nyingi Kemmer, Guy W Neff, University of Cincinnati, Cincinnati, OH 45267-0576, United States
Correspondence to: Bader Alenezi, MD, Thuniyan Al-Ghanim, GI Center, PO Box 2737 Jahra Central, 01029, Kuwait. baderalenezi@hotmail.com
Telephone: +965-97575534 Fax: +965-24575644
Received: May 31, 2007 Revised: November 12, 2008
Accepted: November 19, 2008
Published online: January 7, 2009

## Abstract

**AIM:** To find the current seroepidemiology of hepatitis A virus (HAV) in Kuwait.

**METHODS:** A total of 2851 Kuwaitis applying for new jobs were screened.

**RESULTS:** HAV-positive cases were 28.8%; 59% were males and 41% were females. The highest prevalence was in the Ahmadi area. High prevalence was among the group of non-educated rather than educated parents. This is the first study in Kuwait demonstrating the shifting epidemiology of HAV.

**CONCLUSION:** This study reflects the need of the Kuwaiti population for an HAV vaccine.



**Key words:** Hepatitis A virus; Fulminant Liver Failure; Hepatitis A virus vaccine; Kuwait

**Peer reviewer:** Dr. Paolo Del Poggio, Hepatology Unit, Department of Internal Medicine, Treviglio Hospital, Piazza Ospedale 1, Treviglio BG 24047, Italy

Alkhalidi J, Alenezi B, Al-mufti S, Hussain E, Askar H, Kemmer N, Neff GW. Seroepidemiology of hepatitis A virus in Kuwait. *World J Gastroenterol* 2009; 15(1): 102-105 Available from: URL: http://www.wjgnet.com/1007-9327/15/102.asp DOI: http://dx.doi.org/10.3748/wjg.15.102

## INTRODUCTION

Hepatitis A virus (HAV) infection is often a self-limiting disease that can be associated with fulminant hepatic failure (FHF). The mortality rate tends to increase with age, in particular, when greater than 40 years of age[1]. Various data from the world shows that with the improvement in hygiene and quality of life there is a shift in the epidemiology of HAV to older people, exposing them to the risk of serious HAV infection.

Hepatitis A is often considered a benign disease in our area. The idea is that almost 100% of the adult population has been infected in early childhood[2]. However, there are no available data about the current epidemiology of the disease in Kuwait. The aim of this study was to find out the current sero-prevalence of this disease and to decide the need of the Kuwaiti population for HAV vaccine.

## MATERIALS AND METHODS

### *Study population and serum collection*

The study population was healthy adults attending a medical checkup that was required before applying for a new job. The study was performed in two places in Kuwait; the first one was the General Medical Council, which accepts adults from both sexes and all nationalities applying for civilian jobs. Only Kuwaiti nationals were included in the study. The second place was the Armed Forces Hospital, which accepts Kuwaiti adult males recruited for military service. The study population belong, to the total six governorates of the country of Kuwait, which includes the Capital, Hawali, Farwania, Mubarak, Ahmadi and Jahra.

The study was approved by the Ministry of Health and the local ethical committee of the Kuwait Institution for Medical Specialization (KIMS). Informed consent was obtained from each case. Each individual completed a questionnaire. Thereafter, 5 mL of blood was obtained. The identities of the subjects were kept confidential by assigning a code number for the questionnaire and the blood samples. The study was conducted during May 2003 to May 2004.

### *Laboratory data*

Blood samples were collected from different centers and sent to the Virology Unit-Public Health Laboratories. An Axsym HAVAB 2.0 kit (Abbott Laboratories) was used for the detection of IgG anti-HAV from all samples. The procedure was followed as indicated by the manufacturer. In addition, the samples were tested for anti-hepatitis B surface antigen (HBsAg), anti-hepatitis C virus and anti-HIV.

### *Statistical analysis*

Data are expressed using descriptive statistical methods, namely counts and percentages of screened subjects.

## RESULTS

There were a total of 2851 Kuwaiti cases screened, 2216 were from the Medical Council and the remaining were from the Military Hospital.

### *Residence area*

The screened cases were from the six governorates of Kuwait which included Farwania 528 cases (18.52%), Ahmadi 505 cases (17.71%), Hawali 467 (16.38%), Capital 433 (15.19%), Jahra 411 (14.42%), Mubarak 301 cases (10.56%) and 7.23% were from unidentified areas. Of 2851 cases screened, 816 (28.6%) cases were positive for HAV (Figure 1). The prevalence percentages of HAV in each governorate were higher in Ahmadi, 202 cases (24.5%); Farwania, 170 cases (20.6%); and Jahra, 164 cases (20%), than in others: Capital 89 cases (11%); Hawali, 84 cases (10%); Mubarak, 76 cases (9%); and 31 cases (3.8%) were unidentified. The 28% of cases which tested positive for HAV were in 94% of cases from the Medical Council and in 5% of cases from the Military Hospital.

### *Age and sex distribution*

There were 481 (59%) males and 335 (41%) females. The prevalence of HAV cases in each age group was 24% (561) in the age group 18-27 years, 51% (213 cases) in the age group 28-40 years, and 56.5% (26 cases) in the age group 41-60 years (Table 1).

### *Education level*

Of 2851 subjects screened, 1525 had both parents who were educated, while 412 had non-educated parents. The prevalence of HAV among the group with non-educated parents or a single educated parent was higher than the educated group (42%, 37% and 21%, respectively) (Figure 2). Of the 816 cases who had HAV, single parent education was determined as maternal education in 10%, while paternal education was 90%. Maternal education plays a greater role than paternal in the care of children and the family which is reflected in the social and health standards (Figure 3).

### *Risk factors*

There was no risk factor in 2706 cases, household contacts with hepatitis in one case, surgery in two, blood transfusion in four, and unknown answer in 138 cases.

### *Association with other viral hepatitis*

In this study, 2035 (71%) cases were not immune to HAV, 94% of them had no evidence of HBV or HCV infection. In 4.8% of cases, there was positive HBsAg serology, while 1.5% had positive anti-HCV results. Of 816 (28.6%) having immunity to HAV, 97.7% had no evidence of HBV, HCV or HIV; 1.7% had positive serology for HAV, HBV and HCV; 0.24% had positive serology for HBsAg, while 0.37% had positive serology for HCV. None of these cases were positive for HIV (Tables 2 and 3).

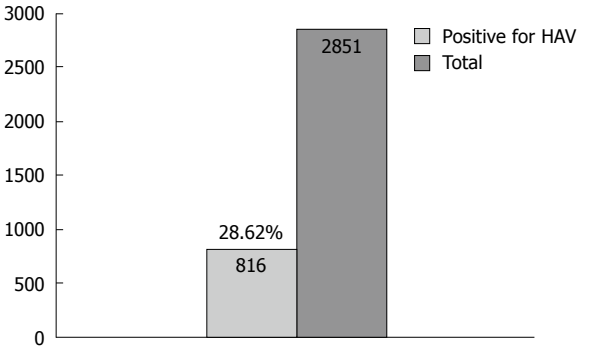


**Figure 1 Patients with HAV+ve.**

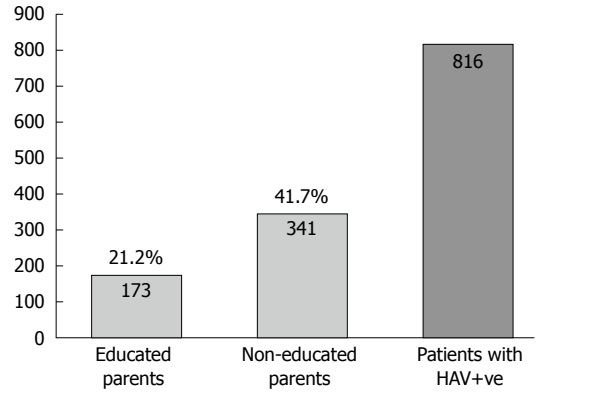


**Figure 2 The prevalence of HAV among the group with non-educated parents or a single educated parent was higher than the educated group.**

## DISCUSSION

HAV is a non-enveloped, RNA-containing virus that belongs to the family Picornaviridae. It is a spherical 27-nm particle that was discovered by Feinstone in 1973[3]. The routes of infection are orofecal and percutaneous. The incubation period is about 28 d. The fecal shedding of virus is at a maximum during the late incubation period, just before or shortly after the onset of symptoms[4].

After oral inoculation of a chimpanzee with HAV, the viral antigen was detected first in the serum on day 14, in the tonsils on day 16 and in the liver on day 21. The viremia lasts for 2 wk[5]. In human studies, HAV RNA is detected for an average of 60 d after onset of clinical symptoms[6]. Risk factors that have been associated with reported HAV infection within the United States include sexual or household contact with another person with hepatitis (25%), contact with children attending a day-care center (15%), international travel (5%) and food or water-borne outbreak (5%). However, in 50% of cases, no risk factor can be identified[7].

In Kuwait, the epidemiology of HAV in the 1980s was similar to developing countries with almost 100% of

**Table 1 Disease prevalence per age group *n* (%)**

| Age category | Pts with HAV+ve |
|---|---|
| Less than 27 yr (2385 cases screened) | 577 (24) |
| 28 to 40 yr (420 cases screened) | 213 (51 ) |
| 41 to 60 yr (46 cases screened) | 26 (56.5 ) |

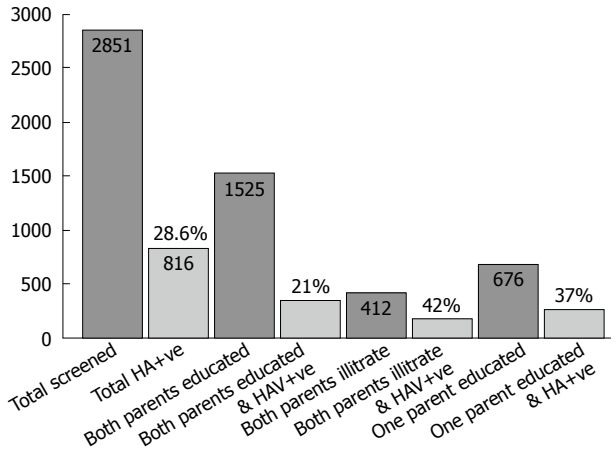


**Figure 3 HAV and level education.**

adults over the age of 20 years testing positive for anti-HAV. At that time, 90% of the screened cases with acute hepatitis A were below the age of 10 years and 70% below the age of 5 years[2]. Retrospective analysis of all charts of Kuwaiti patients presenting with acute HAV infectious between the ages of 0 and 15 years admitted to an infection disease hospital between 2000 and 2002 showed an incidence of 47 per 100 000. One third of these cases were from Jahra region, which is dominated by the Bedouin who live in large extended families. However, in 50% of these cases, there was no identified risk factor. Prolonged jaundice was found in 3% of cases and FHF in 0.4%[8].

There has been a dramatic drop in the incidence of HAV infection in children (47/100 000 *vs* 122/100 000) and a shift toward infection among older children (from 0-4 years to 7-12 years). Our study provides the most recent data about the prevalence of HAV in Kuwait over the last 20 years. The prevalence of HAV was 28% and one quarter of screened individuals below the age of 27 years had positive anti HAV. There were more cases in the areas dominated by Bedouin with extended families. Also, there were more cases among the group with uneducated parents, which reflects the relationship of the disease to low social background. These data show the shifting epidemiology of HAV in Kuwait toward intermediate to low endemicity, leaving 75% of the population below the age of 27 years non-immune, with a risk of exposure to HAV infection at a later age group, with increased morbidity. These changes have resulted from improvement in living standards and socio-economic progress. These data demonstrate the requirement of initiating an HAV vaccine program in Kuwait.

Areas in the Middle East like Qatar, United Arab Emirates and Saudi Arabia show a shifting pattern from high to intermediate endemicity for HAV. In Saudi Arabia, there are existing pockets of high HAV endemicity that may lead to outbreaks[9]. A study by Fathalla *et al*[10] showed that eastern Saudi Arabia still belongs to epidemiological pattern 1, which is characteristic of developing poor countries with low socioeconomic status and the country has a seroprevalence of 99%. The United Arab Emirates data showed that the seroprevalence of HAV was 60% and 90% for the ages of 16 and 40 years respectively which indicates a shift of HAV epidemic with infection towards an adult population[11]. In South-East Asia and China, there is shifting epidemiology of HAV from high to moderate or low endemicity. In China, this is associated with risk of outbreaks as a result of re-introduction of the virus from areas of high endemicity to low endemicity within a non-immune population[12] The incidence of HAV infection varies from high, moderate, low and very low endemicity areas. South-East Asia, India, Africa and Latin America were considered high endemic areas. The epidemiology of HAV is changing due to improvement in water supplies and sanitation conditions. Asian studies from Taiwan and India also show changing seroepidemiology of HAV infection and these countries are considering the use of HAV vaccine. The prevalence of HAV antibodies in Taiwan decreased in 1998 compared to 1992 reflecting the improvement of socioeconomic status and modernization of sanitation[13,14]. Africa is still considered as an area with high endemicity for HAV[9]. In Latin America, the highest anti-HAV seroprevalence rates were found in Mexico and the Dominican Republic. In these countries over the last 15 years, there was a shift towards medium endemicity with the peak of infection occurring in later childhood and adolescence rather than in early childhood. Contaminated water and food supply were the strongest risk factors in Latin America[15].

In European industrialized countries like Italy, there is a markedly lower prevalence of HAV infection,

**Table 2 Association with other viral hepatitis**

| Anti HAV | HBsAg | Anti HCV | Count |
|---|---|---|---|
| Yes | Yes | Yes | 14 |
| Yes | Yes | No | 2 |
| Yes | No | Yes | 3 |
| Yes | No | No | 797 |
| No | Yes | No | 97 |
| No | No | Yes | 30 |
| No | No | No | 1908 |

**Table 3 Hepatitis A positive patients with HB, HC and HIV**

| Anti HAV+ve | HBsAg+ve | Anti HCV+ve | Anti HIV+ve | Number of cases |
|---|---|---|---|---|
| Yes | Yes | Yes | No | 14 |
| Yes | Yes | No | No | 2 |
| Yes | No | Yes | No | 3 |
| Yes | No | No | No | 797 |

especially among a younger age group, due to marked improvements in socioeconomic conditions and hygienic standards. In the same region, small outbreaks of HAV infection were associated with intravenous drug abusers travelling to endemic areas, shellfish consumption and with an increasing number of family members[16].

Patients with negative serology for HAV need HAV vaccine. Also, patients with chronic liver disease who are non-immune to HAV need HAV vaccine[17]. In our study, 2035 (71%) of cases were not immune to HAV, 4.8% of them had positive HBsAg serology, while 1.5% of them had positive anti-HCV serology.

HAV vaccines are highly purified and formalin-inactivated. The vaccine was shown to be safe and effective when tested by Werzberger *et al*[18] among seronegative children 2-16 years of age in Monroe in 1992. Inactivated HAV vaccine (VAQTA, Merck and Co Inc, West Point, PA, USA) is given in two doses (0 and 6-12 mo). The estimated protective efficacy of one or more doses of the vaccine is 98%. HAV vaccine provides long-term immunity lasting probably from 20 to 50 years[19]. The vaccine has been available in the USA since 1995, and is highly effective in preventing disease transmission in a community with recurrent epidemics. The adverse effects of the vaccine are mild and include fever, rash and injection site reaction. It proved safe with no adverse effects among 30 000 vaccine recipients[20]. The HAV vaccine VAQTA given in two doses to a group of infants at the age of 2 years and followed up for 9 years provided long-term protection. It was effective in preventing HAV epidemics in the community in spite of the exposure to sporadic cases in non-vaccinated individuals[21]. HAV vaccine is recommended for persons with increased risk of infection including international travellers, illegal drug users, persons with chronic liver disease, persons who have clotting factor disorders and homosexuals[17]. Vaccination against hepatitis is the most effective means of preventing sexual transmission of hepatitis A and B[22].

## REFERENCES


1 **Vento S**, Garofano T, Renzini C, Cainelli F, Casali F, Ghironzi G, Ferraro T, Concia E. Fulminant hepatitis associated with hepatitis A virus superinfection in patients with chronic hepatitis C. *N Engl J Med* 1998; **338**: 286-290

2 **Nordenfelt E**, Atack W, Al-Kandani S, Al-Nakib W. Hepatitis A in Kuwait. *J Kuwait Med Assoc* 1985; **19**: 103-108

3 **Feinstone SM**, Kapikian AZ, Purceli RH. Hepatitis A: detection by immune electron microscopy of a viruslike antigen associated with acute illness. *Science* 1973; **182**: 1026-1028

4 **Lemon SM**. Type A viral hepatitis. New developments in an old disease. *N Engl J Med* 1985; **313**: 1059-1067

5 **Cohen JI**, Feinstone S, Purcell RH. Hepatitis A virus infection in a chimpanzee: duration of viremia and detection of virus in saliva and throat swabs. *J Infect Dis* 1989; **160**: 887-890

6 **Costa-Mattioli M**, Monpoeho S, Nicand E, Aleman MH, Billaudel S, Ferre V. Quantification and duration of viraemia during hepatitis A infection as determined by real-time RT-PCR. *J Viral Hepat* 2002; **9**: 101-106

7 **Kemmer NM**, Miskovsky EP. Hepatitis A. *Infect Dis Clin North Am* 2000; **14**: 605-615

8 **Husain E**, Husain K. Hepatitis A injection in children in Kuwait. Epidemiology and clinical features 41st Annual meeting of the infectious diseases society of America; 2003 Oct 9-12; San Diego, California

9 **Tufenkeji H**. Hepatitis A shifting epidemiology in the Middle East and Africa. *Vaccine* 2000; **18** Suppl 1: S65-S67

10 **Fathalla SE**, Al-Jama AA, Al-Sheikh IH, Islam SI. Seroprevalence of hepatitis A virus markers in Eastern Saudi Arabia. *Saudi Med J* 2000; **21**: 945-949

11 **Dajani A**, Boloushi S, Kashkosh A. HAV: The risk in UAE. Ashift of epidemicity may improve future concern, 3rd Emirates gastroenterology conference. 1995; Dubai, United Arab Emirates

12 **Barzaga BN**. Hepatitis A shifting epidemiology in South-East Asia and China. *Vaccine* 2000; **18** Suppl 1: S61-S64

13 **Wang SM**, Liu CC, Huang YS, Yang YJ, Lei HY. Change in hepatitis A virus seroepidemiology in southern Taiwan: a large percentage of the population lack protective antibody. *J Med Virol* 2001; **64**: 104-108

14 **Das K**, Jain A, Gupta S, Kapoor S, Gupta RK, Chakravorty A, Kar P. The changing epidemiological pattern of hepatitis A in an urban population of India: emergence of a trend similar to the European countries. *Eur J Epidemiol* 2000; **16**: 507-510

15 **Tanaka J**. Hepatitis A shifting epidemiology in Latin America. *Vaccine* 2000; **18** Suppl 1: S57-S60

16 **Moschen ME**, Floreani A, Zamparo E, Baldo V, Majori S, Gasparini V, Trivello R. Hepatitis A infection: a seroepidemiological study in young adults in North-East Italy. *Eur J Epidemiol* 1997; **13**: 875-878

17 Prevention of varicella: Recommendations of the Advisory Committee on Immunization Practices (ACIP). Centers for Disease Control and Prevention. *MMWR Recomm Rep* 1996; **45**: 1-36

18 **Werzberger A**, Mensch B, Kuter B, Brown L, Lewis J, Sitrin R, Miller W, Shouval D, Wiens B, Calandra G. A controlled trial of a formalin-inactivated hepatitis A vaccine in healthy children. *N Engl J Med* 1992; **327**: 453-457

19 **Wiedermann G**, Kundi M, Ambrosch F, Safary A, D'Hondt E, Delem A. Inactivated hepatitis A vaccine: long-term antibody persistence. *Vaccine* 1997; **15**: 612-615

20 **Averhoff F**, Shapiro CN, Bell BP, Hyams I, Burd L, Deladisma A, Simard EP, Nalin D, Kuter B, Ward C, Lundberg M, Smith N, Margolis HS. Control of hepatitis A through routine vaccination of children. *JAMA* 2001; **286**: 2968-2973

21 **Werzberger A**, Mensch B, Nalin DR, Kuter BJ. Effectiveness of hepatitis A vaccine in a former frequently affected community: 9 years' followup after the Monroe field trial of VAQTA. *Vaccine* 2002; **20**: 1699-1701

22 Sexually transmitted diseases treatment guidelines 2002. Centers for Disease Control and Prevention. *MMWR Recomm Rep* 2002; **51**: 1-78


**S- Editor** Li JL **L- Editor** Logan S **E- Editor** Lin YP

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.106

*World J Gastroenterol* 2009 January 7; 15(1): 106-111
***World Journal of Gastroenterology*** ISSN 1007-9327


*BRIEF ARTICLES*

# Iron homeostasis and H63D mutations in alcoholics with and without liver disease

Mariana Verdelho Machado, Paula Ravasco, Alexandra Martins, Maria Rosário Almeida, Maria Ermelinda Camilo, Helena Cortez-Pinto

Mariana Verdelho Machado, Paula Ravasco, Alexandra Martins, Maria Ermelinda Camilo, Helena Cortez-Pinto, Unidade de Nutrição e Metabolismo, Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, 1649-035, Portugal
Mariana Verdelho Machado, Helena Cortez-Pinto, Departamento de Gastrenterologia do Hospital de Santa Maria, 1649-035, Lisboa, Portugal
Maria Rosário Almeida, Genomed, Instituto de Medicina Molecular 1649-035, Portugal
Author contributions: Machado MV, Cortez-Pinto H designed the research; Machado MV, Martins A, Cortez-Pinto H performed the clinical research; Almeida MR performed the genetic analysis; Machado MV, Ravasco P and Cortez-Pinto H analyzed the data; Machado MV and Cortez-Pinto H wrote the paper; and Camilo ME revised the paper.
Correspondence to: Helena Cortez-Pinto, Professor, Serviço de Gastrenterologia, Hospital de Santa Maria, Av. Prof. Egas Moniz, 1649-035, Lisboa, Portugal. hlcortezpinto@netcabo.pt
Telephone: +351-21-7985187 Fax: +351-21-7985142
Received: July 8, 2008 Revised: August 28, 2008
Accepted: September 4, 2008
Published online: January 7, 2009

## Abstract

**AIM:** To evaluate the prevalence of *HFE* gene mutation and indices of disturbed iron homeostasis in alcoholics with and without liver disease.

**METHODS:** One hundred and fifty-three heavy drinkers (defined as alcohol consumption > 80 g/d for at least 5 years) were included in the study. These comprised 78 patients with liver disease [liver disease alcoholics (LDA)] in whom the presence of liver disease was confirmed by liver biopsy or clinical evidence of hepatic decompensation, and 75 subjects with no evidence of liver disease, determined by normal liver tests on two occasions [non-liver disease alcoholics (NLDA)], were consecutively enrolled. Serum markers of iron status and HFE C282Y and H63D mutations were determined. *HFE* genotyping was compared with data obtained in healthy blood donors from the same geographical area.

**RESULTS:** Gender ratio was similar in both study groups. LDA patients were older than NLDA patients (52 ± 10 years *vs* 48 ± 11 years, $P = 0.03$). One third and one fifth of the study population had serum transferrin saturation (TS) greater than 45% and 60% respectively. Serum iron levels were similar in both groups. However, LDA patients had higher TS (51 ± 27 *vs* 36 ± 13, $P < 0.001$) and ferritin levels (559 ± 607 ng/mL *vs* 159 ± 122 ng/mL, $P < 0.001$), and lower total iron binding capacity (TIBC) (241 ± 88 μg/dL *vs* 279 ± 40 μg/dL, $P = 0.001$). The odds ratio for having liver disease with TS greater than 45% was 2.20 (95% confidence interval (CI): 1.37-3.54). There was no difference in C282Y allelic frequency between the two groups. However, H63D was more frequent in LDA patients (0.25 *vs* 0.16, $P = 0.03$). LDA patients had a greater probability of carrying at least one *HFE* mutation than NLDA patients (49.5% *vs* 31.6%, $P = 0.02$). The odds ratio for LDA in patients with H63D mutation was 1.57 (95% CI: 1.02-2.40).

**CONCLUSION:** The present study confirms the presence of iron overload in alcoholics, which was more severe in the subset of subjects with liver disease, in parallel with an increased frequency of H63D *HFE* mutation.



**Key words:** Alcoholic liver disease; Iron; *HFE* gene; H63D; Hemochromatosis

**Peer reviewer:** Robert Flisiak, PhD, Department of Infectious Diseases, Medical University of Bialystok, 15-540 Bialystok, Zurawia str., 14, Poland

Machado MV, Ravasco P, Martins A, Almeida MR, Camilo ME, Cortez-Pinto H. Iron homeostasis and H63D mutations in alcoholics with and without liver disease. *World J Gastroenterol* 2009; 15(1): 106-111 Available from: URL: http://www.wjgnet.com/1007-9327/15/106.asp DOI: http://dx.doi.org/10.3748/wjg.15.106

## INTRODUCTION

Alcohol consumption and iron overload have long been found to be associated with each other. In 1896, the condition we now recognize as hereditary hemochromatosis was considered a variant of alcoholic cirrhosis[1], and even in the 1960s was believed to be a nutritional disorder related to alcohol intake, in which excess iron originated from the diet and iron content in

red wine[2].

Alcohol may increase iron absorption and cellular iron uptake by several possible mechanisms: (1) increased absorption *via* a non-carrier-mediated paracellular route[3]; (2) iron absorption is stimulated by anemia secondary to ineffective erythropoiesis due to alcohol-induced folic acid deficiency[4,5]; and (3) alcohol consumption is associated with decrease in enterocyte turnover through mitosis inhibition[6], which may reduce the already limited intestinal iron excretion. Recently, it has been shown that alcohol down-regulates hepcidin transcription, which leads to increased duodenal iron absorption *via* a divalent metal transporter-1 (with enhanced luminal import) and ferroportin protein expression (with enhanced basolateral translocation to the circulation)[7,8]. Furthermore, it has been shown that alcohol abolishes the iron-induced up-regulation of both liver hepcidin transcription and the DNA-binding activity of C/EBP alpha[9], thus negating the protective effect of hepcidin.

Suzuki *et al*[10] also demonstrated, an up-regulation of transferrin receptor expression in the hepatocytes of liver disease alcoholics (LDAs), which may promote hepatocyte iron accumulation.

Alcoholic liver disease (ALD) is often associated with elevated serum iron indices and hepatic iron overload[11-14]. Iron is also believed to be central in the pathogenesis of ALD, and some reports show iron overload as a predictive indicator of higher mortality[15], and development of hepatocellular carcinoma[16]. In fact, iron overload and alcohol have a synergistic effect on the production of oxidative stress[17-20].

The fact that only a minority of alcohol abusers, develop advanced liver disease such as steatohepatitis, fibrosis, and cirrhosis, prompted the search for genetic predisposing factors[21], such as C282Y and H63D mutations in the hemochromatosis protein HFE, which increases iron overload. However, no association has been found between C282Y *HFE* gene mutation and ALD, and there are conflicting reports on the association between H63D and ALD[22-27]. On the other hand, it is clear that the phenotypic expression of HFE C282Y homozygosity (the prototype for the genetic hemochromatosis syndrome) is low, and it increases markedly in patients with excessive alcohol consumption[28-30], which suggests that alcohol may act as a potential modifier of the (genetically determined) hemochromatosis phenotype.

The aim of the present study was to evaluate the prevalence of *HFE* mutations, and indices of disturbed iron homeostasis in alcoholics with and without liver disease.

## MATERIALS AND METHODS

The study was approved by the Institutional Ethics Committees and written informed consent was obtained from the study subjects. A total of 284 heavy drinkers, defined as alcohol consumption > 80 g/d for at least five years were included in the study. The subjects consisted of consecutive patients seen in the Liver Unit (ambulatory or hospitalized) of a University Hospital, with suspected ALD; and consecutive referrals to two Alcohol Addiction Units, with psychiatric alcohol dependency, and no previous suspicion of liver disease.

Lifetime alcohol intake was assessed in all subjects, using a semi-structured questionnaire. Subjects were excluded from the study if they had any of the following: serological evidence of hepatitis B virus (HBV) and hepatitis C virus (HCV) infections or autoimmune liver disease, histological evidence of other liver diseases, or "mild" abnormalities of liver tests (bilirubin, aminotransferases, alkaline phosphatase, less than twice the upper limit of normal) in the absence of clinical signs of liver disease. Drinkers without clinical manifestations, and with normal liver tests on one occasion were excluded if it was not possible to obtain a second blood sample for reconfirmation.

Based on the above mentioned criteria, the subjects were divided into two categories: LDA and non-liver disease alcoholics (NLDA). The criteria for inclusion in the LDA group were the presence of either laboratory/clinical evidence of hepatic decompensation (e.g. ascites, varices, and encephalopathy) or liver histology compatible with LDA of severity greater than steatosis. Percutaneous liver biopsy specimens were evaluated blindly according to standard procedures[31]; only 11 subjects underwent this procedure since in patients with evidence of hepatic decompensation, such as ascitis, encephalopathy, or signs of portal hypertension, liver biopsy was considered unnecessary. Inclusion criteria for NLDA consisted of lack of clinical signs of liver disease and normal liver tests on two occasions (aminotransferases, prothrombin time, albumin and bilirubin) with the exception of an isolated rise in γ-glutamyl transferase[32]; no liver biopsy was performed in this group as it was considered unethical.

### *Laboratory tests*

After a 12-h overnight fast, blood samples were collected and biochemical tests were done on the same day by routine methods, in the central pathology laboratory. The tests included: aminotransferases, bilirubin, γ-glutamyl transpeptidase, protein electrophoresis, prothrombin time, renal functions, cholesterol, triglycerides, ceruloplasmin, α-1 antitrypsin, anti-nuclear, anti-mitochondrial, anti-smooth muscle antibodies, and serological markers of HBV and HCV infections. Serum iron indices: iron, ferritin, total iron binding capacity (TIBC) and % transferrin saturation were also determined.

### *Genotyping*

To detect the C282Y and H63D mutations, genomic DNA, extracted from the buffy coat fraction of whole blood was amplified by polymerase chain reaction as previously described[29,33]. The C282Y mutation creates a new *Rsa*I restriction site and the H63D mutation abolishes a *Mbo*I site allowing identification by restriction enzyme digestion.

A sub-group of 11 patients, all belonging to

**Table 1 Clinical and laboratory characteristics of LDA and NLDA**

| | LDA ($n$ = 78) | NLDA ($n$ = 75) | $P$ value |
|---|---|---|---|
| Age (yr) | 52.3 ± 10.1 | 48.5 ± 10.7 | 0.03 |
| Number of men (%) | 66 (85) | 62 (83) | NS |
| Alcohol consumption (g/d) | 217 ± 195 | 327 ± 311 | 0.004 |
| Presence of ascitis (%) | 38 (52.1) | - | - |
| Presence of encephalopathy (%) | 17 (34.7) | - | - |
| Alanine aminotransferase (r.v. 0-37 IU/L) | 53 ± 60 | 17 ± 6 | < 0.05 |
| Aspartate aminotransferase (r.v. 0-41 IU/L) | 81 ± 128 | 15 ± 5 | < 0.05 |
| Alkaline phosphatase (r.v. 40-129 IU/L) | 143 ± 75 | 91 ± 21 | < 0.05 |
| γ-Glutamil transpeptidase (r.v. 8-61 IU/L) | 225 ± 239 | 47 ± 48 | < 0.05 |
| Albumin (g/L) | 36 ± 8 | 43 ± 3 | < 0.05 |
| Bilirubin (mg/dL) | 4.0 ± 7.2 | 0.7 ± 0.9 | < 0.05 |
| Prothrombin time (seconds prolonged from control) | 3.2 ± 2.7 | 0.5 ± 1.2 | < 0.05 |
| Cholesterol (mg/dL) | 160.0 ± 84 | 209.1 ± 41.7 | < 0.05 |
| Triglycerides (mg/dL) | 125.6 ± 111.3 | 162.8 ± 121.0 | NS |
| Glucose (mg/dL) | 119.9 ± 40.5 | 97 ± 13.6 | NS |
| Iron (r.v. 65-175 μg/dL) | 115 ± 64 | 99.4 ± 39 | NS |
| TIBC (r.v. 250-425 μg/dL) | 241 ± 88 | 279 ± 40 | 0.001 |
| Transferrin saturation (%) | 51 ± 27 | 36 ± 13 | < 0.001 |
| Ferritin (r.v. 23-236 ng/mL) | 559 ± 607 | 159 ± 122 | < 0.001 |

r.v.: Reference value; TIBC: Total iron binding capacity.

the LDA, had a liver biopsy; the degree of hepatic parenchymal siderosis was identified by Perl's iron stain, and graded from 0 to 4.

### Statistical analysis

Basic descriptive statistics, means, standard deviation (SD), ranges and percentages, were used to characterize the populations. Categorical variables were analyzed by chi squared test and paired parametric numerical variables were compared, using the Student's $t$ test. Correlations between several variables were evaluated through Spearman correlation coefficient.

Odds ratio analysis was used to explore interactions between iron overload and genetic mutations in the pathogenesis of ALD *vs* non-liver disease: the odds and 95% confidence intervals of having LDA outcome *vs* NLDA outcome were determined. LDA and NLDA were always the dependent variables and transferrin saturation > 45% or the presence of genetic mutations were evaluated as risk factors. All analyses were adjusted for patient's age.

The computer software used was Statistical Program for Social Sciences (SPSS) for Windows 12.0 (SPSS Inc., Chicago, USA, 2004). All $P$ values were two-sided; for all statistics, significance was accepted at the 5% probability level.

## RESULTS

Based on the predefined inclusion and exclusion criteria, 153 heavy drinkers were included, 78 in the LDA group and 75 in the NLDA group. Clinical and biochemical characteristics of the study groups are summarized in Table 1. The gender ratio was similar in both groups; LDA patients were older (52.3 ± 10.1 years *vs* 48.5 ± 10.7 years, $P$ = 0.03); and alcohol consumption was lower in LDA compared to NLDA (217 ± 195 g/d *vs* 327 ± 311 g/d, $P$ = 0.004).

Both groups had similar mean iron concentrations (Table 1), however, LDA patients had lower TIBC (241 ± 88 μg/dL *vs* 279 ± 40 μg/dL, $P$ = 0.001), and higher levels of ferritin (559 ± 607 ng/mL *vs* 159 ± 122 ng/mL, $P$ < 0.001) and serum transferrin saturation (51% *vs* 36%, $P$ < 0.001). Overall, among the 153 heavy drinkers, 33% had serum transferrin saturation greater than 45%, while 20% had greater than 60%; transferrin saturation higher than 45% and higher than 60% were more frequent in LDA patients (47.4% *vs* 18.1%, $P$ < 0.001, and 34.6% *vs* 5.3%, $P$ < 0.001, respectively). Furthermore, in subjects with transferrin saturation higher than 45%, the odds ratio for having LDA was 3.90 (95% confidence interval (CI): 1.59-4.54, $P$ < 0.0001).

In the 11 patients who had a liver biopsy, there was a significant association between serum ferritin levels and the degree of hepatic parenchymal siderosis, as identified by Perl's iron stain ($r$ = 0.692, $P$ = 0.02). Five of seven patients (71%) with Perl's staining > 1, had H63D mutation, compared with two of four (50%) in those with a score of 1 or less ($r$ = 0.217, $P$ = 0.547). The distribution of C282Y and H63D genotypes is shown in Table 2. Allelic frequency of H63D mutation was higher in LDA than in NLDA patients (0.25 *vs* 0.16, $P$ = 0.032). Furthermore, allelic frequencies of H63D mutation in NLDA subjects were similar to that seen in the general population from the same geographical area, based on the data on healthy blood donors[34]. There were no differences in the allelic frequency of C282Y between the two groups.

The odds ratio of having LDA and H63D mutation was 1.75 (95% CI: 1.02-2.40, $P$ < 0.03), while the odds ratio of carrying at least one *HFE* mutation was 1.56 (95% CI: 1.05-2.32, $P$ < 0.03).

The serum transferrin saturation and ferritin levels were higher in subjects carrying at least one *HFE* mutation

**Table 2 Comparison of *HFE* genotypes, with C282Y or H63D allelic frequencies in LDA *vs* NLDA subjects and a control population *n* (%)**

| | LDA (*n* = 78) | NLDA (*n* = 75) | Blood donors[34] (*n* = 133) |
|---|---|---|---|
| wt/wt | 39 (50) | 52 (68) | 92 (69) |
| wt/H63D | 31 (39.7) | 19 (25.3) | 27 (20) |
| wt/C282Y | 3 (3.8) | 2 (3.7) | 7 (5) |
| H63D/H63D | 3 (3.8) | 2 (3.7) | 6 (4) |
| C82Y/H63D | 2 (2.5) | 1 (1.3) | 1 (1) |
| C282Y allelic frequency | 0.032 | 0.02 | 0.03 |
| H63D allelic frequency[1] | 0.25 | 0.16 | 0.15 |
| Any mutation[1] | 39 (50) | 24 (32) | 41 (31) |

wt: Wild type; [1]$P = 0.02$; There were no other statistically significant differences between the various groups.

compared with subjects without *HFE* mutation (49% ± 24% *vs* 39% ± 23%, $P = 0.02$ and 499 ± 600 ng/mL *vs* 258 ± 339 ng/mL, $P = 0.005$, respectively) (Table 3). Moreover, the presence of one H63D mutation in patients with transferrin saturation > 45% increased the odds ratio for having LDA to 2.17 (95% CI: 1.42-3.32, $P < 0.01$).

## DISCUSSION

In the present study, heavy drinking was frequently associated with iron overload, as suggested by elevated serum ferritin levels and transferrin saturation, in the absence of hemochromatosis[35]. Moreover, iron overload was more intense in the presence of liver disease, as shown by higher serum concentrations of ferritin and transferrin saturation.

Although ferritin and transferrin saturation may be questioned as markers of iron overload in the presence of liver disease, since ferritin elevation may result from necroinflammatory activity, and decreased hepatic protein production may occur secondary to liver disease[36], resulting in lower TIBC and higher transferrin saturation, previous studies in patients with liver disease have shown significantly higher ferritin levels in patients with alcohol-related liver disease[12]. Furthermore, in the present study, we observed a positive correlation between serum ferritin and the degree of hepatic iron deposition in patients who had a liver biopsy.

Since iron plays an important pathological role in ALD[37], and alcoholics are more prone to develop iron overload, it is conceivable that alcoholics who tend to absorb and store more iron are at an increased risk of liver disease. The presence of mutations in the hemochromatosis *HFE* gene may serve as a predisposing factor for the development of liver disease. However, five previous studies failed to show a relationship between ALD and the presence of such mutations[22-26]. On the other hand, Ropero Gradilla *et al*[27], in Spain, observed an association between H63D mutation (but not with C282Y mutation) and the risk of advanced liver disease. In the present study, individuals carrying at least one *HFE* mutation had a significantly higher probability of having liver disease, which suggested an association between *HFE* mutation and increased susceptibility to ALD. However, it is possible that our observation of an increased prevalence of *HFE* mutations may be a casual finding (type I error).

**Table 3 Serum iron indices according to *HFE* status**

| | At least one *HFE* mutation (*n* = 63) | No *HFE* mutation (*n* = 90) | *P* value |
|---|---|---|---|
| Iron (μg/dL) (r.v. 65-175) | 118 ± 52 | 100 ± 55 | NS |
| TIBC (μg/dL) (r.v. 250-425) | 262 ± 83 | 259 ± 63 | NS |
| Transferrin saturation (%) | 49 ± 24 | 39 ± 22 | 0.02 |
| Ferritin (ng/mL) (r.v. 23-236) | 499 ± 600 | 258 ± 339 | < 0.001 |

r.v.: Reference value; TIBC: Total iron binding capacity.

The extent to which H63D mutation predisposes to iron overload has been the subject of much debate. Such an association has been observed in homozygosity studies[38,39], and also with the findings that serum transferrin saturation is significantly increased in H63D homozygotes and heterozygotes as compared with wild-type individuals[40]. To reinforce the importance of the *HFE* mutations as risk factors for liver disease, the presence of these mutations should be associated with significantly higher iron parameters. Indeed, the present study showed that transferrin saturation and ferritin concentration were higher in patients with at least one *HFE* mutation, with no difference in the TIBC values. However, even in the sub-group of individuals with increased iron saturation, the presence of H63D mutation was associated with a higher probability of liver disease, suggesting that H63D mutation may be a risk factor independent of the associated iron overload.

In conclusion, the present study has confirmed previous reports of the presence of iron overload in alcoholics, which is more severe in the subset of subjects with liver disease, and is associated with an increased frequency of H63D *HFE* mutation. Our findings indicate that H63D *HFE* mutation, by further increasing iron overload, is a risk factor for liver disease, through the synergistic damaging effects of alcohol and iron. Further research is needed to evaluate if the progression of the liver disease in alcoholic patients with iron overload is associated with a worse prognosis.

## COMMENTS

### *Background*

Alcohol abuse enhances iron absorption and may play a crucial role in the pathogenesis of alcoholic liver disease (ALD). Thus, conditions that enhance iron uptake may have a synergistic role in the development of ALD. Currently, the relevance of hemochromatosis-associated gene mutations and/or iron status in ALD is unclear.

### *Innovations and breakthroughs*

The fact that only a minority of alcohol abusers, develop advanced liver disease such as steatohepatitis, fibrosis, and cirrhosis, prompted the search for genetic predisposing factors, such as C282Y and H63D mutations in the hemochromatosis protein HFE, which increases iron overload. However, no association has been found between C282Y *HFE* gene mutation and ALD, and there are conflicting reports on the association between H63D and ALD. The aim of the present study was to evaluate the prevalence of *HFE* mutations,

and indices of disturbed iron homeostasis in alcoholics with and without liver disease.

### Applications

The research reported that H63D HFE mutation, by further increasing iron overload, is a risk factor for liver disease, through the synergistic damaging effects of alcohol and iron.

### Peer review

The paper is interesting and is focused on original topic. Title reflects the content of the article. Results and discussion provide accurate informations and lead to conclusions

## REFERENCES


1 **Gilbert A**, Grenet A. Cirrhose alcoolique hypertrophique pigmentaire. *Compte Rendus Soc de Biol* 1896; **10**: 1078-1081
2 **MacDonald RA**. Primary hemochromatosis: inherited or acquired? *Prog Hematol* 1966; **5**: 324-353
3 **Duane P**, Raja KB, Simpson RJ, Peters TJ. Intestinal iron absorption in chronic alcoholics. *Alcohol Alcohol* 1992; **27**: 539-544
4 **Celada A**, Rudolf H, Donath A. Effect of experimental chronic alcohol ingestion and folic acid deficiency on iron absorption. *Blood* 1979; **54**: 906-915
5 **Bonkovsky HL**, Lambrecht RW, Shan Y. Iron as a co-morbid factor in nonhemochromatotic liver disease. *Alcohol* 2003; **30**: 137-144
6 **Casini A,** Galli A, Calabro' A, Di Lollo S, Orsini B, Arganini L, Jezequel AM, Surrenti C. Ethanol-induced alterations of matrix network in the duodenal mucosa of chronic alcohol abusers. *Virchows Arch* 1999; **434**: 127-135
7 **Bridle K**, Cheung TK, Murphy T, Walters M, Anderson G, Crawford DG, Fletcher LM. Hepcidin is down-regulated in alcoholic liver injury: implications for the pathogenesis of alcoholic liver disease. *Alcohol Clin Exp Res* 2006; **30**: 106-112
8 **Harrison-Findik DD**, Schafer D, Klein E, Timchenko NA, Kulaksiz H, Clemens D, Fein E, Andriopoulos B, Pantopoulos K, Gollan J. Alcohol metabolism-mediated oxidative stress down-regulates hepcidin transcription and leads to increased duodenal iron transporter expression. *J Biol Chem* 2006; **281**: 22974-22982
9 **Harrison-Findik DD**. Role of alcohol in the regulation of iron metabolism. *World J Gastroenterol* 2007; **13**: 4925-4930
10 **Suzuki Y**, Saito H, Suzuki M, Hosoki Y, Sakurai S, Fujimoto Y, Kohgo Y. Up-regulation of transferrin receptor expression in hepatocytes by habitual alcohol drinking is implicated in hepatic iron overload in alcoholic liver disease. *Alcohol Clin Exp Res* 2002; **26**: 26S-31S
11 **Milman N**, Graudal N, Hegnhøj J, Christoffersen P, Pedersen NS. Relationships among serum iron status markers, chemical and histochemical liver iron content in 117 patients with alcoholic and non-alcoholic hepatic disease. *Hepatogastroenterology* 1994; **41**: 20-24
12 **Bell H**, Skinningsrud A, Raknerud N, Try K. Serum ferritin and transferrin saturation in patients with chronic alcoholic and non-alcoholic liver diseases. *J Intern Med* 1994; **236**: 315-322
13 **Jurczyk K**, Wawrzynowicz-Syczewska M, Boroń-Kaczmarska A, Sych Z. Serum iron parameters in patients with alcoholic and chronic cirrhosis and hepatitis. *Med Sci Monit* 2001; **7**: 962-965
14 **Whitfield JB**, Zhu G, Heath AC, Powell And LW, Martin NG. Effects of alcohol consumption on indices of iron stores and of iron stores on alcohol intake markers. *Alcohol Clin Exp Res* 2001; **25**: 1037-1045
15 **Ganne-Carrié N**, Christidis C, Chastang C, Ziol M, Chapel F, Imbert-Bismut F, Trinchet JC, Guettier C, Beaugrand M. Liver iron is predictive of death in alcoholic cirrhosis: a multivariate study of 229 consecutive patients with alcoholic and/or hepatitis C virus cirrhosis: a prospective follow up study. *Gut* 2000; **46**: 277-282
16 **Lauret E**, Rodríguez M, González S, Linares A, López-Vázquez A, Martínez-Borra J, Rodrigo L, López-Larrea C. HFE gene mutations in alcoholic and virus-related cirrhotic patients with hepatocellular carcinoma. *Am J Gastroenterol* 2002; **97**: 1016-1021
17 **Tsukamoto H**, Horne W, Kamimura S, Niemelä O, Parkkila S, Ylä-Herttuala S, Brittenham GM. Experimental liver cirrhosis induced by alcohol and iron. *J Clin Invest* 1995; **96**: 620-630
18 **Tsukamoto H**, Lin M, Ohata M, Giulivi C, French SW, Brittenham G. Iron primes hepatic macrophages for NF-kappaB activation in alcoholic liver injury. *Am J Physiol* 1999; **277**: G1240-G1250
19 **She H**, Xiong S, Lin M, Zandi E, Giulivi C, Tsukamoto H. Iron activates NF-kappaB in Kupffer cells. *Am J Physiol Gastrointest Liver Physiol* 2002; **283**: G719-G726
20 **Xiong S**, She H, Takeuchi H, Han B, Engelhardt JF, Barton CH, Zandi E, Giulivi C, Tsukamoto H. Signaling role of intracellular iron in NF-kappaB activation. *J Biol Chem* 2003; **278**: 17646-17654
21 **Day CP**. Genes or environment to determine alcoholic liver disease and non-alcoholic fatty liver disease. *Liver Int* 2006; **26**: 1021-1028
22 **Gleeson D**, Evans S, Bradley M, Jones J, Peck RJ, Dube A, Rigby E, Dalton A. HFE genotypes in decompensated alcoholic liver disease: phenotypic expression and comparison with heavy drinking and with normal controls. *Am J Gastroenterol* 2006; **101**: 304-310
23 **Grove J**, Daly AK, Burt AD, Guzail M, James OF, Bassendine MF, Day CP. Heterozygotes for HFE mutations have no increased risk of advanced alcoholic liver disease. *Gut* 1998; **43**: 262-266
24 **Frenzer A**, Rudzki Z, Norton ID, Butler WJ, Roberts-Thomson IC. Heterozygosity of the haemochromatosis mutation, C282Y, does not influence susceptibility to alcoholic cirrhosis. *Scand J Gastroenterol* 1998; **33**: 1324
25 **Campos Franco J**, González Quintela A, Fernández de Trocóniz LL, Barros Angueira F, Pérez-Quintela BV, Pérez Becerra E, Martínez de Rituerto ST, Otero Antón E, Torre Carballada JA. [Mutations in the HFE gene (C282Y, H63D, S65C) in alcoholic patients with finding of iron overload] *Rev Clin Esp* 2002; **202**: 534-539
26 **Sohda T**, Takeyama Y, Irie M, Kamimura S, Shijo H. Putative hemochromatosis gene mutations and alcoholic liver disease with iron overload in Japan. *Alcohol Clin Exp Res* 1999; **23**: 21S-23S
27 **Ropero Gradilla P**, Villegas Martínez A, Fernández Arquero M, García-Agúndez JA, González Fernández FA, Benítez Rodríguez J, Díaz-Rubio M, de la Concha EG, Ladero Quesada JM. C282Y and H63D mutations of HFE gene in patients with advanced alcoholic liver disease. *Rev Esp Enferm Dig* 2001; **93**: 156-163
28 **Bacon BR**, Britton RS. Clinical penetrance of hereditary hemochromatosis. *N Engl J Med* 2008; **358**: 291-292
29 **Fletcher LM**, Dixon JL, Purdie DM, Powell LW, Crawford DH. Excess alcohol greatly increases the prevalence of cirrhosis in hereditary hemochromatosis. *Gastroenterology* 2002; **122**: 281-289
30 **Pietrangelo A**. Hereditary hemochromatosis. *Biochim Biophys Acta* 2006; **1763**: 700-710
31 **Pinto HC**, Baptista A, Camilo ME, Valente A, Saragoça A, de Moura MC. Nonalcoholic steatohepatitis. Clinicopathological comparison with alcoholic hepatitis in ambulatory and hospitalized patients. *Dig Dis Sci* 1996; **41**: 172-179
32 **Wu A**, Slavin G, Levi AJ. Elevated serum gamma-glutamyl-transferase (transpeptidase) and histological liver damage in alcoholism. *Am J Gastroenterol* 1976; **65**: 318-323
33 **Beutler E**, Felitti VJ, Koziol JA, Ho NJ, Gelbart T. Penetrance

of 845G--> A (C282Y) HFE hereditary haemochromatosis mutation in the USA. *Lancet* 2002; **359**: 211-218

34 **Cardoso CS**, Oliveira P, Porto G, Oberkanins C, Mascarenhas M, Rodrigues P, Kury F, de Sousa M. Comparative study of the two more frequent HFE mutations (C282Y and H63D): significant different allelic frequencies between the North and South of Portugal. *Eur J Hum Genet* 2001; **9**: 843-848

35 **Adams PC**. Hemochromatosis case definition: out of focus? *Nat Clin Pract Gastroenterol Hepatol* 2006; **3**: 178-179

36 **Nichols L**, Dickson G, Phan PG, Kant JA. Iron binding saturation and genotypic testing for hereditary hemochromatosis in patients with liver disease. *Am J Clin Pathol* 2006; **125**: 236-240

37 **Kohgo Y**, Ohtake T, Ikuta K, Suzuki Y, Hosoki Y, Saito H, Kato J. Iron accumulation in alcoholic liver diseases. *Alcohol Clin Exp Res* 2005; **29**: 189S-193S

38 **de Diego C**, Opazo S, Murga MJ, Martínez-Castro P. H63D homozygotes with hyperferritinaemia: Is this genotype, the primary cause of iron overload? *Eur J Haematol* 2007; **78**: 66-71

39 **Samarasena J**, Winsor W, Lush R, Duggan P, Xie Y, Borgaonkar M. Individuals homozygous for the H63D mutation have significantly elevated iron indexes. *Dig Dis Sci* 2006; **51**: 803-807

40 **Gochee PA**, Powell LW, Cullen DJ, Du Sart D, Rossi E, Olynyk JK. A population-based study of the biochemical and clinical expression of the H63D hemochromatosis mutation. *Gastroenterology* 2002; **122**: 646-651

**S- Editor** Xiao LL **L- Editor** Anand BS **E- Editor** Zheng XM

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.112

*World J Gastroenterol* 2009 January 7; 15(1): 112-116
***World Journal of Gastroenterology*** ISSN 1007-9327


*BRIEF ARTICLES*

# Use of albendazole sulfoxide, albendazole sulfone, and combined solutions as scolicidal agents on hydatid cysts (*in vitro* study)

Gokhan Adas, Soykan Arikan, Ozgur Kemik, Ali Oner, Nilgun Sahip, Oguzhan Karatepe

Gokhan Adas, Soykan Arikan, Ozgur Kemik, Ali Oner, Nilgun Sahip, Oguzhan Karatepe, Okmeydani Training Hospital, Okmeydani/Sisli, Istanbul 34715, Turkey
Author contributions: Adas G and Karatepe O contributed equally to this work; Adas G and Arikan S performed research; Kemik O and Oner A contributed new reagents/analytic tools; Adas G and Karatepe O wrote the paper; Karatepe O made corrections and performed English editing.
Correspondence to: Dr. Oguzhan Karatepe, Okmeydani Training Hospital, Okmeydani/Sisli, Istanbul 34715, Turkey. drkaratepe99@hotmail.com
Telephone: +90-533-5408070 Fax: +90-216-361214
Received: September 6, 2008 Revised: November 7, 2008
Accepted: November 14, 2008
Published online: January 7, 2009

## Abstract

**AIM:** To establish which scolicidal agents are superior and more suitable for regular use.

**METHODS:** *Echinococcus granulosus* protoscoleces were obtained from 25 patients with liver hydatid cysts. Various concentrations of albendazole sulfone, albendazole sulfoxide, and albendazole sulfone and albendazole sulfoxide mixed together in concentrations of 50 μg/mL, and $H_2O_2$ in a concentration of 4%, NaCl 20%, and 1.5% cetrimide-0.15% chlorhexidine (10% Savlon-Turkey) were used to determine the scolicidal effects. Albendazole (ABZ) derivatives and other scolicidal agents were applied to a minimum of 100 scoleces for 5 and 10 min. The degree of viability was calculated according to the number of living scolices per field from a total of 100 scolices observed under the microscope.

**RESULTS:** After 5 min, ABZ sulfone was 97.3% effective, ABZ sulfoxide was 98.4% effective, and the combined solution was 98.6% effective. When sulfone, sulfoxide and the combined solutions were compared, the combined solution seemed more effective than sulfone. However, there was no difference when the combined solution was compared with sulfoxide. After 10 min, hypertonic salt water, sulfone, sulfoxide, and the combined solution compared to other solutions looked more effective and this was statistically significant on an advanced level. When sulfone, sulfoxide, and the combined solutions were compared with each other, the combined solution appeared more effective than sulfone. When the combined solution was compared with sulfoxide, there was no difference.

**CONCLUSION:** Despite being active, ABZ metabolites did not provide a marked advantage over 20% hypertonic saline. According to these results, we think creating a newly improved and more active preparation is necessary for hydatid cyst treatment.



**Key words:** Hydatid disease; Albendazole; *In vitro* study; Combined solution; Scolicidal agents

**Peer reviewer:** Valentin Fuhrmann, MD, Department of Internal Medicine 4, Intensive Care Unit, Medical University Vienna, Waehringer Guertel 18-20, Vienna A-1090, Austria

Adas G, Arikan S, Kemik O, Oner A, Sahip N, Karatepe O. Use of albendazole sulfoxide, albendazole sulfone, and combined solutions as scolicidal agents on hydatid cysts (*in vitro* study). *World J Gastroenterol* 2009; 15(1): 112-116 Available from: URL: http://www.wjgnet.com/1007-9327/15/112.asp DOI: http://dx.doi.org/10.3748/wjg.15.112

## INTRODUCTION

Treatments of hydatid disease have been suggested in many examples within the literature and text books, including systemic administration of various chemotherapeutic agents, surgery, and the percutaneous approach. The efficacy of systemic chemotherapy is limited[1-4]. Although surgery is the recommended treatment for liver hydatid cyst, percutaneous treatment has been introduced as an alternative treatment, especially in patients who cannot or do not want to undergo surgery[4,5]. There is no completely satisfactory surgical approach, but the operation is best performed by experts. The main object is to remove the cyst completely, without soiling and infecting the peritoneum and with complete obliteration of the resulting dead space. Complete removal of the cyst, with its adventitia, is ideal to avoid spilling the contents. The usual operation is cystectomy with removal of the germinal and laminated layers and preservation of the host derived

ectocyst[6]. Instillation of a scolicidal agent into hepatic hydatid cysts to reduce the risk of spillage of viable protoscolices is an integral part of the surgical technique for many surgeons. For over 20 years, benzoimidazole derivatives have been widely used in the medical treatment of hydatidosis. The current literature suggests that amongst all of these, albendazole (ABZ) is the most effective and useful drug for the medical treatment of hydatid disease[7]. After oral administration, ABZ is oxidized to a sulfoxide, which is in part further oxidized to a sulfone, and ABZ sulfoxide is the main metabolite *in vivo*[8]. The metabolites of ABZ are characterized by their low solubility in water and poor absorption[9]. ABZ has a scolicidal effect via its biologically active metabolites ABZ sulfoxide and ABZ sulfone, the latter being more effective. The first aim of this *in vitro* study was to establish the use of ABZ sulfoxide, ABZ sulfone, and the combined solution as scolicidal agents on the hydatid cyst. The second aim was to compare their effectiveness with other scolicidal agents.

## MATERIALS AND METHODS

### Protoscolex collection

For this *in vitro* study, we used scolex solutions collected from 25 patients who underwent operations for liver hydatid disease at the Haseki Teaching and Research Hospital, Department of General Surgery from 2002 to 2004. All samples were examined and identified at the Istanbul University, Faculty of Medicine, Department of Microbiology and Clinical Microbiology. For this study, ABZ sulfone and ABZ sulfoxide were purchased from Unimark Remedies Ltd Company in India (Certificate of Analysis of working standard ABZ/IMP001/02-Date 17.04.2002).

### Effectiveness of ABZ and other solutions in vitro

ABZ sulfone and ABZ sulfoxide dissolve poorly and slowly in water; however, in order to avoid the effects of other solvents, normal saline (0.9% NaCl) was used as a solvent, so that any scolocidal effect and tissue injury would be due only to ABZ sulfone and ABZ sulfoxide. Preparation of the ABZ sulfone and ABZ sulfoxide solutions was as follows: 10 mg ABZ sulfone and ABZ sulfoxide was dissolved in normal saline and brought to a final volume of 100 mL, and then the solution was mixed for 12 h at room temperature by a magnetic mixer. The solutions were further diluted by normal saline to obtain 50 μg/mL ABZ sulfone and ABZ sulfoxide in the final working solutions. We diluted another solution of ABZ sulfone and ABZ sulfoxide mixed together to a total of 50 μg/mL. All three solutions were sterilized by UV. Moreover, for comparison, we used $H_2O_2$ in 4% concentration, NaCl 20%, and 1.5% cetrimide-0.15% chlorhexidine (10% Savlon-Turkey) solution to determine the scolicidal effects.

The scolices were mixed with each solution for 5 and 10 min. The scolices were separated from the solutions and washed by normal saline. A few drops were smeared on an object glass and a drop of eosin was added; the slide was then covered with a cover glass and evaluated under the light microscope. For each different solution at both 5 and 10 min, the percentages of dead scolices were determined by counting a minumum of 100 scolices. With regard to the viability of cysts, the same criteria were used as defined in a previous report[10]. Scolices that lost their ellipsoid shape and became round, had rostellums rolled in, showed vacuolar degeneration, and took in eosin were considered dead; all four criteria had to be met for a scolex to be accepted as dead.

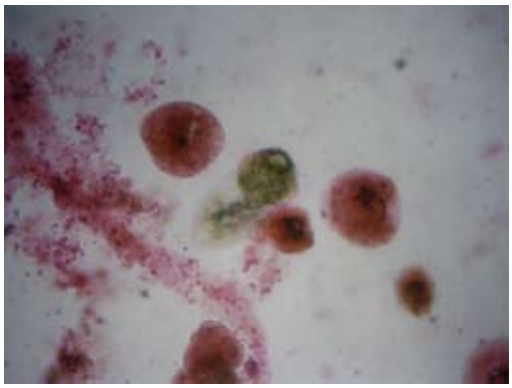

**Figure 1 Light microscopy of *Echinococcus granulosus* microcysts incubated *in vitro* with ABZ.**

### Statistical analysis

All statistical calculations were based on an analysis of comparing means by a paired samples *t* test. Differences were determined using the least significant differences, and $P < 0.05$ was considered to be significiant.

## RESULTS

This *in vitro* study demonstrated that 50 μg/mL ABZ sulfone killed 97.3% of the scolices, ABZ sulfoxide killed 98.4% of the scolices, and the combined solution (sulfone + sulfoxide) killed 98.6% of the scolices in 5 min (Figure 1). The second part of the study showed that the three ABZ solutions respectively killed 98.6%, 99.5%, and 99.6% of the scolices in 10 min. Among the other solutions that were used, hypertonic salt water was seen to be most effective on scolices. Hypertonic saline solution respectively killed 98.2% and 99.5% of the scolices in 5 and 10 min (Table 1). In the first part of the study, the scolicidal effects of hypertonic salt water, the ABZ sulfone, ABZ sulfoxide, and the combined solution were seen to be statistically significant on an advanced level compared to cetrimide and peroxide. When sulfone, sulfoxide, and the combined solution were compared to hypertonic salt water, no statistical significance was seen ($P > 0.05$). When sulfone, sulfoxide, and the combined solutions were compared with each other, the combined solution was seen to be more effective. When comparing the combined solution to sulfoxide there was not any difference.

In the second part of the study, again hypertonic salt water, sulfone, and the combined solution were seen to be more effective compared to other solutions and were

**Table 1 The use of different scolicidal agents at the 5th and 10th min for the treatment of hydatid disease (*in vitro* results)**

| Time | NaCl 20% | $H_2O_2$ | Cetrimide | Sulfone | Sulfoxide | Sulfone + Sulfoxide |
|---|---|---|---|---|---|---|
| 5th min | 98.2% | 90.3% | 86.9% | 97.3% | 98.4% | 98.6% |
| 10th min | 99.5% | 95.7% | 92.6% | 98.6% | 99.5% | 99.6% |

**Table 2 Different scolicidal agents in literature**

| Author | Agents | Results |
|---|---|---|
| Caglar *et al*[26] (2008) | 20% silver nitra (20 min) | 100% death |
| | 50% Dextroz (30 min) | 100% death |
| | 20% NaCl (45 min) | 100% death |
| | 20% Mannitol (45 min) | 100% death |
| Frayha *et al*[27] (1981) | Cetrimide 0.5%-1% (10 min) | 100% death |
| Kayaalp *et al*[28] (2002) | 10%-30% NaCl (3, 6, 75 min) | 100% death |
| Sonişik *et al*[11] (2002) | 10%-30% NaCl ( 3, 6 and 75 min) | 100% death |
| Besim *et al*[16] (1989) | 20% Saline (15 min) | 100% death |
| | 95% Ethyl alcohol (15 min) | 100% death |
| | 10% Polyvinyl pirrolidone iodine (15 min) | 100% death |
| | 3% $H_2O_2$ (15 min) | 100% death |
| Erzurumlu *et al*[20] (1998) | Albendazole sulfoxide 20 μg/mL | 5% death |
| | Albendazole sulfoxide 50 μg/mL | 50% death |
| | Albendazole sulfoxide 100 μg/mL | 100% death |
| Adas *et al* (our study) (2008) | 20% NaCL (5 min) | 98.2% death |
| | 3% $H_2O_2$ (5 min) | 90.3% death |
| | Cetrimide (5 min) | 86.9% death |
| | Albendazole sulfone (5 min) | 97.3% death |

seen to be statistically significant on an advanced level as well. When sulfone, sulfoxide and the combined solution were compared to hypertonic salt water, hypertonic salt water was seen to be statistically significant compared to sulfone, but it was not seen to be significant compared to the others. When sulfone, sulfoxide and the combined solution were compared to each other, the combined solution was seen to be more effective compared to sulfone. Upon comparing the combined solution with sulfoxide, there was not any difference.

When each solution was compared based on 5 and 10 min timing in the first and the second part of the study, the scolicidal effects of each solution in the second part were statistically significant ($P < 0.05$).

## DISCUSSION

There are currently three treatment options for hydatid disease of the liver: surgery, which remains the most efficent treatment, percutaneous aspiration and medical treatment. In general, hydatidosis is a public health problem, especially in our country, and the treatment often is selected depending on the social and medical professional's conditions. Since the 1990s, percutaneous treatment has been used increasingly. Surgery remains the most effective treatment, which aims to treat concurrently the parasitic disease, the cavity, and often the biliary complications. Although surgery is technically demanding and often considered risky, the development of hepatic surgery permits safer performance of this therapeutic option.

Ideally, therapy of liver hydatid disease should be able to cure the disease with a low morbidity. Failure of treatment is defined as recurrence and complications related to the intervention. Protection of the operation field is mandatory before the planned operation on the cyst or before the cyst is emptied. Preoperative destruction of the cyst's contents and preventing infection of the surrounding area has an important role for success of the operation; also, this procedure helps to prevent the illness from returning[11]. For sterilization of the cyst, several parasiticidal substances have been used. Scolicidal solutions remain indispensable in the treatment of hydatid cyst disease. Properties of an ideal solution would be inexpensiveness and the promotion of a rapid and complete scolicidal effect with an absence of local and systemic side effects. From this point of view, no ideal solution and agents have been described yet.

ABZ carbamate is one of the most important benzimidazole derivatives used against liver flukes, tapeworms, and lung and gastrointestinal roundworms. ABZ is normally not detectable in human plasma since it is rapidly metabolized to its major active metabolite as ABZ sulfoxide and ABZ sulfone. ABZ sulfoxide is the main metabolite *in vivo*[8,12-14]. ABZ leads to a decrease in the glycogen content of the cyst wall, inhibits acid phosphatase, ATP, pyruvate kinase, phosphoenolpyruvate kinase, and causes cellular autolysis and degeneration in the microthrics and the microtubuli[15].

Despite ABZ being used preoperatively and postoperatively for hydatid cyst treatment, today there is no comparative study in the literature, especially concerning ABZ metabolites' preoperative scolicidal effectiveness (Table 2). In our study, the ABZ metabolites of ABZ sulfoxide, ABZ sulfone, and the combined form were applied directly on the scolices. In this case, the reason why we chose ABZ metabolites over ABZ was that ABZ was not effective directly on the body. Also, we determined how much time was needed for each of the metabolites to be effective and on how many scolices they were effective.

In both parts of the study, cetrimide was found to be the least effective in killing the scolices. This result showed a dissimilarity from other limited studies performed in the literature. Besim *et al*[16] reported that cetrimide-chlorhexidine was the most potent scolicidal agent *in vitro*. Half-percent cetrimide and 0.05% chlorhexidine combination for 5 min is an effective protoscolicidal agent in labaratory and clinical

studies[16]. Sonişik *et al*[11] further supported the potent scolicidal effect of cetrimide with a clinical study. The disadvantages of cetrimide, namely metabolic acidosis and methemoglobinemia, have been reported[17]. In the literature, hypertonic salt water (20%) was 100% effective on scolices after 6 min, and this is probably the most widely used scolicidal agent in current practice because of its availability and effective scolicidal properties[17]. In our study, this treatment was found to be 98.2% effective at the end of min and 99.5% effective at the end of 10 min[17]. Hypertonic saline can cause acute hypernatremia. $H_2O_2$ was not found to be 100% effective on scolices in our study. Because of the side effects, it is not used in many fields today. Landa García *et al*[18] tested four scolicidal agents (10% $H_2O_2$, 10% providone iodine, praziquantel, 10% hypertonic saline) and reported that 10% $H_2O_2$ and 10% providone iodine were much more potent than the other agents. Even though 10% $H_2O_2$ was defined as a powerful scolicidal agent *in vitro* by Meymerian *et al*[19], fatal air embolism and anaphylactic shock have also been reported with 10% $H_2O_2$.

Today, ABZ and its metabolites are not used as scolicidals for the preoperative routine. ABZ is the most widely used substance for the medical treatment of hydatid cyst disease. It has a scolicidal effect via its biologically active metabolites sulfone and sulfoxide. ABZ sulfoxide is more effective than ABZ sulfone. There is no study examining whether one of the metabolites or combinations are more effective as a preoperative scolicidal. On this topic, there have been two experimental studies by Erzurumlu *et al*[20-22]. He demonstrated that 20 μg/mL ABZ sulfoxide killed 5% of the scolices in 15 min, scolicidal activity was 50% with a 50 μg/mL solution, and it was 100% for a 100 μg/mL solution[20]. The other study showed that 10%, 5% and 1% ABZ solutions had complete scolicidal effects[21]. ABZ was used experimentally for percutaneous treatment of hydatid cysts. Deger *et al*[3] demonstrated that ABZ sulfoxide injection as a scolicidal agent in the percutaneous treatment of cystic echinococcosis seems to be effective in sheep. Yetim *et al*[22] further supported percutaneous treatment with the idea that ABZ solutions are effective as scolicidal solutions on rabbits. Greater scolicidal effects and fewer side effects on the hepatobiliary system are the advantages of ABZ solution[22]. It is known that scolicidal solution injection in the cysts or the biliary system leads to a rise in liver enzyme level. It has also been shown that systemically administered ABZ can lead to the same changes[3,22,23].

When the first and the second part of the study were compared, it was seen that all of the solutions that were used were more effective at the end of 10 min. This result was statistically significant, as it showed us that 5 min of preoperative waiting time was not enough.

In conclusion, none of the solutions killed 100% of the scolices at the end of 5 and 10 min. With the aim of preventing the spread to the operations' surrounding area and the peritoneum, it is necessary to strictly protect the surrounding area of the cyst. Despite the effectiveness of ABZ metabolites, no distinctive advantage was provided compared with 20% hypertonic saline. Its harder obtainability, harder preparation, and high cost are some of the important disadvantages. For this reason, it cannot be seen as an ideal scolicidal agent. An ideal scolicidal agent is defined as being potent in low concentrations, acting in a short period time, being stable in cyst fluid, not affected by dilution with the cyst fluid, being able to kill the scolex in the cyst, being non-toxic, having low viscosity, and being readily avaliable and easily prepared, as well as being inexpensive[24,25]. In conjunction with prevention of cystic fluid spillage, total evacuation and prevention of any contact of the germinative membrane with the peritoneal surface are essential because the germinative membrane can contain viable protoscoleces despite proper cyst fluid inactivation. Walling off the surgical field with laparotomy sponges soaked in scolicidal agents is the most important step in hydatid cyst surgery. If ABZ metabolites can be obtained for commercial use and can be inexpensive, the usage of them could be more common. Combined and sulfoxide solutions were found to be more effective than sulfone. In our clinical practice, it is usually the 20% hypertonic saline solution that is used. However, it presents serious intraoperative problems related to hypernatremia. Therefore, we need less harmful but more effective drugs in hydatid disease treatment. Our study sheds light on the fact that ABZ metabolites may well meet this requirement.

## COMMENTS

### *Background*

Using scolicidal agents is of great importance in hydatid disease. The aim of this study was to find the most effective and appropriate scolicidal agents.

### *Research frontiers*

Albendazole (ABZ) metabolites are highly effective as scolicidal agents. When compared with each other, combined and sulfoxide solutions were found to be more effective than sulfone. When it comes to the comparison of ABZ metabolites with 20% hypertonic saline, no distinctive advantage was provided

### *Applications*

ABZ metabolites, the most effective scolicidal agents, hydatid disease treatment, the comparison of various scolicidal agents.

### *Terminology*

Hydatid disease is a potentially fatal parasitic disease that can affect many animals, including wildlife, commercial livestock and humans. ABZ is a broad spectrum anti-protozoal and anti-helminthic compound used as a drug indicated for the treatment of a variety of worm infestations. ABZ metabolites include sulfone, sulfoxide, and the combined solution.

### *Peer review*

The manuscript deals with the topic with adequate methods and conclusive results. It's very interesting.

## REFERENCES

1 **Ramos G**, Orduña A, García-Yuste M. Hydatid cyst of the lung: diagnosis and treatment. *World J Surg* 2001; **25**: 46-57

2 **Poston JG**, Blumgart HL. Surgical Management of Hepatobiliary and Pancreatic Disorders. In: Tagliacozzo S. Management of hydatid disease of the liver. London: Taylor & Francis, 2003; 215-235

3 **Deger E**, Hokelek M, Deger BA, Tutar E, Asil M, Pakdemirli E. A new therapeutic approach for the treatment of cystic

echinococcosis: percutaneous albendazole sulphoxide injection without reaspiration. *Am J Gastroenterol* 2000; **95**: 248-254
4 **Yorganci K**, Sayek I. Surgical treatment of hydatid cysts of the liver in the era of percutaneous treatment. *Am J Surg* 2002; **184**: 63-69
5 **Akhan O**, Ozmen MN. Percutaneous treatment of liver hydatid cysts. *Eur J Radiol* 1999; **32**: 76-85
6 **Sherlock S**, Dooley J. Diseases of the liver and biliary system. London: Blackwell Science, 1997: 515-520
7 **Erzurumlu K**, Hökelek M, Gönlüsen L, Tas K, Amanvermez R. The effect of albendazole on the prevention of secondary hydatidosis. *Hepatogastroenterology* 2000; **47**: 247-250
8 **Redondo PA**, Alvarez AI, Garcia JL, Larrodé OM, Merino G, Prieto JG. Presystemic metabolism of albendazole: experimental evidence of an efflux process of albendazole sulfoxide to intestinal lumen. *Drug Metab Dispos* 1999; **27**: 736-740
9 **García-Llamazares JL**, Alvarez-de-Felipe AI, Redondo-Cardeña PA, Prieto-Fernández JG. Echinococcus granulosus: membrane permeability of secondary hydatid cysts to albendazole sulfoxide. *Parasitol Res* 1998; **84**: 417-420
10 **Urrea-París MA**, Moreno MJ, Casado N, Rodriguez-Caabeiro F. In vitro effect of praziquantel and albendazole combination therapy on the larval stage of Echinococcus granulosus. *Parasitol Res* 2000; **86**: 957-964
11 **Sonişik M**, Korkmaz A, Besim H, Karayalçin K, Hamamci O. Efficacy of cetrimide-chlorhexidine combination in surgery for hydatid cyst. *Br J Surg* 1998; **85**: 1277
12 **Ingold K**, Bigler P, Thormann W, Cavaliero T, Gottstein B, Hemphill A. Efficacies of albendazole sulfoxide and albendazole sulfone against In vitro-cultivated Echinococcus multilocularis metacestodes. *Antimicrob Agents Chemother* 1999; **43**: 1052-1061
13 **Kitzman D**, Cheng KJ, Fleckenstein L. HPLC assay for albendazole and metabolites in human plasma for clinical pharmacokinetic studies. *J Pharm Biomed Anal* 2002; **30**: 801-813
14 **Okelo GB**, Hagos B, Ng'ang'a JN, Ogeto JO. Pharmacokinetics of albendazole in children with hydatid disease. *East Afr Med J* 1993; **70**: 643-645
15 **Lacey E**. Mode of action of benzimidazoles. *Parasitol Today* 1990; **6**: 112-115
16 **Besim H**, Karayalçin K, Hamamci O, Güngör C, Korkmaz A. Scolicidal agents in hydatid cyst surgery. *HPB Surg* 1998; **10**: 347-351
17 **Urrea-París MA**, Moreno MJ, Casado N, Rodriguez-Caabeiro F. In vitro effect of praziquantel and albendazole combination therapy on the larval stage of Echinococcus granulosus. *Parasitol Res* 2000; **86**: 957-964
18 **Landa García JI**, Alonso E, Gonzalez-Uriarte J, Rodriguez Romano D. Evaluation of scolicidal agents in an experimental hydatid disease model. *Eur Surg Res* 1997; **29**: 202-208
19 **Meymerian E**, Luttermoser GW, Frayha GJ, Schwabe CW, Prescott B. Host-parasite relationships in echinococcosis: x.laboratory evaluation of chemical scollcides as adjuncts to hydatid surgery. *Ann Surg* 1963; **158**: 211-215
20 **Erzurumlu K**, Hokelek M, Baris S, Sahin M, Birinci A, Amanvermez R, Tac K. Effect of albendazole sulfoxide solution on the scolices and the hepatobiliary system. *Eur Surg Res* 1998; **30**: 433-438
21 **Erzurumlu K**, Ozdemir M, Mihmanli M, Cevikbaş U. The effect of intraoperative mebendazole-albendazole applications on the hepatobiliary system. *Eur Surg Res* 1995; **27**: 340-345
22 **Yetim I**, Erzurumlu K, Hokelek M, Baris S, Dervisoglu A, Polat C, Belet U, Buyukkarabacak Y, Guvenli A. Results of alcohol and albendazole injections in hepatic hydatidosis: experimental study. *J Gastroenterol Hepatol* 2005; **20**: 1442-1447
23 **Gil-Grande LA**, Rodriguez-Caabeiro F, Prieto JG,Sánchez-Ruano JJ, Brasa C, Aguilar L, García-Hoz F, Casado N, Bárcena R, Alvarez AI. Randomised controlled trial of efficacy of albendazole in intra-abdominal hydatid disease. *Lancet* 1993; **342**: 1269-1272
24 Guidelines for treatment of cystic and alveolar echinococcosis in humans. WHO Informal Working Group on Echinococcosis. *Bull World Health Organ* 1996; **74**: 231-242
25 **Puryan K**, Karadayi K, Topcu O, Canbay E, Sumer Z, Turan M, Karayalcin K, Sen M. Chlorhexidine gluconate: an ideal scolicidal agent in the treatment of intraperitoneal hydatidosis? *World J Surg* 2005; **29**: 227-230
26 **Caglar R**, Yuzbasioglu MF, Bulbuloglu E, Gul M, Ezberci F, Kale IT. In vitro effectiveness of different chemical agents on scolices of hydatid cyst. *J Invest Surg* 2008; **21**: 71-75
27 **Frayha GJ**, Bikhazi KJ, Kachachi TA. Treatment of hydatid cysts (Echinococcus granulosus) by Cetrimide (R). *Trans R Soc Trop Med Hyg* 1981; **75**: 447-450
28 **Kayaalp C**, Balkan M, Aydin C, Ozgurtas T, Tanyuksel M, Kirimlioglu V, Akoglu M, Oner K, Pekcan M. Hypertonic saline in hydatid disease. *World J Surg* 2001; **25**: 975-979

**S- Editor** Li DL **L- Editor** Logan S **E- Editor** Zheng XM

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.117

*World J Gastroenterol* 2009 January 7; 15(1): 117-120
***World Journal of Gastroenterology*** ISSN 1007-9327


*CASE REPORT*

# An unusual presentation of sclerosing mesenteritis as pneumoperitoneum: Case report with a review of the literature

Sumita Chawla, Satheesh Yalamarthi, Irshad A Shaikh, Veena Tagore, Paul Skaife

Sumita Chawla, Paul Skaife, Department of Colorectal Surgery, University Hospital Aintree, Liverpool L9 7AL, United Kingdom
Satheesh Yalamarthi, Irshad A Shaikh, Department of Colorectal Surgery, Queen Margaret Hospital, Dunfermline, KY12 0SU, United Kingdom
Veena Tagore, Department of Pathology, University Hospital Aintree, Liverpool L9 7AL, United Kingdom
Author contributions: Chawla S, Yalamarthi S contributed equally towards writing the case report and review of the literature as well as undertaking a comprehensive literature search; Shaikh IA contributed towards the literature review and manuscript preparation; Tagore V provided the histopathological information and Skaife P is the main clinical lead.
Correspondence to: Satheesh Yalamarthi, Department of Surgery, Queen Margaret Hospital, Whitefield Road, Dunfermline KY12 0SU, Scotland, United Kingdom. satyalam@aol.com
Telephone: +44-1383-623623 Fax: +44-1383-624156
Received: October 3, 2008 Revised: November 10, 2008
Accepted: November 17, 2008
Published online: January 7, 2009

## Abstract

Sclerosing mesenteritis is a rare condition that involves the small or large bowel mesentery. An unusual presentation of this condition, which led to difficult preoperative assessment and diagnosis, is described. This report is followed by a comprehensive review of the literature.



**Key words:** Mesenteritis; Panniculitis; Small bowel obstruction; Mesentery

**Peer reviewer:** Walter E Longo, Professor, Department of Surgery, Yale University School of Medicine, 205 Cedar Street, New Haven 06510, United States

Chawla S, Yalamarthi S, Shaikh IA, Tagore V, Skaife P. An unusual presentation of sclerosing mesenteritis as pneumoperitoneum: Case report with a review of the literature. *World J Gastroenterol* 2009; 15(1): 117-120 Available from: URL: http://www.wjgnet.com/1007-9327/15/117.asp DOI: http://dx.doi.org/10.3748/wjg.15.117

## INTRODUCTION

Sclerosing mesenteritis is a rare benign condition that affects the intestinal mesentery. Its presentation can be quite varied because of the different histopathological processes involved in the evolution of this condition. Diagnosis can be challenging, as is its management. An unusual presentation of this condition is described, followed by a comprehensive review of the literature.

## CASE REPORT

A 74-year-old man was admitted as an emergency with acute abdominal pain and distension. He previously underwent an abdominoperineal resection 10 years ago for rectal cancer, and most recently had a surveillance colonoscopy about 2 wk prior to admission.

On initial assessment, he had a slightly elevated temperature (37.4℃). There were no signs of peritonitis, but his abdomen was slightly distended and had minimal central abdominal tenderness. His blood investigations, which included a full blood count, C-reactive protein, amylase, serum electrolytes and liver function tests, were all within normal limits. The erect chest X-ray showed pneumoperitoneum on both sides, and the abdominal film showed some distended small bowel loops in the central abdomen.

Although he had evidence of a perforated viscus on the X-ray, he was managed conservatively, in view of his relative clinical wellbeing and lack of hematological and biochemical abnormalities. He was kept nil by mouth, had a nasogastric tube inserted, commenced on intravenous fluids and broad-spectrum antibiotics, and was kept under close observation.

An upper gastrointestinal perforation was excluded by means of a Gastrograffin meal. Over the course of the next few days, his clinical condition improved with conservative management. However, his repeat chest films continued to show free gas under both his diaphragms, and we arranged for a computerized tomography (CT) scan of his abdomen and pelvis, with oral and intravenous contrast. This again confirmed the pneumoperitoneum and a few dilated small bowel loops. There was no extravasation of the contrast material into the peritoneal cavity (both small and large bowel loops

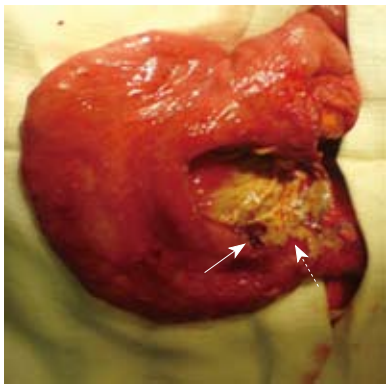

**Figure 1 Intraoperative findings, showing small bowel obstruction from an area of localized thickening (dashed arrow), with a proximal perforation (arrow).** The proximal small bowel is dilated and thickened, suggesting chronic obstruction.

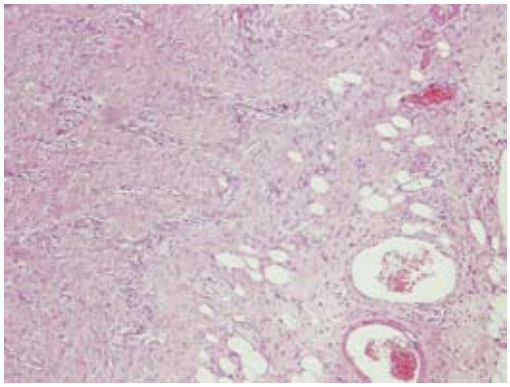

**Figure 2 Histology of the specimen.** Section through the mesentery, showing fibrocollagenous stroma with dilated vessels.

were filled with the contrast agent) or any obvious point of obstruction.

On day 8 following his admission, he started vomiting feculant material, which prompted an urgent midline laparotomy. At operation, there was a small amount of turbid fluid in the peritoneal cavity and an area of near complete obstruction at the mid-part of the small bowel. At this location, there was also a localized collection of small bowel contents. Part of the wall of this collection was made up of the adjacent collapsed loops of small bowel and mesentery. On dissecting this area, the point of obstruction was a 2-3-cm area of circumferentially thickened small bowel, and there was a tiny perforation just proximal to this (Figure 1). Adjacent mesentery was thickened. The proximal bowel wall showed signs of chronic obstruction. The previous colostomy in the left iliac fossa was normal and there was no evidence of any recurrent cancer. As the intraoperative finding was suspicious of a primary small bowel malignancy, nearly 40 cm of the small bowel was resected with wide mesenteric clearance, and a side-to-side hand-sewn jejuno-jejunal anastomosis was carried in two layers.

Postoperatively, the patient developed several complications, such as bilateral pneumonia, adult respiratory distress syndrome, paralytic ileus and renal failure. He unfortunately succumbed to these complications and died after a prolonged stay in the critical care unit.

The histopathological examination of the narrowed segment of the small bowel showed concentric fibrosis of the mesentery, serosa and outer part of the muscularis propria, with a slightly edematous mucosa. The fibrous tissue was arranged in lobules and was composed of fibroblast-like spindle cells arranged in a fascicular pattern. Sections through the thickened mesentery showed marked fibrocollagenous thickening, without fat necrosis or inflammation (Figure 2). No granulomas or foreign-body reaction were seen. The perforated area showed fibrinopurulent exudates, with no ulcers or other lesions. Immunohistochemistry of the young and collagenized fibrous tissue was positive for smooth muscle actin, vimentin and cytokeratin but negative for desmin, S100, CD117 and CD34. The overall appearance was that of fibrosing mesenteritis.

**Table 1 Various nomenclature used to describe this condition[3,5-7]**

| Sclerosing mesenteritis[1] | Xanthogranulomatous mesenteritis |
|---|---|
| Retractile mesenteritis | Mesenteric lipogranuloma |
| Liposclerotic mesenteritis | Systemic nodular panniculitis |
| Mesenteric weber-christian disease | |

[1]Preferred terminology.

**Table 2 Association with other idiopathic inflammatory disorders[1,3,4,7-9]**

| Retroperitoneal fibrosis | Sclerosing cholangitis |
|---|---|
| Riedel thyroiditis | Sclerosing mediastinitis |
| Orbital pseudotumor | Desmoplastic metastatic carcinoma |
| Whipple disease | Sarcoma |
| Mesenteric fibromatosis (intra-abdominal desmoid tumour) | |

## DISCUSSION

Sclerosing mesenteritis is a rare, idiopathic and benign disease of the abdominal mesentery first described by Jura in 1924[1-3]. It is defined as a mass that is found in the lining of the mesentery[4]. Currently, just over 300 cases have been published to date, with the majority being case reports and the rest being very small series.

In addition to sclerosing mesenteritis, other nomenclature has been used (Table 1). In histolo-pathological terms, the preferred terminology is sclerosing mesenteritis[7]. The different terminology used represents the different histological features found, despite the clinical entity being the same. This disorder has been linked with other idiopathic inflammatory disorders (Table 2).

The vast majority of cases of sclerosing mesenteritis are considered as idiopathic. Autoimmunity, ischemia, infection, vasculitis, Gardner's syndrome, carcinoid, trauma and previous abdominal surgery have all been linked[1,3,9-11]. In the series of Daskalogiannaki *et al*[12], sclerosing mesenteritis was related to malignancy in 69% of patients. The association with malignancy may be coincidental or secondary to an autoimmune inflammatory reaction, the mechanism of which has not yet been elucidated. These malignancies included

**Table 3 Different stages of disease and treatment[1-3,5,7,13,15]**

| Stage of disease | Predominant histological feature | Preferred terminology | Treatment |
|---|---|---|---|
| Early stages | Fat necrosis | Mesenteric lipodystrophy | None |
| Intermediate stages | Inflammation | Mesenteric panniculitis | Immunosuppressants[18,19]<br>Colchicine[3,20]<br>Tamoxifen[2]<br>Corticosteroids[3,19,20]<br>Progesterones[2,21] |
| Final stages | Fibrosis | Sclerosing or retractile mesenteritis | Surgery (where indicated) |

lymphoma, breast cancer, lung cancer, melanoma and colon cancer. In our case, previous surgery for rectal cancer may have been a causative factor, despite the fact that the surgery was a decade ago.

Mean age at presentation of sclerosing mesenteritis is in the fifth and sixth decades, and it is seen twice as frequently in males than females[1,8,13]. The disease can present clinically as single or multiple masses, or as a diffuse thickening of the mesentery[1,2,7,8,13]. The most common site of this disease is the small bowel mesentery. However, sole involvement of the mesocolon (the most common site being the rectosigmoid colon)[9] has been found in 20% of cases. Rare sites described are the mesoappendix, peripancreatic area, omentum and pelvis[4,7,9].

Clinical manifestations of this entity are non-specific. Patients may present asymptomatically with an incidental diagnosis. The various clinical features include abdominal pain, vomiting, diarrhea, constipation, anorexia, weight loss, fatigue, fever of unknown origin, ascites, pleural and pericardial effusion[2-4,7-9,13,14]. In our case, the presentation was complex, with the patient presenting with pneumoperitoenum following a recent colonoscopy, which suggested an iatrogenic complication. In retrospect, he also had some features of small bowel obstruction. The combination of these made a preoperative diagnosis difficult.

Diagnosis of sclerosing mesenteritis may be complex and involves appropriate clinical and radiographic analysis. However, for a definitive diagnosis, biopsy and histological confirmation may be necessary. The main histological features are fat necrosis, chronic inflammation and fibrosis, which may all occur together, but there is a predominance of one of these features, as shown in Table 3. The histological diagnosis, as in our case, can be improved with immunohistochemistry techniques using smooth muscle actin, cytokeratin and vimentin[4,9].

Sclerosing mesenteritis has been best diagnosed radiologically with multidetector CT and magnetic resonance imaging (MRI). The two main CT features are the "fat-ring" sign (area of fat around the mesenteric vessels)[8,14-16] and the presence of a tumoral pseudocapsule[1,2,7,8]. The other CT findings include changes of increased attenuation in the mesentery, low-attention foci representing fat necrosis and fibrosis, and development of a solid mass encasing the mesenteric vessel calcification in the center of the necrotic portion of the mass[8,14,16]. The role of CT angiography is also helpful in delineating the relationship of the mass to the mesenteric vasculature. Despite our patient having a CT scan, there were no typical radiological features of this condition noted.

Ghanem *et al*[17] have recently described the role of MRI in finding a fibrous capsule in retractile mesenteritis. The role of fluorine-18 fluorodexoyglucose positron emission tomography has also been reported in the literature as a new and upcoming image modality in the detection of sclerosing mesenteritis[15].

Management of sclerosing mesenteritis is dependent on the stage and hence histological findings of the disease (Table 3). In the early stages, when fat necrosis is the main feature, it tends to settle spontaneously without treatment. As the disease progresses and chronic inflammation predominates with undeveloped fibrosis, various agents have been used alone or in combination. The role of early treatment with cyclophosphamide has been reported in two cases with promising results, without signs of recurrence[18]. A single case study has shown the positive effect of the combination of corticosteroids and azathioprine[19]. Cuff *et al*[3] and Genereau *et al*[20] have demonstrated the effective management of colchicines, together with corticosteroid, in a series of symptomatic patients. In the final stages, when fibrosis overshadows the fat necrosis and chronic inflammation, surgical intervention, which includes partial resection, bypass, and colostomy may become necessary. Overall, surgical treatment should be limited to establishing a diagnosis or treatment of complications[3,7]. In our patient, the complications of small bowel obstruction and a localized perforation occurred, which required resection. It is worthy of note that the intraoperative findings can mimic a malignant process as highlighted in the report by Mathew *et al*[2]. Despite the fact that our patient succumbed to his postoperative complications, the overall prognosis published in the literature is favorable for this condition.

Our case illustrates that the diagnosis of sclerosing mesenteritis can be difficult to make preoperatively, especially when other procedures, such as colonoscopy, have been carried out recently, which by itself has the risk of causing iatrogenic perforation. The presentation and management of this condition can be varied and this depends upon the underlying stage of the histological process.

## REFERENCES

1 **Durst AL**, Freund H, Rosenmann E, Birnbaum D. Mesenteric panniculitis: review of the leterature and presentation of cases. *Surgery* 1977; **81**: 203-211

2 **Mathew J**, McKenna F, Mason J, Haboubi NY, Borghol M. Sclerosing mesenteritis with occult ileal perforation: report of a case simulating extensive intra-abdominal malignancy. *Dis Colon Rectum* 2004; **47**: 1974-1977

3 **Cuff R**, Landercasper J, Schlack S. Sclerosing mesenteritis.

*Surgery* 2001; **129**: 509-510
4 **White B**, Kong A, Chang AL. Sclerosing mesenteritis. *Australas Radiol* 2005; **49**: 185-188
5 **Kelly JK**, Hwang WS. Idiopathic retractile (sclerosing) mesenteritis and its differential diagnosis. *Am J Surg Pathol* 1989; **13**: 513-521
6 **Adachi Y**, Mori M, Enjoji M, Ueo H, Sugimachi K. Mesenteric panniculitis of the colon. Review of the literature and report of two cases. *Dis Colon Rectum* 1987; **30**: 962-966
7 **Emory TS**, Monihan JM, Carr NJ, Sobin LH. Sclerosing mesenteritis, mesenteric panniculitis and mesenteric lipodystrophy: a single entity? *Am J Surg Pathol* 1997; **21**: 392-398
8 **Sabate JM**, Torrubia S, Maideu J, Franquet T, Monill JM, Perez C. Sclerosing mesenteritis: imaging findings in 17 patients. *AJR Am J Roentgenol* 1999; **172**: 625-629
9 **Sharma V**, Martin P, Marjoniemi VM. Idiopathic orbital inflammation with sclerosing mesenteritis: a new association? *Clin Experiment Ophthalmol* 2006; **34**: 190-192
10 **Han SY**, Koehler RE, Keller FS, Ho KJ, Zornes SL. Retractile mesenteritis involving the colon: pathologic and radiologic correlation (case report). *AJR Am J Roentgenol* 1986; **147**: 268-270
11 **Perez-Fontan FJ**, Soler R, Sanchez J, Iglesias P, Sanjurjo P, Ruiz J. Retractile mesenteritis involving the colon: barium enema, sonographic, and CT findings. *AJR Am J Roentgenol* 1986; **147**: 937-940
12 **Daskalogiannaki M**, Voloudaki A, Prassopoulos P, Magkanas E, Stefanaki K, Apostolaki E, Gourtsoyiannis N. CT evaluation of mesenteric panniculitis: prevalence and associated diseases. *AJR Am J Roentgenol* 2000; **174**: 427-431
13 **Ikoma A**, Tanaka K, Komokata T, Ohi Y, Taira A. Retractile mesenteritis of the large bowel: report of a case and review of the literature. *Surg Today* 1996; **26**: 435-438
14 **Katz ME**, Heiken JP, Glazer HS, Lee JK. Intraabdominal panniculitis: clinical, radiographic, and CT features. *AJR Am J Roentgenol* 1985; **145**: 293-296
15 **Nguyen BD**. F-18 FDG PET demonstration of sclerosing mesenteritis. *Clin Nucl Med* 2003; **28**: 670-671
16 **Mata JM**, Inaraja L, Martin J, Olazabal A, Castilla MT. CT features of mesenteric panniculitis. *J Comput Assist Tomogr* 1987; **11**: 1021-1023
17 **Ghanem N**, Pache G, Bley T, Kotter E, Langer M. MR findings in a rare case of sclerosing mesenteritis of the mesocolon. *J Magn Reson Imaging* 2005; **21**: 632-636
18 **Bush RW**, Hammar SP Jr, Rudolph RH. Sclerosing mesenteritis. Response to cyclophosphamide. *Arch Intern Med* 1986; **146**: 503-505
19 **Bala A**, Coderre SP, Johnson DR, Nayak V. Treatment of sclerosing mesenteritis with corticosteroids and azathioprine. *Can J Gastroenterol* 2001; **15**: 533-535
20 **Genereau T**, Bellin MF, Wechsler B, Le TH, Bellanger J, Grellet J, Godeau P. Demonstration of efficacy of combining corticosteroids and colchicine in two patients with idiopathic sclerosing mesenteritis. *Dig Dis Sci* 1996; **41**: 684-688
21 **Mazure R**, Fernandez Marty P, Niveloni S, Pedreira S, Vazquez H, Smecuol E, Kogan Z, Boerr L, Maurino E, Bai JC. Successful treatment of retractile mesenteritis with oral progesterone. *Gastroenterology* 1998; **114**: 1313-1317

**S- Editor** Tian L **L- Editor** Kerr C **E- Editor** Lin YP

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.121

*World J Gastroenterol* 2009 January 7; 15(1): 121-123
***World Journal of Gastroenterology*** ISSN 1007-9327




# Bile duct ligation in rats: A reliable model of hepatorenal syndrome?

Stelios F Assimakopoulos, Constantine E Vagianos

Stelios F Assimakopoulos, Department of Internal Medicine, University Hospital of Patras, Patras 26504, Greece
Constantine E Vagianos, First Surgical Department, "Saint Panteleimon" General Hospital of Nikaia, Piraeus 18454, Greece
Author contributions: Assimakopoulos SF wrote this commentary and Vagianos CE revised it.
Correspondence to: Stelios F Assimakopoulos, MD, PhD, Department of Internal Medicine, School of Medicine, University of Patras, Vironos 18, 26224, Patras, Greece. sassim@upatras.gr
Telephone: +30-2610-346946 Fax: +30-2610-990775
Received: October 18, 2008 Revised: December 6, 2008
Accepted: December 13, 2008
Published online: January 7, 2009

## Abstract

The two most widely used experimental models of advanced liver disease are the administration of carbon tetrachloride, and common bile duct ligation (BDL), however, neither has been systematically evaluated as a model of hepatorenal syndrome (HRS). The BDL model in rats, studied at diverse time points, induced a progressive renal dysfunction without structural changes in the kidney. The authors concluded that BDL is a good model for further studies of HRS and its treatment. However, the renal impairment observed at the acute phase of the BDL model is based on a different pathophysiology than that of HRS. Specifically, in acute obstructive jaundice, cholemia predominates over parenchymal liver disease (reversible at this stage without portal hypertension or cirrhosis) and independently induces negative inotropic and chronotropic effects on the heart, impaired sympathetic vasoconstriction response and profound natriuresis and diuresis that might lead to volume depletion. In addition, systemic endotoxemia contributes to the prerenal etiology of renal impairment and promotes direct nephrotoxicity and acute tubular necrosis. On the other hand, the renal failure observed in the chronic BDL model (with development of biliary cirrhosis, portal hypertension and ascites) shares pathophysiological similarities with HRS, but the accordance of the chronic BDL model to the diagnostic criteria of HRS (e.g. absence of spontaneous bacterial peritonitis, no renal function improvement after plasma volume expansion) should have been confirmed. In conclusion, we think that the BDL model is not suitable for the study of the natural history of HRS, but the chronic BDL model might be valid for the study of established HRS and its potential therapies.



**Key words:** Obstructive jaundice; Rats; Bile duct ligation; Hepatorenal syndrome; Renal failure

Assimakopoulos SF, Vagianos CE. Bile duct ligation in rats: A reliable model of hepatorenal syndrome? *World J Gastroenterol* 2009; 15(1): 121-123 Available from: URL: http://www.wjgnet.com/1007-9327/15/121.asp DOI: http://dx.doi.org/10.3748/wjg.15.121

## TO THE EDITOR

We read with great interest the article recently published in *World J Gastroenterol* by Dr. Pereira *et al*[1], which evaluated the reliability of the bile duct ligation (BDL) model in rats for the study of hepatorenal syndrome (HRS). The authors found that this experimental model induces progressive renal dysfunction without structural changes in the kidney, and they suggested that BDL in rats emerges as a good model for further studies of HRS pathophysiology and treatment. Upon reading this very interesting study, a number of questions a rose as to whether the renal dysfunction observed after BDL really represents HRS.

HRS is defined as a specific type of functional renal failure complicating advanced liver disease (e.g. decompensated cirrhosis, alcoholic hepatitis, or acute liver failure)[2,3]. The pathogenesis of this syndrome is the result of an extreme underfilling of the arterial circulation secondary to an arterial vasodilation located in the splachnic circulation. This underfilling triggers a compensatory response with activation of vasoconstrictor systems leading to intense renal vasoconstriction, especially to the renal cortex, and finally the glomerular filtration rate is decreased in the absence of underlying kidney pathology whilst tubular function is preserved[4]. Consequently, this specific type of functional renal failure observed in advanced liver disease must be differentiated by a number of non-functional causes of renal failure in this setting, e.g., other causes of prerenal azotemia or acute tubular

necrosis[3,4]. The distinction between HRS and other causes of renal failure that may occur in cirrhosis is problematic mainly due to the lack of a specific diagnostic test. A number of commonly used urinary indices (e.g. urinary sodium or osmolarity) cannot reliably distinguish HRS and acute tubular necrosis in the setting of cirrhosis, making this differentiation especially difficult[3,4]. For these reasons, the diagnosis of HRS is currently based on the exclusion of other disorders that could lead to renal failure in cirrhosis including shock (septic or hypovolemic), ongoing bacterial infection, fluid losses and current treatment with nephrotoxic drugs[4]. An additional major criterion that should be fulfilled is that no sustained improvement in renal function occurs after expansion of plasma volume. In their study, the authors have not examined the accordance of the BDL model with these criteria, instead they characterize the BDL-induced renal dysfunction as "HRS" based solely on the absence of histopathological changes in the kidneys and on evaluation of diverse urinary indices. The histological analyses performed in the kidney are not described in detail but it is generally stated that BDL rats exhibited normal renal histology under the light microscope. This finding contradicts most previously published reports, which describe significant histological alterations, predominantly located in tubular epithelial cells, at various intervals of obstructive jaundice[5-10]. Furthermore, as already stated, urinary indices represent only minor and dispensable criteria for the diagnosis of HRS[4]. Taking into consideration these issues, a number of uncertainties are raised regarding the reliability of the BDL model for the study of HRS, which become more evident when considering the pathophysiology of renal failure complicating obstructive jaundice.

In rats, double ligation of the common bile duct with its dissection between the ligatures produces a well established experimental model of: (1) acute obstructive jaundice, studying different time points up to 2 wk after BDL; (2) progression of biliary fibrosis to cirrhosis, studying diverse time intervals up to 4 or 6 wk after BDL; and (3) secondary biliary cirrhosis, at 4 or 6 wk after BDL[11,12]. BDL produces a combined model of cholemia and parenchymal liver disease. The magnitude of contribution of any one factor in remote organ injury and systemic complications depends on the duration of the biliary obstruction. In acute obstructive jaundice, the liver presents typical changes of obstructive cholangiopathy, in the absence of cirrhosis or portal hypertension, which are at least partly reversible if biliary drainage is performed at this stage[13,14]. This initial phase of surgical jaundice is characterized by the effects of severe cholestasis and cholemia due to total obstruction of bile flow, with intestinal barrier failure and decreased reticuloendothelial system function. This predisposes the test subject to portal and systemic endotoxemia and susceptibility to postoperative septic complications and renal dysfunction[15,16]. Severe cholemia, predominantly and independently of liver parenchymal disease, affects the integrity of the cardiovascular system by inducing;s (1) negative inotropic and chronotropic effects on the heart[17,18]; (2) impaired sympathetic vasoconstriction response[19,20]; and (3) profound natriuresis and diuresis that may lead to volume depletion[21,22]. These factors produce systemic hypotension and renal dysfunction, especially when an interventional approach for the release of biliary obstruction is performed[22]. The etiology of renal impairment in this setting is profoundly prerenal and occurs in the absence of advanced and irreversible liver disease. In addition, it has been shown repeatedly that acute obstructive jaundice is universally complicated by extended bacterial translocation with portal and systemic endotoxemia[23-25]. These phenomena activate a systemic inflammatory response characterized by the release of numerous cytokines and proinflammatory mediators, which may lead to the development of the septic syndrome and multiple organ damage[26]. Endotoxin-induced renal injury is not only functional, through induction of a hypotensive response, but endotoxin also exerts direct nephrotoxic effects. The result is renal injury clearly characterized by histological alterations of acute tubular necrosis[27,28]. Given the central role of endotoxemia in obstructive-jaundice-induced systemic complications[16,25], we would expect that renal failure in obstructive jaundice would be accompanied by tubular injury. Despite the authors' findings of normal renal histology in BDL rats, there are numerous previous studies showing that the acute BDL model induces renal histopathological changes, predominantly in the tubular epithelium (acute tubular necrosis)[5-10]. For all these reasons, we think that the pathogenesis and the type of renal dysfunction that is observed during the acute phase of BDL are different from what we mean by the term HRS.

With regard to the chronic phase of BDL (after 4 wk of biliary obstruction), apart from cholemia, the factor severe parenchymal liver disease comes into play, significantly contributing to renal dysfunction[22]. This phase is characterized by development of biliary cirrhosis with portal hypertension, and ascites and more closely resembles clinical conditions complicated by HRS[11]. However, cholemia still exists to a considerable extent and acts on renal hemodynamics. Endotoxemia, with its consecutive systemic inflammatory response and bacterial translocation to remote organs, also exists, interacting with advanced liver disease per se in the development of renal dysfunction. Is the final result on kidney the development of HRS? In order to give a reliable answer to this question we should examine the accordance of the BDL model with the well-established diagnostic criteria of HRS, as we would do in a clinical setting. Rats with BDL for more than 4 wk are cirrhotic with ascites and have increased rates of bacterial translocation; therefore, spontaneous bacterial peritonitis should be excluded as an underlying cause of renal failure. We should also demonstrate the lack of response of renal function to volume repletion by isotonic saline. If these prerequisites are fulfilled, we would be more certain to characterize the renal failure observed in the chronic phase of BDL as HRS.

In conclusion, we think that the BDL model is not

suitable for the study of the natural history of HRS, because at the acute phase of extrahepatic cholestasis, the pathophysiology of the observed renal impairment seems to be different from that of HRS. However, renal failure observed in the chronic BDL model shares pathophysiological similarities with HRS. The accordance of the chronic BDL model to the diagnostic criteria of HRS, if confirmed, may provide us with a valuable experimental model for the study of established HRS and its potential therapies. Another important issue that remains to be elucidated is the comparison of the BDL model with the carbon tetrachloride model of cirrhosis, in order to examine the potential superiority of one model over the other for the study of HRS.

## REFERENCES


1 **Pereira RM**, dos Santos RA, Oliveira EA, Leite VH, Dias FL, Rezende AS, Costa LP, Barcelos LS, Teixeira MM, Simoes e Silva AC. Development of hepatorenal syndrome in bile duct ligated rats. *World J Gastroenterol* 2008; **14**: 4505-4511
2 **Epstein M**. Hepatorenal syndrome: emerging perspectives. *Semin Nephrol* 1997; **17**: 563-575
3 **Gines P**, Guevara M, Arroyo V, Rodes J. Hepatorenal syndrome. *Lancet* 2003; **362**: 1819-1827
4 **Cardenas A**. Hepatorenal syndrome: a dreaded complication of end-stage liver disease. *Am J Gastroenterol* 2005; **100**: 460-467
5 **Ozturk H**, Eken H, Ozturk H, Buyukbayram H. Effects of dexamethasone on small bowel and kidney oxidative stress and histological alterations in bile duct-ligated rats. *Pediatr Surg Int* 2006; **22**: 709-718
6 **Kucuk C**, Sozuer E, Ikizceli I, Avsarogullari L, Keceli M, Akgun H, Muhtaroglu S. Role of oxygen free radical scavengers in acute renal failure complicating obstructive jaundice. *Eur Surg Res* 2003; **35**: 143-147
7 **Yuceyar S**, Gumustas K, Erturk S, Hamzaoglu IH, Uygun N, Ayaz M, Cengiz A, Kafadar Y. The role of oxygen free radicals in acute renal failure complicating obstructive jaundice: an experimental study. *HPB Surg* 1998; **10**: 387-393
8 **Kramer HJ**, Schwarting K, Backer A, Meyer-Lehnert H. Renal endothelin system in obstructive jaundice: its role in impaired renal function of bile-duct ligated rats. *Clin Sci* (Lond) 1997; **92**: 579-585
9 **Fletcher MS**, Westwick J, Kakkar VV. Endotoxin, prostaglandins and renal fibrin deposition in obstructive jaundice. *Br J Surg* 1982; **69**: 625-629
10 **Rodrigo R**, Avalos N, Orellana M, Bosco C, Thielemann L. Renal effects of experimental obstructive jaundice: morphological and functional assessment. *Arch Med Res* 1999; **30**: 275-285
11 **Geerts AM**, Vanheule E, Praet M, Van Vlierberghe H, De Vos M, Colle I. Comparison of three research models of portal hypertension in mice: macroscopic, histological and portal pressure evaluation. *Int J Exp Pathol* 2008; **89**: 251-263
12 **Kountouras J**, Billing BH, Scheuer PJ. Prolonged bile duct obstruction: a new experimental model for cirrhosis in the rat. *Br J Exp Pathol* 1984; **65**: 305-311
13 **Matsumoto Y**, Niimoto S, Katayama K, Hirose K, Yamaguchi A, Torigoe K. Effects of biliary drainage in obstructive jaundice on microcirculation, phagocytic activity, and ultrastructure of the liver in rats. *J Hepatobiliary Pancreat Surg* 2002; **9**: 360-366
14 **Li W**, Chung SC. An improved rat model of obstructive jaundice and its reversal by internal and external drainage. *J Surg Res* 2001; **101**: 4-15
15 **Ding JW**, Andersson R, Soltesz V, Willen R, Bengmark S. Obstructive jaundice impairs reticuloendothelial function and promotes bacterial translocation in the rat. *J Surg Res* 1994; **57**: 238-245
16 **Assimakopoulos SF**, Scopa CD, Vagianos CE. Pathophysiology of increased intestinal permeability in obstructive jaundice. *World J Gastroenterol* 2007; **13**: 6458-6464
17 **Joubert P**. An in vivo investigation of the negative chronotropic effect of cholic acid in the rat. *Clin Exp Pharmacol Physiol* 1978; **5**: 1-8
18 **Liu H**, Ma Z, Lee SS. Contribution of nitric oxide to the pathogenesis of cirrhotic cardiomyopathy in bile duct-ligated rats. *Gastroenterology* 2000; **118**: 937-944
19 **Jacob G**, Said O, Finberg J, Bomzon A. Peripheral vascular neuroeffector mechanisms in experimental cholestasis. *Am J Physiol* 1993; **265**: G579-G586
20 **Bomzon A**, Gali D, Better OS, Blendis LM. Reversible suppression of the vascular contractile response in rats with obstructive jaundice. *J Lab Clin Med* 1985; **105**: 568-572
21 **Heidenreich S**, Brinkema E, Martin A, Dusing R, Kipnowski J, Kramer HJ. The kidney and cardiovascular system in obstructive jaundice: functional and metabolic studies in conscious rats. *Clin Sci* (Lond) 1987; **73**: 593-599
22 **Green J**, Better OS. Systemic hypotension and renal failure in obstructive jaundice-mechanistic and therapeutic aspects. *J Am Soc Nephrol* 1995; **5**: 1853-1871
23 **Deitch EA**, Sittig K, Li M, Berg R, Specian RD. Obstructive jaundice promotes bacterial translocation from the gut. *Am J Surg* 1990; **159**: 79-84
24 **Clements WD**, Erwin P, McCaigue MD, Halliday I, Barclay GR, Rowlands BJ. Conclusive evidence of endotoxaemia in biliary obstruction. *Gut* 1998; **42**: 293-299
25 **Clements WD**, Parks R, Erwin P, Halliday MI, Barr J, Rowlands BJ. Role of the gut in the pathophysiology of extrahepatic biliary obstruction. *Gut* 1996; **39**: 587-593
26 **Deitch EA**. Multiple organ failure. Pathophysiology and potential future therapy. *Ann Surg* 1992; **216**: 117-134
27 **Heemskerk S**, Pickkers P, Bouw MP, Draisma A, van der Hoeven JG, Peters WH, Smits P, Russel FG, Masereeuw R. Upregulation of renal inducible nitric oxide synthase during human endotoxemia and sepsis is associated with proximal tubule injury. *Clin J Am Soc Nephrol* 2006; **1**: 853-862
28 **Memis D**, Hekimoglu S, Sezer A, Altaner S, Sut N, Usta U. Curcumin attenuates the organ dysfunction caused by endotoxemia in the rat. *Nutrition* 2008; **24**: 1133-1138


**S- Editor** Tian L **L- Editor** Stewart GJ **E- Editor** Lin YP

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
www.wjgnet.com

*World J Gastroenterol* 2009 January 7; 15(1): 124
***World Journal of Gastroenterology*** ISSN 1007-9327


*ACKNOWLEDGMENTS*

# Acknowledgments to reviewers of *World Journal of Gastroenterology*

Many reviewers have contributed their expertise and time to the peer review, a critical process to ensure the quality of *World Journal of Gastroenterology*. The editors and authors of the articles submitted to the journal are grateful to the following reviewers for evaluating the articles (including those published in this issue and those rejected for this issue) during the last editing time period.

**Masahiro Arai, MD, PhD**
Department of Gastroenterology, Toshiba General Hospital, 6-3-22 Higashi-ooi, Shinagawa-ku,Tokyo 140-8522, Japan

**Edmund J Bini, Professor**
VA New York Harbor Healthcare System, Division of Gastroenterology (111D), 423 East 23rd Street, New York, NY 10010, United States

**Andrew Seng Boon Chua, MD**
Department of Gastroenterology, Gastro Centre Ipoh, 1, lorong Rani, 31, lebuhraya Tmn Ipoh, Ipoh Garden South, IPOH 30350, Malaysia

**Ana J Coito, Associate Professor of Surgery**
Department of Surgery, The Dumont, UCLA Transplant Center, 77-120 CHS, Box 957054, Los Angeles, CA 90095-7054, United States

**Nikolaus Gassler, Professor**
Institute of Pathology, University Hospital RWTH Aachen, Pauwelsstrasse 30, 52074 Aachen, Germany

**Yik-Hong Ho, Professor**
Department of Surgery, School of Medicine, James Cook University, Townsville 4811, Australia

**Robin Hughes**
Institute of Liver Studies, King's College London School of Medicine, Bessemer Road, London, SE5 9PJ, United Kingdom

**Miran Kim, PhD**
Liver Research Center, Rhode Island Hospital/Brown Medical School, 55 Claverick St. Providence, RI, 02903, United States

**Rupert Leong, Associate Professor, Director of Endoscopy**
Concord Hospital, ACE Unit, Level 1 West, Hospital Rd, Concord NSW 2139, Australia

**Naofumi Mukaida, MD, PhD, Chairperson and Professor**
Division of Molecular Bioregulation, Cancer Research Institute, Kanazawa University, 13-1 Takara-machi, Kanazawa 920-0934, Japan

**Raymund R Razonable, MD**
Division of Infectious Diseases, Mayo Clinic, 200 First Street SW, Rochester, Minnesota 55905, United States

**Dr. Richard A Rippe**
Department of Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7038, United States

**Luis Rodrigo, Professor**
Gastroenterology Service, Hospital Central de Asturias, c/ Celestino Villamil, s.n., Oviedo 33.006, Spain

**Manfred V Singer, Professor**
Department of Medicine II, University Hospital at Mannheim, Theodor-Kutzer-Ufer 1-3, Mannheim 68167, Germany

**Shinji Tanaka, Director**
Department of Endoscopy, Hiroshima University Hospital, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan

**Frank I Tovey, OBE, ChM, FRCS**
Honorary Research Felllow, Department of Surgery, University College London, London, United Kingdom

**Eddie Wisse, Professor**
Irisweg 16, Keerbergen 3140, Belgium

*World J Gastroenterol* 2009 January 7; 15(1): 125
***World Journal of Gastroenterology*** ISSN 1007-9327


# Meetings

## Events Calendar 2008-2009

FALK SYMPOSIA 2008
January 24-25, Frankfurt, Germany
Falk Workshop: Perspectives in Liver
Transplantation

International Gastroenterological Congresses 2008
February 14-16, Paris, France
EASL-AASLD-APASL-ALEH-IASL Conference Hepatitis B and C virus resistance to antiviral therapies
www.easl.ch/hepatitis-conference

February 14-17, Berlin, Germany
8th International Conference on New Trends in Immunosuppression and Immunotherapy
www.kenes.com/immuno

February 28, Lyon, France
3rd Congress of ECCO - the European Crohn's and Colitis Organisation
Inflammatory Bowel Diseases 2008
www.ecco-ibd.eu

February 29, Québec, Canada
Canadian Association of Gastroenterology
E-mail: general@cag-acg.org

March 10-13, Birmingham, UK
British Society of Gastroenterology Annual Meeting
E-mail: BSG@mailbox.ulcc.ac.uk

March 14-15, HangZhou, China
Falk Symposium 163: Chronic Inflammation of Liver and Gut

March 23-26, Seoul, Korea
Asian Pacific Association for the Study of the Liver
18th Conference of APASL: New Horizons in Hepatology
www.apaslseoul2008.org

March 29-April 1, Shanghai, China
Shanghai-Hong Kong International Liver Congress
www.livercongress.org

April 05-09, Monte-Carlo (Grimaldi Forum), Monaco
OESO 9th World Congress, The Gastro-esophageal Reflux Disease: from Reflux to Mucosal Inflammation-Management of Adeno-carcinomas
E-mail: robert.giuli@oeso.org

April 9-12, Los Angeles, USA
SAGES 2008 Annual Meeting - part of Surgical Spring Week
www.sages.org/08program/html/

April 18-22, Buenos Aires, Argentina
9th World Congress of the International Hepato-Pancreato Biliary Association
Association for the Study of the Liver
www.ca-ihpba.com.ar

April 23-27, Milan, Italy
43rd Annual Meeting of the European Association for the Study of the Liver
www.easl.ch

May 2-3, Budapest, Hungary
Falk Symposium 164: Intestinal Disorders

May 18-21, San Diego, California, USA
Digestive Disease Week 2008

May 21-22, California, USA
ASGE Annual Postgraduate Course
Endoscopic Practice 2008: At the Interface of Evidence and Expert Opinion
E-mail: education@asge.org

June 4-7, Helsinki, Finland
The 39th Nordic Meeting of Gastroenterology
www.congrex.com/ngc2008

June 5-8, Sitges (Barcelona), Spain
Semana de las Enfermedades Digestivas
E-mail: sepd@sepd.es

June 6-8, Prague, Czech Republic
3rd Annual European Meeting: Perspectives in Inflammatory Bowel Diseases
E-mail: meetings@imedex.com

June 10-13, Istanbul, Turkey
ESGAR 2008 19th Annual Meeting and Postgraduate Course
E-mail: fca@netvisao.pt

June 11-13, Stockholm, Sweden
16th International Congress of the European Association for Endoscopic Surgery
E-mail: info@eaes-eur.org

June 13-14, Amsterdam, Netherlands
Falk Symposium 165: XX International Bile Acid Meeting. B ile Acid Biology and Therapeutic Actions

June 13-14, Prague, Czech Republic
Central and Eastern European Conference on Colorectal "Cancer" Screening, Prevention and Management
E-mail: idca2008@guarant.cz

June 25-28, Barcelona, Spain
10th World Congress on Gastrointestinal Cancer
Imedex and ESMO
E-mail: meetings@imedex.com

June 25-28, Lodz, Poland
Joint Meeting of the European Pancreatic Club (EPC)
and the International Association of Pancreatology (IAP)
E-mail: office@epc-iap2008.org
www.e-p-c.org
www.pancreatology.org

June 26-28, Bratislava, Slovakia
5th Central European Gastroenterology Meeting
www.ceurgem2008.cz

July 9-12, Paris, France
ILTS 14th Annual International Congress
www.ilts.org

September 10-13, Budapest, Hungary
11th World Congress of the International Society for Diseases of the Esophagus
E-mail: isde@isde.net

September 13-16, New Delhi, India
Asia Pacific Digestive Week
E-mail: apdw@apdw2008.net

III FALK GASTRO-CONFERENCE
September 17, Mainz, Germany
Falk Workshop: Strategies of Cancer Prevention in Gastroenterology

September 18-19, Mainz, Germany
Falk Symposium 166:
GI Endoscopy - Standards & Innovations

September 18-20, Prague, Czech Republic
Prague Hepatology Meeting 2008
www.czech-hepatology.cz/phm2008

September 20-21, Mainz, Germany
Falk Symposium 167:
Liver Under Constant Attack - From Fat to Viruses

September 24-27, Nantes, France
Third Annual Meeting
European Society of Coloproctology
www.escp.eu.com

October 8-11, Istanbul, Turkey
18th World Congress of the International Association of Surgeons,
Gastroenterologists and Oncologists
E-mail: orkun.sahin@serenas.com.tr

October 18-22, Vienna, Austria
16th United European Gastroenterology Week
www.negf.org
www.acv.at

October 22-25, Minnesota, USA
Anstralian Gastroenterology Week 2008
E-mail: gesa@gesa.org.au

October 22-25, Brisbane, Australia
71st Annual Colon and Rectal Surgery Conference
E-mail: info@colonrectalcourse.org

October 31-November 4, Moscone West Convention Center, San Francisco, CA
59th AASLD Annual Meeting and Postgraduate Course
The Liver Meeting
Information: www.aasld.org

November 6-9, Lucerne, Switzerland
Neurogastroenterology & Motility Joint International Meeting 2008
E-mail: ngm2008@mci-group.com
www.ngm2008.com

November 12, Santiago de Chile, Chile
Falk Workshop: Digestive Diseases: State of the Art and Daily Practice

November 28-29, Cairo, Egypt
1st Hepatology and Gastroenterology Post Graduate Course
www.egyptgastrohep.com

December 7-9, Seoul, Korea
6th International Meeting
Hepatocellular Carcinoma: Eastern and Western Experiences
E-mail: sglee@amc.seoul.kr

INFORMATION FOR ALL
FALK FOUNDATION e.V.
E-mail: symposia@falkfoundation.de
www.falkfoundation.de

Advanced Courses - European Institute of Telesurgery EITS - 2008
Strasbourg, France
January 18-19, March 28-29, June 6-7, October 3-4

N.O.T.E.S
April 3-5, November 27-29
Laparoscopic Digestive Surgery

June 27-28, November 7-8
Laparoscopic Colorectal Surgery

July 3-5
Interventional GI Endoscopy Techniques
Contact address for all courses:
E-mail: info@eits.fr

International Gastroenterological Congresses 2009
March 23-26, Glasgow, Scotland
Meeting of the British Society of Gastroenterology (BSG)
E-mail: bsg@mailbox.ulcc.ac.uk

May 17-20, Denver, Colorado, USA
Digestive Disease Week 2009

November 21-25, London, UK
Gastro 2009 UEGW/World Congress of Gastroenterology
www.gastro2009.org


***Global Collaboration for Gastroenterology***

For the first time in the history of gastroenterology, an international conference will take place which joins together the forces of four pre-eminent organisations: Gastro 2009, UEGW/WCOG London. The United European Gastroenterology Federation (UEGF) and the World Gastroenterology Organisation (WGO), together with the World Organisation of Digestive Endoscopy (OMED) and the British Society of Gastroenterology (BSG), are jointly organising a landmark meeting in London from November 21-25, 2009. This collaboration will ensure the perfect balance of basic science and clinical practice, will cover all disciplines in gastroenterology (endoscopy, digestive oncology, nutrition, digestive surgery, hepatology, gastroenterology) and ensure a truly global context; all presented in the exciting setting of the city of London. Attendance is expected to reach record heights as participants are provided with a compact "all-in-one" programme merging the best of several GI meetings. Faculty and participants from all corners of the earth will merge to provide a truly global environment conducive to the exchange of ideas and the forming of friendships and collaborations.

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
www.wjgnet.com

*World J Gastroenterol* 2009 January 7; 15(1): 126-128
***World Journal of Gastroenterology*** ISSN 1007-9327


# Instructions to authors

## GENERAL INFORMATION

*World Journal of Gastroenterology* (*World J Gastroenterol* ISSN 1007-9327 CN 14-1219/R) is a weekly open access peer-reviewed journal supported by an editorial board consisting of 1212 experts in gastroenterology and hepatology from 60 countries.

The biggest advantage of the open access model is that it provides free, full-text articles in PDF and other formats for experts and the public without registration, which eliminates the obstacle that traditional journals possess and usually delays the speed of the propagation and communication of scientific research results. The open access model has been proven to be a true approach that may achieve the ultimate goal of the journals, i.e. the maximization of the value to the readers, authors and society.

Maximization of the value of the readers can be comprehended in two ways. First, the journal publishes articles that can be directly read or downloaded free of charge at any time, which attracts more readers. Second, the readers can apply the knowledge in clinical practice without delay after reading and understanding the information in their fields. In addition, the readers are encouraged to propose new ideas based on those of the authors, or to provide viewpoints that are different from those of the authors. Such discussions or debates among different schools of thought will definitely boost advancements and developments in the fields. Maximization of the value of the authors refers to the fact that these journals provide a platform that promotes the speed of propagation and communication to a maximum extent. This is also what the authors really need. Maximization of the value of the society refers to the maximal extent of the social influences and impacts produced by the high quality original articles published in the journal. This is also the main purpose of many journals around the world.

The major task of *WJG* is to rapidly report the most recent results in basic and clinical research on gastroenterology, hepatology, endoscopy and gastrointestinal surgery fields, specifically including autoimmune, cholestatic and biliary disease, esophageal, gastric and duodenal disorders, cirrhosis and its complications, celiac disease, dyspepsia, gastroesophageal reflux disease, esophageal and stomach cancers, carcinoma of the colon and rectum, gastrointestinal bleeding, gastrointestinal infection, intestinal inflammation, intestinal microflora and immunity, irritable bowel syndrome; liver biology/pathobiology, liver failure, growth and cancer; liver failure/cirrhosis/portal hypertension, liver fibrosis; *Helicobacter pylori*, hepatitis B and C virus, hepatology elsewhere; pancreatic disorders, pancreas and biliary tract disease, pancreatic cancer; transplantation, genetics, epidemiology, microbiology and inflammatory disorders, molecular and cell biology, nutrition; geriatric gastroenterology, pediatric gastroenterology, steatohepatitis and metabolic liver disease; diagnosis and screening, endoscopy, imaging and advanced technology.

The columns in the issues of *WJG* will be adjusted in 2009, which will include: (1) Editorials (contributed by members of the Editorial Board), (2) Topic Highlights (organized by members of the Editorial Board), (3) Observation (invited by members of the Editorial Board), (4) Guidelines for Basic Research (invited by the Editorial Office), (5) Guidelines for Clinical Practice (invited by the Editorial Office), (6) Research Frontier (invited by the Editorial Office), (7) Review Articles (submitted by authors and subject to publication charge), (8) Original Articles (submitted by authors and subject to publication charge), (9) Brief Articles (submitted by authors and subject to publication charge), (10) Case Report (submitted by authors and subject to publication charge), (11) Letters to the Editor (submitted by authors and subject to publication charge), (12) Book Reviews (submitted by authors and subject to publication charge) and (13) Guidelines (submitted by authors and subject to publication charge).

***Indexed and abstracted in***

Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®) and Journal Citation Reports/Science Edition, Index Medicus, MEDLINE and PubMed, Chemical Abstracts, EMBASE/Excerpta Medica, Abstracts Journals, *Nature Clinical Practice Gastroenterology* and *Hepatology*, CAB Abstracts and Global Health. ISI JCR 2003-2000 IF: 3.318, 2.532, 1.445 and 0.993.

***Published by***

The WJG Press and Baishideng

## 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 The WJG Press and Baishideng, 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 International Committee of Medical Journal Editors 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 event 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 corresponding 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://wjg.wjgnet.com/wjg. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/wjg/help/instructions.jsp) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to submission@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 International Committee of Medical Journal Editors, 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: +86-10-59080039, Fax: +86-10-85381893

**Peer reviewers:** All articles received are subject to peer review. Normally, three experts are invited for each article. Decision for acceptance is made only when at least two experts recommend an article for publication. Reviewers for accepted manuscripts are acknowledged in each manuscript, and reviewers of articles which were not accepted will be acknowledged at the end of each issue. To ensure the quality of the articles published in *WJG*, reviewers of accepted manuscripts will be announced by publishing the name, title/position and institution of the reviewer in the footnote accompanying the printed article. For example, reviewers: Professor Jing-Yuan Fang, Shanghai Institute of Digestive Disease, Shanghai, Affiliated Renji Hospital, Medical Faculty, Shanghai Jiaotong University, Shanghai, China; Professor Xin-Wei Han, Department of Radiology, The First Affiliated Hospital, Zhengzhou University, Zhengzhou, Henan Province, China; and Professor Anren Kuang, Department of Nuclear Medicine, Huaxi Hospital, Sichuan University, Chengdu, Sichuan Province, China.

### *Abstract*

There are unstructured abstracts (no more than 256 words) and structured abstracts (no more than 480). The specific requirements for structured abstracts are as follows:

An informative, structured abstracts of no more than 480 words should accompany each manuscript. Abstracts for original contributions should be structured into the following sections. AIM (no more than 20 words): Only the purpose should be included. Please write the aim as the form of "To investigate/study/…; MATERIALS AND METHODS (no more than 140 words); RESULTS (no more than 294 words): 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, $P < 0.001$; CONCLUSION (no more than 26 words). Available from:http://www.wjgnet.com/wjg/help/8.doc

### *Key words*

Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

### *Text*

For articles of these sections, original articles, rapid communication and case reports, 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. The main text format of these sections, editorial, topic highlight, case report, letters to the editors, can be found at: http://www.wjgnet.com/wjg/help/instructions.jsp.

### *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. Figures should be either Photoshop or Illustrator files (in tiff, eps, jpeg formats) at high-resolution. Examples can be found at: http://www.wjgnet.com/1007-9327/13/4520.pdf; http://www.wjgnet.com/1007-9327/13/4554.pdf; http://www.wjgnet.com/1007-9327/13/4891.pdf; http://www.wjgnet.com/1007-9327/13/4986.pdf; http://www.wjgnet.com/1007-9327/13/4498.pdf. 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 printed and 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 1274 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/eid.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 $\chi^2$ (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 23 243 641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/wjg/help/14.doc.

### *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.*

## SUBMISSION OF THE REVISED MANUSCRIPTS AFTER ACCEPTED

Please revise your article according to the revision policies of *WJG*. The revised version including manuscript and high-resolution image figures (if any) should be copied on a floppy or compact disk. The author should send the revised manuscript, along with printed high-resolution color or black and white photos, copyright transfer letter, and responses to the reviewers by courier (such as EMS/DHL).

### Editorial Office

***World Journal of Gastroenterology***

Editorial Department: Room 903, Building D,
Ocean International Center,
No.62 Dongsihuan Zhonglu,
Chaoyang District, Beijing 100025, China
E-mail: wjg@wjgnet.com
http://www.wjgnet.com
Telephone: +86-10-59080039
Fax: +86-10-85381893

### *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; (4) Grade D: rejected. Revised articles should reach Grade A or B.

### *Copyright assignment form*

Please download a Copyright assignment form from http://www.wjgnet.com/wjg/help/10.doc.

### *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/wjg/help/9.doc.

### *Proof of financial support*

For paper supported by a foundation, authors should provide a copy of the document and serial number of the foundation.

### *Links to documents related to the manuscript*

*WJG* will be initiating a platform to promote dynamic interactions between the editors, peer reviewers, readers and authors. After a manuscript is published online, links to the PDF version of the submitted manuscript, the peer-reviewers' report and the revised manuscript will be put on-line. Readers can make comments on the peer reviewer's report, authors' responses to peer reviewers, and the revised manuscript. We hope that authors will benefit from this feedback and be able to revise the manuscript accordingly in a timely manner.

### *Science news releases*

Authors of accepted manuscripts are suggested to write a science news item to promote their articles. The news will be released rapidly at EurekAlert/AAAS (http://www.eurekalert.org). The title for news items should be less than 90 characters; the summary should be less than 75 words; and main body less than 500 words. Science news items should be lawful, ethical, and strictly based on your original content with an attractive title and interesting pictures.

### *Publication fee*

Authors of accepted articles must pay a publication fee.

EDITORIAL, TOPIC HIGHLIGHTS, BOOK REVIEWS and LETTERS TO THE EDITOR are published free of charge.