



WJG

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 14 Number 34
September 14, 2008

World J Gastroenterol

2008 September 14; 14(34): 5233-5360

Online Submissions

wjg.wjgnet.com

www.wjgnet.com

Printed on Acid-free Paper

世界胃肠病学杂志

World Journal of Gastroenterology®

Editorial Board

2007-2009



Published by The WJG Press and Baishideng
Room 903, Ocean International Center, Building D
No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China
Fax: +86-10-8538-1893 E-mail: wjg@wjgnet.com <http://www.wjgnet.com>

The World Journal of Gastroenterology Editorial Board consists of 1208 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 (28), 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 (3), Ireland (4), Israel (8), Italy (96), Japan (176), 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 (14), Spain (38), Sweden (15), Switzerland (13), Turkey (8), United Arab Emirates (1), United Kingdom (83), United States (316) and Uruguay (2).

HONORARY EDITORS-IN-CHIEF

Montgomery Bissell, *San Francisco*
James L Boyer, *New Haven*
Chao-Long Chen, *Kaohsiung*
Ke-Ji Chen, *Beijing*
Li-Fang Chou, *Taipei*
Jacques V Dam, *Stanford*
Martin H Floch, *New Haven*
Guadalupe Garcia-Tsao, *New Haven*
Zhi-Qiang Huang, *Beijing*
Shinn-Jang Hwang, *Taipei*
Ira M Jacobson, *New York*
Derek Jewell, *Oxford*
Emmet B Keefe, *Palo Alto*
Min-Liang Kuo, *Taipei*
Nicholas F LaRusso, *Rochester*
Jie-Shou Li, *Nanjing*
Geng-Tao Liu, *Beijing*
Lein-Ray Mo, *Tainan*
Bo-Rong Pan, *Xi'an*
Fa-Zu Qiu, *Wuhan*
Eamonn M Quigley, *Cork*
David S Rampton, *London*
Rafiq A Sheikh, *Sacramento*
Rudi Schmid, *Kentfield*^[1]
Nicholas J Talley, *Rochester*
Sun-Lung Tsai, *Young-Kang City*
Guido NJ Tytgat, *Amsterdam*
Hsiu-Po Wang, *Taipei*
Jaw-Ching Wu, *Taipei*
Meng-Chao Wu, *Shanghai*
Ming-Shiang Wu, *Taipei*
Jia-Yu Xu, *Shanghai*
Ta-Sen Yeh, *Taoyuan*
Ming-Lung Yu, *Kaohsiung*

PRESIDENT AND EDITOR-IN-CHIEF

Lian-Sheng Ma, *Beijing*

STRATEGY ASSOCIATE

EDITORS-IN-CHIEF

Peter Draganov, *Florida*
Ronnie Fass, *Tucson*
Hugh J Freeman, *Vancouver*
John P Geibel, *New Haven*
Maria Concepción Gutiérrez-Ruiz, *México*
Kazuhiro Hanazaki, *Kochi*
Akio Inui, *Kagoshima*
Kalpesh Jani, *Vadodara*
Sanaa M Kamal, *Cairo*
Ioannis E Koutroubakis, *Heraklion*
Jose JG Marin, *Salamanca*
Javier S Martin, *Punta del Este*
Natalia A Osna, *Omaha*
Jose Sahel, *Marseille*
Ned Snyder, *Galveston*
Nathan Subramaniam, *Brisbane*
Wei Tang, *Tokyo*
Alan BR Thomson, *Edmonton*
Paul Joseph Thuluvath, *Baltimore*
James F Trotter, *Denver*
Shingo Tsuji, *Osaka*
Harry HX Xia, *Hanover*
Yoshio Yamaoka, *Houston*
Jesus K Yamamoto-Furusho, *México*

ASSOCIATE EDITORS-IN-CHIEF

Gianfranco D Alpini, *Temple*
Bruno Annibale, *Roma*

Roger W Chapman, *Oxford*
Chi-Hin Cho, *Hong Kong*
Alexander L Gerbes, *Munich*
Shou-Dong Lee, *Taipei*
Walter E Longo, *New Haven*
You-Yong Lu, *Beijing*
Masao Omata, *Tokyo*

BIostatistical Editor

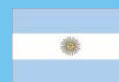
Liang-Ping Hu, *Beijing*

MEMBERS OF THE EDITORIAL BOARD



Albania

Bashkim Resuli, *Tirana*



Argentina

Julio H Carri, *Córdoba*
Carlos J Pirola, *Buenos Aires*
Silvia Sookoian, *Buenos Aires*
Adriana M Torres, *Rosario*



Australia

Leon Anton Adams, *Nedlands*
Minoti V Apte, *Liverpool*
Richard B Banati, *Lidcombe*
Michael R Beard, *Adelaide*
Patrick Bertolino, *Sydney*

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*
 John L Wallace, *Calgary*
 Eric M Yoshida, *Vancouver*



Chile

Silvana Zanlungo, *Santiago*



China

Henry LY Chan, *Hongkong*
 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*
 Soumeiya 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*
 Khalid A Tazi, *Clichy*
 Emmanuel Tiret, *Paris*
 Baumert F Thomas, *Strasbourg*
 Marie-Catherine Vozenin-brotons, *Villejuif*
 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 Miehke, *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

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*
 Antomio 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, *Vareggio*
 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 Ghisetti, *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 Marzoni, *Ancona*
 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 Pranterà, *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*
 Shigeki Kuriyama, *Kagawa*^[2]
 Katsunori Iijima, *Sendai*
 Masato Kusunoki, *Tsu Mie*
 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 Moriawaki, *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*
 Yuji 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*
 Michie 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*
 Tadatashi 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 Serafimovski, *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*
 Vasilij 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*
 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 Lladó, *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, *Mölnådal*
 Hanns-Ulrich Marschall, *Stockholm*
 Lars C Olbe, *Mölnådal*
 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, *Zürich*
 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*
 Hızir 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 Alzarraa, *Manchester*
 Lesley A Anderson, *Belfast*
 Charalambos G Antoniadis, *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*
 Giorgia 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*
 Sakawat 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 Busuttill, *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*
 Magali Fontaine, *Stanford*
 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 Pezzzone, *Pittsburgh*
CS Pitchumoni, *New Brunswick*
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*
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*
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



National Journal Award
2005

World Journal of Gastroenterology®

Weekly Established in October 1995

Volume 14 Number 34
September 14, 2008



Contents

EDITORIAL

- 5233 Heterogeneity of endoscopy negative heartburn: Epidemiology and natural history
Pace F, Casini V, Pallotta S

TOPIC HIGHLIGHTS

- 5237 Capsule endoscopy in non-steroidal anti-inflammatory drugs-enteropathy and miscellaneous, rare intestinal diseases
Gay G, Delvaux M, Frederic M
- 5245 Capsule endoscopy in neoplastic diseases
Pennazio M, Rondonotti E, de Franchis R
- 5254 Esophageal capsule endoscopy
Fernandez-Urien I, Carretero C, Armendariz R, Muñoz-Navas M
- 5261 Role of videocapsule endoscopy for gastrointestinal bleeding
Carretero C, Fernandez-Urien I, Betes M, Muñoz-Navas M
- 5265 Colon capsule endoscopy
Fernandez-Urien I, Carretero C, Borda A, Muñoz-Navas M
- 5269 Patency® and agile® capsules
Caunedo-Álvarez Á, Romero-Vazquez J, Herrerias-Gutierrez JM

COLORECTAL CANCER

- 5274 Active chinese mistletoe lectin-55 enhances colon cancer surveillance through regulating innate and adaptive immune responses
Ma YH, Cheng WZ, Gong F, Ma AL, Yu QW, Zhang JY, Hu CY, Chen XH, Zhang DQ

CLINICAL RESEARCH

- 5282 Incidence of gallstone disease in Italy: Results from a multicenter, population-based Italian study (the MICOL project)
Festi D, Dormi A, Capodicasa S, Staniscia T, Attili AF, Loria P, Pazzi P, Mazzella G, Sama C, Roda E, Colecchia A
- 5290 Ileal lesions in patients with ulcerative colitis after ileo-rectal anastomosis: Relationship with colonic metaplasia
Biancone L, Calabrese E, Palmieri G, Petruzzello C, Onali S, Sica GS, Cossignani M, Condino G, Das KM, Pallone F

- RAPID COMMUNICATION** 5301 Continuous wound infusion of local anaesthetic agents following colorectal surgery: Systematic review and meta-analysis
Karthikesalingam A, Walsh SR, Markar SR, Sadat U, Tang TY, Malata CM
- 5306 mRNA levels of TLR4 and TLR5 are independent of *H pylori*
Garza-González E, Bocanegra-García V, Bosques-Padilla FJ, Flores-Gutiérrez JP, Moreno F, Perez-Perez GI
- 5311 Chemoradiotherapy with twice-weekly administration of low-dose gemcitabine for locally advanced pancreatic cancer
Igarashi H, Ito T, Kawabe K, Hisano T, Arita Y, Kaku T, Takayanagi R
- 5316 Endoscopic findings can predict the efficacy of leukocytapheresis for steroid-naïve patients with moderately active ulcerative colitis
Umehara Y, Kudo M, Kawasaki M
- 5322 Halothane hepatitis in Iran: A review of 59 cases
Eghetesadi-Araghi P, Sohrabpour A, Vahedi H, Saberi-Firoozi M
- 5327 Anti-HBc screening in Indian blood donors: Still an unresolved issue
Dhawan HK, Marwaha N, Sharma RR, Chawla Y, Thakral B, Saluja K, Sharma SK, Thakur MK, Jain A
- 5331 Characteristics of paraesophageal varices: A study with 64-row multidetector computed tomography portal venography
Zhao LQ, He W, Chen G
- 5336 Effect of music on procedure time and sedation during colonoscopy: A meta-analysis
Tam WWS, Wong ELY, Twinn SF
- 5344 Effect of mutant p27^{kip1} gene on human cholangiocarcinoma cell line, QBC939
Luo J, Chen YJ, Wang WY, Zou SQ

CASE REPORT

- 5349 A "false positive" octreoscan in ileal Crohn's disease
Fernandez A, Tabuenca O, Peteiro A

Contents		<i>World Journal of Gastroenterology</i> Volume 14 Number 34 September 14, 2008	
	5353	Inflammatory myoglandular polyp causing hematochezia <i>Hirasaki S, Okuda M, Kudo K, Suzuki S, Shirakawa A</i>	
ACKNOWLEDGMENTS	5356	Acknowledgments to Reviewers of <i>World Journal of Gastroenterology</i>	
APPENDIX	5357	Meetings	
	5358	Instructions to authors	
FLYLEAF	I-VII	Editorial Board	
INSIDE BACK COVER		Online Submissions	
INSIDE FRONT COVER		Online Submissions	
RESPONSIBLE EDITORS FOR THIS ISSUE		Assistant Editor: <i>Hui Li</i> Review Editor: <i>Lin Tian</i> Electronic Page Editor: <i>Wei-Bing Zhang</i> Editor-in-Charge: <i>Lin-Lin Xiao</i> Copy Editor: <i>Dr. Bernardino Rampone</i> Associate Senior Editor: <i>Jian-Xia Cheng</i> Layout Editor: <i>Lian-Sheng Ma</i>	
NAME OF JOURNAL <i>World Journal of Gastroenterology</i> 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 <i>World Journal of Gastroenterology</i> , Room 903, Ocean International Center, Building D, 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., Room 903, Ocean International Center, Building D, 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 September 14, 2008 EDITOR-IN-CHIEF Lian-Sheng Ma, <i>Beijing</i>	SUBSCRIPTION RMB 50 Yuan for each issue, RMB 2400 Yuan for one year CSSN ISSN 1007-9327 CN 14-1219/R HONORARY EDITORS-IN-CHIEF Montgomery Bissell, <i>San Francisco</i> James L. Boyer, <i>New Haven</i> Chao-Long Chen, <i>Kaohsiung</i> Ke-Ji Chen, <i>Beijing</i> Li-Fang Chou, <i>Taipei</i> Jacques V Dam, <i>Stanford</i> Martin H Floch, <i>New Haven</i> Guadalupe Garcia-Tsao, <i>New Haven</i> Zhi-Qiang Huang, <i>Beijing</i> Shinn-Jang Hwang, <i>Taipei</i> Ira M Jacobson, <i>New York</i> Derek Jewell, <i>Oxford</i> Emmet B Keefe, <i>Palo Alto</i> Min-Liang Kuo, <i>Taipei</i> Nicholas F LaRusso, <i>Rochester</i> Jie-Shou Li, <i>Nanjing</i> Geng-Tao Liu, <i>Beijing</i> Lein-Ray Mo, <i>Tainan</i> Bo-Rong Pan, <i>Xi'an</i> Fa-Zu Qiu, <i>Wuhan</i> Eamonn M Quigley, <i>Cork</i> David S Rampton, <i>London</i> Rafiq A Sheikh, <i>Sacramento</i> Rudi Schmid, <i>Kentfield</i> ¹⁾ Nicholas J Talley, <i>Rochester</i> Sun-Lung Tsai, <i>Young-Kang City</i> Guido NJ Tytgat, <i>Amsterdam</i> Hsiu-Po Wang, <i>Taipei</i> Jaw-Ching Wu, <i>Taipei</i> Meng-Chao Wu, <i>Shanghai</i> Ming-Shiang Wu, <i>Taipei</i> Jia-Yu Xu, <i>Shanghai</i> Ta-Sen Yeh, <i>Taoyuan</i> Ming-Lung Yu, <i>Kaohsiung</i> STRATEGY ASSOCIATE EDITORS-IN-CHIEF Peter Draganov, <i>Florida</i> Ronnie Fass, <i>Tucson</i> Hugh J Freeman, <i>Vancouver</i> John P Geibel, <i>New Haven</i> Maria C Gutiérrez-Ruiz, <i>México</i>	Kazuhiro Hanazaki, <i>Kochi</i> Akio Inui, <i>Kagoshima</i> Kalpesh Jani, <i>Vadodara</i> Sanaa M Kamal, <i>Cairo</i> Ioannis E Koutroubakis, <i>Heraklion</i> Jose JG Marin, <i>Salamanca</i> Javier S Martin, <i>Punta del Este</i> Natalia A Osna, <i>Omaha</i> Jose Sahel, <i>Marseille</i> Ned Snyder, <i>Galveston</i> Nathan Subramaniam, <i>Brisbane</i> Wei Tang, <i>Tokyo</i> Alan BR Thomson, <i>Edmonton</i> Paul Joseph Thuluvath, <i>Baltimore</i> James F Trotter, <i>Denver</i> Shingo Tsuji, <i>Osaka</i> Harry HX Xia, <i>Hanover</i> Yoshio Yamaoka, <i>Houston</i> Jesus K Yamamoto-Furusho, <i>México</i> ASSOCIATE EDITORS-IN-CHIEF Gianfranco D Alpini, <i>Temple</i> Bruno Annibale, <i>Roma</i> Roger William Chapman, <i>Oxford</i> Chi-Hin Cho, <i>Hong Kong</i> Alexander L Gerbes, <i>Munich</i> Shou-Dong Lee, <i>Taipei</i> Walter Edwin Longo, <i>New Haven</i> You-Yong Lu, <i>Beijing</i> Masao Omata, <i>Tokyo</i> EDITORIAL OFFICE Director: Jian-Xia Cheng, <i>Beijing</i> Deputy Director: Jian-Zhong Zhang, <i>Beijing</i> LANGUAGE EDITORS Director: Jing-Yun Ma, <i>Beijing</i> Deputy Director: Xian-Lin Wang, <i>Beijing</i> MEMBERS Gianfranco D Alpini, <i>Temple</i> BS Anand, <i>Houston</i> Manoj Kumar, <i>Nepal</i> Patricia F Lalor, <i>Birmingham</i> Ming Li, <i>New Orleans</i> Margaret Lutze, <i>Chicago</i> Sabine Mihm, <i>Göttingen</i> Francesco Negro, <i>Genève</i> Bernardino Rampone, <i>Siena</i> Richard A Rippe, <i>Chapel Hill</i> Stephen E Roberts, <i>Swansea</i>	COPY EDITORS Gianfranco D Alpini, <i>Temple</i> Sujit Kumar Bhattacharya, <i>Kolkata</i> Filip Braet, <i>Sydney</i> Kirsteen N Browning, <i>Baton Rouge</i> Radha K Dhiman, <i>Chandigarh</i> John Frank Di Mari, <i>Texas</i> Shannon S Glaser, <i>Temple</i> Eberhard Hildt, <i>Berlin</i> Patricia F Lalor, <i>Birmingham</i> Ming Li, <i>New Orleans</i> Margaret Lutze, <i>Chicago</i> MI Torrs, <i>Jaén</i> Sri Prakash Misra, <i>Allahabad</i> Giovanni Monteleone, <i>Rome</i> Giovanni Musso, <i>Torino</i> Valerio Nobili, <i>Rome</i> Osman Cavit Ozdogan, <i>Istanbul</i> Francesco Perri, <i>San Giovanni Rotondo</i> Thierry Piche, <i>Nice</i> Bernardino Rampone, <i>Siena</i> Richard A Rippe, <i>Chapel Hill</i> Ross C Smith, <i>Sydney</i> Daniel Lindsay Worthley, <i>Bedford</i> George Y Wu, <i>Farmington</i> Jian Wu, <i>Sacramento</i> COPYRIGHT © 2008 Published by The WJG Press. All rights reserved; no part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise without the prior permission of WJG. Authors are required to grant WJG an exclusive licence to publish. SPECIAL STATEMENT All articles published in this journal represent the viewpoints of the authors except where indicated otherwise. INSTRUCTIONS TO AUTHORS Full instructions are available online at http://www.wjgnet.com/wjg/help/instructions.jsp . If you do not have web access please contact the editorial office. ONLINE SUBMISSION http://wjg.wjgnet.com



Heterogeneity of endoscopy negative heartburn: Epidemiology and natural history

Fabio Pace, Valentina Casini, Stefano Pallotta

Fabio Pace, Department of Clinical Sciences "L. Sacco" University Hospital, Milan 20157, Italy

Valentina Casini, Stefano Pallotta, Division of Gastroenterology, "L. Sacco" University Hospital, Milan 20157, Italy

Author contributions: Pace F had the idea and wrote the outline of the manuscript; Casini V and Pallotta S assisted in editorial work.

Correspondence to: Fabio Pace, Professor, Department of Clinical Sciences "L. Sacco" University Hospital, Milan 20157, Italy. fabio.pace@unimi.it

Telephone: +39-2-39042943 Fax: +39-2-39042337

Received: June 5, 2008 Revised: August 13, 2008

Accepted: August 20, 2008

Published online: September 14, 2008

Abstract

It has now become clear that only about 40% or less of patients with heartburn and/or regurgitation have esophagitis, and that the majority of them lack visible distal esophageal mucosa breaks. These subjects are referred to as non-erosive gastroesophageal reflux disease (NERD) patients. It has been estimated that in the Western world at least one tenth of the general population has at least weekly heartburn. This proportion seems to be lower in Asia, while prevalence is rapidly increasing. Although it would be extremely useful to have prospective information regarding the fate of such patients, the natural history of NERD is largely unknown, and very few studies in the literature have addressed this issue. These studies are for the greater part old, not well conducted, and suffer from methodological drawbacks including ill-defined entry criteria. However, a review of these studies indicates that a consistent minority of NERD patients may develop erosive disease at an approximate rate of about 10% per year.

© 2008 The WJG Press. All rights reserved.

Key words: Gastroesophageal reflux disease; Non-erosive gastroesophageal reflux disease; Esophagitis; Proton pump inhibitor

Peer reviewer: Tomohiko Shimatani, Assistant Professor, Department of General Medicine, Hiroshima University Hospital, 1-2-3 Kasumi, Minami-ku, Hiroshima 7348551, Japan

Pace F, Casini V, Pallotta S. Heterogeneity of endoscopy

negative heartburn: Epidemiology and natural history. *World J Gastroenterol* 2008; 14(34): 5233-5236 Available from: URL: <http://www.wjgnet.com/1007-9327/14/5233.asp> DOI: <http://dx.doi.org/10.3748/wjg.14.5233>

INTRODUCTION

The recently published Montreal Criteria, dealing with a global classification of gastroesophageal reflux disease (GERD), define heartburn as a burning sensation in the retrosternal area (behind the breastbone) (level of agreement = 100%), claim gastroesophageal reflux (GER) as the most common cause of heartburn (level of agreement = 100%), but admit that heartburn can have a number of non-reflux related causes (level of agreement 98%) and that the prevalence of these is unknown^[1]. Moreover, these criteria state that the typical reflux syndrome can be diagnosed on the basis of the presence of characteristic symptoms, i.e. heartburn and regurgitation, without diagnostic testing (level of agreement = 100%). The epidemiology of heartburn shows a clear geographical variation; in North America heartburn occurring at least weekly ranges between 13.2% and 27%; it is slightly lower in Europe ranging between 7.7% and 15%, whereas the prevalence remains definitely lower of in Asia (3.1%)^[2].

It is now clear that only about 40% of patients with heartburn and/or regurgitation have visible distal esophageal mucosal breaks caused by gastroesophageal reflux^[3,4]. The remaining approximately 60% suffer from non-erosive reflux disease (NERD) or, according with the Montreal criteria, a typical reflux syndrome^[2], i.e. the presence of heartburn and/or regurgitation without esophageal injury.

This negative etiologic definition is not satisfactory: it has been suggested that this may lead to a rather heterogeneous group of patients, including both patients with and without pathological esophageal acid exposure^[5]. Thus, subcategorization of NERD relies primarily on the results of 24-h esophageal pH monitoring. Patients with GER symptoms and abnormal esophageal acid exposure during 24-h esophageal pH monitoring can be classified as NERD; additionally, even patients with a normal esophageal acid exposure but a positive symptom-reflux association may be defined as NERD. The remainder patient may be defined as

having “functional heartburn”^[5]. Recently, the Rome III Committee added that functional heartburn patients also have to demonstrate a negative response to standard course of proton pump inhibitor (PPI) treatment^[6].

Since these definitions appear to be useful only at a research setting, and not at a primary care level, in this review we will describe the epidemiology and natural history of NERD patients solely defined on the basis of their symptoms and the absence of endoscopic injury.

EPIDEMIOLOGY

By far, the best study available up to now is the Kalixanda study^[3]. The aim of this study was to estimate the prevalence of, and to identify risk factors for gastroesophageal reflux symptoms and esophagitis in the adult population of two Swedish municipalities, Kalix and Haparanda (“the Kalixanda study”), with roughly 30 000 inhabitants, chosen because the distribution of age and gender in this area was similar to the national average in Sweden. In the two communities, upper endoscopies were provided by both primary and secondary care physicians and by two endoscopy units involved in the study. By using the computerized Swedish national population register, consisting of all citizens in order of date of birth, the adult population living in the two municipalities was identified and defined as the target population ($n = 21\,610$). Subsequently, a systematic sample (every seventh) of the target population (13.9% of the target population) was enrolled as the study population ($n = 3000$), and one-third of them were submitted to an esophago-gastroduodenoscopy (EGD) on a voluntary basis, and this formed the study population, i.e. 1000 individuals in random order, representing 4.6% of the target population. The primary symptom analysis in this study was based on the presence of troublesome heartburn and/or acid regurgitation over the past 3 mo.

Four hundred subjects (40%, CI = 37.0-43.0) reported at the time of the EGD visit that they had been bothered by troublesome heartburn and/or acid regurgitation over the past 3 mo. There was no statistically significant difference in prevalence between the sexes, except in the oldest age group, where women had more symptoms ($P < 0.01$).

Weekly symptoms were reported by 200 (20%, CI = 17.5-22.5, mean age 52.4, 45% M) and daily symptoms by 59 individuals (5.9%, CI = 4.4-7.4, mean age 52.8, 44.1% M). There was no statistically significant difference in age or gender between these two groups. Erosive esophagitis (EE) was found in 155 subjects (15.5%, CI = 13.2-17.7) with a mean age of 52.6 years and was most prevalent in men (22%) especially in the youngest age group (32%), and most often mild esophagitis (L-A grade A or B in 95.5% of cases) was diagnosed. The esophagus was macroscopically normal in 769 subjects (76.9%, CI = 74.3-79.5) in the EGD study sample. These subjects had a mean age of 53.5 years and 340 of them (44.1%) were men. This group also includes 123 individuals who had a hiatus

hernia as the only finding. Overall, a hiatus hernia was observed in 239 individuals (23.9%, CI = 21.2-26.5) with a mean age of 55.6 years, 54.4% being men. Thus, in this study, 40% of subjects reported typical GER symptoms during the last 3 mo (half of them on a weekly basis), and of these 15.5% had esophagitis whereas 76.9% had absence of esophagitis (NERD) at upper endoscopy. Globally, about 10% of the study population had erosive esophagitis ($n = 98$), whereas almost 27% of the sample had typical GER symptoms but no esophagitis ($n = 271$); if only cases with weekly symptoms were considered, the rate cuts down to 12.5% ($n = 125$).

In a preliminary report of an Italian endoscopic study, the Loiano-Monghidoro project, conducted on 892 adult subjects belonging to the general population, the prevalence of esophagitis was 8.2%, and 24.8% of those had no symptoms^[4]. The prevalence of at least weekly heartburn in the same population was 21.5%.

Therefore, from these two population studies, we can estimate that in Europe at least one tenth of the general population has at least weekly heartburn.

NATURAL HISTORY

Evaluating the natural history of NERD is useful for a number of reasons^[7], this knowledge may help (1) to discern the percentage of the population that will progress from non-erosive to erosive disease and possibly to its complications, such as stricture, Barrett's oesophagus, and esophageal adenocarcinoma, or from exclusively esophageal to supraesophageal manifestations, (2) to define, assess, and validate productivity of risk factors for such complicated forms of the disease, (3) to determine if medical or other therapies are able to positively modify the natural course of the disease, and (4) to determine the need for maintenance therapy to prevent complications and persistent symptoms in such patients.

Until recently, patients with NERD were considered to suffer from a milder disease^[8], i.e. requiring less intensive/prolonged treatment and possibly characterized by a better long-term prognosis. This concept was subsequently proven to be incorrect, since the impairment in disease-related quality of life (HRQoL), for example, appears to be similar in GERD patients with or without endoscopic esophagitis and is related in both instances to symptom severity^[9]. Also, the symptomatic acute response to PPI drugs in patients with or without endoscopic mucosal damage seems not to be different, and in fact might be worse in NERD^[10,11]. Finally, after discontinuation of acute treatment, symptomatic relapse within 6 months appears to affect a similarly high proportion of both GERD groups^[12].

We reported one of the first natural history studies of symptomatic GERD patients without endoscopic esophagitis but with a pathological esophageal pH-metry^[13]. In that study we showed that 5 of 33 such patients treated with antacids or prokinetic agents developed endoscopic esophagitis within 6 mo, and that the extent of esophageal acid exposure at entry was not

predictive for this complication. In a subsequent study^[14], we extended the observation of the original patient group up to a median duration of 10 years. The first interesting observation regarding this patient sample is that almost all patients that we were able to trace (28/29) are affected by GERD symptoms when anti-secretory drugs are discontinued, and therefore the majority (75%) were on such therapy due to GERD symptoms. Secondly, a very high proportion (89%) of our patients in whom repeat endoscopy was performed ($n = 18$) showed an erosive esophagitis. Thus, a considerable proportion of the original patient cohort indeed showed a *progression* from non-erosive to erosive disease.

Schindlbeck *et al.*^[15], in a study investigating the fate of GERD patients with and without esophagitis, reported on 16 patients with pH-documented GERD and no esophagitis 3 years after the diagnosis. During this period, four patients (25%) developed reflux esophagitis, while the majority of the patient population, which also included patients with esophagitis at entry, was still taking medications on a daily basis because of their GERD symptoms. Symptoms were rated to be equal or worse than at entry by 70% of patients in the absence of treatment.

In a Finnish study, 57 consecutive referrals with symptoms of GERD were treated by modification of lifestyle/antacids^[16]. Initial assessment included endoscopy and esophageal pH recording, and patients were then followed up for a median of 19.5 years. Of the 30 patients with no evidence of erosive esophagitis at presentation, five (17%) developed grade 1 esophagitis according to Savary-Miller classification. In the study by McDougall *et al.*^[17], 71% of the 17 patients with a pH-metry documented NERD complained of frequent heartburn 3 to 4.5 years after initial diagnosis, 59% were on daily acid suppressive therapy, and 24% of those patients who had repeat endoscopy developed esophagitis. Again, a progression from non-erosive to erosive GERD was observed, at least in a proportion of patients.

More recently, we have performed a study on patients with typical GERD symptoms presenting to our laboratory to undergo 24-h esophageal pH-monitoring. We have analyzed patients ($n = 35$) with a pathological investigation, defined as a 24-h % of GER exceeding 5.0% of the total recording time, and with a negative upper GI endoscopy. These NERD patients have been interviewed by mean of a structured questionnaire on average three years after the initial diagnosis, in order to assess the presence and severity of GERD symptoms, the therapy (if any) received during this period of follow-up, and the results of any subsequent endoscopic examination performed.

The results of this retrospective survey show that 14% of those NERD patients who underwent repeat endoscopy developed erosive esophagitis during the 3-year follow-up, despite the fact that almost all of them received effective symptomatic treatment, i.e. H2-RA or PPI therapy^[18].

Finally, in a recent multicenter trial^[19] conducted on 588 patients with NERD and assessing the effectiveness

of continuous vs on demand PPI maintenance therapy, it was observed that a proportion as high as 5% of patients treated “on-demand” developed erosive changes within 6 mo of study, as compared with 0% in the continuous treatment arm.

A study has been conducted in a cohort of 3894 patients with predominant heartburn, with or without esophagitis, (1717 NERD, 1512 Los Angeles grade A/B and 278 LA grade C/D, and 387 had Barrett's esophagus) under routine clinical care in Germany, Austria, and Switzerland (ProGERD study)^[20]. After initial treatment with esomeprazole, they were followed up for two years, regardless of their response. Medical therapy or endoscopy was initiated at the discretion of their primary care physician, in line with routine care. At two years, endoscopy with biopsy was performed according to the protocol. The results were as follows: 25% of patients who had NERD at baseline progressed to LA A/B and 0.6% to LA C/D. At 2 years, 22% of patients had been off medication for at least 3 mo. The conclusions of the authors were that GERD does not seem to be a categorical disease. Progression and regression (the latter likely due to therapy) between grades was observed in this large cohort of patients under routine clinical care.

Another recent study has examined the possible progression in 47 subjects with symptomatic GERD without endoscopic evidence of esophagitis, out of a group of 497 patients undergoing upper GI endoscopy for various reasons^[21]; all those patients (47 + 450) were endoscopically assessed annually for 5 years. Esophagitis developed in 36.2% of patients with NERD, as compared with 11.3% in the control group, with a hazard ratio of developing esophagitis in the former group of 3.07. The authors concluded that the condition of symptomatic GERD carries a high risk of developing esophagitis, which increases steadily with time and was more frequent in those NERD patients with hiatus hernia, who smoke and drink alcohol, and who are without *H pylori* infection^[21].

All these studies indicate that some patients with NERD may indeed develop erosive disease, at an approximate rate of about 10% per year. If this rate remains stable with time, a substantial proportion of patients with NERD may develop ERD within 10 years, which is a rate close to what we observed in our 10-year follow-up study of NERD patients^[14].

These conclusions are in accordance with results of a recently published systematic review of 22 publications on the endoscopic assessment of erosive or non-erosive GERD over periods larger than 12 months^[22]. In this review, authors conclude that the observed progression rate from NERD to ERD ranges in the literature from 0% to 30%. The variability may be related to the duration of follow-up and other factors as *H pylori* infection.

CONCLUSION

NERD is a heterogeneous condition, presently defined on the basis of the presence of typical GERD symptoms and the absence of esophageal damage as

judged by upper endoscopy. This definition is for various reasons unsatisfactory. The prevalence of at least weekly heartburn in the general population in Europe can be estimated to range from 10% to 20%.

A consistent proportion of this group will develop an erosive esophagitis (progression), even under routine therapeutic care, with a rate probably around 10% per year within a 10-year frame.

REFERENCES

- 1 **Vakil N**, van Zanten SV, Kahrilas P, Dent J, Jones R. The Montreal definition and classification of gastroesophageal reflux disease: a global evidence-based consensus. *Am J Gastroenterol* 2006; **101**: 1900-1920; quiz 1943
- 2 **Dent J**, El-Serag HB, Wallander MA, Johansson S. Epidemiology of gastro-oesophageal reflux disease: a systematic review. *Gut* 2005; **54**: 710-717
- 3 **Ronkainen J**, Aro P, Storskrubb T, Johansson SE, Lind T, Bolling-Sternevald E, Graffner H, Vieth M, Stolte M, Engstrand L, Talley NJ, Agreus L. High prevalence of gastroesophageal reflux symptoms and esophagitis with or without symptoms in the general adult Swedish population: a Kalixanda study report. *Scand J Gastroenterol* 2005; **40**: 275-285
- 4 **Zagari RM**, Fuccio L, Wallander MA, Johansson S, Fiocca R, Casanova S, Farahmand BY, Winchester CC, Roda E, Bazzoli F. Gastro-oesophageal reflux symptoms, oesophagitis and Barrett's oesophagus in the general population: Loiano-Monghidoro study. *Gut* 2008; **57**: 1354-1359
- 5 **Tack J**, Fass R. Review article: approaches to endoscopic-negative reflux disease: part of the GERD spectrum or a unique acid-related disorder? *Aliment Pharmacol Ther* 2004; **19** Suppl 1: 28-34
- 6 **Galmiche JP**, Clouse RE, Balint A, Cook IJ, Kahrilas PJ, Paterson WG, Smout AJ. Functional esophageal disorders. *Gastroenterology* 2006; **130**: 1459-1465
- 7 **Locke GR 3rd**. Natural history of nonerosive reflux disease. Is all gastroesophageal reflux disease the same? What is the evidence? *Gastroenterol Clin North Am* 2002; **31**: S59-S66
- 8 **Quigley EM**, DiBaise JK. Non-erosive reflux disease: the real problem in gastro-oesophageal reflux disease. *Dig Liver Dis* 2001; **33**: 523-527
- 9 **Glise H**, Hallerback B, Wiklund I. Quality of life: a reflection of symptoms and concerns. *Scand J Gastroenterol Suppl* 1996; **221**: 14-17
- 10 **Smout AJPM**. Endoscopy-negative acid reflux disease. *Aliment Pharmacol Ther* 1997; **11** Suppl 2: 81-85
- 11 **Fass R**, Fennerty MB, Vakil N. Nonerosive reflux disease-current concepts and dilemmas. *Am J Gastroenterol* 2001; **96**: 303-314
- 12 **Carlsson R**, Dent J, Watts R, Riley S, Sheikh R, Hatlebakk J, Haug K, de Groot O, van Oudvorst A, Dalvag A, Junghard O, Wiklund I. Gastro-oesophageal reflux disease in primary care: an international study of different treatment strategies with omeprazole. *Eur J Gastroenterol Hepatol* 1998; **10**: 119-124
- 13 **Pace F**, Santalucia F, Bianchi Porro G. Natural history of gastro-oesophageal reflux disease without oesophagitis. *Gut* 1991; **32**: 845-848
- 14 **Pace F**, Bollani S, Molteni P, Bianchi Porro G. Natural history of gastro-oesophageal reflux disease without oesophagitis (NERD)--a reappraisal 10 years on. *Dig Liver Dis* 2004; **36**: 111-115
- 15 **Schindlbeck NE**, Klauser AG, Berghammer G, Londong W, Mueller-Lissner SA. Three year follow up of patients with gastroesophageal reflux disease. *Gut* 1992; **33**: 1016-1019
- 16 **Isolauri J**, Luostarinen M, Isolauri E, Reinikainen P, Viljakka M, Keyrilainen O. Natural course of gastroesophageal reflux disease: 17-22 year follow-up of 60 patients. *Am J Gastroenterol* 1997; **92**: 37-41
- 17 **McDougall NI**, Johnston BT, Collins JS, McFarland RJ, Love AH. Three- to 4.5-year prospective study of prognostic indicators in gastro-oesophageal reflux disease. *Scand J Gastroenterol* 1998; **33**: 1016-1022
- 18 **Pace F**, Pallotta S, Molteni P, Zentilin P, Russo L, Savarino V, Bianchi Porro G, Grossi E, Cuomo R. Natural history of NERD in 3 Italian tertiary referral centres after 5 years of follow up. *Gut* 2006; **55** suppl: A62
- 19 **Bayerdörffer E**, Sipponen P, Bigard M, Weiss W, Mearin F, Rodrigo L, Dominguez-Munoz J, Grundling H, Naclér E, Svedberg L, Keeling N, Eklund S. Esomeprazole 20 mg continuous versus on demand treatment of patients with endoscopy-negative reflux disease (ENRD). *Gut* 2004; **53** (Suppl 4): A106
- 20 **Labenz J**, Nocon M, Lind T, Leodolter A, Jaspersen D, Meyer-Sabellek W, Stolte M, Vieth M, Willich SN, Malfertheiner P. Prospective Follow-Up data from the ProGERD Study Suggest that GERD Is Not a categorical disease. *Am J Gastroenterol* 2006; **101**: 2457-2462
- 21 **Kawanishi M**. Will symptomatic gastroesophageal reflux disease develop into reflux esophagitis? *J Gastroenterol* 2006; **41**: 440-443
- 22 **Fullard M**, Kang JY, Neild P, Poullis A, Maxwell JD. Systematic review: does gastroesophageal reflux disease progress? *Aliment Pharmacol Ther* 2006; **24**: 33-45

S- Editor Li DL L- Editor Mihm S E- Editor Yin DH



Miguel Angel Muñoz-Navas, Professor, Series Editors

Capsule endoscopy in non-steroidal anti-inflammatory drugs-enteropathy and miscellaneous, rare intestinal diseases

Gerard Gay, Michel Delvaux, Muriel Frederic

Gerard Gay, Michel Delvaux, Muriel Frederic, Department of Internal Medicine and Digestive Pathology, Hopitaux de Brabois, CHU of Nancy, Vandoeuvre les Nancy F-54511, France

Correspondence to: Gerard Gay, Professor, Department of Internal Medicine and Digestive Pathology, CHU of Nancy, Hopitaux de Brabois, Allee du Morvan, Vandoeuvre les Nancy F-54511, France. g.gay@chu-nancy.fr

Telephone: +33-383-154366 Fax: +33-383-154012

Received: February 15, 2008 Revised: August 10, 2008

Accepted: August 17, 2008

Published online: September 14, 2008

Abstract

Despite significant advances over the last decade, mucosal lesions of the small bowel are poorly detected by imaging studies such as CT scan, MRI-enteroclysis and contrast-enhanced abdominal ultrasound. Capsule endoscopy (CE) has dramatically changed the diagnostic approach to intestinal diseases. Moreover, the use of CE can be extended to include other conditions. However, it is difficult to assess the positive influence of CE on patient outcomes in conditions involving a small number of patients, or in critically ill and difficult to examine patients. CE has the advantage of diagnosing intestinal lesions and of directing the use of double balloon enteroscopy (DBE) in order to obtain biopsy specimens. Moreover, CE allows repeated assessment in chronic conditions, especially to detect relapse of an infectious disease.

© 2008 The WJG Press. All rights reserved.

Key words: Capsule endoscopy; Non-steroidal anti-inflammatory drugs-enteropathy; Intestinal diseases

Peer reviewer: Giovanni D De Palma, Professor, Department of Surgery and Advanced Technologies, University of Naples Federico II, School of Medicine, Naples 80131, Italy

Gay G, Delvaux M, Frederic M. Capsule endoscopy in non-steroidal anti-inflammatory drugs-enteropathy and miscellaneous, rare intestinal diseases. *World J Gastroenterol* 2008; 14(34): 5237-5244
Available from: URL: <http://www.wjgnet.com/1007-9327/14/5237.asp> DOI: <http://dx.doi.org/10.3748/wjg.14.5237>

INTRODUCTION

Since it was introduced by Iddan and Meron in 2000^[1], capsule endoscopy (CE) has dramatically changed the diagnostic approach to intestinal diseases. Despite significant advances over the last decade, mucosal lesions of the small bowel are poorly detected by techniques such as CT scan, MRI-enteroclysis and contrast-enhanced abdominal ultrasound^[2]. The diagnostic superiority of CE over these methods is related to its ability to provide a complete examination of the small bowel mucosa. On the other hand, the drawback of CE is the inability to obtain biopsies. However, this deficiency has been overcome with the use of double balloon enteroscopy, which permits obtaining biopsies from lesions detected by CE^[3].

Therefore, the diagnostic approach to conditions such as obscure GI bleeding, Crohn's disease and coeliac sprue has been dramatically altered by CE^[4-6]. Moreover, the use of CE has resulted in significant advances in the understanding and diagnosis of several gastrointestinal diseases including assessment of the effect of medications on the small bowel, intestinal lesions secondary to systemic diseases, and some rare conditions^[7].

In this review, the role of CE will be discussed in the following conditions: (I) Intestinal consequences of medications such as non-steroidal anti-inflammatory drugs (NSAIDs). (II) Rare conditions mainly involving the small bowel. (1) Primary lymphangiectasia such as Waldmann's disease. (2) Common variable immunodeficiency disorder. (3) Familial polyposis syndromes with small bowel involvement. (III) Immunological disorders with small bowel involvement. (1) Acute and chronic graft versus host disease. (2) Hypobetalipoproteinaemia. (IV) General diseases with intestinal lesions, such as vasculitides. (V) Infectious intestinal diseases such as Whipple's disease and CMV infection in immunosuppressed patients.

For each of these conditions, the clinical and biological characteristics will be discussed when needed, to understand the role of CE in diagnosis. The typical endoscopic patterns observed with CE will be described, and the use of CE will be integrated in a global diagnostic approach.

CE FOR THE EVALUATION OF INTESTINAL TOXICITY OF MEDICATIONS: THE EXAMPLE OF NSAID-RELATED ENTEROPATHY

CE is a simple and efficient tool to detect intestinal lesions due to NSAIDs

Background: NSAIDs account for 5%-10% of all drug prescriptions in developed countries and about 25% of the reported side effects caused by all classes of medications, including gastroduodenal ulcers seen in 10%-30% patients with intestinal lesions^[8]. The prevalence of severe side effects involving the small bowel and colon is 0.89 per 100 patient-years with naproxen, and 0.41 with rofecoxib, corresponding to 39.4% and 42.0%, respectively, of all the severe gastrointestinal side effects^[9]. NSAIDs alter the intestinal permeability, about 12 hours after intake and result in mucosal inflammation within 10 days. A variety of endoscopic lesions have been described, ranging from asymptomatic enteropathy to severe lesions such as ulcers, perforation, stenosis (Figure 1), diaphragms and villous atrophy. CE may be able to help detect these lesions and clarify their pathophysiology.

Description of the lesions: One of the most interesting studies was reported by Maiden *et al*^[10], who evaluated the number and the type of intestinal lesions induced by NSAIDs in 40 healthy volunteers between the ages of 21 and 61 years, using CE and fecal calprotectin. The fecal calprotectin test and CE were performed before and after a 2-week course of treatment with diclofenac (75 mg) and omeprazole (20 mg twice a day). The CE recordings were read by three independent investigators, and the lesions were regarded as significant if indicated by at least two of them. The lesions were classified into six categories: (1) reddened folds; (2) denuded area with loss of villous architecture; (3) petechiae; (4) mucosal breaks; (5) presence of blood without a lesion being visualized; and (6) other findings, mainly lymphangiectasia and angiodysplasia. Before treatment, lymphangiectasia and arteriovenous malformations were observed in three patients each. After treatment with nonsteroidal anti-inflammatory drugs (NSAIDs), lesions were seen in 27 patients, 15 of whom had more than one lesion: 16 mucosal breaks (40%), including two cases of bleeding; 14 reddened folds (35%); 13 petechiae and red spots (33%); eight denuded areas (20%), including two patients with mucosal breaks; and three patients with blood in the intestinal lumen (8%). After 2 weeks, the fecal calprotectin level increased significantly in 30 subjects (+ 75-82 μg ; $P < 0.0001$) but this increase did not correlate with the number of lesions detected by CE. The authors concluded that short-term NSAID treatment was associated with a high level of intestinal inflammation, and an increased frequency of intestinal lesions.

CE helps understanding the pathophysiology of NSAIDs-enteropathy

NSAID-related lesions were located equally in the

Table 1 Intestinal diseases with lesions similar to Crohn's disease, ranked by order of frequency

	Frequency
Crohn's disease	++++
Drug-related injuries (NSAIDs)	+++
Mesenteric ischaemia	++
Coeliac disease (jejunitis)	++
Cryptogenic multifocal ulcerous stenosing enteritis	+
Radiation enteritis	+
Lymphoma, ulcerated cancer	+
Vasculitides (lupus, polyarthritis, PAN)	+
Behçet's disease	+/-
Eosinophilic enteritis	+/-
Infections (CMV, Whipple, yersinia <i>etc</i>)	+/-

proximal and distal parts of the small bowel. The study by Maiden *et al*^[10] was interesting, as it showed that significant lesions can occur even after a short course of NSAID treatment in healthy individuals. However, the study had several methodological limitations: (1) The interpretation of CE images was often difficult^[11] and the clinical relevance of lesions detected by CE was not always established, since such lesions could be found in the absence of any treatment in healthy individuals^[12]. (2) Other causes of intestinal lesions such as chronic ischemia were not excluded. (3) A standardized terminology allowing a consistent description of the lesions and comparisons between different studies was lacking^[11]. Moreover, DBE provides a direct access to intestinal lesions detected by CE and biopsy specimens can be obtained. Therefore, our understanding of the pathophysiology of early lesions induced by NSAIDs is likely to improve.

CE to evaluate the benefit of medications preventing the toxic intestinal effects of NSAIDs

Shunji and Nakamura showed in a randomized trial on 16 patients that rebamipide significantly decreased the prevalence of diclofenac-induced small intestinal mucosal injury^[13]. These results need to be confirmed in larger studies. On the other hand, the etiological diagnosis of intestinal lesions observed by CE is often difficult. The distinction of lesions related to NSAIDs or those seen in Crohn's disease is particularly difficult, as recently shown by Voderholzer who reported a rate of misdiagnosed lesions in as many as 25% of the 40 patients with 146 intestinal lesions detected by CE^[14].

Table 1 shows how difficult the differential diagnosis is of intestinal erosions and ulcers, since they may be related to a number of pathological conditions and diseases. Therefore, DBE with biopsy of the lesions will often be necessary to obtain a correct diagnosis.

RARE DIGESTIVE DISEASES WITH SMALL BOWEL INVOLVEMENT

Waldmann's disease (Figure 2)

Intestinal lymphangiectasia appears on CE recordings as whitish areas, often diffusely spread over the intestinal mucosa. The endoscopic appearance of

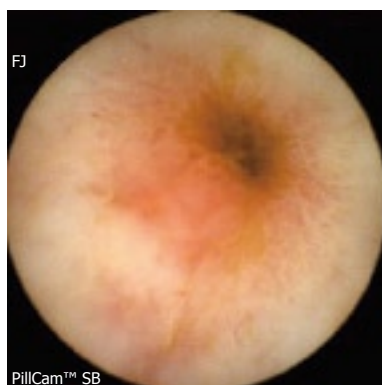


Figure 1 Intestinal stenosis caused by NSAIDs. Capsule endoscopy shows narrowed segment, surrounded by an ulcerated but non-inflammatory mucosa.



Figure 2 Waldmann's disease. The presence of dilated lymphatic vessels in the submucosa gives a whitish appearance to the mucosa. If lymphangiectasia becomes more prominent, it may protrude into the lumen.

lymphangiectasia is the result of an accumulation of chylomicrons in the dilated lymphatic vessels, varying in size from a few millimeter white spots to large white nodular areas. Lymphangiectasia may be diffuse or may involve a localized segment of the small bowel. Clinically, lymphangiectasia is classified as primary, secondary or functional. Functional lymphangiectasia are not clinically relevant and it is often encountered in patients with functional digestive disorders. Secondary lymphangiectasia is a consequence of intestinal or extra-intestinal diseases causing compression of the gut. By contrast, Waldmann's disease is primary idiopathic lymphangiectasia, which results in an exudative enteropathy. This condition may present as a diffuse disease or may involve only a localized segment of the small bowel. Localized lymphangiectasia can be treated surgically, by resection of the pathological segment. However, diffuse disease requires medical treatment (diet containing medium chain fatty acids). DBE shows thickened mucosal folds without villous atrophy in the duodenum, jejunum and ileum. Small size nodules (spots) may be observed as well as large confluent areas^[15]. CT scan may show thickening of the mucosal folds.

Immunoglobulin deficiency: The example of common variable immunodeficiency disorder (CVID, Figure 3)

This condition is characterized by hypogamma-



Figure 3 Common variable immunodeficiency disorder. The characteristic lesions include polypoidal and nodular lesions of variable size, which are disseminated over the intestinal mucosa.

Table 2 Common variable immunodeficiency disorder: Clinical manifestations

Gastrointestinal signs	Extra-intestinal signs
<i>Giardia lamblia</i> infection	Recurrent respiratory tract infections
Small bowel bacterial overgrowth	Increased risk of lymphomas
Viral and infectious diarrhoea	Increased risk of gastric cancer
Celiac disease	
Frequent nodular lymphoid hyperplasia, not premalignant	

globulinaemia, recurrent bacterial infections, mainly pulmonary, and is frequently associated with autoimmune and neoplastic disorders. Table 2 summarizes the clinical features. CE shows small (millimetre size) nodular lesions spread diffusely over the intestinal mucosa. In some cases, the lesions are polypoidal, of variable sizes^[16]. DBE shows similar findings and may also demonstrate areas of atrophic mucosa, in the event of an associated infection. The nodules correspond to hypertrophic lymphoid follicles, without plasma cells.

In most instances, the lesions (either nodules or polyps) are disseminated throughout the gut. There is an increased risk of intestinal lymphoma in these patients. CE allows regular surveillance of the small bowel. When CE reveals a change in the endoscopic findings, DBE with biopsy should be performed or the patient should be referred to a surgeon for intestinal resection.

Familial polyposis syndromes

Background: CE is very effective in detecting intestinal tumours. Intestinal tumours account for 3% to 6% of all digestive neoplasms, less than 2% of which are malignant. The data collected since the introduction of CE, shows a higher frequency of intestinal tumours, between 6.3% and 12.3% in patients investigated for obscure digestive bleeding by CE^[17]. Since the use of CE permits earlier diagnosis, the clinical course of intestinal tumours has changed. These tumours are now seen as ulcerated, haemorrhagic lesions, before the occurrence of obstructive signs. Therefore, one may assume that CE can be used for the detection of intestinal tumours in patients with familial polyposis syndromes. There are

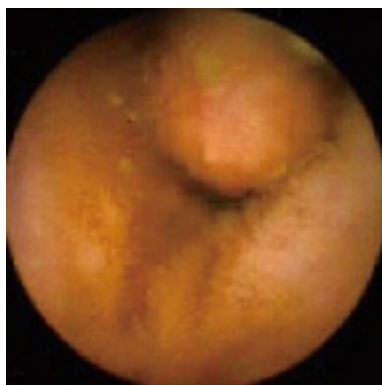


Figure 4 Peutz-Jeghers syndrome. Presence of a large polyp in the ileum, in a young patient with Peutz-Jeghers syndrome. The polyp was ulcerated and required endoscopic resection.



Figure 5 Familial adenomatous polyposis. Multiple polyps of regular shape are present in the ileum.

two conditions that are of particular concern: Peutz-Jeghers syndrome and familial adenomatous polyposis syndrome.

Peutz-Jeghers syndrome (Figure 4): In patients with Peutz-Jeghers syndrome, the prevalence of small bowel cancer is particularly high. Intussusception of the polyps may cause intestinal obstruction, while ulcerated lesions may be responsible for acute or chronic gastrointestinal bleeding. CE can detect polyps in the entire length of the small intestines, with a higher diagnostic yield compared to CT scan and MRI with enteroclysis, particularly for lesions < 1 cm in diameter^[18]. The role of CE has recently been established in the initial work-up, as well as for the follow-up of patients with Peutz-Jeghers syndrome^[18]. However, it is difficult to determine the size of the polyps on capsule recordings, which is an important factor in selecting patients for removal by DBE. It should be noted that polyps selected for removal are of a large size, and those at high risk of ulceration, malignancy and intussusception.

Familial adenomatous polyposis syndrome (FAP, Figure 5): There is currently no established indication for small bowel CE in the initial work-up of patients with FAP. By contrast, CE may be indicated in FAP patients with multiple duodenal polyps (Spiegelmann III or IV)

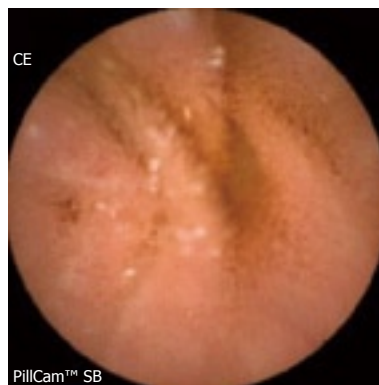


Figure 6 Acute Graft versus Host Disease (GVHD). The lesions correspond to Stage III disease, manifesting as diffuse erythema and oedema of the mucosa, with a few erosions.

as these patients have more frequent intestinal polyps^[19]. Moreover, polyps larger than 10 mm in diameter should be removed because of the risk of malignancy, either by DBE or intra-operative enteroscopy. On the other hand, examination of the duodenum, in particular the area of the papilla is often difficult with CE because of the rapid transit of the capsule through this segment. Therefore, assessment of the duodenum with a lateral viewing endoscope is the preferred approach.

Capsule endoscopy should be used with caution in familial polyposis syndromes, since many of these patients have undergone multiple abdominal surgeries which may delay capsule progression or even cause its retention.

B-cell lymphoma: Finally, in this section on rare intestinal tumours, it is important to mention the role of CE in the investigation of patients with suspected B-cell lymphoma. CE not only allows the diagnosis of such tumours but also helps to evaluate the extent of the intestinal disease^[20]. CE is also useful in evaluating the response to chemotherapy.

IMMUNOLOGICAL DIGESTIVE DISORDERS (PHOTO N° 9 ET 10) (MALADIE DE BORDIGONI)

Acute gastrointestinal graft versus host disease (GVHD) after bone marrow transplantation (Figure 6)

Bone marrow transplantation is widely used for the treatment of a number of haematological diseases. Acute GVHD is a severe complication that requires quick initiation of treatment with immunosuppressive drugs. The small bowel is often involved in acute GVHD, with the development of hematochezia, diarrhoea, abdominal pain, nausea and vomiting. The diagnosis is difficult and GVHD must be distinguished from other conditions sharing a similar clinical picture such as cytomegalovirus enteritis and *Clostridium difficile* infection.

The diagnosis is easier in the presence of multi-system disease, especially when the skin lesions can be biopsied. In patients without skin involvement, an endoscopic

work-up is needed, with esogastroduodenoscopy and ileo-colonoscopy. Until recently, esogastroduodenoscopy was regarded as the gold standard for the examination of the duodenum, with findings of mucosal oedema, denuded mucosa, erosions, erythema and bleeding ulcers. These lesions are classified into 4 grades, grade I refers to focal erythema, grade II diffuse erythema and oedema, grade III severe oedema and friable mucosa with erosions, and grade IV presence of exudate, ulcers and active bleeding^[21].

The clinical usefulness of CE in acute GVHD was demonstrated in two studies. Neumann *et al*^[22] evaluated 14 patients with clinical signs of acute GVHD after bone marrow transplantation, and observed typical intestinal lesions with histological confirmation on biopsy obtained by endoscopy, performed subsequently. This study also showed that the lesions involved the entire length of the small bowel and were more intense in the ileum compared to in the jejunum. The most important finding was the very high negative predictive value of CE since patients with a negative CE did not develop acute GVHD during the 2-month follow-up period after CE. These findings were confirmed by Aghai *et al*^[23] in a study on a larger cohort of patients.

Although it is worth mentioning that CE is well tolerated by such critically ill patients, certain limitations should be noted. In some patients, the capsule may be retained in the stomach. The Rapidview system can be used to monitor the progression of the capsule^[24]. In the event of delayed gastric clearance, erythromycin may be administered. Moreover, it should be emphasized that in some patients, CE was normal but these patients had acute GVHD^[25].

Despite the limited data available in the literature, CE should now be regarded as an alternative to esogastroduodenoscopy in the workup of patients with suspected GVHD, particularly in critically ill patients requiring quick therapeutic decisions. Indeed, CE appears to be as effective as esogastroduodenoscopy with biopsy for the diagnosis of acute GVHD^[25-27].

Hypobetalipoproteinemia (Figure 7)

Background: Hypobetalipoproteinemia is an autosomal dominant disorder caused by a mutation or deletion in the *apoB* gene which produces a truncated apolipoprotein B. The plasma concentrations of the two forms of apolipoprotein B, that is, Apo B-100 synthesized by the liver and Apo-B48 synthesized by the intestine, are undetectable. The biological and clinical picture is similar to that of abetalipoproteinemia. Heterozygous hypobetalipoproteinemia is present in 0.1%-0.8% of the general population, while homozygous hypobetalipoproteinemia is rare. The prognosis of Apo B-related disorders depends mainly on the consequences of the malabsorption syndrome, and on the progression of neurological alterations caused by the deficiency of soluble vitamin E^[28].

Diagnostic procedure: Laboratory tests show hypocholesterolemia, acanthocytosis and undetectable

ApoB lipoprotein. CT scan shows massive and diffuse infiltration of the liver. CE demonstrates diffuse whitish pattern of the intestinal mucosa with occasional yellow areas seen in the entire length of the small bowel, but without any villous atrophy. Enteroscopy, formerly performed with push enteroscopes and currently with DBE shows a similar pattern as CE and moreover, biopsy specimens can be obtained. These lesions are produced by the accumulation of fat vesicles in the enterocytes in the jejunum and ileum, without causing villous atrophy^[29]. Immunohistochemistry shows migration of truncated Apo-B and the absence of normal Apo-B hypobetalipoproteinemia.

INTESTINAL EXPRESSION OF VASCULITIDES

Background

General considerations: Systemic diseases include several very heterogeneous pathological conditions which share some common features: (1) symptoms suggesting the involvement of multiple organs, (2) specific immunological and biological alterations, (3) a tendency to chronicity, and (4) clinical response to immunosuppressive therapy. Systemic diseases are divided into two main categories^[30,31]: connective tissue or collagenous diseases with predominant abnormal production or deregulated synthesis of collagen, and vasculitides with predominant inflammation of the blood vessels resulting in abnormal vascular permeability, thrombosis and tissue ischaemia. These conditions share some pathophysiological mechanisms such as abnormal production of proteins or cytokines, and abnormal humoral or cellular immunity that results in muscular inflammation, atrophy and fragmentation. In the end, the vascular endothelial changes become predominant and are responsible for the muscular, neurological and digestive abnormalities.

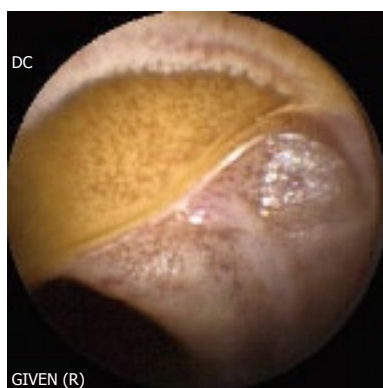
Digestive manifestations of vasculitides and systemic diseases include a number of clinical conditions (Table 3)^[32]. The development of GI symptoms raises three main questions: Do the digestive symptoms correspond to the initial manifestation of the condition or to a new acute phase of the disease? Are the digestive symptoms secondary to a complication of the treatment? Is the observed complication a component of the primary disease? To answer these questions, the clinician may use diagnostic techniques such as CT scan, biological and immunological tests, and CE. The use of CE will be illustrated by a few examples.

Specific characteristics of diseases

Behçet's disease: Behçet's disease is a systemic inflammatory condition, with 10% to 25% patients developing GI manifestations. The symptoms consist mainly of abdominal pain, diarrhoea and acute or chronic bleeding. CE may show erosions and aphthous ulcers in the small bowel, as reported by Fylejk in a series of 20 patients^[33]. Intestinal lesions may be present

Table 3 Frequency of digestive manifestations of vasculitides and connective tissue diseases (adapted from Muller-Ladner^[32])

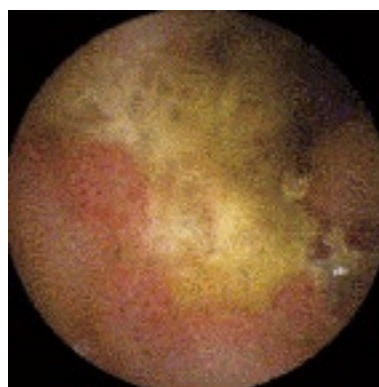
Disease	Frequency of digestive manifestations
Primary vasculitides	
Periarteritis nodosa.	30%-50%
Churg-Strauss Syndrome	25%-50%
Behçet's disease	Up to 30%
Takayasu's arteritis	Up to 15%
Wegener's disease (granulomatosis)	5%-10%
Lymphomatous granulomatosis	1.5%
Horton's disease	1%
Henoch-Schönlein purpura	50%-90%
Secondary vasculitides	
Systemic lupus erythematosus	Up to 50%
Rheumatoid arthritis	Up to 10%
Connective tissue disorders	
Systemic sclerosis	75%-90%
Systemic lupus erythematosus (except vascular lesions)	25%

**Figure 7** Hypobetalipoproteinemia. The endoscopic picture is characterized by a diffuse whitish pattern of the mucosa due to the accumulation of fat vesicles in the enterocytes. The size of the intestinal villi is normal.

in symptomatic and asymptomatic patients, and do not show a specific endoscopic pattern.

Churg et Strauss disease (Figure 8): Churg et Strauss disease is a systemic disease characterized by eosinophilia and NTE manifestations suggesting an allergenic reaction. The small bowel is involved in nearly 50% patients, presenting with gastrointestinal bleeding, diarrhoea and abdominal pain. Intestinal ulcers are not uncommon and often manifest as bleeding or perforation. Sanchez *et al*^[34] have shown that the ulcers detected by CE are deep, involve the jejunum and ileum, and respond well to immunosuppressive treatment. Therefore, CE is also useful in evaluating the response to treatment.

Henoch-Schönlein purpura: The clinical picture of Henoch-Schönlein purpura is characterized by the occurrence of purpuric lesions, abdominal pain, polyarthralgia and renal failure. The disease is a consequence of the deposition of IgA in the blood vessels. CE may reveal intestinal involvement, with

**Figure 8** Churg and Strauss disease. A large ulcer is present in the ileum.

findings of oedematous mucosa, multiple ulcers, fibrin deposits and bleeding. Lesions observed by CE are often diffuse, involving the jejunum and ileum. If the clinical picture suggests Henoch-Schönlein purpura, the presence of intestinal lesions at CE should be regarded as diagnostic, and further endoscopic assessment is not required^[35].

Antral vascular ectasia associated with vasculitides:

Antral vascular ectasia are a common cause of bleeding and anaemia, particularly in patients with liver cirrhosis and portal hypertension. However, portal hypertension is not observed in nearly 70% patients. In these patients, the condition is related to immunological disorders such as systemic sclerosis and rheumatoid arthritis, renal failure and diabetes. Antral vascular ectasia can be diagnosed by CE with a typical endoscopic pattern of "watermelon stomach". Thus, CE is useful not only in diagnosing the cause of anaemia but also for the follow-up of patients treated with endoscopic haemostasis^[36].

INFECTIOUS INTESTINAL DISEASES AND VIRAL INFECTIONS IN IMMUNODEFICIENT PATIENTS

Background

The clinical manifestation of intestinal infection is usually an acute episode of diarrhoea, which resolves spontaneously, and endoscopic assessment is not indicated in most patients. By contrast, patients with persistent diarrhoea or malabsorption require further workup. Recently, there has been an increase in the incidence of certain viral infections in patients with immunodeficiency due to HIV infection or those on immunosuppressive therapy. Endoscopy is the main diagnostic procedure as it allows examination of the mucosa, and biopsy specimens can be obtained for identification of the causal virus. Biopsies are frequently performed during esogastroduodenoscopy and ileocolonoscopy. These procedures have a limited range of examination, since a large part of the small bowel is excluded. CE is a very useful complement to these investigations, as it allows assessment of the intestinal



Figure 9 Cytomegalovirus infection in a heart transplant patient. The disease is characterized by the presence of bleeding ulcers and erosions.

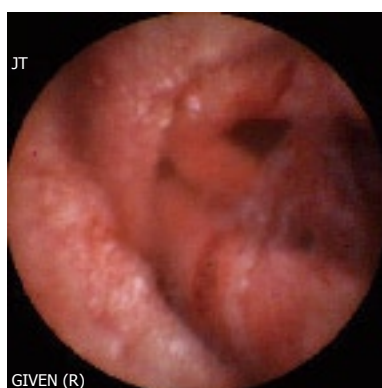


Figure 10 Whipple's disease. The endoscopic picture shows an oedematous and friable mucosa with erosions and serpiginous ulcers. The lesions may involve the entire length of the small bowel.

mucosa with a high diagnostic yield, and can have a direct impact on the management of such patients^[37]. Moreover, lesions detected by CE can be subsequently biopsied during DBE.

Infectious diseases in immunodeficient patients (Figure 9)

CE can detect ulcers involving the intestinal mucosa in patients with viral, fungal or bacterial infections. Cytomegalovirus infection can be diagnosed by the presence of one or two large jejunal ulcers in immunodeficient patients, as for example in transplant patients. These ulcers may bleed and are the source of haematochezia when located in the distal ileum or the colon^[38]. Fungal infection in HIV patients, such as histoplasmosis is characterized by the presence of multiple deep ulcerations accompanied with submucosal nodules protruding into the lumen. The diagnosis is confirmed by the detection of the pathogenic agent on biopsies obtained during enteroscopy^[39]. In patients with tuberculosis, CE may reveal deep ulcers, often appearing as serpiginous. The lesions are difficult to differentiate from those of Crohn's disease. The pathological examination of the biopsies may show a typical granuloma. Studies using CE have shown that the lesions may not be restricted to the ileum but are also seen in the jejunum^[40].

Whipple's disease, an example of diffuse intestinal infection (Figure 10)

Whipple's disease is a multisystem infectious condition caused by *Tropheryma whippelii*. GI symptoms are often present and the diagnosis is made by demonstrating the presence of the infectious agent on duodenal biopsy. The most typical endoscopic finding is the presence of an oedematous, friable and haemorrhagic mucosa with multiple erosions and serpiginous ulcers. CE has shown that the intestinal lesions are diffuse, involving the jejunum and ileum. This is explained by the fact that previous investigative techniques allowed only limited access to the intestinal mucosa, i.e. before CE and DBE became available^[41]. Moreover, CE is also a useful non-invasive method to determine the response to treatment, which has to be maintained for at least two years, and occasionally five years in order to avoid relapse, especially of the neurological manifestations^[42].

CONCLUSION

Since it was introduced in 2000, the use of CE in several clinical situations has been validated. However, the indications for CE can be extended to other diseases, although an assessment of a positive influence of CE on patient outcomes is difficult to obtain in conditions which occur either in a small number of patients, or in critically ill and difficult to examine patients. Clearly, CE has the advantage of detecting lesions in the small intestine and directing DBE to the correct location in order to obtain biopsies. Moreover, repeated assessment can be made in chronic conditions, especially to detect the response to treatment.

REFERENCES

- 1 **Iddan G**, Meron G, Glukhovskiy A, Swain P. Wireless capsule endoscopy. *Nature* 2000; **405**: 417
- 2 **Orjollet-Lecoanet C**, Menard Y, Martins A, Crombe-Ternamian A, Cotton F, Valette PJ. [CT enteroclysis for detection of small bowel tumors] *J Radiol* 2000; **81**: 618-627
- 3 **Gay G**, Delvaux M, Fassler I. Outcome of capsule endoscopy in determining indication and route for push-and-pull enteroscopy. *Endoscopy* 2006; **38**: 49-58
- 4 **Triester SL**, Leighton JA, Leontiadis GI, Fleischer DE, Hara AK, Heigh RI, Shiff AD, Sharma VK. A meta-analysis of the yield of capsule endoscopy compared to other diagnostic modalities in patients with obscure gastrointestinal bleeding. *Am J Gastroenterol* 2005; **100**: 2407-2418
- 5 **Triester SL**, Leighton JA, Leontiadis GI, Gurudu SR, Fleischer DE, Hara AK, Heigh RI, Shiff AD, Sharma VK. A meta-analysis of the yield of capsule endoscopy compared to other diagnostic modalities in patients with non-stricturing small bowel Crohn's disease. *Am J Gastroenterol* 2006; **101**: 954-964
- 6 **Cellier C**, Green PH, Collin P, Murray J. ICCE consensus for celiac disease. *Endoscopy* 2005; **37**: 1055-1059
- 7 **Delvaux M**, Gerard Gay. Capsule endoscopy in 2005: facts and perspectives. *Best Pract Res Clin Gastroenterol* 2006; **20**: 23-39
- 8 **Silverstein FE**, Faich G, Goldstein JL, Simon LS, Pincus T, Whelton A, Makuch R, Eisen G, Agrawal NM, Stenson WF, Burr AM, Zhao WW, Kent JD, Lefkowitz JB, Verburg KM, Geis GS. Gastrointestinal toxicity with celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis

- and rheumatoid arthritis: the CLASS study: A randomized controlled trial. Celecoxib Long-term Arthritis Safety Study. *JAMA* 2000; **284**: 1247-1255
- 9 **Bombardier C**, Laine L, Reicin A, Shapiro D, Burgos-Vargas R, Davis B, Day R, Ferraz MB, Hawkey CJ, Hochberg MC, Kvien TK, Schnitzer TJ. Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. VIGOR Study Group. *N Engl J Med* 2000; **343**: 1520-1528, 2 p following 1528
 - 10 **Maiden L**, Thjodleifsson B, Theodors A, Gonzalez J, Bjarnason I. A quantitative analysis of NSAID-induced small bowel pathology by capsule enteroscopy. *Gastroenterology* 2005; **128**: 1172-1178
 - 11 **Korman LY**, Delvaux M, Gay G, Hagenmuller F, Keuchel M, Friedman S, Weinstein M, Shetzline M, Cave D, de Franchis R. Capsule endoscopy structured terminology (CEST): proposal of a standardized and structured terminology for reporting capsule endoscopy procedures. *Endoscopy* 2005; **37**: 951-959
 - 12 **Goldstein JL**, Eisen GM, Lewis B, Gralnek IM, Aisenberg J, Bhadra P, Berger MF. Small bowel mucosal injury is reduced in healthy subjects treated with celecoxib compared with ibuprofen plus omeprazole, as assessed by video capsule endoscopy. *Aliment Pharmacol Ther* 2007; **25**: 1211-1222
 - 13 **Nakamura M**, Niwa Y, Ohmiya N, Goto H. Protective effect of rebamipide for diclofenac-induced small intestinal mucosal injury: A cross over randomized, double blind, placebo-controlled study. 6th ICCE Report, 2007: 70
 - 14 **Voderholzer W**, Maiden L, Adler S.N, Thjodleifsson B, Lochs H, Bjarnason I. Inter observer variability of wireless capsule endoscopy (WCE) in patients with Crohn's disease and NSAID enteropathy. 6th ICCE Report, 2007: 13
 - 15 **Toth E**, Keuchel M, Riemann JF. Intestinal lymphangiectasia. In: Keuchel M, Hagenmüller F, Fleischer D. Atlas of video capsule endoscopy. Heidelberg: Springer, 2006: 101-106
 - 16 **Mihaly E**, Nemeth A, Zagoni T, Nemet A, Werling K, Racz I, Tulassay Z. Gastrointestinal manifestations of common variable immunodeficiency diagnosed by video- and capsule endoscopy. *Endoscopy* 2005; **37**: 603-604
 - 17 **Bailey AA**, Debinski HS, Appleyard MN, Remedios ML, Hooper JE, Walsh AJ, Selby WS. Diagnosis and outcome of small bowel tumors found by capsule endoscopy: a three-center Australian experience. *Am J Gastroenterol* 2006; **101**: 2237-2243
 - 18 **Caspari R**, von Falkenhausen M, Krautmacher C, Schild H, Heller J, Sauerbruch T. Comparison of capsule endoscopy and magnetic resonance imaging for the detection of polyps of the small intestine in patients with familial adenomatous polyposis or with Peutz-Jeghers' syndrome. *Endoscopy* 2004; **36**: 1054-1059
 - 19 **Schulmann K**, Hollerbach S, Kraus K, Willert J, Vogel T, Moslein G, Pox C, Reiser M, Reinacher-Schick A, Schmiegel W. Feasibility and diagnostic utility of video capsule endoscopy for the detection of small bowel polyps in patients with hereditary polyposis syndromes. *Am J Gastroenterol* 2005; **100**: 27-37
 - 20 **Flieger D**, Keller R, May A, Ell C, Fischbach W. Capsule endoscopy in gastrointestinal lymphomas. *Endoscopy* 2005; **37**: 1174-1180
 - 21 **Thompson B**, Salzman D, Steinhauer J, Lazenby AJ, Wilcox CM. Prospective endoscopic evaluation for gastrointestinal graft-versus-host disease: determination of the best diagnostic approach. *Bone Marrow Transplant* 2006; **38**: 371-376
 - 22 **Neumann S**, Schoppmeyer K, Lange T, Wiedmann M, Golsong J, Tannapfel A, Mossner J, Niederwieser D, Caca K. Wireless capsule endoscopy for diagnosis of acute intestinal graft-versus-host disease. *Gastrointest Endosc* 2007; **65**: 403-409
 - 23 **Yakoub-Agha I**, Maunoury V, Wacrenier A, Couignoux S, Depil S, Desreumaux P, Bauters F, Colombel JF, Jouet JP. Impact of Small Bowel Exploration Using Video-Capsule Endoscopy in the Management of Acute Gastrointestinal Graft-versus-Host Disease. *Transplantation* 2004; **78**: 1697-1701
 - 24 **Delvaux M**, Gay G. Real-Time Viewing of Capsule Endoscopy Recordings: Principle and Clinical Potential. *Techn. Gastrointest Endosc* 2006; **8**: 160-163
 - 25 **Eisen GM**. Using capsule endoscopy to diagnose graft-versus-host disease: seeing is believing? *Gastrointest Endosc* 2007; **65**: 410-411
 - 26 **Terdiman JP**, Linker CA, Ries CA, Damon LE, Rugo HS, Ostroff JW. The role of endoscopic evaluation in patients with suspected intestinal graft-versus-host disease after allogeneic bone-marrow transplantation. *Endoscopy* 1996; **28**: 680-685
 - 27 **Shapira M**, Adler SN, Jacob H, Resnick IB, Slavin S, Or R. New insights into the pathophysiology of gastrointestinal graft-versus-host disease using capsule endoscopy. *Haematologica* 2005; **90**: 1003-1004
 - 28 **Gay G**, Delmotte JS. Abeta and hypobetalipoproteinemias in Atlas of Enteroscopy. Rossini FP, Gay G (Eds). Milan: Springer, 1998: 119-120
 - 29 **Gay G**, Barth E, Keuchel M. Involvement of the small intestine in systemic disease. In Keuchel L, Hagenmüller F, Fleischer D. Atlas of video capsule endoscopy. Heidelberg: Springer, 2006: 136-144
 - 30 **Lie JT**. Nomenclature and classification of vasculitis: plus ca change, plus c'est la meme chose. *Arthritis Rheum* 1994; **37**: 181-186
 - 31 **Jennette JC**, Falk RJ, Andrassy K, Bacon PA, Churg J, Gross WL, Hagen EC, Hoffman GS, Hunder GG, Kallenberg CG. Nomenclature of systemic vasculitides. Proposal of an international consensus conference. *Arthritis Rheum* 1994; **37**: 187-192
 - 32 **Muller-Ladner U**. Vasculitides of the gastrointestinal tract. *Best Practice Res Gastroenterol* 2001; **15**: 59-82
 - 33 **Fylyk S**, Fylyk SN, Safatle-Riberio AV, Neves F, Goncalves CR, Sipahi AM, Ishiok AS, Sakai P, Galvao-Neto M. Small intestine involvement in Behçet's disease: A capsule endoscopy approach. 6th ICCE Report, 2007: 19
 - 34 **Sanchez R**, Aparicio JR, Baeza T, Calero Y. Capsule endoscopy diagnosis of intestinal involvement in a patient with Churg-Strauss syndrome. *Gastrointest Endosc* 2006; **63**: 1082-1084
 - 35 **Skogestad E**. Capsule endoscopy in Henoch-Schonlein purpura. *Endoscopy* 2005; **37**: 189
 - 36 **Tang SJ**, Zanati S, Kandel G, Marcon NE, Kortan P. Gastric intestinal vascular ectasia syndrome: findings on capsule endoscopy. *Endoscopy* 2005; **37**: 1244-1247
 - 37 **Keuchel M**, Soares J, Reddy DN. Infectious diseases of the small intestine. In: Keuchel M, Hagenmüller F, Fleischer DE. Atlas of video capsule endoscopy. Heidelberg: Springer, 2006: 126-135
 - 38 **Bramwell NH**, Davies RA, Koshal A, Tse GN, Keon WJ, Walley VM. Fatal gastrointestinal hemorrhage caused by cytomegalovirus duodenitis and ulceration after heart transplantation. *J Heart Transplant* 1987; **6**: 303-706
 - 39 **Gupta R**, Reddy D. Infectious diseases of the small intestine. *Techn. Gastrointest Endosc* 2006; **8**: 182-187
 - 40 **Reddy DN**, Sriram PV, Rao GV, Reddy DB. Capsule endoscopy appearances of small-bowel tuberculosis. *Endoscopy* 2003; **35**: 99
 - 41 **Fritscher-Ravens A**, Swain CP, von Herbay A. Refractory Whipple's disease with anaemia: first lessons from capsule endoscopy. *Endoscopy* 2004; **36**: 659-662
 - 42 **Gay G**, Roche JF, Delvaux M. Capsule endoscopy, transit times, and Whipple's disease. *Endoscopy* 2005; **37**: 272-273



Miguel Angel Muñoz-Navas, Profesor, Series Editors

Capsule endoscopy in neoplastic diseases

Marco Pennazio, Emanuele Rondonotti, Roberto de Franchis

Marco Pennazio, Division of Gastroenterology 2, Department of Gastroenterology and Clinical Nutrition, S. Giovanni A.S. Hospital, Via Cavour 31, Turin 10123, Italy

Emanuele Rondonotti, Roberto de Franchis, University of Milan, IRCCS Policlinico, Mangiagalli, Regina Elena Foundation, Gastroenterology and Gastrointestinal Endoscopy Unit, Via Pace 9, Milan 20100, Italy

Correspondence to: Dr. Marco Pennazio, Division of Gastroenterology 2, Department of Gastroenterology and Clinical Nutrition, S. Giovanni A.S. Hospital, Via Cavour 31, Turin 10123, Italy. pennazio.marco@gmail.com

Telephone: +39-11-6333594 Fax: +39-11-6333623

Received: February 15, 2007 Revised: July 26, 2008

Accepted: August 2, 2008

Published online: September 14, 2008

remains to be determined through carefully-designed studies.

© 2008 The WJG Press. All rights reserved.

Key words: Capsule endoscopy; Enteroscopy; Obscure gastrointestinal bleeding; Small-bowel tumors; Polyposis syndromes

Peer reviewer: Klaus R Herrlinger, Hepatology and Endocrinology, Robert-Bosch-Hospital, Auerbachstrasse. 110, D-70376 Stuttgart, Germany

Pennazio M, Rondonotti E, de Franchis R. Capsule endoscopy in neoplastic diseases. *World J Gastroenterol* 2008; 14(34): 5245-5253 Available from: URL: <http://www.wjgnet.com/1007-9327/14/5245.asp> DOI: <http://dx.doi.org/10.3748/wjg.14.5245>

Abstract

Until recently, diagnosis and management of small-bowel tumors were delayed by the difficulty of access to the small bowel and the poor diagnostic capabilities of the available diagnostic techniques. An array of new methods has recently been developed, increasing the possibility of detecting these tumors at an earlier stage. Capsule endoscopy (CE) appears to be an ideal tool to recognize the presence of neoplastic lesions along this organ, since it is non-invasive and enables the entire small bowel to be visualized. High-quality images of the small-bowel mucosa may be captured and small and flat lesions recognized, without exposure to radiation. Recent studies on a large population of patients undergoing CE have reported small-bowel tumor frequency only slightly above that reported in previous surgical series (range, 1.6%-2.4%) and have also confirmed that the main clinical indication to CE in patients with small-bowel tumors is obscure gastrointestinal (GI) bleeding. The majority of tumors identified by CE are malignant; many were unsuspected and not found by other methods. However, it remains difficult to identify pathology and tumor type based on the lesion's endoscopic appearance. Despite its limitations, CE provides crucial information leading in most cases to changes in subsequent patient management. Whether the use of CE in combination with other new diagnostic (MRI or multidetector CT enterography) and therapeutic (Push-and-pull enteroscopy) techniques will lead to earlier diagnosis and treatment of these neoplasms, ultimately resulting in a survival advantage and in cost savings,

INTRODUCTION

Tumors of the small intestine present a unique challenge to the clinicians across medical specialties. Although the small bowel represents 75% of the length and 90% of the overall mucosal surface of the alimentary tract and despite its anatomic location between two regions of high cancer risk, the small bowel is generally considered as a rare location for the development of neoplasms, accounting for only 1%-3% of all primary gastrointestinal (GI) tumors^[1-3].

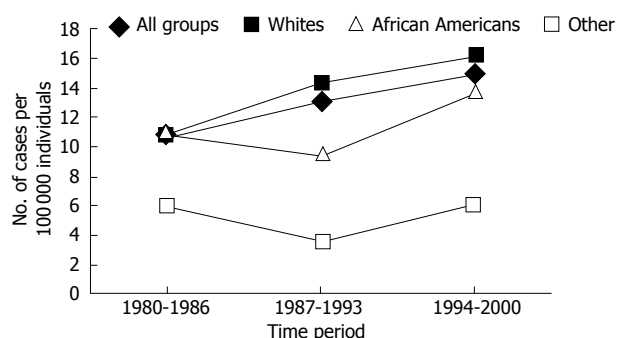
The overall age-adjusted incidence of small-bowel cancers estimated in population based studies in Western countries ranges between 0.9 and 1.4 (Table 1)^[1,4-9]; malignant tumors account for about one half of all new cases of small-bowel tumors reported^[10]. The incidence rate of small-bowel cancer varies among populations: cancer rates are high among the Maori of New Zealand (about 4 cases per 100 000 per year) and among ethnic Hawaiians, and low in India, Romania, and other parts of Eastern Europe^[11]. Some recently published studies reported an increasing incidence of these neoplasms over the last 20 years (Figure 1)^[1,9].

Because small-bowel tumors are relatively rare compared with other neoplasms of the gastrointestinal tract, several factors have been proposed to explain or understand this disparity: (1) a quick transit allowing only short contact of possible carcinogens from food

Table 1 Incidence of small-bowel tumors (modified from Neugut *et al*^[1])

Population/area	Ref.	Time interval	Cases of SB tumor	Incidence per million
Los Angeles County	4	1972-1985	264	-
Nine SEER Registers	5	1973-1982	366	9.6
Cancer register of British Columbia, Alberta, Saskatchewan, Manitoba	6	1975-1989	263	11
Utah Cancer registry	7	1966-1999	442	14
Nine SEER registers	8	1973-1991	892	13
Connecticut Tumor registry	9	1980-2000	1260	8.8

SEER: Surveillance epidemiology and end result.

**Figure 1** Incidence of small-bowel tumors per race in the Connecticut tumor registry per periods of 7 years since 1980 to 2000^[6].

with the intestinal mucosa; (2) the intestinal content is mixed together with a great volume of intestinal juices decreasing the concentration of irritating agents; (3) a decrease in mechanical and/or chemical inflammation of the mucosa because of the liquidity and alkaline pH of the small-bowel contents; (4) the high concentration of lymphatic tissue and of immunoglobulin exerts an effective immune surveillance; (5) the low bacteria concentration in the small intestine processing the intestinal content produces a low amount of carcinogens; (6) the rapid turnover of epithelial cells should decrease the potential growth and development of neoplastic cells^[1,10,11].

Genetics could also play a role in some particular subgroups of patients; subjects affected by familial adenomatous polyposis, hereditary non-polypoid colorectal cancer, Crohn's disease, celiac disease, Peutz-Jeghers syndrome, and several other diseases must be surveyed for the risk of small intestine tumor^[9,12]. A relevant role of genetics has also been described in patients with sporadic gastrointestinal stromal tumors (GISTs) in which four different regions (exon 9, exon 11, exon 13, and exon 17) of the *KIT* gene have found to be mutated^[13].

Approximately 40 different histological types of small intestinal tumors have been identified^[14]. Among malignant tumors, about 30%-50% are adenocarcinomas, 25%-30% are carcinoids, and 15%-20% are lymphomas. A recently published study, including 1260 cases of small-bowel tumor, showed that they seem to be

frequently located in the ileum (about 30% of cases) or in the duodenum (about 25% of cases)^[9]; the sites at highest risk for malignant neoplasms have been reported to be the duodenum for adenocarcinomas and the ileum for carcinoids and lymphomas^[1]. One reason why adenocarcinomas tend to arise in the duodenum may implicate bile or its metabolites in the etiology of the neoplasm at this site^[15]. However, among patients with Crohn's disease, which generally affects the ileum rather than the more proximal small bowel, adenocarcinomas tend to occur in the terminal ileum^[1].

Secondary neoplastic involvement of the small intestine has been reported to be more frequent than primary small intestinal neoplasms. Primary tumors of the colon, ovary, uterus, and stomach can involve the small bowel (by direct invasion or by intraperitoneal spread) whereas primaries from breast, lung, and melanoma metastasize to the small bowel by the hematogenous route^[16]. SB metastases from melanoma have been described in 1.5%-4.4% of patients^[17,18] with previously removed skin melanoma and in 58% of post-mortem specimens^[17].

In the majority of cases, the diagnosis of small-bowel tumors is delayed. This could be due to several factors: (I) Small-bowel tumors grow slowly, extraluminally, remaining asymptomatic for years or presenting insidiously with non-specific complaints such as abdominal pain, diarrhea, iron deficiency anemia, bleeding, extra intestinal symptoms (flushing, paraneoplastic syndromes)^[19]. Obstruction is also a common presentation; indeed, small-bowel tumors are the third most common cause of small-bowel obstruction in the United States^[20]. (II) The rare incidence of small-bowel tumors may contribute to the relatively low index of clinical suspicion for their presence. (III) Routine laboratory tests and other diagnostic tests may frequently be inconclusive; as a consequence, diagnostic laparoscopy or exploratory laparotomy may be indicated not only to deliver an effective treatment but also to reach a definitive diagnosis.

Since the introduction in clinical practice of capsule endoscopy, several case reports describing primary and secondary tumors affecting the small bowel have been published. More recently, a few retrospective studies collecting series of patients in which this technology was able to show the presence of a small-bowel tumor have also been published.

SMALL-BOWEL TUMORS: DIAGNOSTIC TOOLS

Historically the small bowel has been considered a difficult organ to evaluate. For many years, visualization of the small-bowel mucosa and the diagnosis of small-bowel tumors were feasible only in a surgical setting and this organ has been considered a sort of "black box". This situation derived both from the anatomical characteristics of the small bowel and the limitations of available techniques. The length of the small intestine,

the distance between the organ and external orifices (mouth and anus), its sinuousness, its ability to produce huge amounts of fluids and the continuous contractions long hampered accurate inspection of the small-bowel mucosa.

Traditional radiological techniques, including small-bowel follow-through and small-bowel enteroclysis, allow an indirect evaluation of the entire small bowel, however the difficulty to place a specific catheter in the right position (enteroclysis), the low pressure and the dilution of the contrast medium (small-bowel follow-through) contribute to a high miss rate for small and/or flat lesions. Conventional cross-sectional imaging techniques, such as computed tomography (CT) and magnetic resonance imaging (MRI), can be helpful in identifying large small-bowel masses or extraintestinal disease, but are unable to provide precise data about the intestinal wall. Endoscopy has the advantage of visualizing intestinal mucosa directly and, above all, of carrying out targeted biopsies. Upper GI endoscopy, when performed to the ligament of Treitz, is suited for identifying duodenal tumors; however, lesions located distally pose a unique diagnostic challenge. Push enteroscopy (PE) is an effective diagnostic and therapeutic procedure which entails the oral insertion of a dedicated enteroscope; however, it only allows thorough examination of the distal duodenum and proximal jejunum to approximately 50-100 cm beyond the ligament of Treitz. Because of its ability to examine the entire small intestine, sonde enteroscopy has been utilized to diagnose small-bowel tumors^[21]. However, this technically-challenging procedure has been today completely abandoned. Intraoperative enteroscopy (IOE) is the most complete, but also the most invasive means of examining the small bowel. It is a difficult, time-consuming technique, often traumatic to the bowel, with a substantial risk of complications and even mortality.

The development and the introduction in the clinical practice of the capsule endoscopy (CE) has revolutionized the field of small-bowel imaging, not only opening up this sort of “Pandora’s box”, but also stimulating the development of other imaging techniques aimed at studying the small bowel.

Magnetic resonance enteroclysis (MRI-enteroclysis) combines the advantages of cross-sectional resonance with those of volume challenge of conventional enteroclysis in the detection and characterization of small-bowel wall abnormalities, such as initial neoplasms. Small-bowel tumors usually exhibit moderate signal intensity on true-FISP images, as opposed to the high signal intensity of the distended lumen and the mesenteric fat. Post-gadolinium 3D FLASH with fat saturation may be the most important sequence for the identification and characterization of small-bowel tumors by their enhancement pattern. The degree of prestenotic dilatation, the peritoneal extension of the neoplasm and associated lymphadenopathy is well visualized in all MRI-enteroclysis sequences^[22,23]. The multidetector row computed tomography (MRCT) has the potential to provide high-resolution images and

a precise delineation of pathology. The multiplanar reformatted images obtained using MRCT have spatial resolution similar to that of the axial plane without any loss of information. These advantages of MDCT imaging lead to a more accurate demonstration of the site of the tumor and possible complications of underlying small-bowel tumors including small-bowel obstruction, intussusception, perforation and bleeding^[24]. The administration of methyl-cellulose as a neutral luminal contrast material in a 4%-15% water-soluble solution or a diluted (1%) barium solution as positive luminal contrast in patients undergoing MRCT results in a computed tomography enteroclysis (CT-enteroclysis). As previously described for MRI-enteroclysis, this technique combines the advantages of enteral volume challenge and the ability of cross-sectional imaging to depict extra intestinal manifestations of the disease^[25]. Both MRI enteroclysis and CT-enteroclysis require the placement of a specific catheter into the third part of the duodenum (fluoroscopic monitored), administration of medications (anti-motility agents and sedative medications) and small-bowel preparation with laxatives (PEG-based solutions, 2 to 4 L)^[24]. Up to now, there are only few, but promising, publications about the role of these three techniques in the diagnostic algorithm of small-bowel tumors.

In the attempt to design a new scope that would allow a large part of the small-bowel mucosa to be visualized, overcoming the limits of PE and IOE, Yamamoto *et al*^[25] developed a new method of push-and-pull enteroscopy (PPE) using a double-balloon technique. PPE affords inspection of the entire small bowel, combining the oral and anal approaches, with the advantage of enabling biopsies and endoscopic interventions to be performed in all parts of the small bowel without laparotomy. It is, however, invasive, time-consuming, and requires conscious sedation^[26].

CAPSULE ENDOSCOPY IN THE DIAGNOSIS OF SMALL-BOWEL TUMORS

In a recently published paper, the hypothesis of an increased incidence of small-bowel tumors in recent years was put forward, based on the increasing number of cases diagnosed by means a non-invasive methods such as CE and small-bowel ultrasound^[11]. In fact, compared with previously mentioned diagnostic techniques for the study of the small bowel, CE seems to be an ideal tool to recognize the presence of neoplastic lesions along the small bowel. The potential of CE for the diagnosis of small-bowel tumors, as well as for the surveillance of subjects at increased risk of developing them, depends largely on the technical characteristics of this diagnostic device. CE is a non-invasive tool, well accepted by patients, who can allow the visualization of the entire small bowel; high-quality images of the small-bowel mucosa may be captured and small and flat lesions recognized, without exposure to radiation.

In fact, since the introduction of CE in clinical

Table 2 Summary of CE studies for small-bowel tumors

Study ^[ref]	Population	Tumor cases (%)	Mean age of patients with tumors (yr)	Malignant tumors (%)	Tumors leading to capsule retention (%)
Cobrin <i>et al</i> ^[28]	562	50 (8.9)	63	48	0
Bailey <i>et al</i> ^[29]	416	27 (6.3)	61	63	3/26 (11.5)
Urbain <i>et al</i> ^[31]	443	11 (2.5)	63	100	0
Estevez <i>et al</i> ^[30]	320	23 (7.8)	63	NA	NA
Schwartz <i>et al</i> ^[32]	NA	87 (NA)	60	60	NA
Pasha <i>et al</i> ^[33]	1000	16 (1.6)	67	86	4/16 (25)
Rondonotti <i>et al</i> ^[34]	5129	124 (2.4)	59	NA	12/124 (9.7)

NA: Not applicable (these data are not reported in the paper).

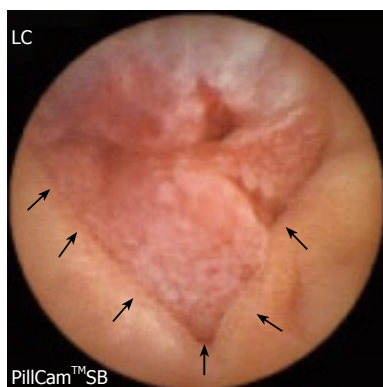


Figure 2 Infiltrating, stenotic, mass (arrows) in the ileum in a patient with hereditary non-polyposis colorectal cancer syndrome. Histology revealed an adenocarcinoma.

practice, some studies have been published^[27-32] reporting a frequency of small-bowel tumors higher than previously expected, ranging between 3.6% and 9%. All these studies were retrospective; each of them collected about 350-500 patients undergoing CE, described the frequency of small-bowel tumors in a highly selected group of patients with symptoms (obscure GI bleeding in the majority of cases) and sometimes the diagnosis was based only on the endoscopic images (one study^[30] reported 35% of lesions described as tumor without histological confirmation). Two recent studies, coming from the USA and Europe, only published in abstract form^[33,34], examined a large population of patients undergoing CE (respectively, 1000^[33] and more than 5000^[34]) in whom the definitive diagnosis was confirmed by means of tissue sampling (Table 2). They reported a small-bowel tumor frequency only slightly above that reported in previous surgical series, ranging from 1.6% to 2.4%, and also confirmed that the main clinical indication to CE in patients with small-bowel tumors is obscure GI bleeding (in about 90% of cases). Other indications for CE in these two studies were: chronic diarrhea, abdominal pain, para-neoplastic syndromes or, in a small group of patients, presence of conditions increasing the risk to develop a small-bowel tumor (such as refractory celiac disease, familial adenomatous polyposis or Peutz-Jeghers syndrome). In some rare cases CE was also used to confirm the presence of a tumor previously suspected by other imaging modalities. Although Cobrin *et al*^[28] underlined that in their study

the percentage of patients with tumor was greater among patients younger than 50 years, the median age of patients enrolled in the above mentioned large studies ranged between 59^[34] and 67 years^[33] (Table 2).

Confirming data previously reported in surgical series^[9,10] the majority of tumors identified by CE (from 63%^[29] to 86%^[33]) are malignant neoplasms and the most frequent histological types are adenocarcinomas and carcinoids (in about 20% of cases each^[28,29,32]), while GISTs represent the most frequently identified benign neoplasm. Of note, this tumor accounted for more than one third of all collected cases in the large multi-center European study^[34]. As far as small-bowel metastases are concerned, these lesions mainly (about 1/3 of cases^[34]) derived from previously removed skin melanomas^[35], but there are also some papers reporting lesions derived from colorectal cancers^[29], from hepatocellular carcinoma or from rare tumors such as seminomas^[34].

Small-bowel tumors appear at CE as masses or polyps in about 70%-80% of cases^[28-34] and as ulcers (sometimes actively bleeding) or stenoses in 20%-30% of cases (Figure 2). Unfortunately, it is very difficult to identify pathology and tumor type based on the capsule endoscopic appearance of lesions^[36]. These tumors are mostly located in the jejunum, 40%-60% of cases, in the ileum, 25%-40% of cases, and less frequently in the duodenum, in 15%-20% of cases^[28-34]. The location of the majority of lesions in the mid-small bowel could be a partial explanation of the extensive (and mainly negative) diagnostic work-up performed in patients enrolled in all these studies. Each patient underwent a mean of 2%-4.6%^[29,32] examinations before CE while, focusing only on exams addressed to evaluate the small bowel (particularly small-bowel series and/or small-bowel follow-through and/or PE and/or CT-enteroclysis), the mean number of examinations performed per patient ranged between 1 and 2^[28,29,32]. Despite the extensive number of examinations performed before CE, this technique was found to have a positive impact on diagnosis (defined as the capability to identify a neoplasm not shown by other diagnostic techniques or as the ability to provide crucial information leading to change the subsequent patient management) in about 65%-80% of cases^[31,34]. Urbain *et al*^[31], trying to evaluate the impact of CE on the therapeutic choices of malignant small-bowel tumors, found that CE may influence directly the therapeutic work-up in about 55% of cases by providing

information about size, location and appearance of the lesion.

Because the early diagnosis and treatment of cancer usually affects outcome, some authors^[28,29] suggest that the capability of CE to discover small-bowel tumors at an early stage may have an impact on prognosis for patients with these lesions. All the papers previously mentioned reported that in patients with small-bowel neoplasm identified by CE, surgery alone or surgery plus chemotherapy is the treatment of choice in about 85%-90% of the cases^[28-30,33,34] but, to date, there is only one published paper describing the follow-up of these patients. Bailey *et al.*^[29] reported that surgical treatment was performed in 88% of patients with small-bowel tumor, in half of the cases with curative aim. None of the patients who underwent a curative resection developed tumor recurrence at follow-up (range, 26-51 mo). These authors also reported that none of the patients with benign tumors discovered by CE and treated according to CE findings had recurrence of either overt or occult obscure GI bleeding at follow-up (3-51 mo).

CAPSULE ENDOSCOPY FOR SPECIFIC SMALL-BOWEL TUMORS

Thanks to its capability to identify a small-bowel lesion in most patients with a prior negative diagnostic work-up, several case reports, but also some small series, aimed at evaluating the possible role of the CE in the diagnosis of specific tumors in particular clinical conditions, have been published over the last few years.

Van Tuyl *et al.*^[37], in a prospective descriptive study, evaluated 20 patients with liver metastases, mesenteric metastases or both, originated from a neuroendocrine tumor (NET) with unknown primary location. All these patients had undergone several examinations including small-bowel enteroclysis, abdominal CT, pentetretotide scintigraphy and laboratory tests. In this particular subset of patients, CE showed a diagnostic yield (60%) significantly higher than enteroclysis and CT scan. Pentetretotide scintigraphy had an even higher diagnostic yield than CE, but without differentiation between intestinal and mesenteric localization. In this study, the absence of findings at CE in patients with abnormalities at nuclear imaging was interpreted to be related to the presence of NET restricted to the mesentery or to a false-negative CE. On the ground of these data, the authors suggested that patients with a metastatic NET and an unknown primary tumor should undergo CE. Conversely, in a small retrospective study of 8 patients^[38], CE detected NETs of the small bowel with high specificity, but slightly lower sensitivity than did CT enteroclysis. It was concluded that CE should not be used as a routine method for diagnosing NET in the small bowel.

As far as small-bowel metastases are concerned, Prakoso and Selby^[35] performed a retrospective analysis of a prospective database identifying 13 patients with

previous or recurrent malignant melanoma referred for CE. The indication for CE were overt GI bleeding in three patients, anemia in six, abnormal imaging in two, abdominal pain in one, and one patient had positive fecal occult blood test. In these patients, CE was able not only to show small-bowel metastases (in 5 patients), but also to provide a different possible explanation of symptoms in three other patients (NSAID-related ulcers, artero-venous malformation or aphthoid lesions). The authors concluded that since the optimal investigation for the detection of small-bowel metastases in patients with melanoma has still to be determined, CE can be considered an ideal method to do so because it appears to be more sensitive than small-bowel follow-through and CT scan.

Flieger *et al.*^[39] explored the potential contribution of CE to the diagnosis and staging of gastrointestinal lymphomas describing capsule endoscopic features of these tumors. They studied with CE a total of 27 consecutive patients with newly diagnosed gastrointestinal lymphoma: 20 patients with histologically confirmed gastric lymphoma and seven patients with intestinal lymphoma. All seven patients with primary intestinal lymphomas were found to have pathological findings at CE (ulcerations, nodules or villous atrophy), while 5 of the 20 patients with gastric lymphoma had pathological findings in the small bowel (including abnormal villi, white nodules or villous atrophy). In this study, the authors found that CE is able to identify pathological intestinal findings in patients with gastrointestinal lymphoma more frequently than previously thought and suggest that knowledge of small-bowel involvement can lead to changes in the therapeutic strategy in individual cases.

Lymphomatous polyposis (LP), first described by Cornes in 1961^[40,41], is a rare condition; however, since the introduction of CE and PPE in clinical practice, a few reports^[42,43] have been published on this topic. LP is defined as polypoid mucosal involvement of long segments of the GI tract by neoplastic lymphoid cells^[40]. For many years LP has been considered the macroscopic appearance of the mantle cell lymphoma, but it has recently been suggested that it can be also the macroscopic manifestation of mucosa-associated lymphoid tissue (MALT) lymphoma and follicular B cell lymphoma^[44]. In patients with LP, CE is a valuable tool because it may recognize the presence of nodules, evaluate the extent of the small-bowel involvement and drive further investigations (i.e. the decision about the PPE approach).

Another peculiar clinical condition is represented by patients with refractory celiac disease. It is known that these patients have an increased risk to develop small-bowel neoplasms, mainly enteropathy associated T-cell lymphoma (EATL). However, in this particular subgroup of patients CE is aimed at identifying not only a malignant neoplasm, but also some other possible complications such as ulcerative jejunitis. To date, two papers have been published on this topic^[45,46] showing that CE is a useful tool in the assessment of complicated

celiac disease, especially in patients with refractory celiac disease type II^[45].

CAPSULE ENDOSCOPY: RISKS AND LIMITATIONS IN PATIENTS WITH SMALL-BOWEL TUMORS

Several papers^[47-49] described risks and limitations related to the use of CE in everyday clinical practice. Some limitations can be present in any procedure performed regardless of the clinical indication (“general limitations”); these limitations are mainly related to the technical characteristics of the device or to the anatomical structure of the small bowel, for example, due to the duration of battery life (about 8 h), the capsule allows an evaluation of the entire small bowel only in 75%-85%^[47,49] of cases. In addition, sometimes the presence of fecal debris, particularly in the distal small bowel, can hamper the accurate visualization of the small-bowel mucosa.

Among general limitations, capsule retention is certainly the more feared one because it can significantly modify the subsequent management of the patient. It is generally recognized that the frequency of capsule retention is mostly dependent on the clinical indication to CE (Table 3), ranging between 0% in healthy subjects to 21% in patients with intestinal obstruction^[50,51]. Patients with small-bowel tumors, which frequently appear as lesions protruding into the small-bowel lumen or as stenoses, in both cases capable of narrowing the lumen of the small bowel, have a high probability to develop capsule retention. However, although capsule retention at the site of the lesion has been described in 10%^[29,34] to 25%^[33] of these patients (Table 2), most authors consider this situation as a minor complication. In fact, although some case reports describing possible acute obstructions due to capsule retained at the site of the tumor^[52,53] exist, none of the 15 patients with capsule retention described in large published series^[29,33,34] developed acute small-bowel obstruction. In these patients the subsequent surgical intervention, allowing capsule retrieval, was planned basically to treat the tumor (or because of symptoms persistence) rather than to retrieve the capsule. We must also keep in mind that surgical intervention aimed to retrieve the capsule can be done in a laparoscopic way^[54] and that PPE can also allow capsule retrieval when surgical intervention is contraindicated or not feasible^[55]. In addition, the recently developed Patency capsule^[56] (given imaging, Yoqneam, Israel) can be used in selected patients as a screening method to prevent capsule retention.

The capsule can also have some problems in sizing lesions because of the shape of its dome, its magnification capability, the lack of air insufflation and of remote orientation. This issue has recently been highlighted in papers addressed to study patients with small-bowel inherited polyposis syndromes^[57,58] in which the authors found that MRI seems to be more accurate and reliable than CE in the estimation of location and

Table 3 Frequency of capsule retention in patients undergoing capsule endoscopy (modified from Pennazio^[50])

Clinical indication	Frequency of capsule retention (%)
Healthy volunteers	0
Obscure GI bleeding	1.5
Suspected Crohn's disease	1.4
Known Crohn's disease	4-13
Small-bowel tumor	10-25
Suspected small-bowel obstruction	21

size of polyps^[58]. The ingestion of “reference granules” of mesalazine 15-20 min before CE has recently been proposed to increase the accuracy of the procedure^[59].

Another general limitation, that can be critical in the field of small-bowel tumors, is the accurate localization of the lesion along the small bowel. To estimate the location of a lesion we can correlate the time when the lesion appears to the small-bowel transit time divided in three equal thirds^[60], or we can refer to the localization system^[61]; both these systems are time-consuming, depend on some reference points established by the reader, are not suitable when the capsule does not reach the ileo-cecal valve during examination time and the localization software is reliable only considering a two dimension plan. Despite all these obvious limitations, in one large study^[33] the capsule was able to correctly estimate the location of the lesion in a surprisingly high percentage of patients (about 85%).

Unfortunately, in the field of small-bowel neoplasms, in addition to these general limitations there are some other related to the intrinsic characteristics of these lesions (“tumor-related limitations”).

Several studies^[30,62-64] reported patients with negative CE in whom further examinations showed small-bowel tumors (false negative capsule endoscopy). Lewis *et al*^[63], analyzing data from an industry-maintained trial database, found that in about 1.5% of patients with small-bowel tumors CE was completely negative. These authors estimated that the miss rate of CE in neoplastic diseases can reach 18.9%. Although this percentage is substantially lower than that reported in the same paper for other diagnostic techniques (63.2%) it remains still alarming, especially if one keeps in mind the clinical relevance of these missing findings. Obviously, there are several reasons contributing to that miss rate, but probably the crucial one is related, in this particular subset of patients, to the fact that sometime it is arduous, on the ground of CE findings, to discriminate masses from bulges. A bulge is defined as a round smooth, large base protrusion in the lumen having an ill defined edge on the surrounding mucosa; it can be a prominent normal fold or the luminal expression of intestinal loop angulation and stiffness, and sometimes it can be virtually indistinguishable from a small submucosal tumor. Some visual clues may help distinguishing masses from bulges (i.e. changes in mucosal characteristics, presence of bridging folds, of transit abnormalities, of repetitive images, and of synchronous lesions), but unfortunately all these are indirect indicators and often

are completely lacking.

Pasha *et al*^[33] described 51 patients with polypoid lesions revealed at CE that were not confirmed at further examinations (false positive capsule endoscopy). This problem, highlighted also in other studies^[30], can significantly influence the subsequent management; in fact a positive CE requires further invasive examinations (PPE or surgical interventions). For this reason, the final interpretation of a finding identified by CE must be done taking into account not only the endoscopic images, but also the patient's clinical history and other diagnostic examinations performed.

CAPSULE ENDOSCOPY IN INHERITED POLYPOSIS SYNDROMES

On the ground of its own technical characteristics (i.e. high-quality endoscopic images of the whole small bowel, no need for radiations) and of the patients' acceptance, CE has also been proposed in patients with inherited polyposis syndromes for both surveillance over time and in case of symptomatic disease.

In Peutz-Jeghers syndrome (PJS) the polyps are chiefly located in the small bowel (Figure 3) and may give rise to complications in the form of intussusception, bleeding and obstruction of the intestine, depending on the number and size of the polyps present, as well as to small-bowel malignancy. Several studies have explored the possible diagnostic role of CE in these patients^[57,58,65,66] showing that this tool seems to be superior to small-bowel follow-through^[57]. Unfortunately, the same studies also underlined that CE (as discussed above) is not reliable for accurate sizing of polyps. At the present time, it is suggested that CE should be performed at diagnosis in all patients with PJS, as the primary surveillance modality every 2-3 years from the age of 10, and as part of the investigation of patients with symptoms^[50]. Additional information to evaluate the size and location of polyps, which is useful for planning the appropriate therapeutic strategy, can be provided by CT/MRI^[57,58]. The coupling of CE with PPE and polypectomy may offer an ideal follow-up and treatment method for these patients, possibly avoiding surgery^[67].

The role of CE is less clear in familial adenomatous polyposis (FAP). CE may miss duodenal/periapillary polyps due to a quick passage of the device in the descending duodenum. In a recently published prospective study, Wong *et al*^[68] compared CE with push enteroscopy and with lower GI endoscopy in 32 patients with FAP. They showed that, in a defined segment of the small bowel, CE diagnosed significantly fewer small-bowel polyps than standard endoscopy, showed only fair agreement with PE in determining polyp counts, and was fairly inaccurate in determining the size of the largest polyp and also in detecting large polyps. For these reasons, CE is not presently recommended when the diagnosis of FAP is well established, but it

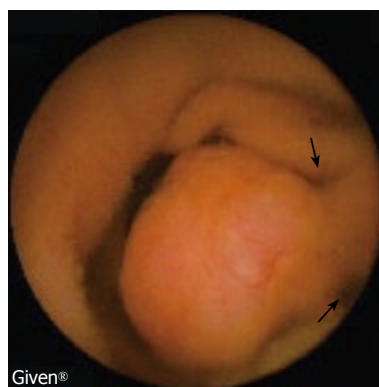


Figure 3 Small sessile, plaque-like, polyp (arrows) in the distal duodenum in a patient with familial adenomatous polyposis.

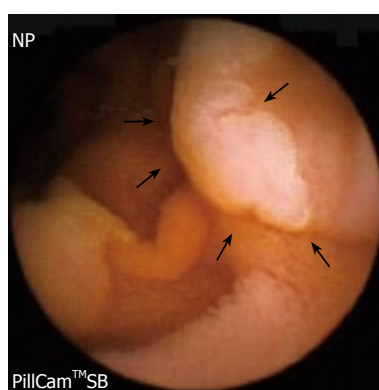


Figure 4 Pedunculated jejunal polyp (arrows denote stalk) in a patient with Peutz-Jeghers syndrome.

may be considered as a part of surveillance for patients with severe duodenal polyposis (Spigelman stage III-IV; Figure 4)^[65,66]. Moreover, in FAP patients with known mesenteric desmoids, caution is recommended before performing CE for the possible risk of capsule retention.

CONCLUSION

Small-bowel tumors are a small, but significant proportion of GI neoplasms. Using new diagnostic modalities, their frequency has been shown to be slightly superior than previously thought. Until recently, diagnosis and management of these tumors were delayed by the difficulty of access to the small bowel and the poor diagnostic capabilities of the available diagnostic techniques. An array of new methods has recently been developed, increasing the possibility of detecting these tumors at an earlier stage. Despite its limitations, CE plays a pivotal role in this setting. Whether the use of CE in combination with other new diagnostic (MRI or multidetector CT enterography) and therapeutic (PPE) techniques will lead to earlier diagnosis and treatment of these neoplasms, ultimately resulting in a survival advantage and in cost savings, remains to be determined through carefully-designed studies.

REFERENCES

- 1 Neugut AI, Jacobson JS, Suh S, Mukherjee R, Arber N. The epidemiology of cancer of the small bowel. *Cancer Epidemiol Biomarkers Prev* 1998; **7**: 243-251
- 2 Rossini FP, Risio M, Pennazio M. Small bowel tumors and polyposis syndromes. *Gastrointest Endosc Clin N Am* 1999; **9**: 93-114
- 3 DiSario JA, Burt RW, Vargas H, McWhorter WP. Small bowel cancer: epidemiological and clinical characteristics from a population-based registry. *Am J Gastroenterol* 1994; **89**: 699-701
- 4 Ross RK, Hartnett NM, Bernstein L, Henderson BE. Epidemiology of adenocarcinomas of the small intestine: is bile a small bowel carcinogen? *Br J Cancer* 1991; **63**: 143-145
- 5 Weiss NS, Yang CP. Incidence of histologic types of cancer of the small intestine. *J Natl Cancer Inst* 1987; **78**: 653-656
- 6 Gabos S, Berkel J, Band P, Robson D, Whittaker H. Small bowel cancer in western Canada. *Int J Epidemiol* 1993; **22**: 198-206
- 7 Chow JS, Chen CC, Ahsan H, Neugut AI. A population-based study of the incidence of malignant small bowel tumours: SEER, 1973-1990. *Int J Epidemiol* 1996; **25**: 722-728
- 8 Sevenson RK, Schenk M, Gurney JG, Weiss LK, Demers RY. Increasing incidence of adenocarcinomas and carcinoid tumors of the small intestine in adults. *Cancer Epidemiol Biomarkers Prev* 1996; **5**: 81-84
- 9 Hatzaras I, Palesty JA, Abir F, Sullivan P, Kozol RA, Dudrick SJ, Longo WE. Small-bowel tumors: epidemiologic and clinical characteristics of 1260 cases from the connecticut tumor registry. *Arch Surg* 2007; **142**: 229-235
- 10 Blanchard DK, Budde JM, Hatch GF 3rd, Wertheimer-Hatch L, Hatch KF, Davis GB, Foster RS Jr, Skandalakis JE. Tumors of the small intestine. *World J Surg* 2000; **24**: 421-429
- 11 Kala Z, Valek V, Kysela P, Svoboda T. A shift in the diagnostics of the small intestine tumors. *Eur J Radiol* 2007; **62**: 160-165
- 12 Ashley SW, Wells SA Jr. Tumors of the small intestine. *Semin Oncol* 1988; **15**: 116-128
- 13 Miettinen M, Lasota J. Gastrointestinal stromal tumors: review on morphology, molecular pathology, prognosis, and differential diagnosis. *Arch Pathol Lab Med* 2006; **130**: 1466-1478
- 14 O'Riordan BG, Vilor M, Herrera L. Small bowel tumors: an overview. *Dig Dis* 1996; **14**: 245-257
- 15 Lowenfels AB. Does bile promote extra-colonic cancer? *Lancet* 1978; **2**: 239-241
- 16 Gill SS, Heuman DM, Mihas AA. Small intestinal neoplasms. *J Clin Gastroenterol* 2001; **33**: 267-282
- 17 Das Gupta TK, Brasfield RD. Metastatic melanoma of the gastrointestinal tract. *Arch Surg* 1964; **88**: 969-973
- 18 Reintgen DS, Thompson W, Garbutt J, Seigler HF. Radiologic, endoscopic, and surgical considerations of melanoma metastatic to the gastrointestinal tract. *Surgery* 1984; **95**: 635-639
- 19 Talamonti MS, Goetz LH, Rao S, Joehl RJ. Primary cancers of the small bowel: analysis of prognostic factors and results of surgical management. *Arch Surg* 2002; **137**: 564-570; discussion 570-571
- 20 Fitzgibbons RJ, Filippi CJ, Quinn TH. Inguinal hernias. In: Brunnicardi FC, Andersen DK, Billiar TR, Dunn DL, Hunter JG, Pollock RE. Schwartz's Principles of Surgery. 8th ed. New York: McGraw-Hill, 2005: 230-246
- 21 Lewis BS, Kornbluth A, Waye JD. Small bowel tumours: yield of enteroscopy. *Gut* 1991; **32**: 763-765
- 22 Semelka RC, John G, Kelekis NL, Burdeny DA, Ascher SM. Small bowel neoplastic disease: demonstration by MRI. *J Magn Reson Imaging* 1996; **6**: 855-860
- 23 Gourtsoyiannis N, Papanikolaou N, Grammatikakis J, Prassopoulos P. MR enteroclysis: technical considerations and clinical applications. *Eur Radiol* 2002; **12**: 2651-2658
- 24 Ramachandran I, Sinha R, Rajesh A, Verma R, Maglinte DD. Multidetector row CT of small bowel tumours. *Clin Radiol* 2007; **62**: 607-614
- 25 Yamamoto H, Kita H, Sunada K, Hayashi Y, Sato H, Yano T, Iwamoto M, Sekine Y, Miyata T, Kuno A, Ajibe H, Ido K, Sugano K. Clinical outcomes of double-balloon endoscopy for the diagnosis and treatment of small-intestinal diseases. *Clin Gastroenterol Hepatol* 2004; **2**: 1010-1016
- 26 May A, Nachbar L, Schneider M, Ell C. Prospective comparison of push enteroscopy and push-and-pull enteroscopy in patients with suspected small-bowel bleeding. *Am J Gastroenterol* 2006; **101**: 2016-2024
- 27 de Franchis R, Rondonotti E, Abbiati C, Beccari G, Signorelli C. Small bowel malignancy. *Gastrointest Endosc Clin N Am* 2004; **14**: 139-148
- 28 Cobrin GM, Pittman RH, Lewis BS. Increased diagnostic yield of small bowel tumors with capsule endoscopy. *Cancer* 2006; **107**: 22-27
- 29 Bailey AA, Debinski HS, Appleyard MN, Remedios ML, Hooper JE, Walsh AJ, Selby WS. Diagnosis and outcome of small bowel tumors found by capsule endoscopy: a three-center Australian experience. *Am J Gastroenterol* 2006; **101**: 2237-2243
- 30 Estevez E, Gonzalez-Conde B, Vazquez-Iglesias JL, Alonso PA, Vazquez-Millan Mde L, Pardeiro R. Incidence of tumoral pathology according to study using capsule endoscopy for patients with obscure gastrointestinal bleeding. *Surg Endosc* 2007; **21**: 1776-1780
- 31 Urbain D, De Looze D, Demedts I, Louis E, Dewit O, Macken E, Van Gossum A. Video capsule endoscopy in small-bowel malignancy: a multicenter Belgian study. *Endoscopy* 2006; **38**: 408-411
- 32 Schwartz GD, Barkin JS. Small-bowel tumors detected by wireless capsule endoscopy. *Dig Dis Sci* 2007; **52**: 1026-1030
- 33 Pasha SF, Sharma VK, Carey EJ, Shiff AD, Heigh RI, Gurudu SR, Erickson PJ, Post JK, Hara AK, Fleischer DE, Leighton JA. Utility of video capsule endoscopy in the detection of small bowel tumors. A single center experience of 1000 consecutive patients. Proceedings of the 6th International Conference on Capsule Endoscopy, 2007 June 8-10; Madrid, Spain, 2007: 45
- 34 Rondonotti E, Pennazio M. Small bowel tumors detected by capsule endoscopy (VCE): preliminary data from the ECEG (European Capsule Endoscopy Group) database. *Gastrointest Endosc* 2007; **65**: AB90-AB371
- 35 Prakoso E, Selby WS. Capsule endoscopy in patients with malignant melanoma. *Am J Gastroenterol* 2007; **102**: 1204-1208
- 36 Pennazio M. Small-intestinal pathology on capsule endoscopy: tumors. *Endoscopy* 2005; **37**: 1008-1017
- 37 van Tuyl SA, van Noorden JT, Timmer R, Stolk MF, Kuipers EJ, Taal BG. Detection of small-bowel neuroendocrine tumors by video capsule endoscopy. *Gastrointest Endosc* 2006; **64**: 66-72
- 38 Johanssen S, Boivin M, Lochs H, Voderholzer W. The yield of wireless capsule endoscopy in the detection of neuroendocrine tumors in comparison with CT enteroclysis. *Gastrointest Endosc* 2006; **63**: 660-665
- 39 Flieger D, Keller R, May A, Ell C, Fischbach W. Capsule endoscopy in gastrointestinal lymphomas. *Endoscopy* 2005; **37**: 1174-1180
- 40 Cornes JS. Multiple lymphomatous polyposis of the gastrointestinal tract. *Cancer* 1961; **14**: 249-257
- 41 Yatabe Y, Nakamura S, Nakamura T, Seto M, Ogura M, Kimura M, Kuhara H, Kobayashi T, Taniwaki M, Morishima Y, Koshikawa T, Suchi T. Multiple polypoid lesions of primary mucosa-associated lymphoid-tissue lymphoma of colon. *Histopathology* 1998; **32**: 116-125
- 42 Higuchi K, Komatsu K, Wakamatsu H, Kawasaki H, Murata M, Miyazaki K, Oikawa K, Ohwada M, Nanjo H, Otaka M, Watanabe S, Komatsu K. Small intestinal follicular lymphoma with multiple tumor formations diagnosed by double-balloon enteroscopy. *Intern Med* 2007; **46**: 705-709

- 43 **Esaki M**, Matsumoto T, Nakamura S, Suekane H, Ohji Y, Yao T, Iida M. Capsule endoscopy findings in intestinal follicular lymphoma. *Endoscopy* 2007; **39** Suppl 1: E86-E87
- 44 **Kodama T**, Ohshima K, Nomura K, Taniwaki M, Nakamura N, Nakamura S, Kohno S, Yamamoto J, Karube K, Yamasita Y, Shirakusa T, Kikuchi M. Lymphomatous polyposis of the gastrointestinal tract, including mantle cell lymphoma, follicular lymphoma and mucosa-associated lymphoid tissue lymphoma. *Histopathology* 2005; **47**: 467-478
- 45 **Daum S**, Wahnschaffe U, Glasenapp R, Borchert M, Ullrich R, Zeitz M, Faiss S. Capsule endoscopy in refractory celiac disease. *Endoscopy* 2007; **39**: 455-458
- 46 **Culliford A**, Daly J, Diamond B, Rubin M, Green PH. The value of wireless capsule endoscopy in patients with complicated celiac disease. *Gastrointest Endosc* 2005; **62**: 55-61
- 47 **Ho KK**, Joyce AM. Complications of capsule endoscopy. *Gastrointest Endosc Clin N Am* 2007; **17**: 169-178, viii-ix
- 48 **Cheifetz AS**, Lewis BS. Capsule endoscopy retention: is it a complication? *J Clin Gastroenterol* 2006; **40**: 688-691
- 49 **Rondonotti E**, Herreras JM, Pennazio M, Caunedo A, Mascarenhas-Saraiva M, de Franchis R. Complications, limitations, and failures of capsule endoscopy: a review of 733 cases. *Gastrointest Endosc* 2005; **62**: 712-716; quiz 752, 754
- 50 **Pennazio M**. Capsule endoscopy: where are we after 6 years of clinical use? *Dig Liver Dis* 2006; **38**: 867-878
- 51 **Cave D**, Legnani P, de Franchis R, Lewis BS. ICCE consensus for capsule retention. *Endoscopy* 2005; **37**: 1065-1067
- 52 **Strosberg JR**, Shibata D, Kvols LK. Intermittent bowel obstruction due to a retained wireless capsule endoscope in a patient with a small bowel carcinoid tumour. *Can J Gastroenterol* 2007; **21**: 113-115
- 53 **Lin OS**, Brandabur JJ, Schembre DB, Soon MS, Kozarek RA. Acute symptomatic small bowel obstruction due to capsule impaction. *Gastrointest Endosc* 2007; **65**: 725-728
- 54 **Dominguez EP**, Choi Y, Raijman IL, Sweeney JF. Laparoscopic approach for the retrieval of retained video capsule endoscopy. *JLS* 2006; **10**: 496-498
- 55 **Tanaka S**, Mitsui K, Shirakawa K, Tatsuguchi A, Nakamura T, Hayashi Y, Sakamoto C, Terano A. Successful retrieval of video capsule endoscopy retained at ileal stenosis of Crohn's disease using double-balloon endoscopy. *J Gastroenterol Hepatol* 2006; **21**: 922-923
- 56 **Spada C**, Shah SK, Riccioni ME, Spera G, Marchese M, Iacopini F, Familiari P, Costamagna G. Video capsule endoscopy in patients with known or suspected small bowel stricture previously tested with the dissolving patency capsule. *J Clin Gastroenterol* 2007; **41**: 576-582
- 57 **Brown G**, Fraser C, Schofield G, Taylor S, Bartram C, Phillips R, Saunders B. Video capsule endoscopy in peutz-jeghers syndrome: a blinded comparison with barium follow-through for detection of small-bowel polyps. *Endoscopy* 2006; **38**: 385-390
- 58 **Caspari R**, von Falkenhausen M, Krautmacher C, Schild H, Heller J, Sauerbruch T. Comparison of capsule endoscopy and magnetic resonance imaging for the detection of polyps of the small intestine in patients with familial adenomatous polyposis or with Peutz-Jeghers' syndrome. *Endoscopy* 2004; **36**: 1054-1059
- 59 **Racz I**, Janoki M, Kovacs V. Measurement of small-bowel polyp size in patients with Peutz-Jeghers syndrome by using reference granules during video capsule endoscopy. *Endoscopy* 2007; **39** Suppl 1: E41
- 60 **Biagi F**, Rondonotti E, Campanella J, Villa F, Bianchi PI, Klersy C, De Franchis R, Corazza GR. Video capsule endoscopy and histology for small-bowel mucosa evaluation: a comparison performed by blinded observers. *Clin Gastroenterol Hepatol* 2006; **4**: 998-1003
- 61 **Fischer D**, Schreiber R, Levi D, Eliakim R. Capsule endoscopy: the localization system. *Gastrointest Endosc Clin N Am* 2004; **14**: 25-31
- 62 **Mehdizadeh S**, Ross A, Gerson L, Leighton J, Chen A, Schembre D, Chen G, Semrad C, Kamal A, Harrison EM, Binmoeller K, Waxman I, Kozarek R, Lo SK. What is the learning curve associated with double-balloon enteroscopy? Technical details and early experience in 6 U.S. tertiary care centers. *Gastrointest Endosc* 2006; **64**: 740-750
- 63 **Lewis BS**, Eisen GM, Friedman S. A pooled analysis to evaluate results of capsule endoscopy trials. *Endoscopy* 2005; **37**: 960-965
- 64 **Chong AK**, Chin BW, Meredith CG. Clinically significant small-bowel pathology identified by double-balloon enteroscopy but missed by capsule endoscopy. *Gastrointest Endosc* 2006; **64**: 445-449
- 65 **Schulmann K**, Hollerbach S, Kraus K, Willert J, Vogel T, Moslein G, Pox C, Reiser M, Reinacher-Schick A, Schmiegel W. Feasibility and diagnostic utility of video capsule endoscopy for the detection of small bowel polyps in patients with hereditary polyposis syndromes. *Am J Gastroenterol* 2005; **100**: 27-37
- 66 **Burke CA**, Santisi J, Church J, Levinthal G. The utility of capsule endoscopy small bowel surveillance in patients with polyposis. *Am J Gastroenterol* 2005; **100**: 1498-1502
- 67 **Ohmiya N**, Taguchi A, Shirai K, Mabuchi N, Arakawa D, Kanazawa H, Ozeki M, Yamada M, Nakamura M, Itoh A, Hirooka Y, Niwa Y, Nagasaka T, Ito M, Ohashi S, Okamura S, Goto H. Endoscopic resection of Peutz-Jeghers polyps throughout the small intestine at double-balloon enteroscopy without laparotomy. *Gastrointest Endosc* 2005; **61**: 140-147
- 68 **Wong RF**, Tuteja AK, Haslem DS, Pappas L, Szabo A, Ogara MM, DiSario JA. Video capsule endoscopy compared with standard endoscopy for the evaluation of small-bowel polyps in persons with familial adenomatous polyposis (with video). *Gastrointest Endosc* 2006; **64**: 530-537

S- Editor Zhong XY L- Editor Rippe RA E- Editor Lin YP



TOPIC HIGHLIGHT

Miguel Angel Muñoz-Navas, Profesor, Series Editors

Esophageal capsule endoscopy

Ignacio Fernandez-Urien, Cristina Carretero, Raul Armendariz, Miguel Muñoz-Navas

Ignacio Fernandez-Urien, Cristina Carretero, Raul Armendariz, Miguel Muñoz-Navas, Department of Gastroenterology, University of Navarra, Pamplona 31080, Spain

Author contributions: Fernandez-Urien I and Carretero C wrote the paper; Armendariz R reviewed bibliography and selected pictures; Muñoz-Navas M reviewed the paper.

Correspondence to: Ignacio Fernandez-Urien, MD, Department of Gastroenterology, University of Navarra, Av. Pio XII, 36, Pamplona 31080, Spain. ifurien@unav.es

Telephone: +34-948-255400 **Fax:** +34-948-296500

Received: February 26, 2008 **Revised:** August 23, 2008

Accepted: August 30, 2008

Published online: September 14, 2008

Abstract

Capsule endoscopy is now considered as the first imaging tool for small bowel examination. Recently, new capsule endoscopy applications have been developed, such as esophageal capsule endoscopy and colon capsule endoscopy. Esophageal capsule endoscopy in patients with suspected esophageal disorders is feasible and safe, and could be also an alternative procedure in those patients refusing upper endoscopy. Although large-scale studies are needed to confirm its utility in GERD and cirrhotic patients, current results are encouraging and open a new era in esophageal examination.

© 2008 The WJG Press. All rights reserved.

Key words: Capsule endoscopy; Esophagus; Gastroesophageal reflux disease; Varices; Esophagoscopy; PillCam ESO

Peer reviewer: Giovanni D De Palma, Professor, Department of Surgery and Advanced Technologies, University of Naples Federico II, School of Medicine, Naples 80131, Italy

Fernandez-Urien I, Carretero C, Armendariz R, Muñoz-Navas M. Esophageal capsule endoscopy. *World J Gastroenterol* 2008; 14(34): 5254-5260 Available from: URL: <http://www.wjgnet.com/1007-9327/14/5254.asp> DOI: <http://dx.doi.org/10.3748/wjg.14.5254>

INTRODUCTION

Since its introduction by Iddan *et al*^[1], capsule endoscopy

(CE) has acquired a well-established role in the investigation of suspected small bowel (SB) diseases. In fact, over 500 000 CE-procedures have been performed worldwide. Demands for CE are hoped to increase because of its proven superiority to conventional techniques for small bowel examination^[2-11] and emerging indications, such as esophageal capsule endoscopy (ECE) and colon capsule endoscopy. Currently, upper endoscopy (EGD) is considered by most authors as the best method to explore the esophagus. However, because of the discomfort of intubation, conscious sedation is usually required resulting in increased costs, risks and patients acceptability^[12-14]. In fact, because of these concerns, some patients are reluctant to undergo EGD even when it is indicated. So, it seems that there is a need for an alternative, simple and less invasive diagnostic tool for the evaluation of patients with known or suspected esophageal disorders. The esophageal capsule (PillCam™ ESO), which was approved by the FDA in November 2004, allows direct visualization of the esophagus without the need of sedation. Advantages include also its invasiveness and painless nature, the ability to pursue normal daily activities after the procedure and patients acceptability. Clinical data on its use and current indications, although quite limited, have opened a promising era for esophageal endoscopic examination.

PILLCAM™ ESO

The PillCam™ ESO (Given Imaging Ltd. Yoqneam, Israel) is an ingestible and disposable capsule measuring 11 mm × 26 mm (similar to PillCam™ SB) that acquires video images from both ends at a combined rate of 14 frames per second (7 from each side of the capsule) during its natural passage through the esophagus (Figure 1). The battery expires in 20-30 min approximately, resulting in more than 15 000 images captured per procedure, which are usually enough to explore the entire esophagus and sometimes, part of the stomach. The images, transmitted via digital radio frequency communication channel to the data recorder unit located outside the body, are captured by an antenna array located on the upper chest and the abdominal wall of the patient. Upon completion of the procedure, the images are transmitted to the Rapid® Workstation for processing and interpretation, which takes only a few minutes. Recently, a new complementary tool has been developed by Given Imaging: the RAPID® Access



Figure 1 PillCam™ ESO.

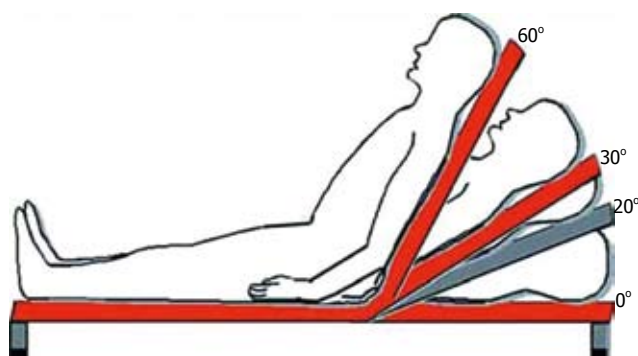


Figure 2 Ingestion protocol.

RT. During the ECE-procedure, the RAPID® Access RT allows real time visualization of capsule images. This is extremely useful in certain circumstances as the physician can intervene to optimize the procedure by changing patient position or administering medications such as laxatives depending on the images obtained in real time.

PROCEDURE

ECE is a quite simplex procedure, which can take only 4-5 min to physicians. This procedure requires implementation of a specific ingestion procedure to assure effective coverage of the esophagus (Figure 2). After a 6 h fast and before capsule ingestion, the patient is asked to drink swiftly a small amount of water (100 mL) in a standing position to clear saliva from the esophagus. Then, the capsule is swallowed in the supine position helped by a small sip of water (10 mL) if required. The patient must remain in this position for 2 min and then must be rose to an inclination of 30 degrees. The patient must remain in this position for 2 min and then the inclination must be increased to 60 degrees. One minute later, the patient is asked to drink a small sip of water (10 mL) and then, allowed to sit upright and to drink again 10 mL of water. In this moment, the patient is allowed to get up and walk in the waiting room for 15-20 min. During procedure in bed, the patient is instructed not to talk. Once the batteries have expired, the procedure is over and downloading

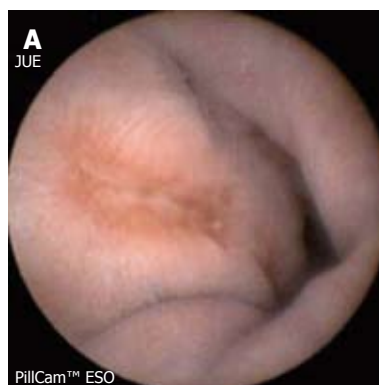


Figure 3 A: PillCam™ ESO image of erosive esophagitis; B: Upper endoscopy image of distal esophagus in the same patient.

process begins (4-5 min). Recently, an article published by Gralnek *et al.*^[15] has evaluated a new simplified ingestion procedure (SIP) in healthy volunteers who swallowed the capsule in the right supine position. Although esophageal transit time was shorter in comparison with the original ingestion procedure (mean: 38 s *vs* 225 s, respectively; $P < 0.001$), results showed that the SIP provides significantly improved visualization of the Z-line (visualization of ≥ 2 quadrants of the Z-line in 100% *vs* 75% of the cases; $P = 0.025$). Therefore, the authors recommend testing the proposed SIP in patients undergoing ECE.

ECE IN GERD PATIENTS (TABLE 1, FIGURES 3 AND 4)

Persistent heartburn is one of the most frequent gastrointestinal symptoms in western countries. Symptomatic GERD affects at least 5%-7% of the global population and in western countries, up to 30% of the population is affected by this disorder^[16-18]. Complications of GERD include erosive esophagitis, ulcers, strictures or Barrett's (BE) esophagus. Up to 30% of subjects with GERD are found to have esophagitis while ulcers or strictures occur in 5% of patients^[19]. Barrett's esophagus, which carries a risk of 0.5% per patient-years of esophageal adenocarcinoma, may occur in up to 10% of patients with chronic GERD^[20]. Therefore, international guidelines recommend screening EGD in all GERD patients. However, as demonstrated, its cost and invasiveness limits its utilization in many patients^[21].

Table 1 ECE in GERD patients: results in published studies

Author	Yr	n	Indication	Capsule	Ingestion	S (%)	E (%)	PPV (%)	NPV (%)
Neu ^[22]	2003	8	Esophagitis	SB	Supine	37.5	(-)	(-)	(-)
Ramirez ^[23]	2005	50	BE	String SB	Standing	100	100	100	100
Eliakim ^[24]	2004	17	Esophagitis BE	ESO 4-fps	Supine	100	80	92	100
Eliakim ^[25]	2005	93	Esophagitis	ESO 4-fps	Supine	89	99	97	94
Eliakim ^[25]	2005	13	BE	ESO 4-fps	Supine	97	99	97	99
Koslowsky ^[26]	2006	25	Esophagitis BE	ESO 4-fps	Supine	81	61	74	79
Koslowsky ^[26]	2006	25	Esophagitis BE	ESO 14-fps	Supine	100	74	100	77
Sharma ^[27]	2007	53	BE (suspected-known)	ESO 14-fps	Supine	67-79	87-78	60-94	90-44
Sharma ^[27]	2007	41	Esophagitis	ESO 14-fps	Supine	50	90	56	88
Lin ^[28]	2007	90	BE	ESO 14-fps	Supine	67	84	22	98

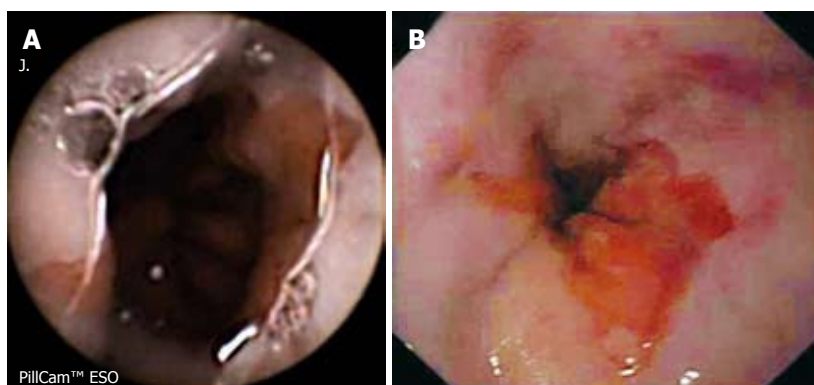


Figure 4 A: PillCam™ ESO image of short segment Barrett's esophagus; B: Subsequent upper endoscopy image which confirms capsule findings.

In 2003, Neu *et al*^[22] published the first article regarding ECE. Using SB capsules (single camera at a frame rate of 2/s), they evaluated the accuracy of capsule endoscopy in 8 patients with known esophagitis. All patients swallowed the capsule in the supine position. The capsule detected only 3 of 8 (37.5%) patients with esophagitis and an adequate visualization of 50% and 100% of the Z-line circumference was achieved in 12.5% and 37.5% of the patients, respectively. They also evaluated the quality of images obtained by the capsule in 58 patients examined for suspected small bowel pathology with poor results (0% of 100% of the Z-line circumference visualization), due to the short esophageal transit time (these patients swallowed the capsule in standing position). They concluded that distal esophageal assessment by SB capsules was not feasible.

Few months later, Ramirez *et al*^[23] used SB capsules attached to strings allowing capsule control up and down the esophagus. Fifty patients with Barrett's esophagus were enrolled in this study. The mean recording time in this study was much longer than in the previous study by Neu *et al* (7.9 min *vs* 3 s). All 50 patients with BE were detected by the capsule. The majority of patients (92%) preferred string-capsule endoscopy to EGD because usually, none or minimal discomfort was associated with capsule ingestion. They concluded that string esophageal capsule endoscopy is feasible, safe and highly acceptable by patients with esophageal disorders.

In 2004, Eliakim *et al*^[24] published a pilot study of ECE using specifically-designed capsules for esophageal examination (double camera at a frame rate of 4 per second). They compared the diagnostic yield of ECE to EGD (used as gold standard) in 17 patients with

suspected esophageal disorders. All patients swallowed the capsule in supine position to avoid rapid esophageal transit of the capsule. All patients with positive findings at EGD (12/17) were detected by the capsule. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 100%, 80%, 92% and 100%, respectively. Of 15 patients asked, 12 (80%) preferred the capsule experience to EGD which was performed under sedation with Midazolam 2.5-5 mg. They concluded that ECE with the new device proposed is an accurate, convenient, safe and well-tolerated method for patients with esophageal disorders. These results were encouraging but the small sample size of this pilot study was an obstacle to elaborate solid conclusions. Then, largescale studies seemed to be necessary.

A similar study conducted at 7 sites in 2005^[25] evaluated the new developed PillCam™ ESO (double camera at a frame rate of 4 per second) compared to EGD in 106 patients (93 with GERD and 13 with BE). All patients swallowed the capsule in the supine position. Sensitivity, specificity, PPV and NPV for esophagitis were 89%, 99%, 97% and 94%, respectively, and 97%, 99%, 97% and 99%, respectively, for BE. ECE was preferred over EGD by all patients. These results were consistent with those obtained by Eliakim *et al*^[24] in 2004. They concluded that ECE was a convenient and sensitive method for visualization of esophageal mucosal pathology and may provide an effective method to evaluate patients for esophageal disease.

However, some experiences have shown that the speed of the capsule in the proximal esophagus can reach up to 20 cm per second. It means that using the

Table 2 ECE in portal hypertension: Results in published studies

Author	Yr	n	Indication	Capsule	Ingestion	S	E	PPV	NPV
Ramirez ^[40]	2005	30	Varices	String SB	Standing	96%	100%	100%	83.3%
Eisen ^[41]	2006	32	Varices	ESO 14-fps	Supine	100%	89%	(-)	(-)
Eisen ^[41]	2006	32	PHG	ESO 14-fps	Supine	100%	77%	(-)	(-)
Lapalus ^[42]	2006	21	Varices	ESO 14-fps	Supine	81.2%	100%	100%	57%

4-frame per second capsule, only one image could be taken per 10 cm length in some instances. Koslowsky *et al*^[26] speculated in 2006 that the diagnostic yield of the ECE might be improved by using a 14-frame per second capsule. Fifty patients (42 suffering from GERD symptoms and 8 with confirmed BE) were included in this study and all of them swallowed the capsule in the supine position: 25 underwent ECE with the 4-frame per second capsule and 25 underwent ECE with the 14-frame per second capsule. Using EGD as gold standard, the 4-frame per second capsule sensitivity, specificity, PPV and NPV were 81%, 61%, 74% and 79%, respectively, and the 14-frame per second capsule sensitivity, specificity, PPV and NPV were 100% ($P < 0.02$), 74%, 100% and 77%, respectively. The upper esophageal sphincter and the entire esophagus were assessed by the 4-fps capsule in 25% and 12% of the cases, respectively, and in 81% ($P < 0.01$) and 76% ($P < 0.01$) of the cases by the 14-fps capsule, respectively. They concluded that ECE using the 14-fps capsule has a greater sensitivity and allows better visualization of the entire esophagus than the 4-fps capsule.

Recently, two prospective, blinded and well-designed studies have compared the diagnostic accuracy of ECE using the 14-fps capsule *vs* EGD in both GERD and BE. Sharma *et al*^[27] included 100 patients with GERD and BE. Ninety-four of these patients swallowed the capsule in the supine position. Results reported showed a higher diagnostic accuracy for BE than for erosive esophagitis. The sensitivity, specificity, PPV and NPV for BE in GERD patients were 67%, 87%, 60% and 90%, respectively, and for known Barrett's esophagus in patients undergoing surveillance were 79%, 78%, 94% and 44%, respectively. The diagnostic accuracy of ECE for long Barrett's segment esophagus (LSBE) was greater than for short Barrett's segments esophagus (SSBE). For erosive esophagitis, the sensitivity was 50%, the specificity 90%, the PPV 56% and the NPV 88%. These results were quite different than those obtained in previous studies. These differences might be attributed in part, to the diagnostic skills of examiners and to the ingestion protocol in the supine position. Anyway, the authors require for the future an improvement in technology and learning curve assessments. The other study, published at the same time by Lin *et al*^[28], included 96 patients with chronic gastroesophageal reflux and BE undergoing surveillance. Again, the selected ingestion protocol was in the supine position. ECE sensitivity, specificity, PPV and NPV for BE were 67%, 84%, 22% and 98%, respectively. There were no differences between SSDE and LSBE detection. These results were

similar to the study by Sharma *et al*^[27] but again, quite different to those showed in previous studies with the 4-fps capsule. The authors attributed these differences to the patient adjudication process in previous studies (unblinded investigators). They conclude that ECE is not, at present, suitable as a primary screening tool for BE but may be used in patients unwilling to undergo EGD. Precisely, one study by Sanchez-Yague *et al*^[29] published in 2006, reviewed 30 cases of ECE in patients refusing conventional endoscopy. They demonstrated that ECE is an adequate alternative diagnostic method for the study of patients with suspected esophageal diseases.

ECE IN PATIENTS WITH PORTAL HYPERTENSION (TABLE 2, FIGURES 5 AND 6)

The presence of esophageal varices is one of the most common complications of portal hypertension in cirrhotic patients. Although they are present in about 50% of the patients when cirrhosis is diagnosed, most of these patients develop varices during their lifetime^[30,31]. Severe upper gastrointestinal bleeding as a complication of portal hypertension occurs in about 30%-40% of cirrhotic patients and in most cases because of the presence of esophageal varices^[32,33]. Despite recent improvements in the diagnosis and treatment of esophagogastric variceal haemorrhage, the mortality rate of first variceal haemorrhage remains high (20%-35%)^[33-36]. The risk of bleeding is related to the hepatic venous pressure, the Child- Pugh class and the endoscopic appearance of the varices^[37]. Therefore, one of the challenges is to identify those cirrhotic patients who have esophageal varices and are also at risk of bleeding. Recently, the Baveno III Consensus Conference on portal hypertension recommended that all cirrhotic patients should be screened by means of upper endoscopy for esophageal varices when liver cirrhosis is diagnosed, at 2-3 years intervals in compensated patients without varices and at 1-2 years intervals in compensated patients with previous small varices^[38]. However, sedation during EGD in cirrhotic patients carries increased risks of cardiopulmonary complications because they are more susceptible to oversedation than those with normal liver function^[12,39].

At the moment, three published studies have evaluated the role of ECE in portal hypertension. The first study was published in 2005 by Ramirez *et al*^[40] who used the string-capsule endoscopy to evaluate portal

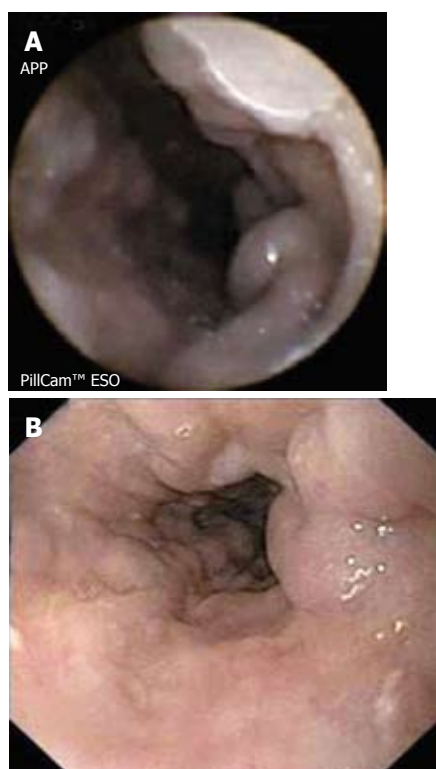


Figure 5 A: PillCam ESO™ image showing esophageal varices; B: Upper endoscopy image of distal esophagus in the same patient.

hypertension in 30 cirrhotic patients. They reported an overall accuracy of 96.7% for varices (sensitivity of 96%, specificity of 100%, PPV of 100% and NPV of 83.3%, respectively) and all patients preferred string-capsule endoscopy to EGD.

On the other hand, two comparative studies have been recently published. Both of them used the new 14-fps capsule, which were swallowed in the supine position, and compared ECE *vs* EGD for portal hypertension in cirrhotic patients. Eisen *et al*^[41] included 32 cirrhotic patients who were undergoing EGD for varices screening or surveillance. They reported a sensitivity of 100%, specificity of 89%, positive likelihood ratio of 9.1 and negative likelihood ratio of 0.0 for esophageal varices detection in comparison with EGD. There was complete agreement in the grading of varices in 65% of the cases and in 95% of the cases within one grade. They also evaluated the accuracy of the capsule to detect portal hypertension gastropathy (PHG). Sensitivity, specificity, positive likelihood ratio and negative likelihood ratio for PHG were 100%, 77%, 4.3 and 0.0, respectively. This pilot study led to a multicenter study with more than 300 patients included which is now finished but not yet published. The other comparative study by Lapalus *et al*^[42], simultaneously published, included 21 cirrhotic patients who were undergoing unsedated EGD for varices screening. Results showed that ECE accurately assessed the presence of esophageal varices in 85% of the cases and correctly indicated a need for primary prophylaxis in 100% of the cases. All patients preferred ECE to unsedated EGD which was performed with a small-diameter upper gastrointestinal endoscope.

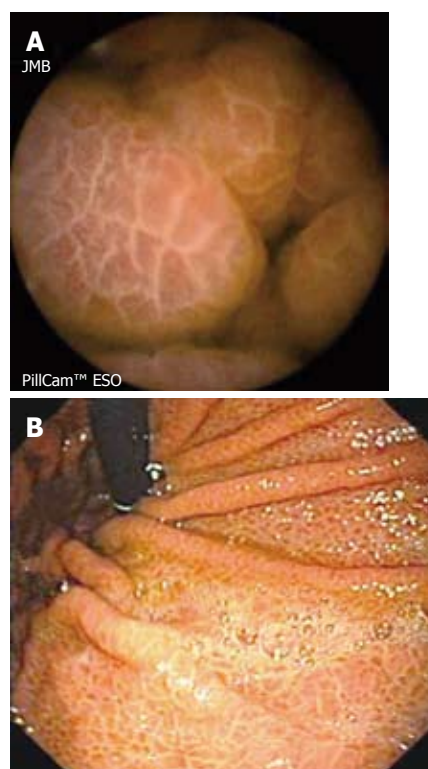


Figure 6 A: PillCam ESO™ image of the gastric wall showing portal hypertension gastropathy; B: Subsequent upper endoscopy image which confirms capsule findings.

CONCLUSION

Capsule endoscopy has opened a new era in small bowel examination. Its indications are now well-defined and currently, wireless capsule endoscopy is considered as the first-line imaging tool for the diagnosis of small bowel diseases. ECE has been shown to be feasible, safe (no ECE-related complications have been reported with the PillCam™ ESO) and a good alternative technique in patients refusing conventional endoscopy. Although results reported in both GERD and cirrhotic patients are encouraging, great differences in terms of accuracy (particularly in GERD patients) have been found in published studies. These differences have been attributed to study designs, the lack of adequate experience and inconvenience of ingestion protocols. In summary, more large-scale studies evaluating the new 14-fps capsule, adequate ECE-experience and new modified ingestion protocols are still needed.

REFERENCES

- 1 Iddan G, Meron G, Glukhovskiy A, Swain P. Wireless capsule endoscopy. *Nature* 2000; **405**: 417
- 2 Eli C, Remke S, May A, Helou L, Henrich R, Mayer G. The first prospective controlled trial comparing wireless capsule endoscopy with push enteroscopy in chronic gastrointestinal bleeding. *Endoscopy* 2002; **34**: 685-689
- 3 Lewis BS, Swain P. Capsule endoscopy in the evaluation of patients with suspected small intestinal bleeding: Results of a pilot study. *Gastrointest Endosc* 2002; **56**: 349-353
- 4 Mylonaki M, Fritscher-Ravens A, Swain P. Wireless capsule endoscopy: a comparison with push enteroscopy in patients with gastroscopy and colonoscopy negative gastrointestinal bleeding. *Gut* 2003; **52**: 1122-1126

- 5 **Saurin JC**, Delvaux M, Gaudin JL, Fassler I, Villarejo J, Vahedi K, Bitoun A, Canard JM, Souquet JC, Ponchon T, Florent C, Gay G. Diagnostic value of endoscopic capsule in patients with obscure digestive bleeding: blinded comparison with video push-enteroscopy. *Endoscopy* 2003; **35**: 576-584
- 6 **Hartmann D**, Schilling D, Bolz G, Hahne M, Jakobs R, Siegel E, Weickert U, Adamek HE, Riemann JF. Capsule endoscopy versus push enteroscopy in patients with occult gastrointestinal bleeding. *Z Gastroenterol* 2003; **41**: 377-382
- 7 **Eliakim R**, Fischer D, Suissa A, Yassin K, Katz D, Guttman N, Migdal M. Wireless capsule video endoscopy is a superior diagnostic tool in comparison to barium follow-through and computerized tomography in patients with suspected Crohn's disease. *Eur J Gastroenterol Hepatol* 2003; **15**: 363-367
- 8 **de Franchis R**, Rondonotti E, Abbiati C, Beccari G, Merighi A, Pinna A, Villa E. Capsule enteroscopy in small bowel transplantation. *Dig Liver Dis* 2003; **35**: 728-731
- 9 **Appleyard M**, Fireman Z, Glukhovsky A, Jacob H, Shreiver R, Kadirkamanathan S, Lavy A, Lewkowicz S, Scapa E, Shofti R, Swain P, Zaretsky A. A randomized trial comparing wireless capsule endoscopy with push enteroscopy for the detection of small-bowel lesions. *Gastroenterology* 2000; **119**: 1431-1438
- 10 **Costamagna G**, Shah SK, Riccioni ME, Foschia F, Mutignani M, Perri V, Vecchioli A, Brizi MG, Picciocchi A, Marano P. A prospective trial comparing small bowel radiographs and video capsule endoscopy for suspected small bowel disease. *Gastroenterology* 2002; **123**: 999-1005
- 11 **Liangpunsakul S**, Chadalawada V, Rex DK, Maglinte D, Lappas J. Wireless capsule endoscopy detects small bowel ulcers in patients with normal results from state of the art enteroclysis. *Am J Gastroenterol* 2003; **98**: 1295-1298
- 12 **Lapalus MG**, Saurin JC. [Complications of gastrointestinal endoscopy: gastroscopy and colonoscopy] *Gastroenterol Clin Biol* 2003; **27**: 909-921
- 13 **Daneshmend TK**, Bell GD, Logan RF. Sedation for upper gastrointestinal endoscopy: results of a nationwide survey. *Gut* 1991; **32**: 12-15
- 14 **Froehlich F**, Gonvers JJ, Fried M. Conscious sedation, clinically relevant complications and monitoring of endoscopy: results of a nationwide survey in Switzerland. *Endoscopy* 1994; **26**: 231-234
- 15 **Gralnek IM**, Rabinovitz R, Afik D, Eliakim R. A simplified ingestion procedure for esophageal capsule endoscopy: initial evaluation in healthy volunteers. *Endoscopy* 2006; **38**: 913-918
- 16 **Johanson JF**. Epidemiology of esophageal and supraesophageal reflux injuries. *Am J Med* 2000; **108** Suppl 4a: 99S-103S
- 17 **Locke GR 3rd**, Talley NJ, Fett SL, Zinsmeister AR, Melton LJ 3rd. Prevalence and clinical spectrum of gastroesophageal reflux: a population-based study in Olmsted County, Minnesota. *Gastroenterology* 1997; **112**: 1448-1456
- 18 **Farup C**, Kleinman L, Sloan S, Ganoczy D, Chee E, Lee C, Revicki D. The impact of nocturnal symptoms associated with gastroesophageal reflux disease on health-related quality of life. *Arch Intern Med* 2001; **161**: 45-52
- 19 **Frazzoni M**, De Micheli E, Savarino V. Different patterns of oesophageal acid exposure distinguish complicated reflux disease from either erosive reflux oesophagitis or non-erosive reflux disease. *Aliment Pharmacol Ther* 2003; **18**: 1091-1098
- 20 **Sharma P**, Falk GW, Weston AP, Reker D, Johnston M, Sampliner RE. Dysplasia and cancer in a large multicenter cohort of patients with Barrett's esophagus. *Clin Gastroenterol Hepatol* 2006; **4**: 566-572
- 21 **Inadomi JM**, Sampliner R, Lagergren J, Lieberman D, Fendrick AM, Vakil N. Screening and surveillance for Barrett esophagus in high-risk groups: a cost-utility analysis. *Ann Intern Med* 2003; **138**: 176-186
- 22 **Neu B**, Wettschureck E, Rosch T. Is esophageal capsule endoscopy feasible? Results of a pilot. *Endoscopy* 2003; **35**: 957-961
- 23 **Ramirez FC**, Shaukat MS, Young MA, Johnson DA, Akins R. Feasibility and safety of string, wireless capsule endoscopy in the diagnosis of Barrett's esophagus. *Gastrointest Endosc* 2005; **61**: 741-746
- 24 **Eliakim R**, Yassin K, Shlomi I, Suissa A, Eisen GM. A novel diagnostic tool for detecting oesophageal pathology: the PillCam oesophageal video capsule. *Aliment Pharmacol Ther* 2004; **20**: 1083-1089
- 25 **Eliakim R**, Sharma VK, Yassin K, Adler SN, Jacob H, Cave DR, Sachdev R, Mitty RD, Hartmann D, Schilling D, Riemann JF, Bar-Meir S, Bardan E, Fennerty B, Eisen G, Faigel D, Lewis BS, Fleischer DE. A prospective study of the diagnostic accuracy of PillCam ESO esophageal capsule endoscopy versus conventional upper endoscopy in patients with chronic gastroesophageal reflux diseases. *J Clin Gastroenterol* 2005; **39**: 572-578
- 26 **Koslowsky B**, Jacob H, Eliakim R, Adler SN. PillCam ESO in esophageal studies: improved diagnostic yield of 14 frames per second (fps) compared with 4 fps. *Endoscopy* 2006; **38**: 27-30
- 27 **Sharma P**, Wani S, Rastogi A, Bansal A, Higbee A, Mathur S, Esquivel R, Camargo L, Sampliner RE. The diagnostic accuracy of esophageal capsule endoscopy in patients with gastroesophageal reflux disease and Barrett's esophagus: a blinded, prospective study. *Am J Gastroenterol* 2008; **103**: 525-532
- 28 **Lin OS**, Schembre DB, Mergener K, Spaulding W, Lomah N, Ayub K, Brandabur JJ, Bredfeldt J, Drennan F, Gluck M, Jiranek GC, McCormick SE, Patterson D, Kozarek RA. Blinded comparison of esophageal capsule endoscopy versus conventional endoscopy for a diagnosis of Barrett's esophagus in patients with chronic gastroesophageal reflux. *Gastrointest Endosc* 2007; **65**: 577-583
- 29 **Sanchez-Yague A**, Caunedo-Alvarez A, Garcia-Montes JM, Romero-Vazquez J, Pellicer-Bautista FJ, Herrerias-Gutierrez JM. Esophageal capsule endoscopy in patients refusing conventional endoscopy for the study of suspected esophageal pathology. *Eur J Gastroenterol Hepatol* 2006; **18**: 977-983
- 30 **Lay CS**, Tsai YT, Teg CY, Shyu WS, Guo WS, Wu KL, Lo KJ. Endoscopic variceal ligation in prophylaxis of first variceal bleeding in cirrhotic patients with high-risk esophageal varices. *Hepatology* 1997; **25**: 1346-1350
- 31 **D'Amico G**, Pagliaro L, Bosch J. Pharmacological treatment of portal hypertension: an evidence-based approach. *Semin Liver Dis* 1999; **19**: 475-505
- 32 **Conn HO**, Lindenmuth WW, May CJ, Ramsby GR. Prophylactic portacaval anastomosis. *Medicine (Baltimore)* 1972; **51**: 27-40
- 33 **Pagliaro L**, D'Amico G, Sorensen TI, Lebrech D, Burroughs AK, Morabito A, Tine F, Politi F, Traina M. Prevention of first bleeding in cirrhosis. A meta-analysis of randomized trials of nonsurgical treatment. *Ann Intern Med* 1992; **117**: 59-70
- 34 **Kovacs TOG**, Jensen DM. Initial management of UGI hemorrhage in patients with portal hypertension. In: Rutherford RB, ed. Vascular surgery. 5th ed. Philadelphia: Saunders, 1999: 1554-1566
- 35 **Sarin SK**, Lamba GS, Kumar M, Misra A, Murthy NS. Comparison of endoscopic ligation and propranolol for the primary prevention of variceal bleeding. *N Engl J Med* 1999; **340**: 988-993
- 36 **D'Amico G**, Pagliaro L, Bosch J. The treatment of portal hypertension: a meta-analytic review. *Hepatology* 1995; **22**: 332-354
- 37 **Jensen DM**. Endoscopic screening for varices in cirrhosis: findings, implications, and outcomes. *Gastroenterology* 2002; **122**: 1620-1630
- 38 **D'Amico G**, Garcia-Tsao G, Cale's P, Escorsell A, Nevens F, Cestari R, Caletti G. Diagnosis of portal hypertension: how and when. In: De Franchis R, ed. Proceedings of the Third

- Baveno International Consensus Workshop on Definitions, Methodology and Therapeutic Strategies. Oxford: Blackwell Science, 2001: 36-63
- 39 **Madhotra R**, Mokhashi M, Willner I, Hawes RH, Reuben A. Prospective evaluation of a 3.1-mm battery-powered esophagoscope in screening for esophageal varices in cirrhotic patients. *Am J Gastroenterol* 2003; **98**: 807-812
- 40 **Ramirez FC**, Hakim S, Tharalson EM, Shaukat MS, Akins R. Feasibility and safety of string wireless capsule endoscopy in the diagnosis of esophageal varices. *Am J Gastroenterol* 2005; **100**: 1065-1071
- 41 **Eisen GM**, Eliakim R, Zaman A, Schwartz J, Faigel D, Rondonotti E, Villa F, Weizman E, Yassin K, deFranchis R. The accuracy of PillCam ESO capsule endoscopy versus conventional upper endoscopy for the diagnosis of esophageal varices: a prospective three-center pilot study. *Endoscopy* 2006; **38**: 31-35
- 42 **Lapalus MG**, Dumortier J, Fumex F, Roman S, Lot M, Prost B, Mion F, Ponchon T. Esophageal capsule endoscopy versus esophagogastroduodenoscopy for evaluating portal hypertension: a prospective comparative study of performance and tolerance. *Endoscopy* 2006; **38**: 36-41

S- Editor Zhong XY E- Editor Lin YP



Miguel Angel Muñoz-Navas, Profesor, Series Editors

Role of videocapsule endoscopy for gastrointestinal bleeding

Cristina Carretero, Ignacio Fernandez-Urien, Maite Betes, Miguel Muñoz-Navas

Cristina Carretero, Ignacio Fernandez-Urien, Maite Betes, Miguel Muñoz-Navas, Department of Gastroenterology, University of Navarra, Pamplona 31008, Spain

Author contributions: Carretero C and Fernandez-Urien I contributed equally to this paper, both performing research and writing the paper; Betes M and Muñoz-Navas M reviewed the paper.

Correspondence to: Cristina Carretero, MD, Department of Gastroenterology, University of Navarra, Av. Pio XII, 36, Pamplona 31008, Spain. ccarretero@unav.es

Telephone: +34-948-255400 **Fax:** +34-948-296500

Received: March 31, 2008 **Revised:** July 26, 2008

Accepted: August 2, 2008

Published online: September 14, 2008

Abstract

Obscure gastrointestinal bleeding (OGIB) is defined as bleeding of an unknown origin that persists or recurs after negative initial upper and lower endoscopies. Several techniques, such as endoscopy, arteriography, scintigraphy and barium radiology are helpful for recognizing the bleeding source; nevertheless, in about 5%-10% of cases the bleeding lesion cannot be determined. The development of videocapsule endoscopy (VCE) has permitted a direct visualization of the small intestine mucosa. We will analyze those techniques in more detail. The diagnostic yield of CE for OGIB varies from 38% to 93%, being in the higher range in those cases with obscure-overt bleeding.

© 2008 The WJG Press. All rights reserved.

Key words: Capsule endoscopy; Bleeding; Small bowel; Obscure; Hemorrhage

Peer reviewer: Ronan A Cahill, Department of General Surgery, Waterford Regional Hospital, Waterford, Cork, Ireland

Carretero C, Fernandez-Urien I, Betes M, Muñoz-Navas M. Role of videocapsule endoscopy for gastrointestinal bleeding. *World J Gastroenterol* 2008; 14(34): 5261-5264 Available from: URL: <http://www.wjgnet.com/1007-9327/14/5261.asp> DOI: <http://dx.doi.org/10.3748/wjg.14.5261>

INTRODUCTION

Obscure gastrointestinal bleeding (OGIB) is defined as

bleeding of an unknown origin that persists or recurs after negative initial upper and lower endoscopies^[1]. It can be subclassified as either obscure-occult, detected only by positive fecal occult blood tests (FOBT) and/or iron deficiency anemia (IDA), or obscure-overt with recurrent or persistent visible episodes of bleeding^[2].

Approximately 5% of all gastrointestinal bleeding^[3] are of obscure origin, and the most frequent causes are found among these: esophagitis, Cameron ulcers, Dieulafoy lesions, angiodysplasias, GAVE, portal hypertensive gastropathy, small bowel neoplasms, hemobilia, Meckel diverticulum, Crohn's disease, medication-induced mucosal lesions and a few others^[2].

Several techniques, such as endoscopy, arteriography, scintigraphy and barium radiology, are helpful for recognizing the bleeding source; nevertheless, in about 5%-10% of cases the bleeding lesion cannot be determined^[4]. Before 2001, the study of OGIB was deficient as the small bowel could not be reviewed reliably in its whole, but the development of videocapsule endoscopy (VCE) has permitted a direct visualization of the small intestine mucosa.

DIAGNOSTIC TECHNIQUES

Capsule endoscopy (CE)

Capsule endoscopy (CE) is a disposable 26 mm × 11 mm plastic capsule (Figure 1) consisting of an optical dome, 4 light-emitting electrodes, a sensor, 2 batteries and a micro transmitter. It acquires and transmits 2 frames per second (until the battery expires after 7 h ± 1 h^[5]) to a sensor array attached to the patient^[2]. Image features include a 140-degree field of view, 1:8 magnification, 1 to 30 mm depth of field and a minimum size for detection of about 0.1 mm^[5].

It is passively propelled by peristalsis and it captures images of the entire length of the small intestine. The main limitations are the lack of air insufflation, the unavailability of rinsing, taking biopsies or/and treating lesions^[2].

The incidence of capsule retention accounts of less than 1%^[2] and is generally related to the presence of endoluminal narrowing. For that reason, and intending to predict the risk of retention, patients with high suspicion of bowel narrowing (history of NSAID's intake, Crohn's Disease, occlusive symptoms or ischemic bowel disease) should undergo capsule endoscopy exploration after performing other techniques such as CT scan, small



Figure 1 Hand holding a small bowel PillCam.

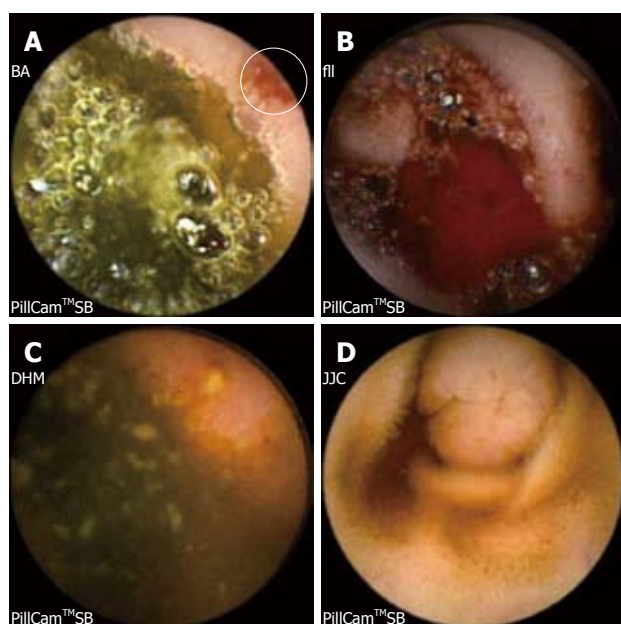


Figure 2 Lesions in the small bowel suspicious of being responsible for bleeding that can be found on CE: Angioectasia (A); Active bleeding (B); Ileal ulceration (C); Polypoid lesion (D).

bowel series or M2A patency capsule.

The diagnostic yield of CE for OGIB varies from 38% to 93%^[5], being in the higher range in those cases with obscure-overt bleeding^[3]. The ability to exclude bleeding lesions is between 82.6% and 100%^[2]; nevertheless, in up to 35% of cases the capsule doesn't reach the cecum, probably due to slow gastric transit^[2]. The most commonly detected lesions in the small bowel suspicious of being responsible for bleeding, that can be found on CE, are: angioectasia (Figure 2A), fresh blood (Figure 2B), ulceration (Figure 2C), polypoid or tumoral lesions (Figure 2D) and varices^[5], and it seems that there is no significant difference in the diagnostic yield of CE in obscure-overt and obscure-occult bleeding^[5,6]. One of the difficulties while reading the CE is to determine what has to be considered a positive finding with clinical significance, and a consensus still has to be reached^[3]. In general, nonspecific mucosal changes (red spots in Figure 3, white spots, *etc*) are not considered positive



Figure 3 Red spot, with no relevant clinical significance.



Figure 4 Ulcerated polyp.

findings; while angioectasias, tumors (Figure 4), masses or mucosal breaks should be included as positive findings.

This wide range of diagnostic yield can be related to several reasons, and one that has been suggested is the performance of CE within 15 days which can improve it (91% *vs* 34%, $P < 0.001$)^[4]. Despite this improvement, some bleedings are still from an unknown origin. It has been suggested that a repeated CE can come across new findings in about 75% of cases, leading to changes in patient management in 62.5%^[7] of cases. One of the main reasons to repeat a CE is the limited visualization that happens in about 44% of cases^[7]. Recurrent bleeding is another reason for repeating CE. It can be helpful if the lesion responsible for bleeding is present intermittently or there was no bleeding source recognized in the first CE^[7].

However, not only is it important to have a high diagnostic yield, but also to know if patient outcomes are improved after performing a CE. Carey *et al* considered measures of patient outcome the number of hospitalizations, units of blood transfused and the number of tests or procedures related to GI bleeding. Considering these measures, patient outcome appeared to improve after CE^[3].

Lai *et al*^[8] reported, in 2006, the results of a long-term follow-up of patients with obscure gastrointestinal bleeding. In 63.3% of cases, CE was able to determine the bleeding source and 32.7% of patients presented re-bleeding within the follow-up (median follow-up period: 19 mo)^[8]. Patients with angiodysplasia were more susceptible to re-bleed (58.3% of cases were due to this condition), followed by patients with active

bleeding during the CE procedure with no identified bleeding source (53.8%)^[8]. They found that the probability of re-bleeding was significantly higher in patients with positive CE than in those with negative CE ($P = 0.003$)^[8].

Repeat upper endoscopy and colonoscopy

Between 35% to 75% of patients can be under-diagnosed at the initial endoscopic study^[2]. Missed lesions occur due to their size, location, presence of clots or the absence of active bleeding while the endoscopy is being performed. Other possible causes are anemia, volume contraction, the effect of sedatives, as they can result in paleness of vascular lesions, and the timing of the endoscopy, as it is more probably to identify the bleeding source if the endoscopy is performed within 48 hours of the acute event^[2].

It has been recommended to repeat upper and lower endoscopy ("second-look endoscopy")^[2], nevertheless, about 5% to 10% of patients will remain undiagnosed.

Push enteroscopy

Enteroscopy consists of a peroral insertion into the jejunum of a long endoscope, using either a pediatric colonoscope or an enteroscope, and with or without the use of an overtube in order to avoid gastric looping^[2]. This technique allows the examination of 15 to 160 cm beyond the ligament of Treitz^[5] and it is generally considered the next diagnostic step after upper and lower negative endoscopic studies.

In 38%-75% of cases, the lesion causing OGIB can be identified with this technique^[2,5]. However, in 28% to 75% of cases, it is reachable with a gastroscope^[5]. Common findings are angiodysplasias (20%-46%), peptic ulcer disease, benign and malignant jejunal tumors, diverticulum, esophagitis and varices^[2]. A meta-analysis has been published showing that the yield of CE for all findings is 63% *vs* 28% for Push Enteroscopy, with an incremental yield of 35%, $P < 0.00001$ ^[9]. Yield of clinically significant findings is 56% for CE *vs* 26% for Push Enteroscopy^[9].

Small bowel series and enteroclysis

Before the development of CE, a radiologic study of the small bowel was mandatory for the study of OGIB. The diagnostic yield is about 6%^[2,5], and the most common missing lesions include angioectasia, ulcers and erosions^[5]. The diagnostic yield can be improved (diagnostic yield of 10%-20%)^[5] by performing enteroclysis. This technique consists in the introduction of a catheter into the small intestine followed by the injection of barium and methylcellulose. The barium coats the intestine and the methylcellulose distends the lumen to give a double contrast exam that allows for fluoroscopic visualization of the entire small bowel. The sensitivity of enteroclysis for small bowel tumors is higher than small bowel series^[5].

A recent meta-analysis has shown that the diagnostic yield for all findings for CE is 67%, compared with an 8% for small bowel series. Diagnostic yield for

significant findings is 42% for CE *vs* 6% for small bowel radiography^[9].

Bleeding scanning with technetium-99-labeled RBC and angiography

This test can be useful if active bleeding is present as it may detect the source of hemorrhage if the bleeding rate ranges from 0.1 to 0.4 mL/min. Angiography can also be performed for diagnosis, as it is able to detect bleeding rates over 0.5 mL/min^[2]. If the bleeding scanning is positive, an angiography should be performed to detect bleeding lesions and treat them if possible^[2]. The diagnostic yield of these techniques varies from 44% to 68%^[2].

Computed tomographic angiography (CTA)

Angiographic images can be obtained, not only by routine angiographic techniques, but also by CT scan. CTA is noninvasive and potentially useful for the diagnosis of GI bleeding, as it avoids the risks of standard angiography^[10]. The CTA is able to identify the bleeding source in 24% of patients^[10]. As has been recently published, CE is able to identify the bleeding source in a higher proportion of patients than CTA (72% *vs* 24%)^[10].

Intraoperative enteroscopy (IE)

IE is the final diagnostic procedure for OGIB. It consists of performing an enteroscopy with the help of the surgeon, who helps by pushing the bowel over the enteroscope as the endoscopist examines it. The insertion can be done transoral or directly through a small enterotomy, but the last is more likely to achieve a complete examination of the small bowel. One of the drawbacks of this technique is that the manipulation of the bowel can create artifacts, which can be considered as bleeding sources although they are really not^[2]. The direct effect of this handicap is that the examination of the mucosa has to be done while the intubation and not while the withdrawal of the endoscope. IE can achieve a diagnostic yield of a 70 to 93 which is comparable to CE^[9].

Double balloon enteroscopy (DBE)

DBE is a novel endoscopic technique that allows for visualization of the entire small bowel, tissue sampling and therapeutic interventions. It consists of an enteroscope with 2 latex balloons, one attached to the endoscope and the other attached to an overtube. The procedure is performed by inflating and deflating the balloons, allowing the deep intubation through the small bowel. The route of insertion (perorally or transanally) selected depends on the patients' symptoms (hematochezia, melena *etc*).

A potential bleeding cause can be found in 75.7% of patients^[11] and approximately a quarter of patients might need both antegrade and retrograde approaches^[11]. DBE can change patient management in about 83.5% of cases, with an average therapeutic endoscopic intervention of 15.7%^[11]. Hadithi *et al*^[12] have published that CE is able to detect the presence of a possible

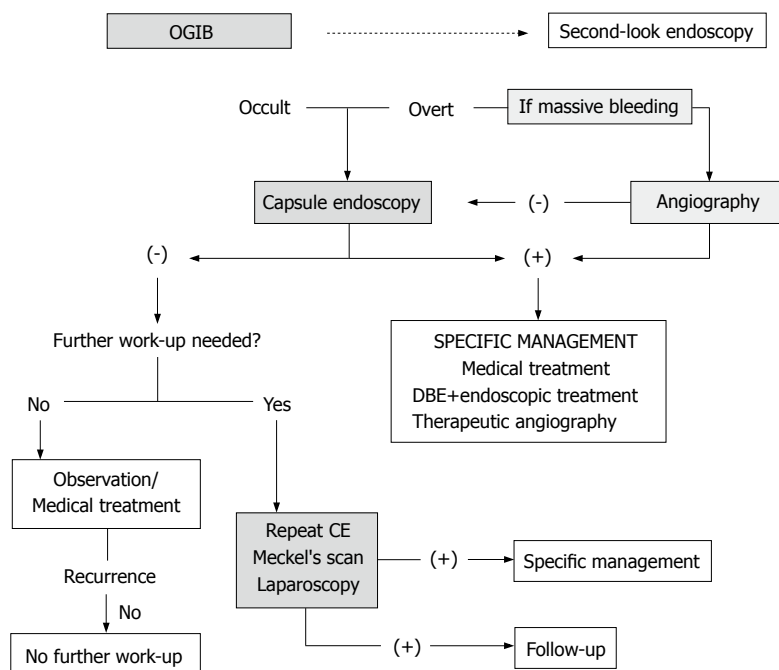


Figure 5 Suggested algorithm for OGIB.

bleeding source in a higher proportion of patients than DBE (80% *vs* 60%).

CONCLUSION

As explained before, there are some diagnostic techniques that can be used for the study of OGIB. With so many different tools, it is important to establish which should be performed first, not only taking into account the diagnostic yield but also the cost effectiveness of each one. Finally the suggested algorithm for OGIB by the International Consensus on Capsule Endoscopy is shown in Figure 5.

REFERENCES

- 1 Zuckerman GR, Prakash C, Askin MP, Lewis BS. AGA technical review on the evaluation and management of occult and obscure gastrointestinal bleeding. *Gastroenterology* 2000; **118**: 201-221
- 2 Concha R, Amaro R, Barkin JS. Obscure gastrointestinal bleeding: diagnostic and therapeutic approach. *J Clin Gastroenterol* 2007; **41**: 242-251
- 3 Carey EJ, Leighton JA, Heigh RI, Shiff AD, Sharma VK, Post JK, Fleischer DE. A single-center experience of 260 consecutive patients undergoing capsule endoscopy for obscure gastrointestinal bleeding. *Am J Gastroenterol* 2007; **102**: 89-95
- 4 Bresci G, Parisi G, Bertoni M, Tumino E, Capria A. The role of video capsule endoscopy for evaluating obscure gastrointestinal bleeding: usefulness of early use. *J Gastroenterol* 2005; **40**: 256-259
- 5 Tang SJ, Haber GB. Capsule endoscopy in obscure gastrointestinal bleeding. *Gastrointest Endosc Clin N Am* 2004; **14**: 87-100
- 6 Ben Soussan E, Antonietti M, Herve S, Savoye G, Ramirez S, Lecleire S, Ducrotte P, Lerebours E. Diagnostic yield and therapeutic implications of capsule endoscopy in obscure gastrointestinal bleeding. *Gastroenterol Clin Biol* 2004; **28**: 1068-1073
- 7 Jones BH, Fleischer DE, Sharma VK, Heigh RI, Shiff AD, Hernandez JL, Leighton JA. Yield of repeat wireless video capsule endoscopy in patients with obscure gastrointestinal bleeding. *Am J Gastroenterol* 2005; **100**: 1058-1064
- 8 Lai LH, Wong GL, Chow DK, Lau JY, Sung JJ, Leung WK. Long-term follow-up of patients with obscure gastrointestinal bleeding after negative capsule endoscopy. *Am J Gastroenterol* 2006; **101**: 1224-1228
- 9 Triester SL, Leighton JA, Leontiadis GI, Fleischer DE, Hara AK, Heigh RI, Shiff AD, Sharma VK. A meta-analysis of the yield of capsule endoscopy compared to other diagnostic modalities in patients with obscure gastrointestinal bleeding. *Am J Gastroenterol* 2005; **100**: 2407-2418
- 10 Saperas E, Dot J, Videla S, Alvarez-Castells A, Perez-Lafuente M, Armengol JR, Malagelada JR. Capsule endoscopy versus computed tomographic or standard angiography for the diagnosis of obscure gastrointestinal bleeding. *Am J Gastroenterol* 2007; **102**: 731-737
- 11 Sun B, Rajan E, Cheng S, Shen R, Zhang C, Zhang S, Wu Y, Zhong J. Diagnostic yield and therapeutic impact of double-balloon enteroscopy in a large cohort of patients with obscure gastrointestinal bleeding. *Am J Gastroenterol* 2006; **101**: 2011-2015
- 12 Hadithi M, Heine GD, Jacobs MA, van Bodegraven AA, Mulder CJ. A prospective study comparing video capsule endoscopy with double-balloon enteroscopy in patients with obscure gastrointestinal bleeding. *Am J Gastroenterol* 2006; **101**: 52-57

S- Editor Zhong XY L- Editor Rippe RA E- Editor Lin YP



Miguel Angel Muñoz-Navas, Profesor, Series Editors

Colon capsule endoscopy

Ignacio Fernandez-Urien, Cristina Carretero, Ana Borda, Miguel Muñoz-Navas

Ignacio Fernandez-Urien, Cristina Carretero, Ana Borda, Miguel Muñoz-Navas, Department of Gastroenterology, University of Navarra, Pamplona 31080, Spain

Author contributions: Fernandez-Urien I and Carretero C wrote the paper; Borda A performed picture selection; Muñoz-Navas M reviewed the paper.

Correspondence to: Ignacio Fernandez-Urien, MD, Department of Gastroenterology, University of Navarra, Av. Pio XII, 36, Pamplona 31080, Spain. ifurien@unav.es

Telephone: +34-948-255400 Fax: +34-948-296500

Received: August 2, 2008 Revised: August 18, 2008

Accepted: August 25, 2008

Published online: September 14, 2008

Abstract

Wireless capsule endoscopy has become the first imaging tool for small bowel examination. Recently, new capsule endoscopy applications have been developed, such as esophageal capsule endoscopy and colon capsule endoscopy. Clinical trials results have shown that colon capsule endoscopy is feasible, accurate and safe in patients suffering from colonic diseases. It could be a good alternative in patients refusing conventional colonoscopy or when it is contraindicated. Upcoming studies are needed to demonstrate its utility for colon cancer screening and other indications such as ulcerative colitis. Comparative studies including both conventional and virtual colonoscopy are also required.

© 2008 The WJG Press. All rights reserved.

Key words: Capsule endoscopy; Colon; Polyp; Colon cancer; Screening; Colonoscopy

Peer reviewers: Burton I Korelitz, MD, Department of Gastroenterology, Lenox Hill Hospital, 100 East 77th Street, 3 Achelis, New York, N.Y 10021, United States; Francesco Costa, Dr, Dipartimento di Medicina Interna-U.O. di Gastroenterologia Università di Pisa-Via Roma, 67-56122-Pisa, Italy

Fernandez-Urien I, Carretero C, Borda A, Muñoz-Navas M. Colon capsule endoscopy. *World J Gastroenterol* 2008; 14(34): 5265-5268 Available from: URL: <http://www.wjgnet.com/1007-9327/14/5265.asp> DOI: <http://dx.doi.org/10.3748/wjg.14.5265>

INTRODUCTION

Colorectal cancer (CRC) is the second most frequent cause of cancer-related death in western countries -skin tumors excluded-, after lung cancer in men and breast cancer in women. One out of three patients suffering from CRC will not survive^[1]. Nevertheless, it can be considered as a preventable and curable condition. Firstly a preventable condition because in most cases, it develops from colonic adenomas. In fact, colonic adenomas are found in 11% to 40% of average risk population^[2-4]. And secondly, a curable condition, because the 5-year survival rate in early stages can reach 90%^[1]. For these reasons, conventional colonoscopy is suggested to be the optimal technique to be used for CRC screening programs in high-risk population, allowing a 90% decrease in CRC incidence^[5]. However, it has to be considered that no more than 25% of compliance has been achieved in screening programs^[5]. This low compliance can be explained by the drawbacks of conventional colonoscopy, such as being painful, patient's embarrassment or the need of sedation. Non-invasive techniques for colonoscopy, such as CT colonography^[6-8] and Colon Capsule Endoscopy^[9-11] are currently being evaluated as alternatives to conventional colonoscopy in order to improve the compliance to screening programs.

PILLCAM™ COLON CAPSULE

A large number of clinical trials have been performed testing different capsule designs in healthy volunteers. Finally, Given Imaging Ltd. has developed the final prototype for colon examination, which is called PillCam™ Colon. The PillCam™ Colon capsule has some differences from those used to study the small bowel and the esophagus. It measures 31 mm in length (4 mm longer than the PillCam™ ESO and SB) and 11 mm in diameter (the same as PillCam™ ESO and SB).

Figure 1 shows some morphologic differences between the three capsules commercially available. The PillCam™ Colon capsule has also some technical improvements, such as being equipped with cameras on both ends taking 4 images per second (2 images per camera). Each camera contains an automatic lighting control and has improved optics, which capture more



Figure 1 PillCam™ SB, ESO and Colon.

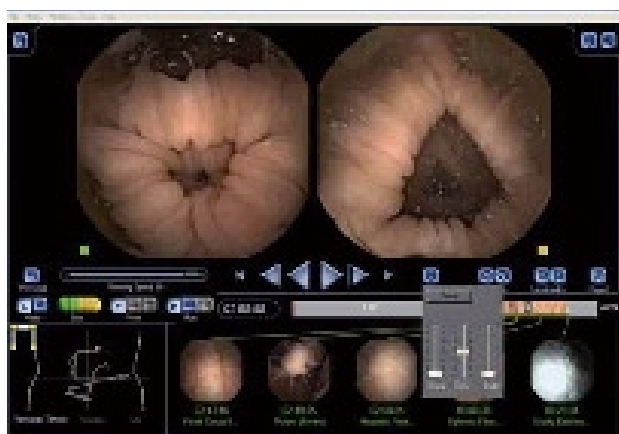


Figure 2 New Software for Colon Capsule reading: RAPID® 5 Scientific Edition.

than twice the coverage area and depth of field of PillCam™ SB resulting in a superior observation field. Other specific features are the presence of a longer battery (lasts 9-10 h on average), which can also “hibernate” minutes to hours after ingestion in order to conserve power before the capsule enters into the colon.

The accessory devices (sensor arrays and Data Recorder) are similar to those ones used by the PillCam™ ESO and SB. The RAPID® software used for images visualization during first clinical trials is a scientific version very similar to RAPID® 4, which includes (I) a larger image display (round-rectangular shape), (II) a complementary capsule localization system and an (III) image enhancement (IE) features (Figure 2). The localization display is similar to the one already in use for the small bowel, but it also includes a schematic diagram of the colon that helps the physician to identify the location of findings, i.e., right, transverse and left colon segments once the main anatomic landmarks (first cecal image, the hepatic flexure, the splenic flexure and the body exit) have been selected. Moreover, this software allows the physician to enhance the appearance of the image by changing their color, brightness and sharpness.

Table 1 Procedure protocols

	Eliakim ^[10]	Schoofs ^[9]	Lewis ^[11]
Day-2	Low fiber diet	(-)	(-)
Day-1	19:00-20:00 PEG 2 L	18:00-21:00 PEG 3 L	18:00-21:00 PEG 3 L
Day 0 ¹	07:00-08:00 PEG 1 L + 08:15 Tegaserod 6 mg + 08:30 Capsule ingestion + 10:30 NaP 30 mL ² + 13:00 Tegaserod 6 mg + 14:00 NaP 15 mL + 16:30 Bisacodyl suppository 10 mg	06:00-07:00 PEG 1 L + 07:45 Motilium 20 mg + 08:00 Capsule ingestion + 10:00 NaP 45 mL ² + 14:00 NaP 30 mL + 16:30 Bisacodyl suppository 10 mg	07:00-08:00 PEG 1 L + 08:15 Tegaserod 6 mg + 08:30 Capsule ingestion + 10:30 NaP 30 mL ² + 13:00 Tegaserod 6 mg + 14:00 NaP 15 mL + 16:30 Bisacodyl suppository 10 mg

¹If the capsule was excreted, the regimen was discontinued; ²Only if the capsule has exit the stomach.

RAPID® Access RT by Given Imaging allows real time visualization of capsule images. This is extremely useful in certain circumstances as the physician can intervene to optimize the procedure by changing patient position or administering medications such as laxatives depending on the images obtained in real time. In the PillCam™ Colon procedure, the importance of the real time viewer is that -as we will see later in more detail- 2 h post PillCam™ Colon Capsule ingestion, the patient has to drink a small amount of Sodium Phosphate. It is well known that Sodium Phosphate can delay gastric emptying time; therefore before giving it to the patient, it is recommended to check if the capsule has left the stomach, which can be easily done with the real time viewer.

PROCEDURE AND CLEANLINESS

The procedure of bowel cleansing until capsule ingestion is similar to that used for traditional colonoscopy. It usually begins one day before capsule ingestion, with the administration of laxatives to the patient. Patients are usually asked to maintain a low fiber diet 2 d before capsule ingestion. After the capsule has been ingested additional laxative and prokinetic agents are provided to the patient in order to (I) maintain the cleanliness of the colon throughout the transit of the capsule and (II) enhance capsule propulsion and excretion within 9-10 h post ingestion. The laxative and prokinetic agents are commercially available, and are provided within their permitted dose. Detailed information of the prep and procedure regimen used in recent trials^[9-11] is shown in Table 1.

First results using the same prep as conventional colonoscopy showed low capsule excretion rates (about

Table 2 Results of PillCam™ Colon trials

	Yr	n	S	E	PPV	NPV
Results for polyps (any size)						
Eliakim ^[9]	2006	91	69%	81%	74%	78%
Schoofs ^[10]	2006	41	76%	64%	83%	54%
Results for significant polyps (> 6 mm or > 3 polyps > 3 mm)						
Eliakim ^[9]	2006	91	63%	94%	67%	91%
Schoofs ^[10]	2006	41	60%	73%	46%	83%
Results for other lesions						
Eliakim ^[9]	2006	91	78%	76%	47%	93%
Schoofs ^[10]	2006	41	76%	63%	82%	52%

S: Sensitivity; E: Specificity; PPV: Positive predictive value; NPV: Negative predictive value.

20%) which meant low rates of complete colonoscopies. Changes in prep regimens were then introduced (see Table 1) and higher excretion rates were reported by Eliakim *et al.*^[9], Schoofs *et al.*^[10] and Lewis *et al.*^[11] (78%, 84% and 90%, respectively). Moreover, the colon cleansing level reported by Eliakim *et al.*^[9] and Schoofs *et al.*^[10] was good to excellent in 84.4% and 88% of the patients, respectively. Recently, an undergoing European multicenter study published in abstract form^[12] has reported a capsule excretion rate of 93% and good to excellent colon cleansing level in 71% of the patients. All these results are consistent with those obtained by conventional colonoscopy. As the goal of colon capsule endoscopy is to improve patient compliance to CRC screening, other simplified ingestion regimens including Moviprep® as the main laxative product or capsule procedures during the night are currently under evaluation.

LESIONS DETECTION

The long term primary objective of the PillCam™ Colon capsule is the average risk population undergoing CRC screening. In order to evaluate the accuracy of the new capsule device, it is being tested in those patients with known or suspected lesions (i.e. polyps or tumors). At the moment, encouraging results has been reported. Two European feasibility studies^[9,10] including a total of 132 patients and one American study^[11] published in abstract form including 25 patients, have recently evaluated the role of the PillCam™ Colon capsule in detecting colonic lesions. In all of these studies, conventional colonoscopy was considered the gold standard and the American^[11] study included also the virtual colonoscopy as an additional comparative procedure. Preliminary results from these studies are resumed in Table 2. The European studies showed a capsule sensitivity (S) for polyps of any size of 69% and 76%, specificity (E) of 81% and 64%, positive predictive value (PPV) of 74% and 83% and negative predictive value (NPV) of 78% and 54%, respectively. Those polyps greater than 6 mm or 3 polyps of 3 mm were considered significant lesions. The accuracy of the colon capsule for significant lesions was very similar as well as for inflammatory lesions (i.e. diverticula, ulcerative colitis, *etc.*). These results are consistent with those obtained in the American study

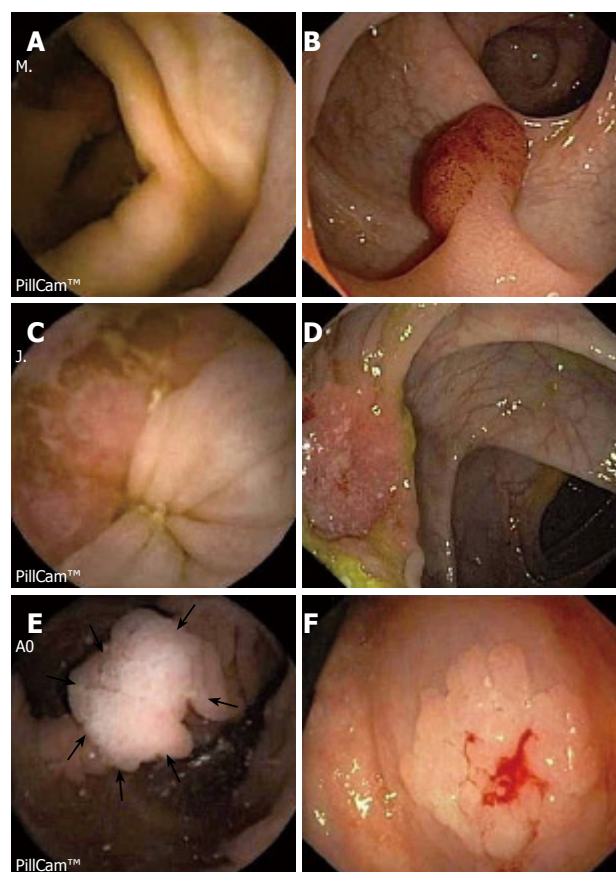


Figure 3 Images captured by the Pillcam™ Colon and conventional colonoscopy. **A** and **B**: Pedunculated polyp in the sigmoid colon; **C** and **D**: Ulcerated tumor in the transverse colon; **E** and **F**: Flat adenoma in the ascending colon.

which also showed that conventional colonoscopy was more accurate than colon capsule endoscopy and virtual colonoscopy (81%, 63% and 54%, respectively). In the European multicenter study^[12], S, E, PPV and NPV for significant lesions were 66%, 82%, 72% and 77%, respectively; S, E, PPV and NPV for polyps > 6 mm were 64%, 84%, 60% and 86%, respectively and S, E, PPV and NPV for polyps > 10 mm were 60%, 98%, 83% and 93%, respectively. These results are very similar to those obtained by previous studies. On the other hand, the Z line is clearly visualized in 60% of cases by the capsule, even if the capsule is ingested in the standing position^[10]. It means that patients undergoing CRC screening by PillCam™ Colon capsule endoscopy could be also screened for Barrett's esophagus. Figure 3 shows some images from PillCam™ Colon capsule endoscopy.

SAFETY

The capsule colonoscopy seems to be a safe procedure. Capsule or laxatives-related complications during procedures has nor been reported by first feasibility studies^[9-11]. On the other hand, 2 of 126 patients (1.6%) were unable to swallow the capsule in the study by Eliakim *et al.*^[9]. However, in these patients, the capsule can be easily introduced into the stomach or duodenum by means of the capsule deliver system (US Endoscopy).

COST-EFFECTIVENESS

As demonstrated by several studies, patients' compliance for CRC screening is still much lower than for other common neoplastic diseases such as breast and prostate cancer. Therefore, alternative procedures such as colon capsule endoscopy or CT colonography, which may increase patients' compliance, are welcome. In fact, colon capsule endoscopy is an attractive non-invasive method for CRC screening, especially for those patients who are non-compliant to current screening procedures. Whether colon capsule endoscopy will be cost-effective has not been widely evaluated. However, a recent paper by Hassan *et al*^[13] based on a mathematical Markov model concludes that colon capsule endoscopy may be cost-effective compared with colonoscopy if a 30% patients' compliance increase is achieved. Moreover, as polyp detection by capsule endoscopy is expected to be more accurate in the future, it may be cost-effective even if compliance rates achieved remains lower than 30%.

CONCLUSION

Based on current available studies, PillCam™ Colon capsule colonoscopy is a feasible, effective and safe procedure that allows the visualization of the entire colon in most of the cases. It may be complementary to conventional colonoscopy and could be an appropriate exam for those patients who have received incomplete colonoscopy, contraindicated or are unwilling to undergo conventional colonoscopy. Further studies are needed to confirm these results and the possibilities of this new modality for endoscopic examination of the colon and for CRC screening. As colon capsule endoscopy has still some limitations (cannot insufflate air, clean or take biopsies), future capsule prototypes seem to be necessary. Moreover, it is anticipated that future procedures with modified regimens that may be performed at home, possibly over the weekend, can offer a unique method and further enhance patient compliance.

REFERENCES

- Ibanez MB, Ribon CC, de la Torre FT, Munoz-Navas M. Evidencia científica en cribado del cáncer colorrectal: manual de actuación. Madrid: International Marketing & Communication, 2006
- Betes M, Munoz-Navas MA, Duque JM, Angos R, Macias E, Subtil JC, Herraiz M, De La Riva S, Delgado-Rodriguez M, Martinez-Gonzalez MA. Use of colonoscopy as a primary screening test for colorectal cancer in average risk people. *Am J Gastroenterol* 2003; **98**: 2648-2654
- Betes Ibanez M, Munoz-Navas MA, Duque JM, Angos R, Macias E, Subtil JC, Herraiz M, de la Riva S, Delgado-Rodriguez M, Martinez-Gonzalez MA. Diagnostic value of distal colonic polyps for prediction of advanced proximal neoplasia in an average-risk population undergoing screening colonoscopy. *Gastrointest Endosc* 2004; **59**: 634-641
- Levine JS, Ahnen DJ. Clinical practice. Adenomatous polyps of the colon. *N Engl J Med* 2006; **355**: 2551-2557
- Winawer SJ, Zauber AG, Ho MN, O'Brien MJ, Gottlieb LS, Sternberg SS, Waye JD, Schapiro M, Bond JH, Panish JF. Prevention of colorectal cancer by colonoscopic polypectomy. The National Polyp Study Workgroup. *N Engl J Med* 1993; **329**: 1977-1981
- Silva AC, Wellnitz CV, Hara AK. Three-dimensional virtual dissection at CT colonography: unraveling the colon to search for lesions. *Radiographics* 2006; **26**: 1669-1686
- Johnson KT, Carston MJ, Wentz RJ, Manduca A, Anderson SM, Johnson CD. Development of a cathartic-free colorectal cancer screening test using virtual colonoscopy: a feasibility study. *AJR Am J Roentgenol* 2007; **188**: W29-W36
- Chaoui AS, Barish MA. Virtual colonoscopy: a new tool for colorectal cancer screening. *Curr Opin Gastroenterol* 2001; **17**: 78-85
- Eliakim R, Fireman Z, Gralnek IM, Yassin K, Waterman M, Kopelman Y, Lachter J, Koslowsky B, Adler SN. Evaluation of the PillCam Colon capsule in the detection of colonic pathology: results of the first multicenter, prospective, comparative study. *Endoscopy* 2006; **38**: 963-970
- Schoofs N, Deviere J, Van Gossum A. PillCam colon capsule endoscopy compared with colonoscopy for colorectal tumor diagnosis: a prospective pilot study. *Endoscopy* 2006; **38**: 971-977
- Lewis B, Rex D, Lieberman D. Capsule Colonoscopy: An Interim Report of a Pilot 3 Arm, Blinded Trial of Capsule Colonoscopy, Virtual Colonoscopy and Colonoscopy. *Am J Gastroenterol* 2006; **101**: S545-S561 (A1470)
- Deviere J, Munoz-Navas M, Fernandez-Urien I, Carretero C, Gay G, Delvaux M, Lapalus MG, Ponchon T, Costamagna G, Riccioni ME, Spada C, Neuhaus H, Philipper M, Frazer DM, Postgate A, Fitzpatrick A, Hagenmuller F, Keuchel M, Schoofs N, Van Gossum AM. PillCam® Colon Capsule Endoscopy Compared to Colonoscopy in Detection of Colon Polyps and Cancers. *Gastroenterology* 2008; **134**: 282 (A38)
- Hassan C, Zullo A, Winn S, Morini S. Cost-effectiveness of capsule endoscopy in screening for colorectal cancer. *Endoscopy* 2008; **40**: 414-421

S- Editor Zhong XY E- Editor Lin YP



Miguel Angel Muñoz-Navas, Profesor, Series Editors

Patency[®] and agile[®] capsules

Ángel Caunedo-Álvarez, Javier Romero-Vazquez, Juan M Herreras-Gutierrez

Ángel Caunedo-Álvarez, Javier Romero-Vazquez, Juan M Herreras-Gutierrez, Gastroenterology Service, "Virgen Macarena" University Hospital, Av Dr. Fedriani, Seville 41071, Spain

Correspondence to: Ángel Caunedo Álvarez, MD, Gastroenterology Service, "Virgen Macarena" University Hospital, Avda. Dr. Fedriani, Seville 41071, Spain. acaunedoa@meditex.es

Telephone: +34-955-008801 Fax: +34-955-008805

Received: February 15, 2008 Revised: July 26, 2008

Accepted: August 2, 2008

Published online: September 14, 2008

Abstract

Small bowel strictures can be missed by current diagnostic methods. The Patency capsule is a new non-endoscopic dissolvable capsule which has as an objective of checking the patency of digestive tract, in a non-invasive manner. The available clinical trials have demonstrated that the Patency[®] capsule is a good tool for assessment of the functional patency of the small bowel, and it allows identification of those patients who can safely undergo a capsule endoscopy, despite clinical and radiographic evidence of small-bowel obstruction. Some cases of intestinal occlusion have been reported with the Patency[®] capsule, four of them needed surgery. So, a new capsule with two timer plugs (Agile[®] capsule) has been recently developed in order to minimize the risk of occlusion. This new device starts its dissolution process earlier (30 h after ingestion) and its two timer plugs have been designed to begin the disintegration even when the device is blocked in a tight stricture.

© 2008 The WJG Press. All rights reserved.

Key words: Capsule endoscopy; Patency capsule; Agile capsule; Small bowel strictures

Peer reviewer: Amado S Peña, Professor, Department of Pathology, Immunogenetics, VU University Medical Centre, De Boelelaan 1117, PO Box 7057, Amsterdam 1007 MB, The Netherlands

Caunedo-Álvarez Á, Romero-Vazquez J, Herreras-Gutierrez JM. Patency[®] and agile[®] capsules. *World J Gastroenterol* 2008; 14(34): 5269-5273 Available from: URL: <http://www.wjgnet.com/1007-9327/14/5269.asp> DOI: <http://dx.doi.org/10.3748/wjg.14.5269>

INTRODUCTION AND DEVICE DESCRIPTION

Since Iddan *et al*^[1] reported the new wireless endoscopy system the capsule endoscopy (CE) has become one of the most significant technical innovations of Gastroenterology in recent years. Regarding the complications of the techniques, some incidental cases of impaction in a Meckel's diverticulum^[2], a Zenker's diverticulum^[3], or in the cricopharyngeal muscle^[4], as well as aspiration of the capsule^[5-7], solved without complications, have been reported. However, the most frequent side effect is undoubtedly the non-natural excretion (NNE) of the capsule due to a stricture or a tumor in the small bowel. The retrospective analysis of some series indicates that the incidence of NNE depends on the indication for the capsule exam: 0% in healthy controls^[8], 1.4% in obscure gastrointestinal bleeding^[9-13], 1.48% in suspected Crohn's disease^[14-17], 5%-13% in known Crohn's disease^[17-19] and 21% in suspected small bowel obstruction^[20].

In the vast majority of cases, capsule retention, regardless of its cause, is asymptomatic, being evidenced by the absence of excretion and confirmed by means of radiology. On the other hand, capsule retention frequently allows for the identification of the bowel pathology which caused the symptoms in the patient and which could not have been diagnosed by means of standard methods, furthermore facilitating the location of the stricture or the tumor for the surgeon after "milking" the capsule through the bowel. For these reasons, many authors have described NNE as a "therapeutic complication" of the CE^[21-23]. However, although this consideration is valid for patients with tumors or those with stricture due to NSAIDs, radiotherapy or previous abdominal surgery, it may not be valid in patients with stenoses secondary to Crohn's disease. Unlike the previously mentioned groups, in patients with Crohn's disease, surgery is not curative and may present more complications, thus in this case, NNE of the capsule can become more an "undesirable circumstance" than a "therapeutic complication". The same can be said of those patients showing high surgical risk or who would not be willing to undergo surgery in the event that NNE occurs.

Current imaging techniques can show long or medium stenoses, with great reduction of the lumen size; however, short stenoses usually cannot be detected by standard methods. This fact explains that in most

Table 1 Summary of the main series on Patency Capsule (%)

Author	Inclusion criteria	n	Diagnosis known or suspected before PillCam	Capsule integrity at egestion	Pts with uneventful PillCam after PC excreted intact
Spada <i>et al</i> ^[24] 2005	Suspected or confirmed small bowel stricture based on radiological exams	34	Crohn's disease: 30/34 (88.23) Adhesional syndrome: 3/34 (8.82) Ischemic enteritis: 1/34 (2.94)	30/34 (88.23)	10/10 (100)
Boivin <i>et al</i> ^[25] 2005	Obstructive small bowel symptoms, and/or radiographic evidence of structuring small bowel disease	22	Crohn's disease: 15/22 (68.18) Adhesional syndrome: 4/22 (18.18) Others: 3/22 (13.64)	16/22 (72.73)	13/13 (100)
Delvaux <i>et al</i> ^[26] 2005	Suspected or confirmed small bowel stricture based on either clinical background or radiological exams	22	Crohn's disease: 12/22 (54.54) NSAIDs stricture: 3/22 (13.64) Tumors: 3/22 (13.64) Others: 4/22 (18.18)	16/22 (72.73)	5/5 (100)
Signorelli <i>et al</i> ^[27] 2006	Risk of capsule retention because of clinical background or radiological exams	32	Crohn's disease: 18/32 (56.25) Intestinal surgery: 7/32 (21.87) Others: 7/32 (21.87)	26/32 (81.25)	25/25 (100)
Caunedo <i>et al</i> ^[28] 2003	Suspected or confirmed small bowel stricture based on radiological exams	29	Crohn's disease: 15/29 (51.72) Adhesional syndrome: 6/29 (20.69) Tumors: 3/29 (10.34) Others: 5/29 (17.24)	13/29 (44.83)	12/12 (100)
Spada <i>et al</i> ^[31] 2007	Radiologic findings suggesting small bowel stricture without clinical evidence of obstruction	27	Crohn's disease: 24/27 (88.89) Adhesional syndrome: 2/27 (7.41) Ischemic enteritis: 1/27 (3.70)	15/27 (55.55)	15/15 (100)
Total		166	Crohn's disease: 114/166 (68.67) Adhesional syndrome: 15/166 (9.04) Tumors: 6/166 (3.61) Intestinal surgery: 7/166 (4.22) Others: 24/166 (14.46)	116/166 (69.88)	80/80 (100)

of the reported cases of non-natural excretion (NNE) of the capsule, the previous performance of the usual radiological studies was not capable of diagnosing the intestinal strictures which the capsule clearly showed^[19,11-13,19-21]. It is, therefore, proven that the lack of findings in radiological techniques does not rule out the existence of a bowel stenosis.

The manufacturing company of the PillCamSB has recently developed a new system (Given® M2A Patency System) which has as an objective of checking the patency of digestive tract, in a non-invasive manner. The Patency Capsule (PC) consists of a small identification tag (RFID), detectable by radiofrequency, which is surrounded by an absorbable material with a small amount of barium, all this covered by an external cover (Figure 1). PC has the same dimensions (11.4 mm × 26.4 mm) and the same shape as the standard capsule. PC is designed to remain intact in the gastrointestinal tract for about 80 h. After this period, if still within the body, it spontaneously disintegrates, except for the identification tag, whose small size (3 mm × 13 mm) allows it to pass through a stenosis of a very reduced lumen size. The persistence of the PC inside the organism can be verified by means of radiology, or with a radiofrequency emitting external detector device locating the identification tag.

CLINICAL TRIALS WITH PATENCY CAPSULE

Clinical experience with PC is still limited. A prospective,

multi-center trial was designed to assess the clinical usefulness and safety of the PC capsule in patients with intestinal strictures suspected from clinical and/or radiological data. The global data of this trial have not been published, but the results of four of these centers have been reported (Table 1)^[24-27]. In the series reported by Spada *et al*^[24], 30 out of 34 (88.2%) patients with known or suspected small bowel stricture retrieved the capsule in the stool. After the excretion, the PC was intact in 20 cases (median transit time 22 h), and disintegrated in 10 patients (median transit time 53 h). Ten patients underwent video capsule endoscopy following the patency capsule examination. In all of these the video capsule passed through the small-bowel stricture without complication. The rate of patients with the PC excreted intact was similar in the series of Boivin *et al*^[25] (16/22, 73%), Delvaux *et al*^[26] (16/22, 73%), Signorelli *et al*^[27] (26/32, 81%). In our center, the percentage of cases with PC excreted intact was significant lower (13/29, 45%), probably because of patient selection^[28]. In all the series, the patency system scanner showed a good agreement (94%-100%) with fluoroscopy findings in identifying the presence of the tag in the body and may be used to detect the presence of the patency capsule without the need for radiology.

All these authors conclude that PC was unable to detect the presence of a small bowel stricture as previously defined by radiological techniques, but it added crucial information on the functional patency of the stenoses, and this information could allow a distinction between rigid fibrotic strictures and flexible ones. Boivin *et al*^[25] found that passage of an intact capsule that is

Table 2 Complications reported in the main series on Patency Capsule (%)

Author	Pts with abdominal pain during procedure	Severity of adverse event (abdominal pain)	Action taken
Spada <i>et al</i> ^[24] 2005	6/34 (17.64)	Mild: 5/34 (14.71) Moderate: 0/34 (0) Severe: 1/34 (2.94)	Nothing: 5/34 (14.71) Medical therapy: 1/34 (2.94) Surgery: 0/34 (0)
Boivin <i>et al</i> ^[25] 2005	6/22 (27.27)	Mild: 1/22 (4.54) Moderate: 1/22 (4.54) Severe: 4/22 (18.18)	Nothing or medical therapy: 5/22 (22.73) Surgery: 1/22 (4.54)
Delvaux <i>et al</i> ^[26] 2005	3/22 (13.64)	Mild: 1/22 (4.54) Moderate: 0/22 (0) Severe: 2/22 (9.09)	Nothing: 1/22 (4.54) Medical therapy: 0/22 (0) Surgery: 2/22 (9.09)
Signorelli <i>et al</i> ^[27] 2006	2/32 (6.25)	Mild: 2/32 (1.44) Moderate: 0/32 (0) Severe: 0/32 (0)	Nothing: 2/32 (1.44) Medical therapy: 0/32 (0) Surgery: 0/32 (0)
Caunedo <i>et al</i> ^[28] 2003	10/29 (34.48)	Mild: 4/29 (13.79) Moderate: 4/29 (13.79) Severe: 2/29 (6.90)	Nothing: 3/29 (10.35) Medical therapy: 7/29 (24.14) Surgery: 0/29 (0)
Spada <i>et al</i> ^[31] 2007	6/27 (22.22)	Mild: 5/27 (18.52) Moderate: 0/27 (0) Severe: 1/27 (3.70)	Nothing or medical therapy: 5/27 (18.52) Surgery: 1/27 (3.70)
Total	33/166 (19.88)	Mild: 18/166 (10.84) Moderate: 5/166 (3.01) Severe: 10/166 (6.02)	Nothing or medical therapy: 29/166 (17.47) Surgery: 4/166 (2.41)

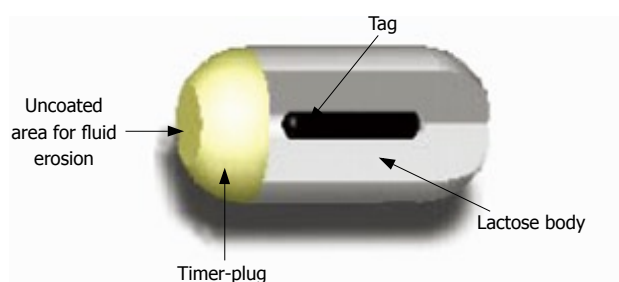


Figure 1 Schematic drawing of M2A® patency Capsule.

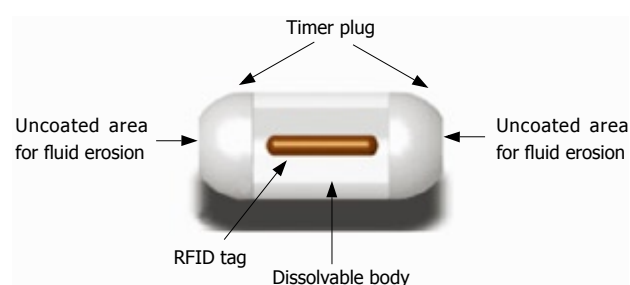


Figure 2 Schematic drawing of AGILE® patency Capsule.

accompanied by severe pain, similar to disintegration of the capsule with or without pain, seems indicative of a clinically relevant small-bowel stricture and is associated with a high probability of surgery. This observation seems to be confirmed by a recent retrospective analysis of 42 patients who underwent PC with known or suspected small bowel stricture^[29]. In this study, the rate of patients who need to be operated in a period of three months was significantly higher in those with a delayed excretion, with the capsule excreted deformed, or with pain during the procedure. Moreover, in patients where painless natural expulsion of the intact PC occurred, CE could be applied without problems despite radiographic evidence of small-bowel strictures. This is an important finding since it might open the path to CE for about 60% of patients where video capsule investigation would otherwise be denied on grounds of history and radiological findings.

Abdominal pain during the procedure seems to be the main complication of PC (Table 2), observed in almost 20% of the cases reported to date. Probably, this pain is secondary to symptomatic intestinal occlusion, and it resolves spontaneously when the disintegration process concludes. However, at least four cases of occlusion did not respond adequately to conservative treatment and

needed surgery. According to Gay *et al*^[30], the problem could be that when the PC entrapped in a very tight stenosis it might not have been in contact with fluids and, therefore, only started to dissolve 48 h later, after moving back to the enlarged intestinal loop where it encountered fluids. Another possible factor is proposed by Gay *et al*^[30]; PC is mainly made of lactose and the presence of lactase, an enzyme produced by intestinal mucosal cells, may be of importance in initiating the dissolution process. As lactase is mainly produced in the jejunum, one may also assume the enzyme is massively destroyed before the intestinal content reaches the ileum and that in the cases of occlusion, the enzyme could not have interacted with the capsule material. These authors^[26,31] conclude that the start of dissolution at 40 h after ingestion is too slow to prevent episodes of intestinal occlusion, and so, it should be used cautiously under clinical surveillance in patients with Crohn's disease.

THE AGILE® PATENCY CAPSULE

In order to reduce the risk of obstruction, a new dissolvable capsule with two timer plugs, one at each end, has been recently developed (Figure 2). With these

two timer plugs, the dissolution process starts earlier (30 h), and the contact with intestinal fluids is ensured even if the device is blocked in a tight stricture^[32]. The new capsule, named Agile[®] Patency Capsule, has been evaluated in a multicenter clinical trial^[33] designed to assess its safety in patients with known strictures and its ability to help physicians identify which patients may safely undergo CE. In this study, the intestinal tract was considered to be sufficiently patent if the capsule was excreted intact, or if the capsule was not detected by the scanner at 30 h after ingestion. If patency was established, then the patient underwent CE. Fifty-nine out (56%) of the 106 included patients excreted the Agile[®] capsule intact and subsequently underwent CE. There were no cases of retention of the video capsule and no Agile[®] capsules were found to have dissolved before 30 h after ingestion. Significant findings on CE were found in 24 patients (41%). A total of 17 (17/106) subjects had an adverse event, of these, 11 (11/106) consisted of abdominal pain. The pain resolved with conservative management within 48 h in all except for a patient with Crohn's disease who need surgery (1/106). This patient developed obstruction after ingestion of the AGILE capsule and underwent surgery with resection of the terminal ileum and proximal colon, no remnants of the capsule were found at surgery. The physicians involved felt that the capsule did not lead to the obstruction. All of the other adverse events resolved within 48 h with conservative management.

In summary, the AGILE Patency capsule seems to be a useful, non-invasive tool to identify which patients with suspected strictures could safely ingest the standard video capsule. It has been designed to minimize the risk of intestinal occlusion.

REFERENCES

- 1 Iddan G, Meron G, Glukhovskiy A, Swain P. Wireless capsule endoscopy. *Nature* 2000; **405**: 417
- 2 Gortzak Y, Lantsberg L, Odes HS. Video Capsule entrapped in a Meckel's diverticulum. *J Clin Gastroenterol* 2003; **37**: 270-271
- 3 Feitoza AB, Gostout CJ, Knipschild MA, Rajan E. Video capsule endoscopy: Is the recording time ideal? *Am J Gastroenterology* 2002; **97**: S307
- 4 Fleischer DE, Heigh RI, Nguyen CC, Leighton JA, Sharma VK, Musil D. Videocapsule impaction at the cricopharyngeus: a first report of this complication and its successful resolution. *Gastrointest Endosc* 2003; **57**: 427-428
- 5 Schneider AR, Hoepffner N, Rosch W, Caspary WF. Aspiration of an M2A capsule. *Endoscopy* 2003; **35**: 713
- 6 Sinn I, Neef B, Andus T. Aspiration of a capsule endoscope. *Gastrointest Endosc* 2004; **59**: 926-927
- 7 Buchkremer F, Herrmann T, Stremmel W. Mild respiratory distress after wireless capsule endoscopy. *Gut* 2004; **53**: 472
- 8 Goldstein JL, Eisen GM, Lewis B, Gralnek IM, Zlotnick S, Fort JG. Video capsule endoscopy to prospectively assess small bowel injury with celecoxib, naproxen plus omeprazole, and placebo. *Clin Gastroenterol Hepatol* 2005; **3**: 133-141
- 9 Barkin JS, Friedman S. Wireless capsule endoscopy requiring surgical intervention: The world's experience. *Am J Gastroenterol* 2002; **97**: A83
- 10 Pennazio M, Santucci R, Rondonotti E, Abbiati C, Beccari G, Rossini FP, De Franchis R. Outcome of patients with obscure gastrointestinal bleeding after capsule endoscopy: report of 100 consecutive cases. *Gastroenterology* 2004; **126**: 643-653
- 11 Sears DM, Avots-Avotins A, Culp K, Gavin MW. Frequency and clinical outcome of capsule retention during capsule endoscopy for GI bleeding of obscure origin. *Gastrointest Endosc* 2004; **60**: 822-827
- 12 Rondonotti E, Herreras JM, Pennazio M, Caunedo A, Mascarenhas-Saraiva M, de Franchis R. Complications, limitations, and failures of capsule endoscopy: a review of 733 cases. *Gastrointest Endosc* 2005; **62**: 712-716; quiz 752, 754
- 13 Caunedo A, Rodriguez-Tellez M, Garcia-Montes JM, Gomez-Rodriguez BJ, Guerrero J, Herreras JM Jr, Pellicer F, Herreras JM. Usefulness of capsule endoscopy in patients with suspected small bowel disease. *Rev Esp Enferm Dig* 2004; **96**: 10-21
- 14 Fireman Z, Mahajna E, Broide E, Shapiro M, Fich L, Sternberg A, Kopelman Y, Scapa E. Diagnosing small bowel Crohn's disease with wireless capsule endoscopy. *Gut* 2003; **52**: 390-392
- 15 Eliakim R, Fischer D, Suissa A, Yassin K, Katz D, Guttman N, Migdal M. Wireless capsule video endoscopy is a superior diagnostic tool in comparison to barium follow-through and computerized tomography in patients with suspected Crohn's disease. *Eur J Gastroenterol Hepatol* 2003; **15**: 363-367
- 16 Herreras JM, Caunedo A, Rodriguez-Tellez M, Pellicer F, Herreras JM Jr. Capsule endoscopy in patients with suspected Crohn's disease and negative endoscopy. *Endoscopy* 2003; **35**: 564-568
- 17 Cheifetz AS, Kornbluth AA, Legnani P, Schmelkin I, Brown A, Lichtiger S, Lewis BS. The risk of retention of the capsule endoscope in patients with known or suspected Crohn's disease. *Am J Gastroenterol* 2006; **101**: 2218-2222
- 18 Mow WS, Lo SK, Targan SR, Dubinsky MC, Treyzon L, Abreu-Martin MT, Papadakis KA, Vasilias EA. Initial experience with wireless capsule enteroscopy in the diagnosis and management of inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2004; **2**: 31-40
- 19 Buchman AL, Miller FH, Wallin A, Chowdhry AA, Ahn C. Videocapsule endoscopy versus barium contrast studies for the diagnosis of Crohn's disease recurrence involving the small intestine. *Am J Gastroenterol* 2004; **99**: 2171-2177
- 20 Cheifetz AS, Lewis BS. Capsule endoscopy retention: is it a complication? *J Clin Gastroenterol* 2006; **40**: 688-691
- 21 Cave D, Legnani P, de Franchis R, Lewis BS. ICCE consensus for capsule retention. *Endoscopy* 2005; **37**: 1065-1067
- 22 Brandt LJ. Deformation of the anus: an alternative to rectal air suctioning for patient comfort after colonoscopy. *Gastrointest Endosc* 2004; **59**: 461
- 23 Baichi MM, Arifuddin RM, Mantry PS. What we have learned from 5 cases of permanent capsule retention. *Gastrointest Endosc* 2006; **64**: 283-287
- 24 Spada C, Spera G, Riccioni M, Biancone L, Petruzzello L, Tringali A, Familiari P, Marchese M, Onder G, Mutignani M, Perri V, Petruzzello C, Pallone F, Costamagna G. A novel diagnostic tool for detecting functional patency of the small bowel: the Given patency capsule. *Endoscopy* 2005; **37**: 793-800
- 25 Boivin ML, Lochs H, Voderholzer WA. Does passage of a patency capsule indicate small-bowel patency? A prospective clinical trial? *Endoscopy* 2005; **37**: 808-815
- 26 Delvaux M, Ben Soussan E, Laurent V, Lerebours E, Gay G. Clinical evaluation of the use of the M2A patency capsule system before a capsule endoscopy procedure, in patients with known or suspected intestinal stenosis. *Endoscopy* 2005; **37**: 801-807
- 27 Signorelli C, Rondonotti E, Villa F, Abbiati C, Beccari G, Avesani EC, Vecchi M, de Franchis R. Use of the Given Patency System for the screening of patients at high risk for capsule retention. *Dig Liver Dis* 2006; **38**: 326-330

- 28 **Caunedo A**, Rodriguez-Tellez M, Romero J, Hernandez-Duran M, Romero R, Pellicer FJ, Herrerias JM. Evaluation of the M2A Patency capsule in the gastrointestinal tract: one-centre preliminary data from a multicentre prospective trial. *Endoscopy* 2003; **35**: A182
- 29 **Caunedo-Alvarez A**, Romero-Vázquez J, Gomez-Rodriguez BJ, Sanchez-Yague A, Castro-Laria L, Herrerias-Gutierrez JM. Prognostic Factors of Short-Term Surgery in Patients with Known or Suspected Stricture Undergone to Patency Capsule. *Gastrointes Endoscopy* 2007; **65**: AB340
- 30 **Gay G**, Delvaux M, Laurent V, Reibel N, Regent D, Grosdidier G, Roche JF. Temporary intestinal occlusion induced by a "patency capsule" in a patient with Crohn's disease. *Endoscopy* 2005; **37**: 174-177
- 31 **Spada C**, Shah SK, Riccioni ME, Spera G, Marchese M, Iacopini F, Familiari P, Costamagna G. Video capsule endoscopy in patients with known or suspected small bowel stricture previously tested with the dissolving patency capsule. *J Clin Gastroenterol* 2007; **41**: 576-582
- 32 **Caunedo-Alvarez A**, Romero-Vazquez J, Gomez-Rodriguez BJ, Sanchez-Yague A, Castro-Laria L, Herrerias-Gutierrez JM. Evaluation of a new double-headed biodegradable device (AGILE Patency Capsule) for detecting functional patency of the small intestine: A prospective clinical trial. Proceedings of the 5th International Conference on Capsule. *Endoscopy* 2006
- 33 **Herrerias JM**, Leighton JA, Costamagna G, Infantolino A, Eliakim R, Fischer D, Rubin DT, Manten HD, Scapa E, Morgan DR, Bergwerk AJ, Koslowsky B, Adler SN. Agile patency system eliminates risk of capsule retention in patients with known intestinal strictures who undergo capsule endoscopy. *Gastrointest Endosc* 2008; **67**: 902-909

S- Editor Zhong XY L- Editor Rippe RA E- Editor Lin YP



COLORECTAL CANCER

Active chinese mistletoe lectin-55 enhances colon cancer surveillance through regulating innate and adaptive immune responses

Yan-Hui Ma, Wei-Zhi Cheng, Fang Gong, An-Lun Ma, Qi-Wen Yu, Ji-Ying Zhang, Chao-Ying Hu, Xue-Hua Chen, Dong-Qing Zhang

Yan-Hui Ma, Wei-Zhi Cheng, Fang Gong, An-Lun Ma, Qi-Wen Yu, Ji-Ying Zhang, Chao-Ying Hu, Shanghai Jiao Tong University School of Medicine, Shanghai Institute of Immunology, Shanghai 200025, China

Xue-Hua Chen, Shanghai Institute of Digestive Surgery, Ruijin Hospital, Shanghai 200025, China

An-Lun Ma, Centre de recherche du CHUM, Hôpital Notre-Dame, Université de Montréal, Montréal, Québec H2L 4M1, Canada

Author contributions: Ma YH and Cheng WZ contributed equally to this work; Zhang DQ, Ma YH and Cheng WZ designed research; Ma YH, Cheng WZ, Gong F, Ma AL, Yu QW, Zhang JY, Hu CY and Chen XH performed research; Chen XH contributed new reagents/analytic tools; Ma YH, Cheng WZ and Zhang DQ wrote the paper.

Supported by The National Natural Science Foundation of China, No. 30471593 and No. 30670939; the Shanghai Leading Academic Discipline Project, No. T0206; the Shanghai Commission of Science and Technology, No. 07JC14033; the Shanghai Institute of Immunology Project, No. 07-A02

Correspondence to: Dong-Qing Zhang, Shanghai Institute of Immunology, Shanghai Jiao Tong University School of Medicine, Shanghai 200025,

China. dqzhang1333@yahoo.com.cn

Telephone: +86-21-64453049 Fax: +86-21-64453049

Received: May 26, 2008 Revised: July 17, 2008

Accepted: July 24, 2008

Published online: September 14, 2008

Balb/c mice *in vivo*. Treatment with ACML-55 enhanced both Ag specific activation and proliferation of CD4+ and CD8+ T cells, and increased the number of tumor Ag specific CD8+ T cells. It was more important to increase the frequency of tumor Ag specific IFN- γ producing-CD8+ T cells. Interestingly, ACML-55 treatment also showed increased cell number of NK, and $\gamma\delta$ T cells, indicating the role of ACML-55 in activation of innate lymphocytes.

CONCLUSION: Our results demonstrate that ACML-55 therapy can enhance function in immune surveillance in colon cancer-bearing mice through regulating both innate and adaptive immune responses.

© 2008 The WJG Press. All rights reserved.

Key words: Active chinese mistletoe lectin-55; Colon cancer; Immune surveillance; Tumor therapy; Ag-specific-CD8+ T cell

Peer reviewer: Yoshiharu Motoo, MD, PhD, FACP, FACP, Professor and Chairman, Department of Medical Oncology, Kanazawa Medical University, 1-1 Daigaku, Uchinada, Ishikawa 920-0293, Japan

Ma YH, Cheng WZ, Gong F, Ma AL, Yu QW, Zhang JY, Hu CY, Chen XH, Zhang DQ. Active chinese mistletoe lectin-55 enhances colon cancer surveillance through regulating innate and adaptive immune responses. *World J Gastroenterol* 2008; 14(34): 5274-5281 Available from: URL: <http://www.wjgnet.com/1007-9327/14/5274.asp> DOI: <http://dx.doi.org/10.3748/wjg.14.5274>

Abstract

AIM: To investigate the potential role of Active Chinese mistletoe lectin-55 (ACML-55) in tumor immune surveillance.

METHODS: In this study, an experimental model was established by hypodermic inoculating the colon cancer cell line CT26 (5×10^5 cells) into BALB/c mice. The experimental treatment was orally administered with ACML-55 or PBS, followed by the inoculation of colon cancer cell line CT26. Intracellular cytokine staining was used to detect IFN- γ production by tumor antigen specific CD8+ T cells. FACS analysis was employed to profile composition and activation of CD4+, CD8+, $\gamma\delta$ T and NK cells.

RESULTS: Our results showed, compared to PBS treated mice, ACML-55 treatment significantly delayed colon cancer development in colon cancer -bearing

INTRODUCTION

Mistletoe (*Viscum album*) is a semiparasitic plant with many unusual properties. It was used as a kind of herbal remedy in the ancient Chinese Pharmacopoeia and has been used in traditional Chinese medicine for diseases, such as gonorrhea, syphilis, hypertension and rheumatism for thousands of years. The aqueous extract of European Mistletoe (EM) has been used in conventional cancer therapy for decades^[1]. Therapeutic efficacy is mostly attributed to the mistletoe lectins

(ML) ML-I, ML-II, ML-III, that belong to the “toxic lectin family” and represent ribosome deactivating proteins class II. They consist of one N-glycosidase (A chain) and one galactoside binding lectin (B chain) linked by a disulfide bridge. The lectins ML-I and ML-III preferentially bind to galactoside or N-acetylgalactosamine groups while ML-II can bind to both carbohydrates^[2].

EM has recently been found to act through several distinct bioactivities as a potent immune modulator. First, EM exerts its broad immunostimulatory activity by activating different types of cells^[3-5] *in vivo* and *in vitro*. Incubation of lymphocytes with EM could result in anti-tumoral cytotoxic T lymphocytes bearing phosphorylated mistletoe ligands^[6,7]. Second, EM favors bridging of natural killer tumor cell conjugates, enhancing its efficiency of killing^[8-10]. Third, it has been found that EM could activate immune responses by modulating the complex network of cytokines that regulate leukocyte functions. EM caused increased secretion of tumor necrosis factor (TNF)- α , interleukin (IL)-1, and IL-6 from isolated human mononuclear cells *in vitro*^[11,12]. Finally, EM has also been described as an inducer of cell apoptosis. In the presence of EM, human mononuclear cells and many cell lines underwent apoptosis^[1,13].

While the EM has been studied intensively, less is known about the Chinese mistletoe as an anti-cancer drug. In our prior study, a mistletoe lectin was purified from Chinese mistletoe and the effect of the active Chinese mistletoe lectin-55 (ACML-55) on human $\gamma\delta$ T cell cytotoxicity, apoptosis and modulation of the cytokine network was reported^[14,15]. Although these investigations suggest that ACML-55 may modulate the immune response against tumor development, the precise mechanism by which ACML-55 regulates the immune function has not been studied systematically. In this study, we demonstrate that ACML-55 enhances tumor immune surveillance against colon cancer formation by regulating both innate and adaptive immune responses. Our results suggest that ACML-55 may be a useful complementary therapy for treating colon cancer.

MATERIALS AND METHODS

Mice

BALB/c mice were purchased from Shanghai Experimental Animal Center, Chinese Academy of Science and were used at 6-8 wk of age in all experiments. All mice were maintained under specific pathogen-free conditions at Shanghai Jiao Tong University School of Medicine.

Administration of ACML-55

ACML-55 was dissolved in PBS at final concentration of 2 g/L. Mice were treated with ACML-55 or PBS orally (200 μ L/mouse) once a day for 2 wk. Oral administration was achieved by gavage to ensure all mice

received the entire dose.

Reagents

Recombinant murine IL-2 was purchased from R&D Systems (Minneapolis, MN, USA). Anti-mouse antibodies (CD3, NK1.1, CD4, CD8, CD62L, CD44, anti- $\alpha\beta$, anti- $\gamma\delta$ and IFN- γ) used for phenotypic and cytokine analysis were purchased from BD Biosciences (San Jose, CA, USA).

Preparation of mistletoe lectins

Mistletoe lectins were isolated from extract of Chinese mistletoe, a subspecies of *V. album* according to previously described methods^[14] with our own modifications. Briefly, the air-dried mistletoe (3 kg), collected from Sichuan province, China, was crushed and purified twice with 20 L methanol/water (1:1, V/V). The homogenate was filtered through a nylon cloth. After filtration, with its volume reduced to 2 liters, the aqueous phase was successively partitioned with cyclohexane, dichloromethane and ethyl acetate. Ethanol was added to the concentrated aqueous phase to a final concentration of 85% (V/V). A precipitate was obtained and separated from the supernatant by centrifugation (8000 *g*, 20 min). The supernatant was concentrated and ethanol was added to 85% (V/V). After centrifugation, the precipitate was collected and combined with the former precipitate. The final yield of ML extract was 100 g from 3 kg mistletoe. All the precipitate was dissolved in 100 mL phosphate buffer (10 mmol/L, pH 6.5) and the stock solution of mistletoe extract was stored at -80°C.

Purification of mistletoe lectins

To obtain the pure Chinese mistletoe protein, extract was further purified by CM-Sepharose column chromatography^[14]. The aqueous layer (1 mL) was applied to a column of CM-Sepharose (1.5 cm \times 20 cm) equilibrated with 10 mmol/L phosphate buffer (pH 6.5). After washing with 10 mmol/L phosphate buffer (pH 6.5) and 100 mmol/L NaCl in the same buffer at a rate of 0.5 mL/min, a peak eluted with 500 mmol/L NaCl in the same buffer was dialyzed with PBS (pH 7.4). The fractions containing hemagglutinating protein were collected and then applied to a column of Con A column (1.5 cm \times 20 cm) equilibrated with 10 mmol/L PBS (pH 7.4). The column was washed with PBS (pH 7.4) and eluted with 300 mmol/L glucose in the same buffer. Fractions were subject to sodium dodecyl sulphate (SDS)-electrophoresis and fractions containing 55 kDa protein were pooled, dialyzed against water and freeze-dried.

SDS-polyacrylamide gel electrophoresis (PAGE)

The molecular mass and purity of ACML-55 was determined by SDS-PAGE. Twelve percent polyacrylamide gel was used as resolving gel and 5% was used as stacking gel. To further denature the proteins by reducing disulfide linkages, the samples were heated at 100°C for 3 min in the presence of a reducing agent,

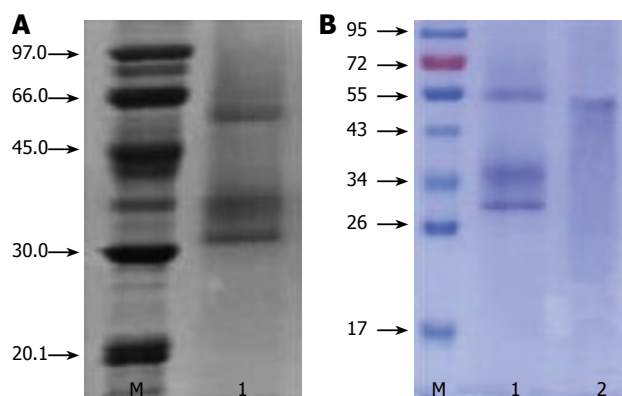


Figure 1 A: SDS-PAGE profiles of ACML-55. ACML-55 was determined by SDS-PAGE; ACML-55. B: ACML-55 was also determined by SDS-PAGE. ACML-55 in presence (lane 1) and absence (lane 2) of reducing agent.

then electrophoresed using electrophoresis system at 200 V for 75 min and lastly the gel was stained with Coomassie brilliant R-250 to show bands.

Tumor models

Colon cancer cell line CT26 and OVA-expressing EG7 cell line were purchased from ATCC (Manassas, VA, USA). For tumor induction, colon cancer cell line CT26 cells (5×10^5 cells/mouse) were injected subcutaneously, and tumor growth was monitored and recorded daily for over 3 wk as described in our previous studies^[15]. For some experiments, EG7 tumor cells were also administered intraperitoneally (1.0×10^6 tumor cells/mouse).

IFN- γ production of tumor antigen specific CD8 $^+$ T cells

Mice were treated with ACML-55 or PBS for 2 wk as described above. These treated mice ($n = 5$ for each group) were immunized with 200 μ g of CT26 tumor lysate emulsified in CFA in the hind footpad, as described in previous studies^[16]. On day 7 post-immunization, draining lymph node cells were harvested, lymphocytes were cultured with comptumor air ratio RPMI-1640 containing 200 μ g/mL CT26 tumor lysate for 24 h, with brefeldin A added for the last 3 h. Cells were then used for intracellular cytokine staining as described below.

Intracellular cytokine staining

Cultured draining lymph node cells were stained with FITC-anti-CD8 antibody followed by fixation with 2% formaldehyde and permeabilization with 0.5% saponin (w/v) for intracellular IFN- γ staining, using PE-anti-IFN- γ as described previously^[17]. PE-conjugated rat IgG2a (BD Pharmingen) was used as an isotype control. Gating was performed on CD8 $^+$ T cells and the percentage of IFN- γ $^+$ cells was reported.

Detecting tumor antigen specific CD8 $^+$ T cells

Both ACML-55 treated mice ($n = 4$) and control mice ($n = 4$) were inoculated intraperitoneally with 1.0×10^6 EG7 tumor cells. On day 10, splenocytes from these

mice were isolated and stained with FITC-anti-CD8 and PE-tetramer antibodies for OVA. Percentage of tetramer positive CD8 $^+$ T cells was shown by FACS analysis.

Analysis of cell composition and activation

Both ACML-55 treated mice ($n = 4$) and control mice ($n = 4$) were inoculated intraperitoneally with 5×10^5 CT26 cells. On day 10, splenocytes from these mice were isolated and stained with one of the following antibody combinations: FITC-anti-CD3 and PE-anti-NK1.1; FITC-anti- $\gamma\delta$ and PE-anti- $\alpha\beta$; PE-anti-CD62L, CyChrome- anti-CD44, FITC-anti-CD8a and APC-anti-CD4. Percentages of different cell subpopulations were shown by FACS analysis.

Statistical analysis

Statistical significance was evaluated by two tailed unpaired Student's test or non-parametric analysis if SDs were significantly different between two compared groups using InStat 2.03 for Macintosh software (Graph Pad Software). The incidence of tumor development was compared and analyzed using the log rank test, performed by Graph Pad Prism Version 3.0a for Macintosh (Graph Pad Software). $P < 0.05$ was used to denote statistical significance.

RESULTS

SDS-PAGE

Chinese ML extractions were analyzed by SDS-PAGE. In the presence of the reducing agent, it showed an estimated 55 kDa band consisting of two bands of a 30 kDa A chain and a 34 kDa B Chain (Figure 1).

Oral administration of ACML-55 enhances tumor immunesurveillance

Based on the findings that EM reduces the metastasis of rat mammary adenocarcinomas and its ability to modulate immune functions^[18,12], we hypothesized that ACML-55 might enhance tumor immune surveillance. To assess the effect of ACML-55 on tumor development, ACML-55 or PBS was administered to sex- and age-matched BALB/c mice by gavage daily for 2 wk, followed by subcutaneous inoculation of CT26 cells (5×10^5 cells/mouse). Tumor growth was observed and recorded daily as previously described^[16,19]. Compared to control group (PBS treated), ACML-55 treated mice showed delayed tumor development (Figure 2A) as well as reduced tumor size (Figure 2B). ACML-55 treated mice were much more resistant to tumor cell growth upon subcutaneous tumor inoculation (Figure 2C). The results indicate that ACML-55 significantly enhances tumor surveillance.

ACML-55 increases tumor specific activation of CD4 $^+$ and CD8 $^+$ T cells

To define the underlying molecular mechanisms of ACML-55 mediated anti-tumor immune response, we first tested the effect of ACML-55 on the adaptive immune response. Sex- and age- matched BALB/c mice

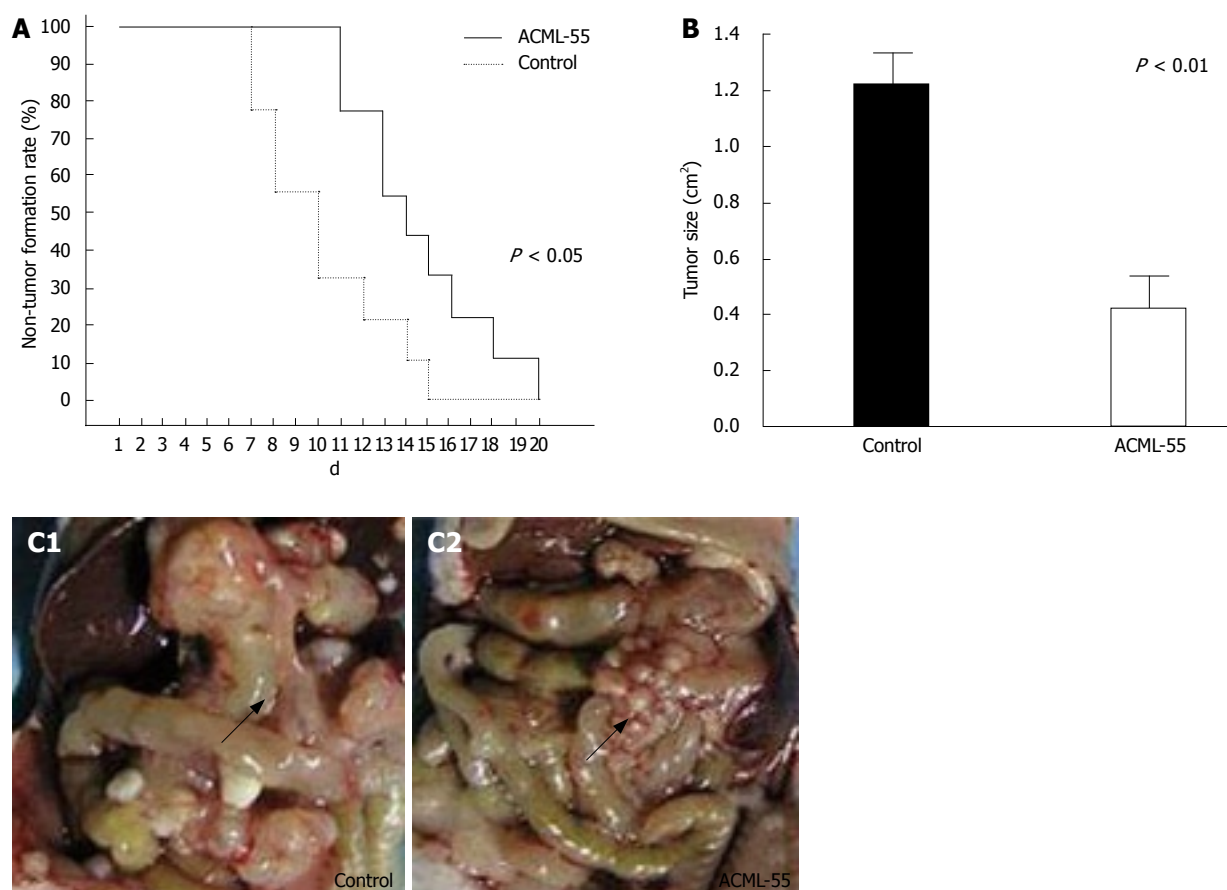


Figure 2 ACML-55 enhances tumor surveillance. **A:** ACML-55 treatment delays CT26 melanoma tumor formation. Sex- and age-matched BALB/c mice were administered orally either with 2 mg/mL (200 μ L/mouse) ACML-55 or equivalent volume of PBS (control) daily for fifteen days ($n = 20$ for each group), followed by subcutaneous inoculation of CT26 melanoma tumor cells (5×10^5 /mouse) on day 7 after the initiation of ACML-55 or PBS treatment. Tumor growth was recorded daily. Tumor size $> 5 \text{ mm} \times 5 \text{ mm}$ was considered positive. Data represents three independent experiments ($P < 0.01$). **B:** ACML-55 treatment inhibits tumor growth. The mean tumor size from ACML-55 and PBS-treated mice at day 20 is shown in this figure ($P < 0.01$). **C:** ACML-55 treatment significantly reduces intraperitoneal tumor formation. BALB/c mice were treated with ACML-55 or PBS followed by CT26 tumor cell inoculation intraperitoneally ($n = 20$ for each group) as described above, and tumor growth was monitored. A representative example of tumor formation is provided. Arrows point to intraperitoneal tumor.

were given ACML-55 or PBS daily for 2 wk, followed by intraperitoneal inoculation with EG7 tumor cells (EG7 tumor cells expressing OVA, 1.0×10^6 cell/mouse)^[20]. On day 10 post-inoculation, harvested splenocytes were used for analysis of CD4⁺ and CD8⁺ T cell activation using specific activation markers. ACML-55 treatment significantly increased the number of activated CD4⁺ and CD8⁺ T cells. According to our findings, the percentage of CD62L^{low} CD44^{high} population in the spleen for each T cell subset was significantly higher in ACML-55 treated mice compared to those treated with PBS [14.29 ± 4.3 *vs* 7.63 ± 2.95 for CD4⁺ T cells, and 6.79 ± 1.41 *vs* 3.95 ± 1.97 for CD8⁺ T cells (Figure 3A), $P = 0.0008$ for CD4 and $P = 0.0002$ for CD8]. Representative data of the FACS profile for CD4⁺ and CD8⁺ T cells from ACML-55 or PBS treated mice are represented in Figure 3B.

ACML-55 promotes IFN- γ production in CD8⁺ T cells

IFN- γ has been shown previously to be a critical cytokine in tumor immunosurveillance^[21]. To define the effect of ACML-55 on tumor antigen specific IFN- γ production, ACML-55 or PBS was administered to sex-

and age- matched BALB/c mice ($n = 6$ for each group) as mentioned above for 2 wk, and then immunized in the hind footpad with CT26 tumor lysate in CFA. Eight days post-immunization, lymphocytes from the draining lymph nodes were isolated, cultured with 200 μ g/mL tumor lysate for 24 h, with brefeldin A added during the last 3 h of culture. These cells were then fixed and permeabilized with 0.5% saponin for intracellular cytokine staining. The percentage of IFN- γ producing CD8⁺ T cells from ACML-55 treated mice (mean \pm SD) was significantly higher than that of PBS treated mice (10.05 ± 2.3 and 2.30 ± 1.013 , respectively, $P = 0.0001$; Figure 4A). An example of FACS analysis is represented in Figure 4B. In the same cultures, the percentage of IFN- γ producing CD4⁺ T cells from ACML-55 treated mice was also higher than those from PBS treated mice, although it did not reach significance (data not shown). ACML-55 treatment did not enhance the percentage of IFN- γ producing CD8⁺ T cells responding to control tumor lysate (different tumor cell line, data not shown). Our results demonstrate that ACML-55 not only enhances activation and proliferation of T cells, but also increases their capacity to produce

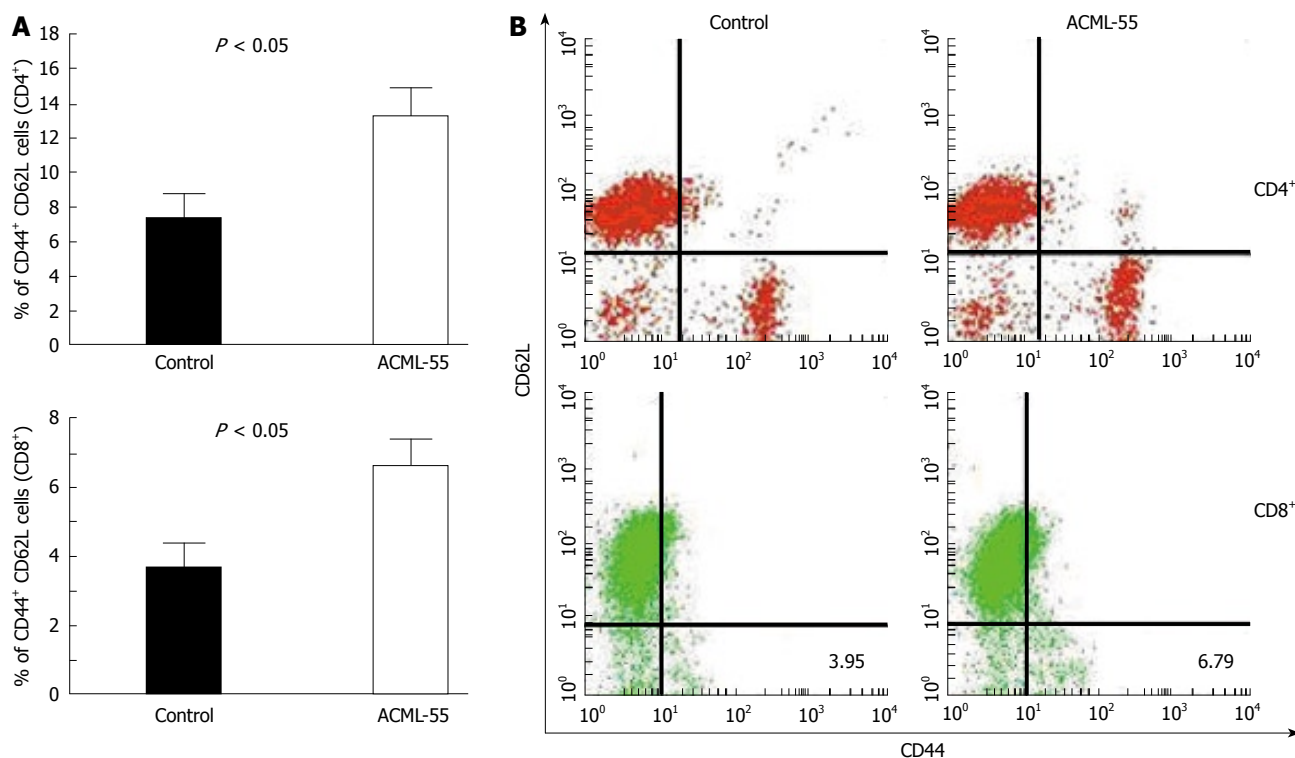


Figure 3 ACML-55 enhances CD4⁺ and CD8⁺ T cell activation. Sex- and age- matched BALB/c mice were administered orally with 2 mg/mL (200 μ L/mouse) ACML-55 or equivalent volume of PBS daily for 15 days ($n = 5$ for each group), and on day 7, received an intraperitoneal inoculation with EG7 tumor cells (1×10^6 cells/mouse). Ten days postinoculation, splenocytes were stained with antibodies against different surface molecules and analyzed by FACS. **A:** The percentage of activated CD4⁺ and CD8⁺ T cells (mean \pm SD) is shown ($P < 0.05$); **B:** An example of the FACS profile for CD4⁺ and CD8⁺ T cells is given.

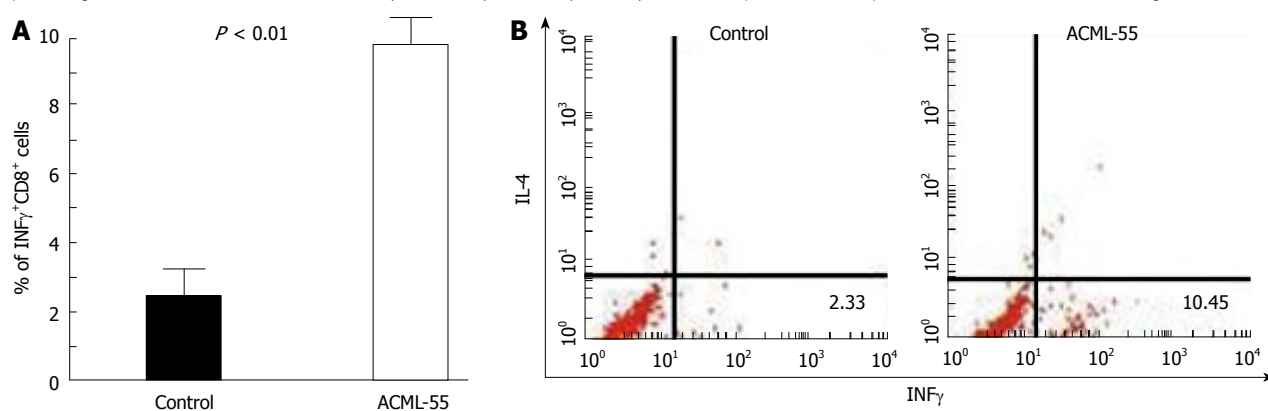


Figure 4 ACML-55 increases the number of IFN- γ +CD8⁺ T cells. Sex- and age- matched BALB/c mice were administered orally with 2 mg/mL (200 μ L/mouse) ACML-55 or equivalent volume of PBS daily ($n = 5$ for each group) for fourteen days, followed by immunization with 200 μ g of CT26 tumor lysate emulsified in CFA. After seven days, lymphocytes recovered from draining lymph nodes of immunized mice were cultured with 200 μ g/mL of tumor lysate for 24 h, with the addition of brefeldin A to the culture for the remaining 3 h. Cells were then fixed with 2% formaldehyde and permeabilized with 0.5% saponin for intracellular IFN- γ staining. **A:** The percentage of IFN- γ producing cells (mean \pm SD) from CD8⁺ T cells is shown; **B:** An example of intracellular cytokine staining upon gating on CD8⁺ T cells is given.

IFN- γ cytokine.

ACML-55 increases the number of both NK and $\gamma\delta$ T cells

Both NK cells and $\gamma\delta$ T cells play a critical role in tumor immune surveillance. To test the effect of ACML-55 on these cell types, sex- and age- matched BALB/c mice were treated with ACML-55 or PBS ($n = 6$ for each group) as above for 2 wk, and the percentages of NK and $\gamma\delta$ T cells in the spleen were analyzed by flow cytometry. Treatment with ACML-55 significantly increased the numbers of splenic NK cells and $\gamma\delta$ T cells, with the percentage (mean \pm SD) of NK1.1+

cells in ACML-55 treated mice *vs* control being 6.28 ± 0.90 *vs* 3.48 ± 0.77 , and the percentage of CD3+ $\gamma\delta$ + cells for ACML-55 *vs* control being 6.51 ± 0.59 *vs* 3.85 ± 0.59 , (Figure 5A, $P = 0.0001$). A representative result of FACS analysis for CD3 and NK1.1 as well as $\alpha\beta/\gamma\delta$ T cell staining is presented in Figure 5B. Splenocytes from ACML-55 or PBS treated mice were cultured with anti-CD3 and anti-CD28 antibodies in the presence of brefeldin A for 6 h, and the percentage of IFN- γ + $\gamma\delta$ + T cells was analyzed by intracellular cytokine staining after gating TCR $\gamma\delta$ positive cells. The results showed there is no significant difference in the percentage of

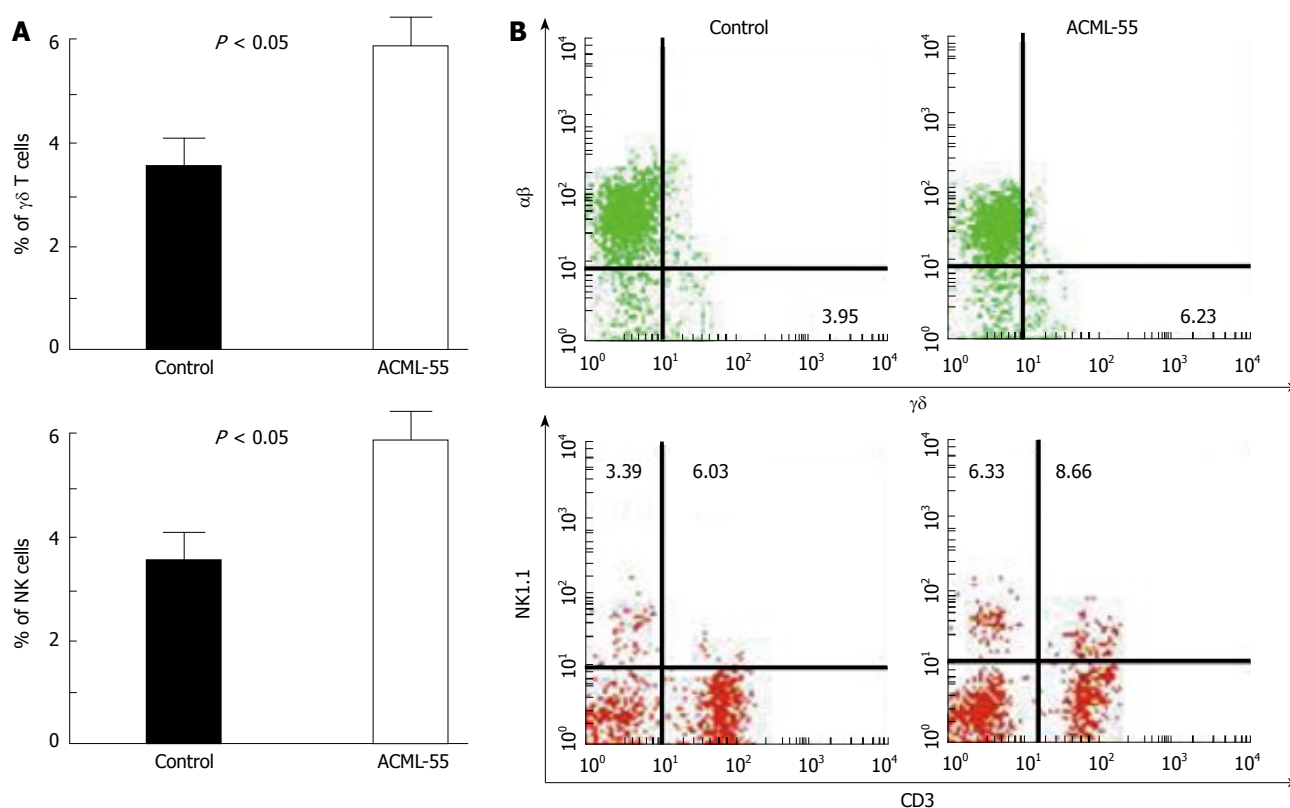


Figure 5 ACML-55 treatment increases the number of NK and $\gamma\delta$ T cells. Sex- and age- matched BALB/c mice were administered orally with 2 mg/mL (200 μ L/mouse) ACML-55 or equivalent volume of PBS daily ($n = 5$ for each group) for fourteen days, followed by inoculation with CT26 tumor cells intraperitoneally. Ten days post-inoculation, splenocytes were used for analysis of NK, NKT, $\alpha\beta$, and $\gamma\delta$ T cells. **A:** The percentage of NK and $\gamma\delta$ T cells (mean \pm SD) is shown ($P < 0.05$); **B:** An example of the FACS analysis is given.

IFN- γ producing $\gamma\delta$ T cells between two groups (data not shown). NKT cells (CD3+ NK1.1+) were also higher in ACML-55 treated mice compared to controls, although it did not reach significance. These results indicate that ACML-55 may enhance the antitumor immune response not only through modulating the adaptive immune response, but also working on innate immunity.

DISCUSSION

Extracts from European mistletoe are used widely in the treatment of cancer, but the mechanism of antitumor properties has not yet been clearly elucidated. Consumers often use EM as a complementary therapy for cancer treatment, and in some cases as an alternative to conventional cancer treatment^[22,23]. Although Korean mistletoe, a subspecies of European mistletoe, has been used as a medicinal herb and shown to be cytotoxic against tumor cells as well^[24], there are fewer systemic controlled studies to define the effect of EM in tumor immunity. In this study, we demonstrate that ACML-55 enhances tumor immune surveillance against both melanoma and lymphoma by regulating both innate and adaptive immune responses. We first illustrated that oral administration of ACML-55 prior to tumor inoculation could significantly delay the tumor growth and reduce tumor size (Figure 2A and B). The anti-tumor effect of ACML-55 is not limited to melanoma.

It has been shown that lymphocytes and IFN- γ both are essential components of tumor immune surveillance^[25,26]. Different subsets of lymphocytes contribute to anti-tumor immune responses at different stages. CD4+ and CD8+ T cells are critical elements for the adaptive anti-tumor immune response. CD4+ T cells, especially Th1 subsets, produce IFN- γ to facilitate innate and adaptive immune responses^[27]. These cells are also in favor of CD8+ T cells to develop memory response, whereas CD8+ T cells provide cytokines (IFN- γ and TNF- α) and cytotoxicity to exert their function to kill tumor cells directly. To explain the molecular mechanisms through which ACML-55 could mediate anti-tumor immune response, we sought to determine whether ACML-55 modulates the adaptive immune response. Our results indicate that ACML-55 treatment enhances activation and proliferation of both CD4+ and CD8+ T cells (Figure 3A and B). Moreover, administration of ACML-55 significantly increases the frequency of tumor antigen specific CD8+ T cells and their ability to produce higher level of IFN- γ (Figure 4A and 3B). Finally, ACML-55 treatment can make antigen specific CD8+ T cells expand more actively. The increasing number of CD8+ T cells partially contributes to the tumor resistance of ACML-55 treated mice. However, it is unclear how ACML-55 enhances the function of these T cells. It is possible that the mixture of polysaccharides in ACML-55 may activate the innate immune response

through undefined signaling pathways, such as Toll-like receptors and the downstream NF- κ B pathway, which in turn helps to regulate the adaptive immune response. Consistently, it has been reported that ACML-55 enhances IL-12 production from macrophages^[10] and increases nitric oxide concentration^[12]. Further studies are needed to clarify the underlying mechanisms that mediate the effect of ACML-55 on the adaptive immune response.

A potential target of ACML-55 modulation within the innate immune system may be $\gamma\delta$ T cells. These cells belong to a unique subset of T cells. They recognize protein or peptide independent of antigen presentation and function as innate like cells^[28]. Our earlier studies have demonstrated that $\gamma\delta$ T cells predominantly produce IFN- γ upon activation^[12,29] and play a critical role in tumor immune surveillance by providing an early source of IFN- γ ^[14]. Interestingly, ACML-55 treatment significantly increases the number of $\gamma\delta$ T cells compared to those of PBS-treated mice (Figure 5). Since ACML-55 was given orally, it is possible that the effective components in ACML-55 might directly encounter $\gamma\delta$ T cells lining the epithelial layer of the intestine resulting in their activation. In addition to $\gamma\delta$ T cells, it has been well established that NK cells play an essential role in tumor immune surveillance. Interestingly, we found that treatment with ACML-55 also upregulates the number of NK cells upon tumor inoculation. Although the changes of NK T cells did not reach significance, the trend is clear. These results indicate that ACML-55 has multiple effects on the immune system.

Given the findings that ACML-55 could efficiently enhance several immune parameters (CD4+, CD8+ and $\gamma\delta$ T cells), which were shown previously to be positive for tumor immune surveillance, it is likely that ACML-55 mediates its potential effects on tumor surveillance, at least in part, by upregulation these particular parameters. Future studies using different T cell subset from deficient mice will help to illuminate these questions.

In summary, we have presented clear picture that, as an active lectin from Chinese mistletoe, ACML-55 enhances tumor immune surveillance by regulating both the innate and the adaptive immune responses. Further studies are needed to define the molecular mechanisms mediating the effect of ACML-55 in tumor immunity.

COMMENTS

Background

Mistletoe is a semiparasitic plant with many unusual properties. In our prior study, a mistletoe lectin was purified from Chinese mistletoe and the effect of the active Chinese mistletoe lectin-55 (ACML-55) on human $\gamma\delta$ T cell cytotoxicity, apoptosis and modulation of the cytokine network was reported. Although these investigations suggest that ACML-55 may modulate the immune response against tumor development, the precise mechanism by which ACML-55 regulates the immune function has not been studied systematically.

Research frontiers

In this study, an experimental model was established by hypodermically inoculating the colon cancer cell line CT26 into Balb/c mice. Intracellular cytokine staining used to detect tumor antigen specific CD8+ T cell IFN- γ production. The FACS profile for CD4+ and CD8+ T cells and NK or $\gamma\delta$ T Cells composition and activation.

Innovations and breakthroughs

This study investigates the potential effect of active Chinese mistletoe lectin-55 to enhance colon cancer immune surveillance through regulating both innate and adaptive immune responses.

Applications

The results demonstrate that ACML-55 can enhance colon cancer immune surveillance through regulating both innate and adaptive immune responses and also suggest that ACML-55 may be a useful complementary therapy for treating colon cancer.

Peer review

The study investigates the potential effect of ACML-55 to enhance colon cancer immune surveillance through regulating both innate and adaptive immune responses. Its scientific contents can reflect the advanced levels of basic research and the first report on Chinese mistletoe lectin-55.

REFERENCES

- 1 Bussing A, Suzart K, Bergmann J, Pfuller U, Schietzel M, Schweizer K. Induction of apoptosis in human lymphocytes treated with Viscum album L. is mediated by the mistletoe lectins. *Cancer Lett* 1996; **99**: 59-72
- 2 Hajto T. Immunomodulatory effects of iscador: a Viscum album preparation. *Oncology* 1986; **43** Suppl 1: 51-65
- 3 Ribereau-Gayon G, Dumont S, Muller C, Jung ML, Poindron P, Anton R. Mistletoe lectins I, II and III induce the production of cytokines by cultured human monocytes. *Cancer Lett* 1996; **109**: 33-38
- 4 Beuth J. Clinical relevance of immunoactive mistletoe lectin-I. *Anticancer Drugs* 1997; **8** Suppl 1: S53-S55
- 5 Stein GM, Berg PA. Mistletoe extract-induced effects on immunocompetent cells: in vitro studies. *Anticancer Drugs* 1997; **8** Suppl 1: S39-S42
- 6 Fischer S, Scheffler A, Kabelitz D. Activation of human gamma delta T-cells by heat-treated mistletoe plant extracts. *Immunol Lett* 1996; **52**: 69-72
- 7 Fischer S, Scheffler A, Kabelitz D. Stimulation of the specific immune system by mistletoe extracts. *Anticancer Drugs* 1997; **8** Suppl 1: S33-S37
- 8 Hauer J, Anderer FA. Mechanism of stimulation of human natural killer cytotoxicity by arabinogalactan from Larix occidentalis. *Cancer Immunol Immunother* 1993; **36**: 237-244
- 9 Mueller EA, Anderer FA. A Viscum album oligosaccharide activating human natural cytotoxicity is an interferon gamma inducer. *Cancer Immunol Immunother* 1990; **32**: 221-227
- 10 Mueller EA, Anderer FA. Synergistic action of a plant rhamnogalacturonan enhancing antitumor cytotoxicity of human natural killer and lymphokine-activated killer cells: chemical specificity of target cell recognition. *Cancer Res* 1990; **50**: 3646-3651
- 11 Mockel B, Schwarz T, Zinke H, Eck J, Langer M, Lentzen H. Effects of mistletoe lectin I on human blood cell lines and peripheral blood cells. Cytotoxicity, apoptosis and induction of cytokines. *Arzneimittelforschung* 1997; **47**: 1145-1151
- 12 Thies A, Nügel D, Pfuller U, Möll I, Schumacher U. Influence of mistletoe lectins and cytokines induced by them on cell proliferation of human melanoma cells in vitro. *Toxicology* 2005; **207**: 105-116
- 13 Harmsma M, Gromme M, Ummelen M, Dignef W, Tusenius KJ, Ramaekers FC. Differential effects of Viscum album extract IscadorQu on cell cycle progression and apoptosis in cancer cells. *Int J Oncol* 2004; **25**: 1521-1529
- 14 Lee HS, Kim YS, Kim SB, Choi BE, Woo BH, Lee KC. Isolation and characterization of biologically active lectin from Korean mistletoe, Viscum album var. Coloratum. *Cell Mol Life Sci* 1999; **55**: 679-682
- 15 Gong F, Ma Y, Ma A, Yu Q, Zhang J, Nie H, Chen X, Shen B, Li N, Zhang D. A lectin from Chinese mistletoe increases gammadelta T cell-mediated cytotoxicity through induction of caspase-dependent apoptosis. *Acta Biochim Biophys Sin (Shanghai)* 2007; **39**: 445-452
- 16 Gao Y, Yang W, Pan M, Scully E, Girardi M, Augenlicht LH,

- Craft J, Yin Z. Gamma delta T cells provide an early source of interferon gamma in tumor immunity. *J Exp Med* 2003; **198**: 433-442
- 17 **Yin Z**, Zhang DH, Welte T, Bahtiyar G, Jung S, Liu L, Fu XY, Ray A, Craft J. Dominance of IL-12 over IL-4 in gamma delta T cell differentiation leads to default production of IFN-gamma: failure to down-regulate IL-12 receptor beta 2-chain expression. *J Immunol* 2000; **164**: 3056-3064
 - 18 **Jung ML**, Baudino S, Ribereau-Gayon G, Beck JP. Characterization of cytotoxic proteins from mistletoe (*Viscum album* L.). *Cancer Lett* 1990; **51**: 103-108
 - 19 **Gao Y**, Tao J, Li MO, Zhang D, Chi H, Henegariu O, Kaech SM, Davis RJ, Flavell RA, Yin Z. JNK1 is essential for CD8+ T cell-mediated tumor immune surveillance. *J Immunol* 2005; **175**: 5783-5789
 - 20 **Gorelik L**, Flavell RA. Immune-mediated eradication of tumors through the blockade of transforming growth factor-beta signaling in T cells. *Nat Med* 2001; **7**: 1118-1122
 - 21 **Ikeda H**, Old LJ, Schreiber RD. The roles of IFN gamma in protection against tumor development and cancer immunoediting. *Cytokine Growth Factor Rev* 2002; **13**: 95-109
 - 22 **deVere White RW**, Hackman RM, Soares SE, Beckett LA, Li Y, Sun B. Effects of a genistein-rich extract on PSA levels in men with a history of prostate cancer. *Urology* 2004; **63**: 259-263
 - 23 **deVere White RW**, Hackman RM, Soares SE, Beckett LA, Sun B. Effects of a mushroom mycelium extract on the treatment of prostate cancer. *Urology* 2002; **60**: 640-644
 - 24 **Khil LY**, Kim W, Lyu S, Park WB, Yoon JW, Jun HS. Mechanisms involved in Korean mistletoe lectin-induced apoptosis of cancer cells. *World J Gastroenterol* 2007; **13**: 2811-2818
 - 25 **Dunn GP**, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. *Immunity* 2004; **21**: 137-148
 - 26 **Li NL**, Nie H, Yu QW, Zhang JY, Ma AL, Shen BH, Wang L, Bai J, Chen XH, Zhou T, Zhang DQ. Role of soluble Fas ligand in autoimmune diseases. *World J Gastroenterol* 2004; **10**: 3151-3156
 - 27 **Shankaran V**, Ikeda H, Bruce AT, White JM, Swanson PE, Old LJ, Schreiber RD. IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. *Nature* 2001; **410**: 1107-1111
 - 28 **Carding SR**, Egan PJ. Gammadelta T cells: functional plasticity and heterogeneity. *Nat Rev Immunol* 2002; **2**: 336-345
 - 29 **Yin Z**, Chen C, Szabo SJ, Glimcher LH, Ray A, Craft J. T-Bet expression and failure of GATA-3 cross-regulation lead to default production of IFN-gamma by gammadelta T cells. *J Immunol* 2002; **168**: 1566-1571

S- Editor Zhong XY **L- Editor** Negro F **E- Editor** Zhang WB



CLINICAL RESEARCH

Incidence of gallstone disease in Italy: Results from a multicenter, population-based Italian study (the MICOL project)

Davide Festi, Ada Dormi, Simona Capodicasa, Tommaso Staniscia, Adolfo F Attili, Paola Loria, Paolo Pazzi, Giuseppe Mazzella, Claudia Sama, Enrico Roda, Antonio Colecchia

Davide Festi, Giuseppe Mazzella, Claudia Sama, Enrico Roda, Antonio Colecchia, Department of Internal Medicine and Gastroenterology, University of Bologna, Bologna 40138, Italy

Ada Dormi, Department of Clinical Medicine and Biotechnology, University of Bologna, Bologna 40138, Italy

Simona Capodicasa, Tommaso Staniscia, Department of Medicine and Aging, University of Chieti, Chieti 66100, Italy

Adolfo F Attili, Department of Gastroenterology, University "La Sapienza", Roma 00010, Italy

Paola Loria, Department of Internal Medicine, University of Modena, Modena 41100, Italy

Paolo Pazzi, Department of Internal Medicine, S. Anna Hospital, Ferrara 44100, Italy

Author contributions: Festi D, Attili AF, Pazzi P, Loria P, Sama C, Roda E designed research; Festi D, Attili AF, Pazzi P, Loria P, Colecchia A performed research; Capodicasa S, Staniscia T, Dormi A analyzed data; Festi D wrote the paper.

Correspondence to: Davide Festi, MD, Dipartimento di Medicina Interna e Gastroenterologia, Policlinico S. Orsola-Malpighi, Via Massarenti 9, Bologna 40138, Italy. davide.festi@unibo.it

Telephone: +39-51-6364123 Fax: +39-51-6364123

Received: January 3, 2008 Revised: August 13, 2008

Accepted: August 20, 2008

Published online: September 14, 2008

Increasing age and pain in the right hypochondrium in men and increasing age in females were identified as predictors of gallstones. Pain in the epigastrium/right hypochondrium was the only symptom related to gallstones; furthermore, some characteristics of pain (forcing to rest, not relieved by bowel movements) were significantly associated with gallstones. No correlation was found between gallstone characteristics and clinical manifestations, while increasing age in men and increasing age and BMI in females were predictors of pain.

CONCLUSION: Increasing age and BMI represent true risk factors for gallstone disease (GD); pain in the right hypochondrium and/or epigastrium is confirmed as the only symptom related to gallstones.

© 2008 The WJG Press. All rights reserved.

Key words: Gallstone disease; Ultrasonography; Epidemiology; Prevalence; Incidence; Abdominal pain; Cholecystectomy; Body mass index

Peer reviewer: Serdar Karakose, PhD, Professor, Department of Radiology, Meram Medical Faculty, Selcuk University, Konya 42080, Turkey

Festi D, Dormi A, Capodicasa S, Staniscia T, Attili AF, Loria P, Pazzi P, Mazzella G, Sama C, Roda E, Colecchia A. Incidence of gallstone disease in Italy: Results from a multicenter, population-based Italian study (the MICOL project). *World J Gastroenterol* 2008; 14(34): 5282-5289 Available from: URL: <http://www.wjgnet.com/1007-9327/14/5282.asp> DOI: <http://dx.doi.org/10.3748/wjg.14.5282>

Abstract

AIM: To evaluate gallstone incidence and risk factors in a large population-based study.

METHODS: Gallstone incidence and risk factors, were evaluated by structured questionnaire and physical examination, respectively, in 9611 of 11 109 (86.5%) subjects who were gallstone-free at the cross-sectional study.

RESULTS: Six centers throughout Italy enrolled 9611 subjects (5477 males, 4134 females, aged 30-79 years), 9517 of whom were included into analysis: 424 subjects (4.4%) had gallstones and 61 (0.6%) had been cholecystectomized yielding a cumulative incidence of 0.67% per year (0.66% in males, 0.81% in females). Increasing age, a high body mass index (BMI), a history of diabetes, peptic ulcer and angina, and low cholesterol and high triglyceride levels were identified as risk factors in men while, in females, the only risk factors were increasing age and a high BMI.

INTRODUCTION

Gallstone disease (GD) is a very common gastrointestinal disorder, presents mainly in the Western world^[1,2]. Although this disease has a low mortality rate, its economic and health impact is significant due to its high morbidity. In fact, GD is one of the most common abdominal conditions for which patients in developed countries are admitted to hospitals^[3], and this frequency has increased in Western countries since the 1950s^[4]. GD is considered "a surgical disease"

since only a cholecystectomy is capable of definitively curing the disease^[5]. However, since the introduction of laparoscopic cholecystectomy in the early 90s, which is considered a safe treatment for GD^[6], a possible unjustified increase in surgical procedures has been observed^[7]. Therefore, there is the need for more knowledge of the epidemiological characteristics of GD in order to better identify therapeutic strategies.

The availability of ultrasonography (US) as an accurate tool for gallstone diagnosis has allowed the evaluation of gallstone prevalence by means of epidemiological surveys of the general population, both in Eastern and Western countries^[8,9]. Furthermore, these studies, as well as case-control studies, have allowed the identification of the factors most frequently associated with GD, i.e. increasing age, female sex, familial history of GD, number of pregnancies, obesity, or type 2 diabetes^[10].

However, only a few prospective US surveys, mainly in Europe^[11-14], have been carried out which aimed measuring the gallstone incidence rate and risk factors for the disease. The knowledge of disease risk factors is crucial to carrying out primary or secondary preventive programs.

The Multicenter Italian Study on Epidemiology of Cholelithiasis (the MICOL project) is a population-based cross-sectional study which began in 1985 and extended since 1998 and it was designed to obtain an overview of the distribution of GD in Italy according to different regions and ages^[15]. Results on prevalence distribution, associated factors, and clinical manifestations have been extensively reported in previous articles^[15-17].

This article reports the incidence rate and risk factors for GD which were evaluated in six units belonging to the MICOL project.

MATERIALS AND METHODS

Study design

The MICOL project is a population-based, cross-sectional study carried out in 8 Italian regions by different operative units. Complete details on the study protocol have been published elsewhere^[15]. The project plan includes 2 cross-sectional surveys; the first began in 1985 and was completed in 1988 and the second survey was carried out on the same subjects 10 years later in order to estimate the incidence of GD as well as its natural history. Seven of the original operative units were able to complete the second survey.

Subjects

After 10 years, all participants in the first survey were invited to a follow-up examination. Preliminarily, using the electoral lists, subjects who had died or moved were identified. The remaining subjects received a standardized invitation for a general health examination and an US examination of the upper abdomen. Subjects who did not respond were invited again; those who refused to participate or those who did not respond at all were contacted by telephone.

Screening protocol

The re-examination took place between 1995 and 1998. Subjects who were found to have had gallstones or a previous cholecystectomy for gallstones at the first survey were studied separately, the major aim of this re-examination being evaluation of the natural history of gallstones.

Similarly to what had been carried out in the first survey^[15], the screening protocol included upper abdominal US, physical examination, fasting blood specimen collection, and administration of a precoded questionnaire. This questionnaire inquired about family and personal history, dietary habits, past and current use of medications, and the presence of comorbidities (diabetes, chronic heart disease, liver cirrhosis, peptic ulcer); the questionnaire also included a specific section aimed at assessing the occurrence/presence of abdominal symptoms (details of the structure of the questionnaire as well as details on its items have been published elsewhere^[17]). The questionnaire was administered by trained interviewers selected from the medical staff of each operative unit. Upper abdominal US was performed thereafter by independent physicians.

Statistical analysis

The incidence rate for new-onset gallstones was determined from baseline to the second examination, after adjustment for age and gender. Logistic regression analysis was carried out providing risk factors and Odds Ratios (OR) for developing gallstones; Cox proportional hazard regression was used to calculate hazard ratios.

According to previous results regarding associated factors obtained from the gallstone prevalence study^[15] and from available literature data^[8,10,18], different factors were evaluated by means of logistic regression analysis as possible risk factors for GD including age, gender, educational level, family history of gallstones, body mass index (BMI) (measured both at the prevalence and the incidence studies), number of pregnancies, history of diets, diabetes, peptic ulcer, inflammatory bowel disease, liver cirrhosis, smoking, coffee drinking, and serum levels of total cholesterol, HDL-cholesterol, and triglycerides.

To identify the possible role of the disease status on clinical presentation, patients were subsequently classified into 4 groups according to their disease status: gallstone-free subjects (GF), patients with gallstones not previously diagnosed (GNPD), patients with previously diagnosed gallstones (GPD), and patients with a history of cholecystectomy for gallstones (CC).

Logistic regression models were created for the latter 3 groups, using the gallstone-free subjects as the comparison group.

As a consequence of the results of the logistic regression models, patients referring pain located at the right hypochondrium or epigastrium were selected and further evaluated.

A multivariate analysis was subsequently carried out using logistic regression and, for each gallstone group, a model was created using the gallstone-free subjects

as the comparison group. SPSS (ver.9.0 for windows) statistical software was used. A probability of $P < 0.05$ was considered significant.

RESULTS

Subjects

Out of the 12709 potentially enrolled subjects belonging to the seven participating units, 502 died and 1098 transferred. Among the dead subjects, one case of gallbladder cancer was observed, which translates in a mortality rate for this cause of 2.5/10000 per year.

Of the 11109 enrolled subjects, 9611 (5477 males, 4134 females, aged 30-79 years) were evaluated (86.5%). The attendance rate was different in different age classes, greater in younger than in older subjects (83% in the 40-60 years old group and 74% in the > 60 years old group) while no difference was documented between the sexes (78% in males, 89% in females). The mean follow-up period was 8 years.

On the basis of US diagnosis, 9517 (5428 males, 4089 females) were suitable for analysis since, in 94 cases, the US diagnosis was biliary sludge (35 cases) or gallbladder polyps (40 cases); in 19 cases, the US diagnosis was uncertain. These 94 cases were excluded from the study, since no definitive data are available in literature regarding these aspects as true gallstones^[5,10,11].

Four hundred and twenty-four subjects (206 males, 218 females) (4.4%) were found to have gallstones and 61 (26 males, 35 females) (0.6%) had been cholecystectomized for gallstones during the follow-up period.

The cumulative incidence rate of GD thus was 0.67% per year [0.66% per year in males, 0.81% per year in females (Table 1)]. Table 1 also shows the incidence rate among the different operative units.

Table 2 reports the morphological characteristics of gallstones; incident gallstones were more frequently small in size (77.9% less than 1.5 cm); no difference was observed between males and females in terms of gallstone number or size.

According to the disease status evaluation, of the 9517 subjects enrolled in the study, 9032 (94.5%) were GF; 312 (3.3%) were GNP, 112 (1.2%) were GPD, and 61 (0.6%) were CC.

Risk factors and predictors for GD

Risk factors for GD: The results of the logistic regression analysis carried out to identify the risk factors for GD are shown in Table 3.

Among the factors considered, in males, increasing age ($P < 0.0001$), a high BMI ($P < 0.006$), a history of diabetes ($P < 0.01$) and of peptic ulcer ($P < 0.01$), low levels of total ($P < 0.03$) and HDL ($P < 0.04$) cholesterol, and high levels of triglycerides ($P < 0.007$) were identified as risk factors.

In females, only increasing age ($P < 0.00001$) and a high BMI ($P < 0.0001$) were identified as risk factors for GD.

Table 1 Incidence of GD in the different operative units of the MICOL project

Operative unit	Overall % yr	Male	Female
Bologna Loiano	0.50	0.50	0.50
Bologna Brisighella	0.61	0.60	0.80
Bolzano	0.82	0.50	1.10
Castellana Grotte	0.75	0.70	0.80
Tivoli	0.64	0.50	0.70
Modena San Lazzaro	0.43	0.40	0.40
Modena Madonnina	0.86	0.60	1.20
Overall	0.67	0.66	0.81

Table 2 Characteristics of newly developed gallstones

Characteristics		%	P
Overall			
Number	Single	48.90	< 0.0001
	Multiple	51.10	
Size	≤ 1.5 cm	77.90	< 0.0001
	≥ 1.5 cm	22.10	
Male			
Number	Single	44.80	< 0.0001
	Multiple	55.20	
Size	≤ 1.5 cm	78.70	< 0.0001
	≥ 1.5 cm	21.30	
Female			
Number	Single	52.30	< 0.0001
	Multiple	47.70	
Size	≤ 1.5 cm	77.10	< 0.0001
	≥ 1.5 cm	22.90	

Table 3 Risk factors for GD

Factor	Coefficient	SE	P
Men			
Age	0.0405	0.0071	< 0.000
BMI	0.0536	0.0195	< 0.006
HDL cholesterol	-0.0118	0.0059	< 0.040
Cholesterol	-0.0034	0.0016	< 0.030
Triglycerides	0.0004	0.0001	< 0.007
Diabetes	0.5293	0.2220	< 0.010
Peptic ulcer	0.4378	0.1753	< 0.010
Women			
Age	0.0279	0.0073	< 0.000
BMI	0.0178	0.0147	< 0.000

Logistic regression analysis: regression coefficients, corresponding SE, and P values for single factors and interactions, significantly associated with gallstones.

When evaluating the risk factor distribution according to the classification of the patient's clinical status (Table 4), no differences were observed in terms of age, BMI, and biochemical parameters while the presence of comorbidities increased among the three groups; in particular in males, diabetes, peptic ulcer, and myocardial infarction while, in females, peptic ulcer and myocardial infarction.

Predictors of GD: The evaluation of predictive factors for the presence of GD (Table 5) in patients with abdominal pain indicated that these were increasing age ($P < 0.04$) and pain in the right hypochondrium ($P < 0.03$) in males with gallstones and only increasing age

Table 4 Risk factors for GD

Factor OR (95% CI)	Gallstones not previously diagnosed (n = 312)	Gallstones previously diagnosed (n = 112)	Cholecystectomized (n = 61)
Men			
Age	1.03 (1.01-1.05)	1.05 (1.02-1.07)	1.01 (0.97-1.06)
BMI	1.06 (1.01-1.11)	1.03 (0.97- 1.10)	1.07 (0.96-1.06)
HDL cholesterol	0.99 (0.97-1.004)	0.98 (0.96-1.00)	1.00 (0.97-1.03)
Cholesterol	0.99 (0.99-1.00)	0.99 (0.98-0.99)	1.00 (0.99-1.01)
Triglycerides	1.00 (0.99-1.00)	1.00 (1.00-1.01)	0.99 (0.99-1.00)
Diabetes	1.75 (1.04-2.96)	1.60 (0.77-3.32)	2.72 (0.89-8.33)
Peptic ulcers	1.11 (0.69-1.77)	2.53 (1.52-4.20)	3.38 (1.48-7.72)
Myocardial infarction	0.99 (0.99-1.00)	1.27 (0.38-4.23)	1.43 (0.18-11.18)
Women			
Age	1.01 (1.00-1.03)	1.04 (1.02-1.07)	0.98 (0.94-1.02)
BMI	1.08 (1.04-1.12)	1.05 (1.00-1.11)	1.07 (0.99-1.15)
HDL cholesterol	1.00 (0.99-1.01)	0.98 (0.97-1.00)	0.98 (0.95-1.00)
Cholesterol	0.99 (0.99-1.00)	1.00 (0.99-1.00)	1.00 (1.00-1.01)
Triglycerides	1.00 (0.99-1.00)	0.99 (0.99-1.00)	0.99 (0.99-1.00)
Diabetes	1.10 (0.58-2.09)	0.48 (0.14-1.58)	1.00 (0.22-4.49)
Peptic ulcers	0.67 (0.34-1.34)	0.70 (0.28-1.76)	3.55 (1.57-8.04)
Myocardial infarction	1.83 (0.99-1.00)	3.15 (0.70-14.18)	12.82 (2.69-60.94)

OR calculated by logistic regression analysis; regression coefficients, corresponding standard errors, and *P* values for single factors and interactions, significantly associated with gallstones.

Table 5 Predictors of GD in patients with abdominal pain

Variables	Gallstones			Cholecystectomized		
	<i>P</i>	OR	95% CI	<i>P</i>	OR	95% CI
Male						
Age	0.04	1.033	1.00-1.06	0.02	1.05	1.00-1.10
Pain in the right hypochondrium	0.03	2.35	1.04-5.3	0.62		
Pain in the epigastrium	0.68	1.19		0.66		
Female						
Age	0.000	1.053	1.02-1.08	0.50		
Pain in the right hypochondrium	0.36			0.01	14.64	1.83-116.81
Pain in the epigastrium	0.09			0.002	24.19	3.18-183.92

Table 6 Predictors of GD according to the presence of concomitant diseases

Variables	Gallstones			Cholecystectomized		
	<i>P</i>	OR	95% CI	<i>P</i>	OR	95% CI
Male						
Age	0.0000	1.03	1.02-1.05	0.385		
Diabetes	0.0008	2.09	1.36-3.22	0.055		
Peptic ulcer	0.04	1.44	1.01-2.05	0.004	3.28	1.45-7.41
Cirrhosis	0.002	1.79	1.08-2.97	0.000	5.73	2.13-15.42
Female						
Age	0.0000	1.03	1.01-1.04	0.409		
Diabetes	0.522			0.208		
Peptic ulcer	0.186			0.004	3.19	1.43-7.08
Cirrhosis	0.388			0.742		

($P < 0.0001$) in females with gallstones; in cholecystectomized male patients, the only factor was increasing age ($P < 0.02$) while, in cholecystectomized females, pain in the right hypochondrium ($P < 0.01$) and the epigastrium ($P < 0.002$) were the predictive factors.

Considering only the effect of comorbidities, the evaluation of predictive factors for the presence of GD indicated that, in males with gallstones, these factors were increasing age ($P < 0.0001$), the presence of diabetes ($P < 0.001$), peptic ulcer ($P < 0.04$), and liver cirrhosis ($P < 0.002$) while, in females with gallstones, only increasing age ($P < 0.0001$) (Table 6) was a predictor of GD; in cholecystectomized male patients peptic ulcer ($P < 0.004$) and liver cirrhosis ($P < 0.0001$) were predictors while, in female patients, only peptic ulcer ($P < 0.004$) appeared as a predictor of the disease.

Clinical manifestation of GD

The distributions of abdominal symptoms in the four groups are reported in Table 7. No differences among

the groups were documented in terms of frequency for non-specific symptoms, with the exception of nausea, while pain in the epigastrium and the right hypochondrium were related to GD and varied according to the disease categories.

In Table 8, the characteristics of abdominal pain in the considered groups are reported: some characteristics resulted significantly associated with GD, showing a progressive increase in the ORs throughout the disease categories; pain necessitating rest, pain not relieved by bowel movements and the presence of clinical signs of gallstone complications (jaundice, fever, dark urine).

Predictive factors for biliary pain

No correlation was found between US gallstone characteristics (number and size) and their presence or clinical manifestation.

In males with GD, increasing age was the only predictor of biliary pain (mainly pain in the right hypochondrium) while, in females, BMI was also related to pain (mainly pain in the epigastrium). No correlation was found between comorbidities and GD.

Table 7 Distribution of symptoms (and unadjusted ORs) in gallstone-free subjects, patients with gallstones not previously diagnosed, patients with a previous diagnosis of gallstones, and patients with a history of cholecystectomy for gallstones

Symptoms (%) OR (95% CI)	Gallstone-free subjects (<i>n</i> = 9001)	Gallstones not previously diagnosed (<i>n</i> = 312)	Gallstones previously diagnosed (<i>n</i> = 112)	Cholecystectomized (<i>n</i> = 61)
Belching	19.1	19.5 1.03 (0.75-1.39)	27.2 1.27 (0.86-1.89)	23.1 0.92 (0.48-1.75)
Heartburn	32.2	33.1 1.12 (0.85-1.39)	37.5 0.89 (0.61-1.30)	44.2 1.29 (0.72-2.03)
Nausea	12.3	14.3 1.22 (0.82-1.82)	18.4 1.48 (0.90-2.42)	26.9 2.40 (1.19-4.83)
Vomiting	7.2	7.7 0.99 (0.59-1.64)	9.6 0.79 (0.41-1.53)	9.6 0.97 (0.40-2.33)
Bloated feeling after meals	37.2	38.6 1.24 (0.95-1.61)	44.9 1.12 (0.78-1.63)	48.1 1.24 (0.69-2.21)
Epigastric discomfort	33.8	33.1 0.96 (0.72-1.26)	40.4 1.26 (0.87-1.83)	38.5 0.87 (0.48-1.59)
Bitter taste in the morning	36.4	35.4 0.93 (0.71-1.21)	41.2 0.88 (0.61-1.26)	44.2 1.08 (0.61-1.88)
Heavy feeling on the right side	19.7	18.8 0.94 (0.68-1.29)	25.7 1.14 (0.77-1.71)	28.8 1.09 (0.58-2.05)
Heavy feeling in the epigastrium	22.7	21.3 0.83 (0.60-1.15)	25.0 0.81 (0.53-1.24)	26.9 0.67 (0.34-1.33)
Pain in the epigastrium	11.2	24.7 1.5 (1.17-2.4)	39.0 3.0 (2.6-5.7)	51.2 29.9 (7.0-221.3)
Pain in the right hipocondrium	15.8	23.7 1.7 (1.3-2.8)	39.0 3.9 (3.1-7.4)	46.5 32.1 (9.3-238.9)
Intolerance to fatty or fried foods	24.0	5 0.99 (0.63-1.55)	4.4 0.94 (0.19-1.79)	3.4 1.04 (0.5-2.14)

DISCUSSION

The present study evaluated incidence and risk factors for GD in a large population from various Italian regions, thus providing a more detailed picture of the epidemiological characteristics of this disease. Incidence was higher in females than in males and increased with age. In our population, the risk factors for GD in males were increasing age, BMI, concomitant diseases such as diabetes, liver cirrhosis, peptic ulcer, coronary disease, HDL and total cholesterol, and high levels of triglycerides while, in females, only increasing age and BMI. Increasing age, pain in the right hypochondrium/epigastrium, and the presence of concomitant diseases are predictors of GD. Pain in the right hypochondrium or epigastrium was the only symptom associated with GD; symptom severity increased as a function of the natural history of the disease. Increasing age in men and aging and BMI in females were the only predictive factors for the eventual presence of symptoms.

GD is a very common gastrointestinal disorder mainly in the Western world^[1,2]; although this disease has a low mortality rate, its economic and health impact is significant due to its high morbidity. In fact, GD is one of the most common abdominal conditions for which patients are admitted to hospitals in developed countries^[3]. Knowledge of disease epidemiology is therefore crucial in managing this disorder, not only for planning preventive programs, but also for the identification of the best therapeutic strategy. Several US-based surveys have been carried out in Europe^[19-23] and in North^[24,25] and South^[26] America as well as in Asia^[27,28], indicating prevalence rates for GD ranging

from 5.9%^[20] to 21.9%^[22]. However, few studies^[11-14] have been carried out to evaluate incidence and risk factors of GD, mainly due to the difficulties in following up large populations for several years. The MICOL study was designed to obtain a general overview of GD in Italy, investigating GD in terms of prevalence, incidence, risk factors, and natural history^[15]. In the present study, a large general population was evaluated with the objective of identifying gallstone incidence and risk factors as well as the morphological and clinical characteristics of newly developed gallstones. Incidence was higher than that measured in previous Italian studies^[12-14]; these differences could be related to the small population sample evaluated in the earlier studies. Furthermore, prevalence was also higher than in Denmark (4.5% and 5.8%, in males and females, respectively)^[11]. Differences in research design may justify these differences even if it is not possible to exclude the role of environmental factors.

In the present study, the response rate was higher (79%) than that reported in other GD incidence surveys performed in the north (63.7%)^[12] and in the centre (73.5%)^[14] of Italy and similar to that observed in Denmark (82.8%)^[11] and in southern Italy (87.7%)^[13]; this percentage indicates a high adherence of the target population to the epidemiological study.

The participation rate was also higher in the present incidence study (79%) than in the previous study evaluating GD prevalence (64.4%)^[15]; this difference could be related to a possible self-selection of patients or to an effect of dilution in the prevalence study since, in that study, 14 units participated while, in the present study, only 7 units were able to adhere to the protocol.

Table 8 Distribution of pain characteristics (and unadjusted ORs) in subjects reporting abdominal pain

Characteristics of Pain	Gallstone-free subjects	Gallstones not previously diagnosed (n = 312)	Gallstones previously diagnosed (n = 112)	Cholecystectomized (n = 61)
Radiation				
Not radiated (%)	53.0	55.4	43.9	53.5
Radiated (%)	37.4	41.5	45.6	46.5
OR (95% CI)		0.97 (0.63-1.50)	1.11 (0.74-1.66)	0.94 (0.57-1.53)
Duration ¹				
< ½ h (%)	30.3	35.5	7.8	16.3
½-1 h (%)	21.1	19.4	21.6	16.3
1-3 h (%)	16.0	19.4	31.4	18.6
> 3 h (%)	32.6	25.8	39.2	48.8
OR (95% CI)		0.89 (0.71-1.10)	1.35 (1.05-1.75)	1.27 (0.96-1.69)
Tollerability				
Not forced to rest (%)	80.5	68.3	62.0	25.6
Forced to rest (%)	19.5	31.7	38.0	74.4
OR (95% CI)		1.98 (1.11-3.51)	2.26 (1.22-4.18)	9.88 (4.82-20.27)
Relationship with meals				
Yes (%)	13.2	9.7	10.2	9.5
No (%)	86.8	90.3	89.8	90.5
OR (95% CI)		1.84 (0.64-5.23)	1.30 (0.47-3.63)	1.29 (0.37-4.47)
During meals				
Yes (%)	2.6	1.7	6.1	
No (%)	97.4	98.3	93.9	100
OR (95% CI)		0.94 (0.12-7.32)	0.38 (0.10-1.48)	129.09 (0.90-4.62)
Soon after meals				
Yes (%)	32.3	24.6	43.1	40.5
No (%)	67.7	75.4	56.9	59.5
OR (95% CI)		1.61 (0.84-3.09)	0.70 (0.37-1.31)	0.63 (0.31-1.25)
After heavy meals				
Yes (%)	24.5	23.8	26.0	23.8
No (%)	75.5	76.2	74.0	76.2
OR (95% CI)		1.02 (0.55-1.91)	0.73 (0.38-1.38)	1.82 (0.77-4.33)
Relieved by bowel movements				
Yes (%)	41	30	28	12.2
No (%)	59	70	72	87.8
OR (95% CI)		2.02 (1.07-3.82)	1.76 (0.99-3.10)	5.35 (2.07-13.76)
Clinical signs of gallstone complications				
Yes (%)	29.9	36.4	47.7	69.4
No (%)	70.1	63.6	52.3	30.7
OR (95% CI)		1.31 (0.69-2.46)	2.13 (1.16-3.94)	5.28 (2.56-10.9)

¹The category "< ½ h" has been taken as baseline.

We documented variability between the different units in terms of incidence; this difference could be related to the role of environmental factors (life style, dietary habit, *etc.*) even if we were unable to identify any possible difference between the different operative units.

Gender, increasing age, and BMI were confirmed as true risk factors for GD; in males, low levels of cholesterol, high levels of triglycerides, and the presence of co-morbidities such as diabetes, peptic ulcer, angina, and liver cirrhosis represented additional risk factors. These results further confirm the importance of environmental factors in gallstone development, possibly related to an unhealthy life style. In fact, recent epidemiological studies have suggested that GD may be included in those disorders which characterize the metabolic syndrome^[29,30]. In particular, a close relationship between obesity and cardiovascular disease (two of the more characteristic features of the metabolic syndrome) and GD has been identified^[31,32].

We did not confirm a family history of gallstones, dieting, the number of pregnancies, and the use of

contraceptive pills, which were found to be significantly associated with GD in the prevalence study as risk factors^[16]. We are unable to interpret the significance of this result; however, the data available on some risk factors for GD are frequently conflicting^[8,10]. The prospective cohort design of the present study and the use of US as the diagnostic tool have reduced the possibility of a recall and other information bias; furthermore, the high response rate makes selection bias unlikely.

An important result of the present study is related to the clinical manifestation of incident gallstones. In fact, we have confirmed the observation made in the prevalence study^[15,17] that pain in the right hypochondrium and/or epigastrium is the only symptom significantly associated with gallstones while the so-called "non-specific biliary symptoms", i.e. dyspeptic symptoms, showed the same frequency in gallstone-free subjects and GD patients.

We have also confirmed our previous observation^[17] concerning the usefulness of splitting the subjects

enrolled in the study into 4 categories reproducing the different stages of GD (absence of disease, silent disease, overt disease, severe disease); in fact, the frequency and severity of the clinical symptoms and the signs of GD increased throughout the 3 disease categories.

Furthermore, for those characteristics that are an expression of the degree of pain severity (pain forcing to rest, presence of clinical signs of gallstone complications) they progressively increased from silent gallstones to symptomatic gallstones to cholecystectomized patients for gallstones, suggesting that the natural history of GD moves from a silent to a clinically evident stage; this is also true for newly developed gallstones. This information may be useful in choosing the best therapeutic strategy in gallstone patients since surgical treatment is indicated only in true symptomatic gallstone patients^[5,33].

Finally, we were unable to identify any predictive factor for the presence of biliary pain in terms of gallstone characteristics (number and size), while increasing age in males and a high BMI in females were related to the presence of biliary pain. These results are in agreement with some^[34], but in disagreement with others^[35,36].

In conclusion, this study provides data on the gallstone incidence and risk factors for GD in a large free-living population; the incidence rate is higher in females and it increases with age. Increasing age and BMI represent true risk factors for GD; pain in the right hypochondrium and/or epigastrium is confirmed as the only symptom related to GD; pain, as well as its characteristics of disease severity, increases in severity and frequency throughout the different stages of GD (from silent to severe disease). This information may help physicians in clinical decision making.

COMMENTS

Background

Gallstone incidence and risk factors are poorly understood and the relationship between gallstone presence and its clinical manifestations is debated.

Research frontiers

Gallstone incidence, risk factors, and clinical manifestations.

Innovations and breakthroughs

The present paper provides important information on gallstone incidence and risk factors and clearly identifies the clinical manifestations of the disease.

Applications

The results of the present paper can be useful for the early recognition of gallstones and for deciding the most appropriate therapeutic management according to the clinical presentation. Furthermore, preventive strategies can be identified and planned according to these results.

Peer review

This manuscript is a multicenter Italian study giving information about the incidence of gallstone disease in Italy. The results of this study can provide information for the comparison of Italian population results with the other nations' results.

REFERENCES

- 1 Sandler RS, Everhart JE, Donowitz M, Adams E, Cronin K, Goodman C, Gemmen E, Shah S, Avdic A, Rubin R. The

- burden of selected digestive diseases in the United States. *Gastroenterology* 2002; **122**: 1500-1511
- 2 Aerts R, Penninckx F. The burden of gallstone disease in Europe. *Aliment Pharmacol Ther* 2003; **18** Suppl 3: 49-53
- 3 Russo MW, Wei JT, Thiny MT, Gangarosa LM, Brown A, Ringel Y, Shaheen NJ, Sandler RS. Digestive and liver diseases statistics, 2004. *Gastroenterology* 2004; **126**: 1448-1453
- 4 Bateson MC. Gallstones and cholecystectomy in modern Britain. *Postgrad Med J* 2000; **76**: 700-703
- 5 Roda E, Festi D, Lezoche E, Leushner U, Paumgartner G, Sauerbruch T. Strategies in the treatment of biliary stones. *Gastroenterol Int* 2000; **13**: 7-15
- 6 National Institutes of Health Consensus Development Conference Statement: Gallstones and Laparoscopic Cholecystectomy. September 14-16, 1992. *J Laparoendosc Surg* 1993; **3**: 77-90
- 7 Legorreta AP, Silber JH, Costantino GN, Kobylinski RW, Zatz SL. Increased cholecystectomy rate after the introduction of laparoscopic cholecystectomy. *JAMA* 1993; **270**: 1429-1432
- 8 Shaffer EA. Epidemiology and risk factors for gallstone disease: has the paradigm changed in the 21st century? *Curr Gastroenterol Rep* 2005; **7**: 132-140
- 9 Kratzner W, Mason RA, Kachele V. Prevalence of gallstones in sonographic surveys worldwide. *J Clin Ultrasound* 1999; **27**: 1-7
- 10 Diehl AK. Epidemiology and natural history of gallstone disease. *Gastroenterol Clin North Am* 1991; **20**: 1-19
- 11 Jensen KH, Jorgensen T. Incidence of gallstones in a Danish population. *Gastroenterology* 1991; **100**: 790-794
- 12 Barbara L, Sama C, Morselli Labate AM, Taroni F, Rusticali G, Festi D. A 10-year incidence of gallstone disease: the Sirmione study. *J Hepatol* 1993; **18** Suppl 1: 104A
- 13 Misciagna G, Leoci C, Elba S, Petruzzi J, Guerra V, Mossa A, Noviello M, Coviello A, Capece-Minutolo M, Giorgiot T. The epidemiology of cholelithiasis in southern Italy. *Eur J Gastroenterol Hepatol* 1994; **6**: 937-941
- 14 Angelico F, Del Ben M, Barbato A, Conti R, Urbinati G. Ten-year incidence and natural history of gallstone disease in a rural population of women in central Italy. The Rome Group for the Epidemiology and Prevention of Cholelithiasis (GREPCO). *Ital J Gastroenterol Hepatol* 1997; **29**: 249-254
- 15 Attili AF, Carulli N, Roda E, Barbara B, Capocaccia L, Menotti A, Okolicsanyi L, Ricci G, Capocaccia R, Festi D. Epidemiology of gallstone disease in Italy: prevalence data of the multicenter Italian study on cholelithiasis (MICOL). *Am J Epidemiol* 1995; **141**: 158-165
- 16 Attili AF, Capocaccia R, Carulli N, Festi D, Roda E, Barbara L, Capocaccia L, Menotti A, Okolicsanyi L, Ricci G, Lalloni L, Mariotti S, Sama C, Scafato E. Factors associated with gallstone disease in the MICOL experience. Multicenter Italian Study on Epidemiology of Cholelithiasis. *Hepatology* 1997; **26**: 809-818
- 17 Festi D, Sottili S, Colecchia A, Attili A, Mazzella G, Roda E, Romano F. Clinical manifestations of gallstone disease: evidence from the multicenter Italian study on cholelithiasis (MICOL). *Hepatology* 1999; **30**: 839-846
- 18 Misciagna G, Leoci C, Guerra V, Chiloire M, Elba S, Petruzzi J, Mossa A, Noviello MR, Coviello A, Minutolo MC, Mangini V, Messa C, Cavallini A, De Michele G, Giorgio I. Epidemiology of cholelithiasis in southern Italy. Part II: Risk factors. *Eur J Gastroenterol Hepatol* 1996; **8**: 585-593
- 19 Barbara L, Sama C, Morselli Labate AM, Taroni F, Rusticali AG, Festi D, Sapio C, Roda E, Banterle C, Puci A. A population study on the prevalence of gallstone disease: the Sirmione Study. *Hepatology* 1987; **7**: 913-917
- 20 Loria P, Dilengite MA, Bozzoli M, Carubbi F, Messori R, Sassatelli R, Bertolotti M, Tampieri A, Tartoni PL, Cassinadri M. Prevalence rates of gallstone disease in Italy. The Chianciano population study. *Eur J Epidemiol* 1994; **10**: 143-150

- 21 **Jorgensen T.** Prevalence of gallstones in a Danish population. *Am J Epidemiol* 1987; **126**: 912-921
- 22 **Glambek I, Kvaale G, Arnesjo B, Soreide O.** Prevalence of gallstones in a Norwegian population. *Scand J Gastroenterol* 1987; **22**: 1089-1094
- 23 **Heaton KW, Braddon FE, Mountford RA, Hughes AO, Emmett PM.** Symptomatic and silent gall stones in the community. *Gut* 1991; **32**: 316-320
- 24 **Everhart JE, Khare M, Hill M, Maurer KR.** Prevalence and ethnic differences in gallbladder disease in the United States. *Gastroenterology* 1999; **117**: 632-639
- 25 **Everhart JE, Yeh F, Lee ET, Hill MC, Fabsitz R, Howard BV, Welty TK.** Prevalence of gallbladder disease in American Indian populations: findings from the Strong Heart Study. *Hepatology* 2002; **35**: 1507-1512
- 26 **Miquel JF, Covarrubias C, Villaroel L, Mingrone G, Greco AV, Puglielli L, Carvallo P, Marshall G, Del Pino G, Nervi F.** Genetic epidemiology of cholesterol cholelithiasis among Chilean Hispanics, Amerindians, and Maoris. *Gastroenterology* 1998; **115**: 937-946
- 27 **Khuroo MS, Mahajan R, Zargar SA, Javid G, Sapru S.** Prevalence of biliary tract disease in India: a sonographic study in adult population in Kashmir. *Gut* 1989; **30**: 201-205
- 28 **Nomura H, Kashiwagi S, Hayashi J, Kajiyama W, Ikematsu H, Noguchi A, Tani S, Goto M.** Prevalence of gallstone disease in a general population of Okinawa, Japan. *Am J Epidemiol* 1988; **128**: 598-605
- 29 **Mendez-Sanchez N, Chavez-Tapia NC, Motola-Kuba D, Sanchez-Lara K, Ponciano-Rodriguez G, Baptista H, Ramos MH, Uribe M.** Metabolic syndrome as a risk factor for gallstone disease. *World J Gastroenterol* 2005; **11**: 1653-1657
- 30 **Grundy SM.** Cholesterol gallstones: a fellow traveler with metabolic syndrome? *Am J Clin Nutr* 2004; **80**: 1-2
- 31 **Tsai CJ, Leitzmann MF, Willett WC, Giovannucci EL.** Prospective study of abdominal adiposity and gallstone disease in US men. *Am J Clin Nutr* 2004; **80**: 38-44
- 32 **Mendez-Sanchez N, Bahena-Aponte J, Chavez-Tapia NC, Motola-Kuba D, Sanchez-Lara K, Conciano-Rodriguez G, Ramos MH, Uribe M.** Strong association between gallstones and cardiovascular disease. *Am J Gastroenterol* 2005; **100**: 827-830
- 33 **Guidelines for the treatment of gallstones.** American College of Physicians. *Ann Intern Med* 1993; **119**: 620-622
- 34 **Gruppo Romano per la Epidemiologia e la Prevenzione della Colelitiasi (GREPCO).** Radiologic appearance of gallstones and its relationship with biliary symptoms and awareness of having gallstones. Observations during epidemiological studies. Rome Group for the Epidemiology and Prevention of Cholelithiasis (GREPCO). *Dig Dis Sci* 1987; **32**: 349-353
- 35 **Ros E, Valderrama R, Bru C, Bianchi L, Teres J.** Symptomatic versus silent gallstones. Radiographic features and eligibility for nonsurgical treatment. *Dig Dis Sci* 1994; **39**: 1697-1703
- 36 **Juvonen T, Niemela O, Makela J, Kairaluoma MI.** Characteristics of symptomatic gallbladder disease in patients with either solitary or multiple cholesterol gallstones. *Hepatogastroenterology* 1994; **41**: 263-266

S- Editor Li DL L- Editor Mihm S E- Editor Yin DH



CLINICAL RESEARCH

Ileal lesions in patients with ulcerative colitis after ileo-rectal anastomosis: Relationship with colonic metaplasia

Livia Biancone, Emma Calabrese, Giampiero Palmieri, Carmelina Petruzzello, Sara Onali, Giuseppe Sigismondo Sica, Marta Cossignani, Giovanna Condino, Kiron Moy Das, Francesco Pallone

Livia Biancone, Emma Calabrese, Carmelina Petruzzello, Sara Onali, Marta Cossignani, Giovanna Condino, Francesco Pallone, Cattedra di Gastroenterologia, Dipartimento di Medicina Interna, Università di "Tor Vergata", Roma 00133, Italy
Giampiero Palmieri, Cattedra di Anatomia ed Istologia Patologica, Università di "Tor Vergata", Roma 00133, Italy
Giuseppe Sigismondo Sica, Cattedra di Chirurgia Generale, Università di "Tor Vergata", Roma 00133, Italy

Kiron Moy Das, GI Unit, Department of Gastroenterology & Hepatology, University of Medicine & Dentistry of New Brunswick, New Brunswick NJ 08903, United States

Author contributions: Biancone L designed research, performed endoscopies and wrote the paper; Calabrese E, Petruzzello C, Onali S, Condino G performed the clinical assessment and recruitment of patients; Cossignani M performed research and collected the data; Palmieri G performed immunohistochemistry; Sica GS gave his surgical support; Pallone F contributed to writing the paper; Das KM designed research, provided reagents and contributed to wrote the paper.

Supported by The Fondazione Umberto Di Mario, Largo Marchiafava n. 1, ZIP code 00161, Roma, Italy

Correspondence to: Livia Biancone, MD, PhD, Cattedra di Gastroenterologia, Dipartimento di Medicina Interna, Università "Tor Vergata", Via Montpellier, 1, Rome 00133, Italy. biancone@med.uniroma2.it

Telephone: +39-6-20903737 Fax: +39-6-20903738

Received: May 2, 2008 Revised: June 20, 2008

Accepted: June 27, 2008

Published online: September 14, 2008

RESULTS: Stenosing adenocarcinoma of the rectal stump was detected in 1 UC patient. The neo-terminal ileum was therefore investigated in 10/11 UC patients. Ileal ulcers were detected in 7/10 UC, associated with colonic metaplasia in 4/7 (57.1%) and Das-1 and CG3 reactivity in 3/4 UC. In controls, recurrence occurred in 4/6 CD, associated with colonic metaplasia in 3/4 and reactivity with Das-1 and CG3 in 2/3.

CONCLUSION: Present findings suggest that in UC, ileal lesions associated with changes towards colonic epithelium may develop also after IRA. Changes of the ileal content after colectomy may contribute to the development of colonic metaplasia, leading to ileal lesions both in the pouch and in the neo-terminal ileum after IRA.

© 2008 The WJG Press. All rights reserved.

Key words: Ulcerative colitis; Ileo-rectal anastomosis; Ileal lesions; Colonic metaplasia

Peer reviewer: Jay Pravda, MD, Inflammatory Disease Research Center, Gainesville, Florida 32614-2181, United States

Biancone L, Calabrese E, Palmieri G, Petruzzello C, Onali S, Sica GS, Cossignani M, Condino G, Das KM, Pallone F. Ileal lesions in patients with ulcerative colitis after ileo-rectal anastomosis: Relationship with colonic metaplasia. *World J Gastroenterol* 2008; 14(34): 5290-5300 Available from: URL: <http://www.wjgnet.com/1007-9327/14/5290.asp> DOI: <http://dx.doi.org/10.3748/wjg.14.5290>

Abstract

AIM: To assess whether in ulcerative colitis (UC) patients with ileo-rectal anastomosis (IRA), ileal lesions may develop in the neo-terminal-ileum and their possible relation with phenotypic changes towards colonic epithelium.

METHODS: A total of 19 patients with IRA under regular follow up were enrolled, including 11 UC and 8 controls (6 Crohn's disease, CD; 1 familial adenomatous polyposis, FAP; 1 colon cancer, colon K). Ileal lesions were identified by ileoscopy with biopsies taken from the ileum (involved and uninvolved) and from the rectal stump. Staining included HE and immunohistochemistry using monoclonal antibodies against colonic epithelial protein CEP (Das-1) and human tropomyosin isoform 5, hTM5 (CG3). Possible relation between development of colonic metaplasia and ileal lesions was investigated.

INTRODUCTION

Inflammatory changes of the distal ileum in ulcerative colitis (UC) may be observed in backwash ileitis^[1-3] and after total proctocolectomy with ileal pouch ("pouchitis")^[4-8]. Chronic inflammation of the ileal pouch has been described not only in patients with UC^[9-13], but also after total proctocolectomy for other conditions, including Familial Adenomatous Polyposis (FAP). The frequency of pouchitis is more frequent in UC (60%-70% at 1 years)^[14-16] than in FAP (< 50% at 1 years), thus suggesting that the underlying UC may be involved in the pathogenesis of pouchitis^[8,14,17]. Common bacterial flora also appears to play a major role in

pouchitis, as suggested by both experimental and clinical evidences, supporting the efficacy of probiotics in subsiding symptoms of pouchitis in UC^[18-22]. Although the etiology of pouchitis is unknown, the development of changes of the ileal mucosa lining the pouch, including flattening, reduced number and/or complete villar atrophy has been involved in UC^[4,23,24]. These changes of the ileum, becoming similar to the colonic epithelium (“colonic metaplasia”) have been reported more frequently associated with pouchitis. Changes of the resident bacterial flora after proctocolectomy have therefore been involved in the development of both colonic metaplasia and pouchitis.

Proctocolectomy represented the procedure of choice in severe UC over the past 30 years^[25], and permanent ileostomy^[26,27] is still a valid option in an elderly patient in whom either an ileal pouch or an ileo-rectal anastomosis (IRA) is contraindicated. Nowadays, colectomy with IRA^[28-30] must be compared with the more recent sphincter saving procedure, namely restorative proctocolectomy. Ravitch and Sabiston^[31] are rightly given the credit for the development of total colectomy and ileo-anal pouch, representing the preferred surgical option in referral IBD surgical unit^[32].

IRA for UC determines the persistence of the diseased rectal stump requiring local treatment and cancer surveillance, thus being rarely performed in UC, due to technical feasibility or clinical requirements. It is conceivable that not only after ileal pouch, but also after IRA, the neo-terminal ileum may develop inflammatory changes.

In order to investigate this issue, we aimed to assess whether inflammatory changes of the ileum in UC may also be observed after IRA. We also explored whether these changes of the neo-terminal ileum are associated with the development of colonic metaplasia of the ileum itself. For this purpose, colonic metaplasia of the ileum was investigated in biopsy samples taken from the neo-terminal ileum of patients with IRA for UC, by both conventional histological assessment and immunohistochemistry, using monoclonal antibodies (MoAb) against colonic epithelial antigens (CEP and hTM5)^[23,33-36]. In one UC patient, ileal lesions were also assessed by wireless capsule endoscopy (WCE). As controls, a small group of patients with IRA for Crohn's disease (CD), FAP or colon cancer (colon K) were also investigated as patients with IRA for UC.

MATERIALS AND METHODS

Patients

A total of 19 patients with IRA in regular follow up at the University “Tor Vergata” of Rome, Italy, were studied. Clinical characteristics of each patient are reported in Table 1. The study population included patients with IRA for UC ($n = 11$), and, as control group, 6 patients with IRA for CD, 1 for FAP and 1 for adenocarcinoma of the sigmoid colon (colon K). In all UC patients and controls the diagnosis was confirmed by macroscopic and histological analysis of the surgical

Table 1 Demographic and clinical characteristics of patients with IRA for UC, CD, FAP and colon cancer

Patient	Sex	Age	Disease duration before surgery (yr)	Indication for surgery
UC				
DLE	M	50	7	Refractory UC
LRA	F	25	7	CS-Refractory
DFML	F	49	2	CS-Refractory
ML	M	37	1	Refractory UC
BM	F	46	1	Toxic megacolon
TE	M	47	8	Refractory UC
SC	F	39	1	Refractory UC
CF	M	36	8	Toxic megacolon
LP	F	50	20	Refractory UC
NF	M	63	8	Refractory UC
TR	F	47	11	Toxic megacolon
CD				
DAD	F	37	5	Colonic stenosis
MA	M	83	0	Colonic stenosis
CP	F	51	24	Pelvic abscess
DCE	M	30	5	Obstruction
DLLV	M	22	1	Refractory CD
CRL	F	59	1	Recto-vaginal fistula
FAP				
ZG	F	57	16	FAP
Colon K				
ML	F	68	1	Colon cancer

CS-Refractory: Steroid-refractory disease.

specimen. Clinical history, endoscopic, surgical and histological findings were reviewed for the possibility that endoscopic findings after surgery would suggest possible changes of previous diagnosis (i.e. ileal lesions in UC). The study group included 11 UC patients (5 M; median age 46; range 25-63), with a median UC duration of 7 years (range 1-20), a median time interval from surgery of 4 years (range 1-12). Indication for surgery was adenocarcinoma in the rectal stump ($n = 1$), medical intractability ($n = 8$), toxic megacolon ($n = 2$). Among the 8 controls, there were 6 patients with CD (3 M, median age 51, range 22-83), with a median CD duration of 3 years (range 0-24), a median time interval from surgery of 4 years (range 1-20). Indication was stenosis of the sigmoid colon ($n = 2$), recto-vaginal fistula ($n = 1$), obstruction ($n = 1$), pelvic abscess ($n = 1$), refractory CD ($n = 1$). Control group also included 2 patients with IRA for FAP (F, age 57) ($n = 1$) and adenocarcinoma of the sigmoid colon (F, age 68) ($n = 1$).

Study protocol

Endoscopy: Endoscopy was performed according to clinical criteria, including possible treatment changes, cancer surveillance, or recurrence assessment (in CD). According to these criteria, 9 IBD patients underwent repeated endoscopic assessments of the rectum and ileum, in a median follow up of 4 years (range 1-20). The numbers of endoscopic assessment were: 21 in the 11 UC, 12 in the 6 CD, and 1 in the only FAP and colon cancer patients. Persistence or healing of the ileal lesions was searched in patients undergoing repeated endoscopies. All endoscopies were performed by the same gastroenterologist (LB), with pictures taken from



Figure 1 Endoscopic view of the rectal stump from one UC patient (TR) showing a hard and ulcerated mass inducing stenosis of the anastomosis, not passed by the colonoscope. Histological analysis of the lesion detected an adenocarcinoma of the rectal stump.

the rectum, anastomosis and neo-terminal ileum. In UC, the endoscopic degree of inflammation was graded according to the Mayo score (0-4)^[37]. The presence and degree of ileal lesions visualized by the endoscope was searched and described as: erosions, aphthoid or deep ulcers, strictures and/or stenosis. The extent of the lesions (cm), the number of ulcerations (few or diffuse: < 5 or \geq 5) and macroscopic aspect of the ileum between ulcers were also reported. In CD, endoscopic recurrence was graded according to Rutgeerts *et al* (from 0 to 4)^[38].

Histology: In all patients biopsies ($n = 2$) were taken from the neo-terminal ileum (uninvolved and involved area), anastomosis and rectal stump. In all patients, at least 10 cm of the neo-terminal ileum were visualized. When considering all biopsy samples taken during repeated endoscopies, 90 biopsy samples (180 biopsies) were taken from the ileum, anastomotic area and rectum. In UC, biopsies were taken from the neo-terminal ileum (macroscopically uninvolved $n = 17$, involved $n = 12$), anastomosis ($n = 3$) and rectum ($n = 18$). In controls, biopsies were taken: in CD from the ileum (uninvolved $n = 8$; involved $n = 8$), anastomosis ($n = 6$) and rectal stump ($n = 11$), in FAP ($n = 1$) and colon cancer patients ($n = 1$) from the uninvolved $n = 2$ or involved $n = 1$ ileum, anastomosis ($n = 2$) and rectum ($n = 2$). Biopsy samples were kept in 10% formalin. Paraffin blocks were used for routine histology by HE staining and for immunohistochemistry by immunoperoxidase staining. Histological assessment was made by one single histopathologist (GP), in order to: (1) Confirm the diagnosis of UC or CD; (2) Assess the presence and degree of inflammation; (3) Detect dysplasia/neoplasia in the rectal stump; (4) Detect changes of the epithelium lining the ileum towards colonic epithelium. Inflammatory changes were assessed according to conventional criteria^[4,23,39]. Histologic elements of inflammatory and colonic metaplasia were assessed according to Fruin *et al*^[24], including: (1) An inflammatory score considering histological characteristics of the villi epithelia, crypt epithelia, stroma and ulceration (A score:

range 0-28); (2) Colonic metaplasia score considering characteristics of villous atrophy and crypt hyperplasia (B score: range 0-6).

Immunohistochemistry: In order to detect possible ileal changes towards colonic epithelium, the expression of colonic antigens was assessed by immunoperoxidase using 2 MoAbs against human tropomyosin isoform 5 (hTM5) (CG3) and against the > 200 kDa colonic epithelial antigen (Das-1)^[33-36]. Sections were stained as reported previously^[33-36].

Clinical assessment: Clinical assessment was made according to the Mayo score in UC^[37] and to the CD Activity Index (CAI) in CD^[40].

WCE: In one compliant UC patient, the presence and extent of the lesions in the neo-terminal ileum was also investigated by WCE. WCE examination was performed using the Given M2A capsule (Given Imaging, Yoqneam, Israel^[41,42]) as described^[43], by using bowel preparation with PEG (2 L). Exclusion criteria included: low compliance, diverticula, blind loop, pace-maker, neurological disorders, intestinal strictures. WCE images were assessed by one gastroenterologist. The detection of the following lesions was reported: erosions, ulcers, strictures or stenosis. Any other lesion was also reported.

The study was approved by the Local Ethic committee.

RESULTS

UC patients

In one UC patient, an anastomotic stricture that could not be passed by the endoscope was detected. A hard and ulcerated area was present in the rectal stump, compatible with adenocarcinoma, confirmed by histology (Figure 1). The ileum was therefore visualized in 10/11 patients. Figure 2A shows the percentage of UC patients with endoscopic lesions in the neo-terminal ileum, together with changes towards colonic epithelium, as detected by both conventional histology and staining using CG3 and Das-1 MoAbs. As indicated, ileal lesions were detected at first endoscopy in 7/10 patients. Histological analysis of the macroscopically involved ileum detected changes towards colonic epithelium in 4/7 patients. CG3 and Das-1 MoAbs staining were observed in ileal samples from 3 out of these 4 patients. The endoscopic and histological views of the neo-terminal ileum and rectal stump from 2 UC patients with lesions associated or not with changes towards colonic epithelium are shown in Figures 3 and 4, respectively.

Clinical assessment: At first endoscopy, UC was clinically active (Mayo score) in 5 and inactive in 6 patients.

Endoscopy: As the neo-terminal ileum was not visualized in 1 UC patient due to an adenocarcinoma of

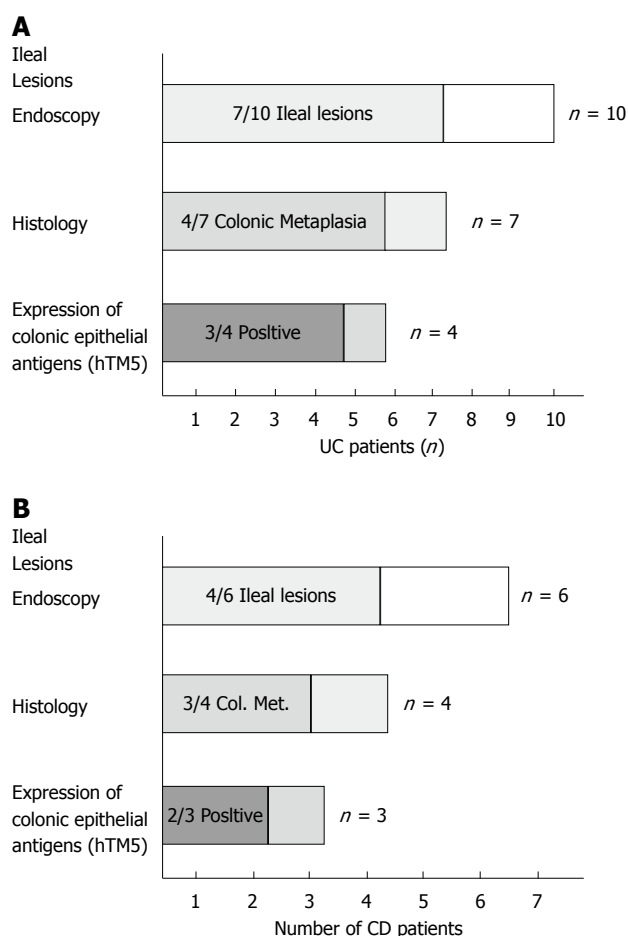


Figure 2 Histograms indications. **A:** The number of UC patients after IRA showing: Lesions in the neo-terminal ileum as assessed by endoscopy; Histological findings compatible with colonic metaplasia; Expression of hTM5-related colon epithelial antigens; **B:** The number of CD patients with IRA showing ileal recurrence at endoscopy, inflammatory changes at histology (including those compatible with colonic metaplasia) and expression of hTM5-related colon epithelial antigens.

the rectal stump (Figure 1), the ileum was visualized in 10/11 patients. Among these 10 patients, macroscopic lesions were detected in the rectal stump in 8, in the anastomosis in 6, in the ileum in 7. Rectal lesions in the 8 patients included erosions ($n = 2$) and ulcers ($n = 6$). In 2/6 patients with anastomotic lesions, a stricture that could be passed by the endoscope was observed. Table 2 shows the endoscopic and histological findings in each of the 11 UC patients studied. Among the 10 patients, 7 showed macroscopic lesions in the ileum proximal to the IRA at first endoscopy. Lesions were localized within 15 cm above the anastomosis in all patients, including ulcers, sporadic (< 5) in 5 (apthoid, $n = 3$; deep, $n = 2$) and diffuse (> 5) in 2 UC. In all 7 patients, ileal lesions were surrounded by macroscopically uninvolved areas, while the rectal stump showed typical UC lesions. All 6 UC with anastomotic lesions also showed ileal lesions, while 1 patient with ileal lesions showed a normal anastomosis.

Histology: HE staining. Adenocarcinoma of the rectal stump was detected in 1 patient (Figure 1) and

inflammatory changes in the remaining 10. In the macroscopically uninvolved ileum, biopsies were taken from 7/10 UC, showing inflammatory changes ($n = 2$), lamina propria oedema ($n = 3$) or no lesions ($n = 2$) (Table 2). In the macroscopically involved ileum, histology detected inflammation in all 7 UC (acute and chronic inflammation $n = 3$; villous atrophy $n = 4$). The anastomotic area showed inflammation in 3/6 UC.

Assessment of changes of the ileal epithelium toward colonic epithelium. Changes towards colonic epithelium are observed in the macroscopically uninvolved ileum from 7/10 UC, showing an inflammatory infiltrate in 3 (A1, $n = 1$; A2, $n = 2$), no inflammation in 4 and colonic metaplasia in 1 (B2) (Table 2). Changes toward colonic epithelium were found in the 7/10 UC with ileal lesions, showing inflammatory changes in 4 (A12, $n = 1$; A10, $n = 1$; A6, $n = 2$). Ileal changes towards colonic epithelium have been detected in the 4/7 UC with ileal lesions (A12, $n = 1$; A4, $n = 1$; A3, $n = 1$; A2, $n = 1$).

Figures 3A to C and 4A to C show the endoscopic, histologic and immunohistochemistry analysis of biopsy samples taken from 4 additional UC patients with IRA.

Immunohistochemistry: Expression of hTM5-related antigens was seen in the uninvolved ileum from 6/10 UC, showing CG3 staining in all 6 and Das-1 staining in 5/6 UC. The involved ileum showed CG3 staining in 5/6 UC and Das-1 staining in 3/6 UC.

Longitudinal study: Endoscopy. Among the 7 patients with ileal lesions, 6 underwent repeated endoscopies ($n = 5$ in 1; $n = 2$ in 4; $n = 3$ in 1). Ileal lesions were detected at all endoscopies in 3/6 UC, at first but not at second endoscopy in 2, healing at third endoscopy in 1 UC. Figure 5 shows the endoscopic, histologic and immunohistochemical analysis of the rectum, involved and uninvolved ileum from one UC patient at 3 consecutive endoscopies.

Histology. Ileal lesions at endoscopy were confirmed by histology in all UC patients. One patient showed ileal changes toward colonic epithelium at all endoscopies (Figure 3).

Immunohistochemistry. In UC, biopsy samples were taken during repeated endoscopies from the uninvolved ($n = 11$) and involved ileum ($n = 10$). In the uninvolved ileum, 10/11 biopsies showed CG3 staining (strong in 4) and 8/11 Das-1 staining (glandular $n = 5$; epithelial cells $n = 3$). In the involved ileum 9/10 biopsies showed CG3 staining (glandular $n = 2$; epithelial cells $n = 7$, strong in 3) and 6/10 showed Das-1 staining (glandular $n = 5$; epithelial cells, $n = 1$).

WCE. In the only UC patient studied by WCE, this procedure confirmed the endoscopic findings, showing multiple erosions and ulcers covered by fibrin in the distal 10 cm of the neo-terminal ileum (Figure 3D). These lesions appeared focal, being surrounded by macroscopically uninvolved mucosa. No other lesions, active bleeding or strictures were detected by WCE in the entire small bowel.

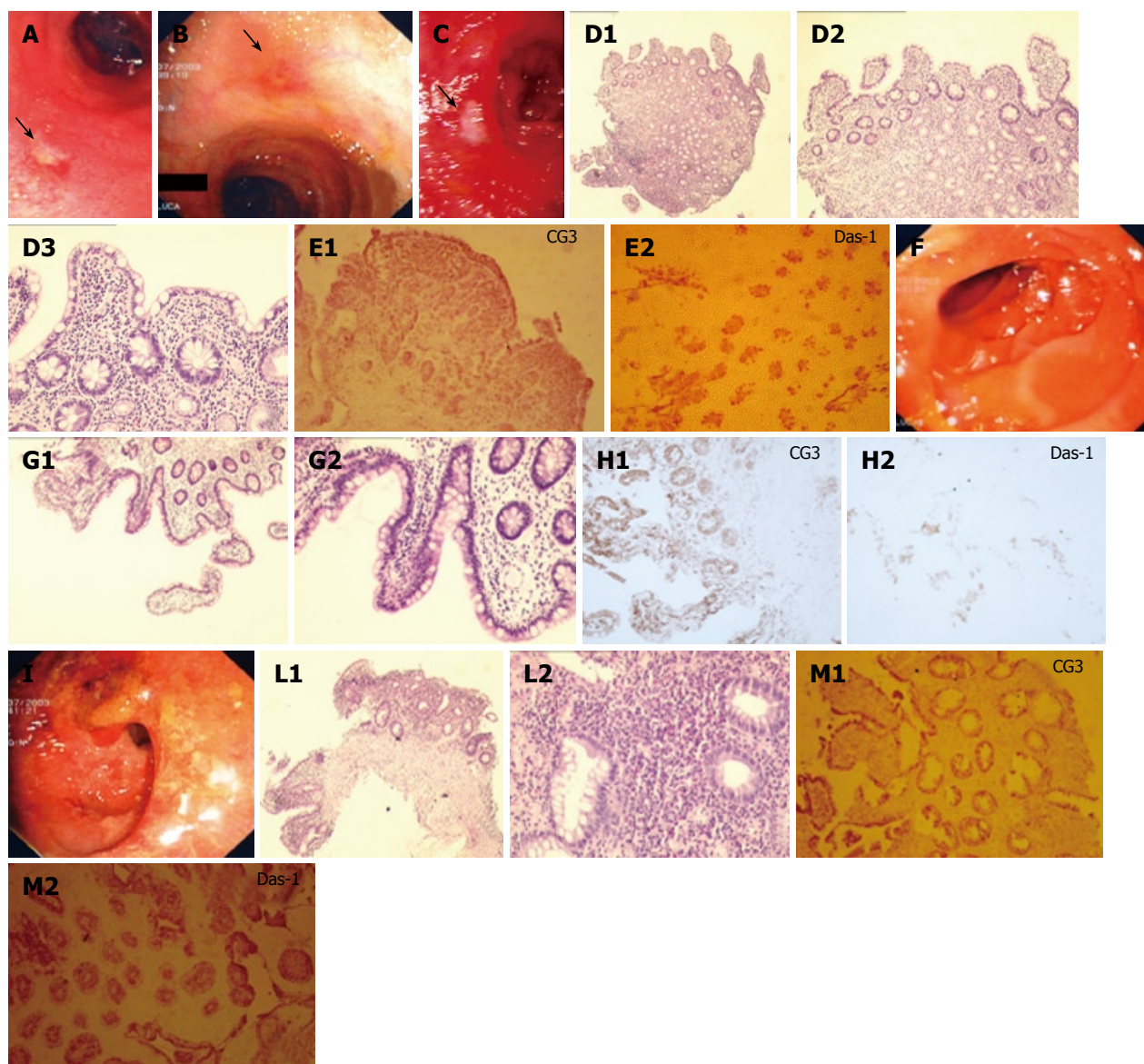


Figure 3 Endoscopic view, histological analysis and immunohistochemistry against CG3 and Das-1 MoAbs of biopsy samples taken from the neo-terminal ileum and rectal stump from a second UC patient (DLE) with IRA. **A-C**: Endoscopic views of the neo-terminal ileum at 3 endoscopies, showing focal erosions and ulcers at all times; **D**: Histological analysis of ileal biopsy samples, compatible with changes towards colonic epithelium, including villous atrophy; **E**: Immunohistochemistry showing staining against CG3 (**E1**) and Das-1 (**E2**) MoAbs in the same ileal samples. **F-H**: Endoscopic view (**F**), histological (**G1**, **G2**) and immunohistochemical analysis against CG3 (**H1**) and Das-1 MoAbs (**H2**) of the uninvolved ileum, showing no inflammation or colonic metaplasia. **I-M**: Endoscopic view (**I**), histological (**L1**, **L2**) and immunohistochemical analysis using CG3 (**M1**) and Das-1 MoAbs (**M2**) of the rectal stump, showing typical UC lesions and staining against hTM5-related antigens.

Controls with IRA

CD patients: Figure 2B shows the percentage of CD patients with ileal recurrence detected by endoscopy and histology, together with changes towards colonic epithelium assessed by histology and immunohistochemistry. Recurrence was detected in 4 out of 6 patients: histology detected changes towards colonic epithelium in 3 out of these 4 patients, associated with the expression of colonic epithelial antigens in 2 out of 3 patients.

Clinical assessment. At first endoscopy, disease was active in 3 (CDAI > 150) and inactive in 3 patients.

Endoscopy. Lesions were detected in the rectum in 2/6 patients, in the anastomosis in 5/6 and in the ileum in 4/6 patients. Lesions included CD recurrence of grade 0 ($n = 2$), 2 ($n = 2$), 3 ($n = 1$) or 4 ($n = 1$)

(Figures 6 and 7).

Histology. (1) HE staining: In rectal samples, inflammatory changes were detected in 3/6 CD. The macroscopically uninvolved ileum from 4/6 patients showed changes towards colonic metaplasia ($n = 1$), inflammation and villous atrophy ($n = 2$) or no lesions ($n = 1$) (Figures 6 and 7). Among the 4 patients with ileal recurrence, inflammation was detected in 3 (with villous atrophy in 2) and colonic metaplasia in 1. The anastomosis showed inflammation in 3 (glandular hyperplasia $n = 1$, villous atrophy $n = 1$, colonic metaplasia $n = 1$). (2) Assessment of changes of the ileal epithelium toward colonic epithelium: In 4/6 CD, biopsy samples were taken from the macroscopically uninvolved ileum, showing inflammation in 3/4 (A2, $n = 1$; A4, $n = 2$) and changes towards colonic metaplasia

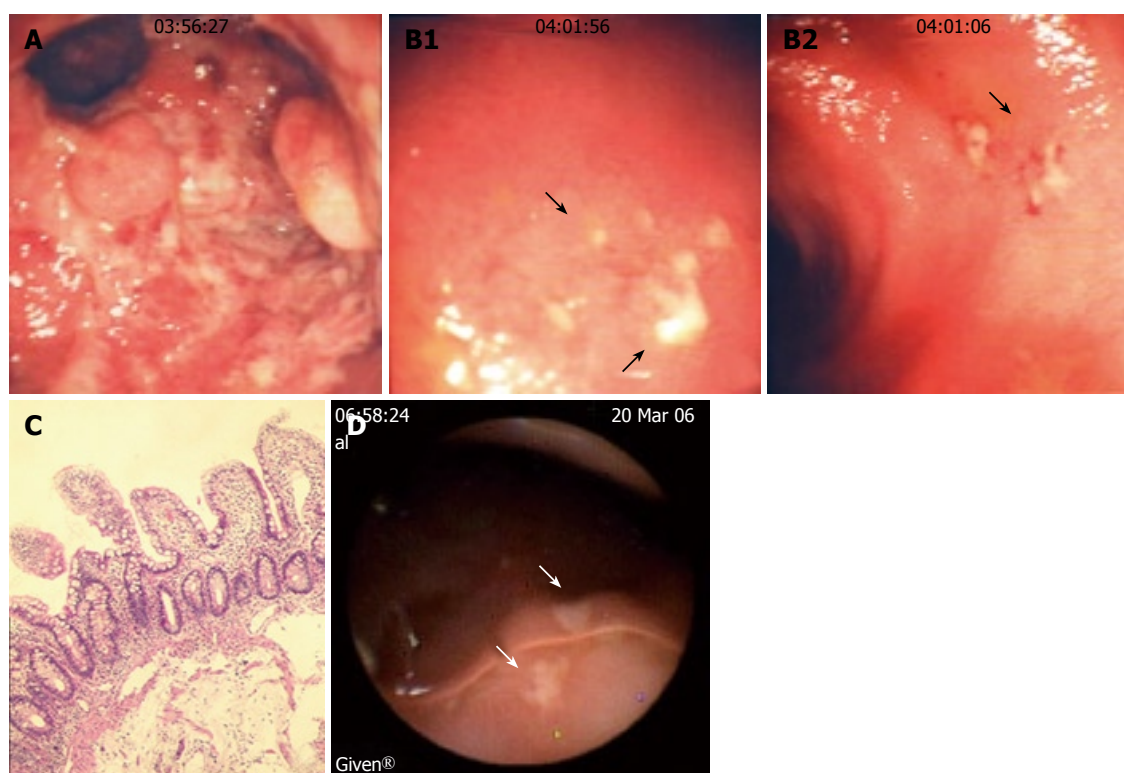


Figure 4 Endoscopic view, histological analysis and WCE images of the rectum and neo-terminal ileum from a third UC patient (LRA) with IRA. **A:** Endoscopic view of the rectal stump showing erosions and pseudopolyps; **B:** Endoscopic views of the ileum showing small focal ulcers above the anastomosis; **C:** Ileal biopsy sample from the same patient showing no villous atrophy or colonic metaplasia by histology; **D:** WCE images showing in the neo-terminal ileum above the anastomosis, 2 ulcers covered by fibrin (arrows) surrounded by macroscopically normal ileum.

Table 2 Endoscopic and histologic findings at different time intervals in each patient with IRA for UC

Patient	Endo (n)	Ileal lesions at endoscopy	Histology									
			Uninvolved ileum					Involved ileum				
			Conventional histology ^[23]			Immunohistochemistry		Conventional histology ^[23]			Immunohistochemistry	
			A	B	Colonic metaplasia	CG3 MoAb	Das-1 MoAb	A	B	Colonic metaplasia	CG3 MoAb	Das-1 MoAb
LRA	1	Y	1	0	N	-	-	ND	ND	ND	ND	ND
	2	Y	0	0	N	+	+	12	12	Y	+	+
	3	Y	2	0	N	+	+	6	1	N	+	+
	4	Y	0	0	N	+	+	6	0	N	+	+
	5	Y	0	0	N	+	+	8	0	N	+	+
DFML	1	Y	ND	ND	ND	ND	ND	0	0	N	+	-
	2	N	0	0	N	+	+	ND	ND	ND	ND	ND
SC	1	N	2	0	N	+	+	ND	ND	ND	ND	ND
ML	1	Y	0	0	N	+	+	10	4	Y	+	+
	2	N	0	0	N	ND	ND	ND	ND	ND	ND	ND
TE	1	Y	ND	ND	ND	ND	ND	0	0	Y	+	-
LP	1	N	0	0	N	ND	ND	ND	ND	ND	ND	ND
BM	1	N	0	0	N	ND	ND	ND	ND	ND	ND	ND
	2	N	1	0	N	+	-	ND	ND	ND	ND	ND
	3	N	0	0	N	ND	ND	ND	ND	ND	ND	ND
DLE	1	Y	0	0	N	+	-	6	2	Y	+	-
	2	Y	0	2	Y	+	+	0	2	Y	+	+
	3	N	0	0	N	ND	ND	ND	ND	ND	ND	ND
CF	1	Y	ND	ND	ND	ND	ND	2	0	N	-	-
	2	Y	0	0	N	ND	ND	ND	ND	ND	ND	ND
NF	1	Y	ND	ND	ND	ND	ND	6	3	Y	ND	ND

in 2/4 (B2, $n = 1$; B4, $n = 1$) (Table 3). Ileal lesions at endoscopy were confirmed by histology in all 4 CD (A2, $n = 1$; A8, $n = 1$; A10, $n = 1$; A12, $n = 1$) showing colonic metaplasia in 3/4 patients (B3, $n = 1$; B4, $n =$

2). (3) Immunohistochemistry: hTM5-related antigens expression in the uninvolved ileum was searched in 2 CD, showing staining against CG3 in both and against Das-1 in 1 patient. Biopsies from the involved ileum

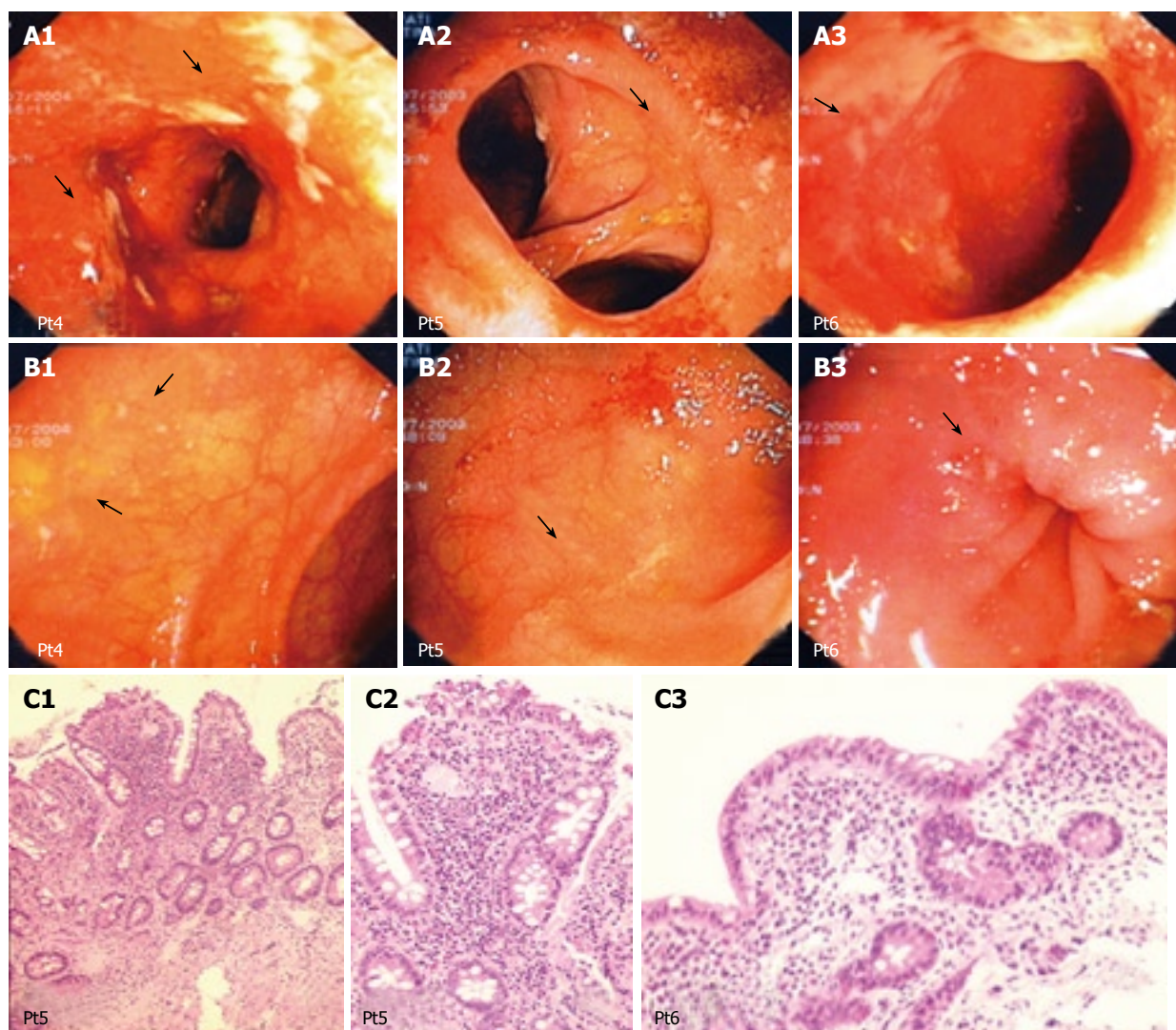


Figure 5 Endoscopic view and histological analysis of the anastomosis and neo-terminal ileum from 3 UC patients with IRA. **A:** Endoscopic views of the anastomosis from 3 UC patients showing focal small ulcers; **B:** Endoscopic views of the neo-terminal ileum from the same 3 UC patients, scattered ulcerations surrounded by macroscopically uninvolved ileum; **C:** Histological analysis of biopsy samples taken from the involved neo-terminal ileum from patients 5 and 6, showing changes towards colonic metaplasia.

were taken from 3 CD, showing CG3 staining in all 3, and Das-1 staining in 2/3 patients.

Longitudinal study: Endoscopy: Repeated endoscopies ($n = 4$ in 1; $n = 3$ in 2) were performed in 3/6 patients, showing persistent lesions. Histology: In 5/6 patients, ileal inflammatory changes were detected at all endoscopies. Immunohistochemistry: The only patient with repeated immunohistochemical analysis showed persistent CG3 positivity and Das-1 negativity.

Patient with FAP

Endoscopy: One anastomotic ulcer was detected. Histology: Inflammation were detected only in the anastomosis (A = 0; B = 2).

Patient resected for colon cancer

Endoscopy: No lesions were detected. Histology: Mild inflammation was detected in the rectum only.

DISCUSSION

In UC, inflammatory changes of the ileum in UC may be observed in pouchitis^[4-8] and in backwash ileitis^[1-3]. In pouchitis, ileal lesions have been related to the development of changes of the epithelium towards colonic epithelium ("colonic metaplasia")^[4,23,24]. Although the etiology of pouchitis remains unknown, the resident bacterial flora is involved in the pathogenesis of this condition^[15,18,19,21,22]. In particular, the efficacy "*in vivo*" of probiotic preparations in UC patients with pouchitis suggests a pathogenetic role for changes of the resident bacterial flora after proctocolectomy^[18,19,21]. As proctocolectomy is required for both ileal pouch and IRA, we aimed to assess whether UC patients may develop inflammatory changes of the ileum not only in the ileal pouch, but also above the IRA. We also explored whether these changes are associated with the development of colonic metaplasia of the epithelium lining the ileum. Ileal changes have been examined by

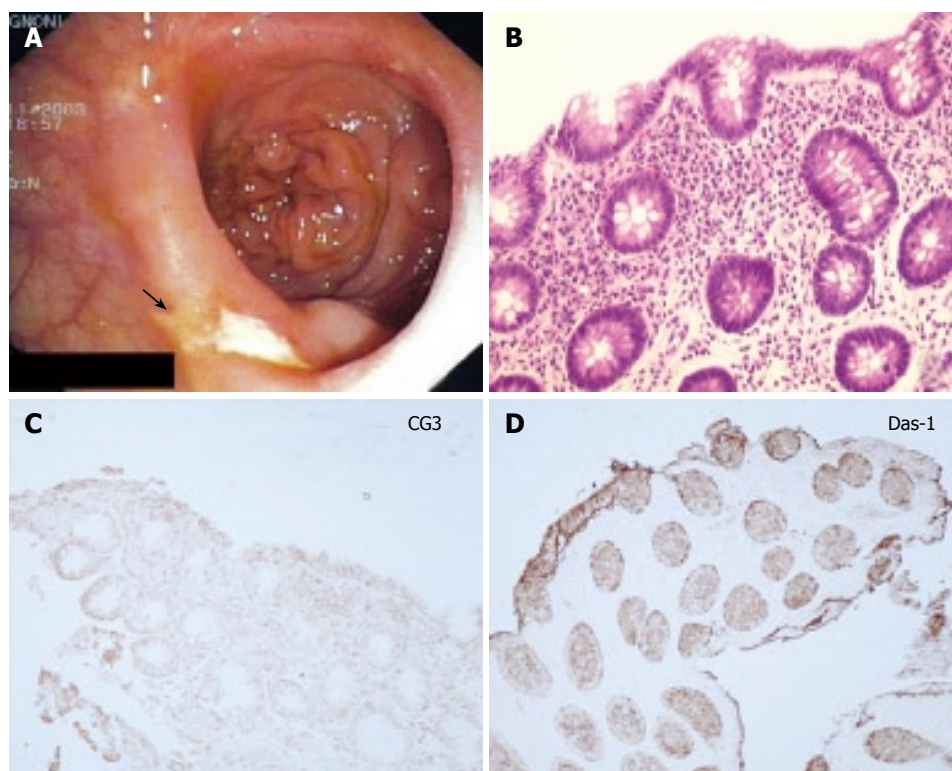


Figure 6 Endoscopic view (A), histological (B) and immunohistochemical analysis using CG3 (C) and Das-1 MoAbs (D) of the neo-terminal ileum from one CD patient. Endoscopy shows CD recurrence (grade 2), histology lesions compatible with changes of the ileum towards colonic epithelium and immunohistochemistry staining against CG3 and Das-1 MoAbs.

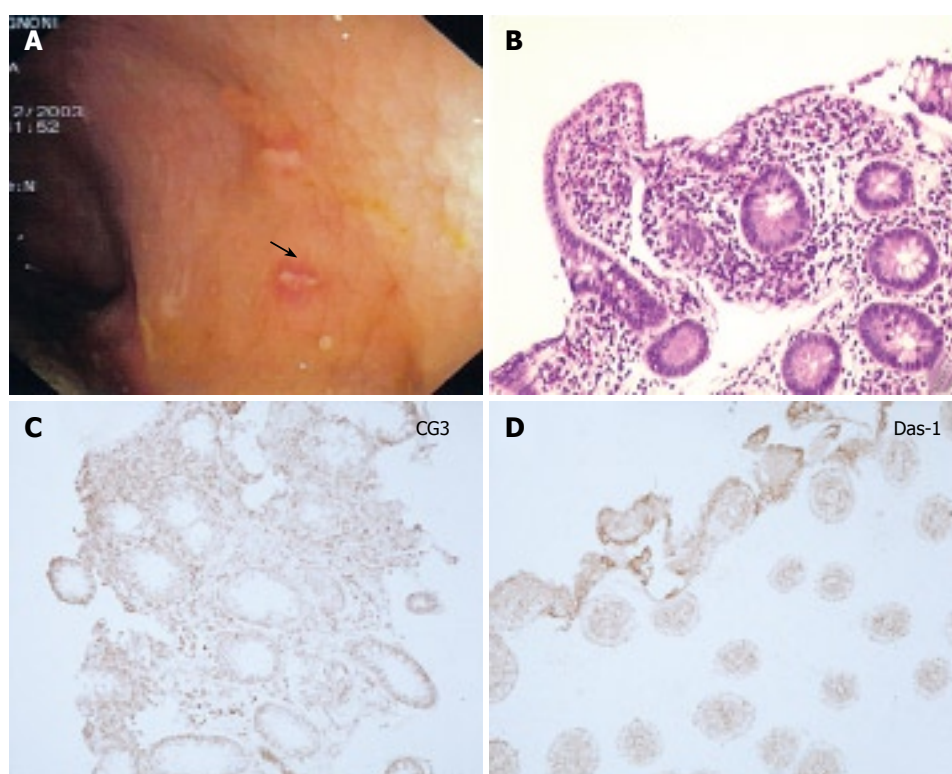


Figure 7 Endoscopic view (A), routine histochemical (B) and immunohistochemistry analysis using CG3 (C) and Das-1 MoAbs (D) of the neo-terminal ileum from a second CD patient. Endoscopy detected recurrence of the anastomosis and neo-terminal ileum (grade 3), histology lesions compatible with changes of the ileal epithelium towards colonic epithelium and immunohistochemistry the expression of colonic epithelial antigens, compatible with colonic metaplasia.

histology and immunohistochemistry using MoAbs against the major cytoskeletal microfilament protein, tropomyosin isoform 5 (hTM5) in colon epithelium^[33-36]. Evidence indicates that bacterial-host interactions may induce the expression of cryptic cytoskeletal proteins on human cells surface^[23,44]. Cytoskeletal proteins include a family of intracytoplasmatic proteins (α -actinin, talin, ezrin, villin, F-actin, myosin II, calpactin, gelsolin, laminin, tropomyosin), modulating the structure,

shape, and motility of several cell types, including human colonic epithelial cells^[45]. The enteropathogenic *Escherichia coli* binds to enterocytes by injecting a translocated intimin receptor in the host cells membrane, linking to the intimin receptor of the bacterium itself. This binding is followed by a rearrangement of the cytoskeletal proteins within the cytoplasm of the enterocytes (α -actinin, talin, ezrin, villin, F-actin, myosin II, TMs), thus forming pedestals linking the bacterium

Table 3 Endoscopic and histologic findings of patients with IRA for CD, FAP or colonic K

Patient	Endo (n)	Ileal lesions at endoscopy	Histology									
			Uninvolved ileum					Involved ileum				
			Conventional histology ^[23]			Immunohistochemistry		Conventional histology ^[23]			Immunohistochemistry	
			Score	Score	Colonic metaplasia	CG3 MoAb	Das-1 MoAb	Score	Score	Colonic metaplasia	CG3 MoAb	Das-1 MoAb
DAD (CD-1)	1	Y	ND	ND	ND	ND	ND	4	0	N	+	-
	2	Y	ND	ND	ND	ND	ND	0	0	N	+	-
	3	Y	0	0	N	+	-	0	0	N	+	-
	4	Y	0	0	N	+	-	8	4	Y	+	-
MA (CD-2)	1	N	4	4	Y	+	+	0	3	Y	+	+
DCE (CD-3)	1	N	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
CP (CD-4)	1	N	0	0	N	ND	ND	ND	ND	ND	ND	ND
	2	N	2	0	N	ND	ND	ND	ND	ND	ND	ND
	3	Y	1	1	N	ND	ND	2	1	N	ND	ND
DLV (CD-5)	1	Y	ND	ND	ND	ND	ND	4	1	Y	+	+
	2	N	2	2	Y	ND	ND					
	3	Y	4	2	Y	ND	ND	12	4	Y	ND	ND
CRL (CD-6)	1	Y	ND	ND	ND	ND	ND	10	3	Y	ND	ND
ZG (FAP)	1	N	0	0	N	ND	ND	8	2	N	ND	ND
ML (K)	1	N	0	0	N	ND	ND	ND	ND	ND	ND	ND

itself to colonic epithelial cells^[44,45]. These observations prompted us to also assess whether hTM5 may be expressed on the epithelial cells lining the neo-terminal ileum in UC patients with IRA, due to changes of the ileum towards colonic epithelium after colectomy and the possible relation between the development of colonic metaplasia and ileal lesions. Our findings from a limited number of patients with IBD suggest that ileal lesions may be observed after IRA, although more frequently in UC (7 out of 10 patients). The presence of scattered erosions and ulcers in the neo-terminal ileum above the anastomosis was confirmed by WCE images, acquired as described^[41-43], from the only patient performing this procedure. Despite the focal inflammation of the ileum, histological analysis of the surgical colonic specimen confirmed the diagnosis of UC in all 7 patients. Ileal lesions were associated with epithelial changes towards colonic epithelium in 4 out of these 7 patients, associated with the expression of hTM5-related antigens in 3 out of these 4 patients. The same findings were not detected in any of the 7 UC patients with no ileal lesions. Surprisingly, colonic metaplasia and the expression of colonic epithelial antigens were also observed in some of the few tested patients with IRA for CD. Although “colonization” of the ileum after total colectomy for any indication has been described, its possible relation with the development of ileal lesions is unknown. The score from Fruin *et al*^[24] was used for assessing colonic metaplasia, as for pouchitis. Major limits of our study include the low number of patients and the cross-sectional study plan, not allowing comparisons before versus after surgery. Although it is said that colectomy cures UC, this study underscores the fact that surgery is not the final answer due to the high

incidence of pouchitis and other functional problems experience by these patients. Of particular concern is the asymptomatic neoplasia that can arise in the residual rectal stump required for these surgical procedures, which was seen in one of the study patients.

Although not conclusive, our findings suggest that lesions may be observed in the neo-terminal ileum of UC and CD patients following IRA. These changes are towards colonic epithelium phenotype and with the expression of colonic epithelial antigens in some patients. Longitudinal studies are ongoing for further characterization of the molecular mechanisms leading to ileal changes in UC. Present findings suggest that changes of the ileal content after colectomy may contribute to the development of colonic type of metaplasia, leading to ileal lesions both in the pouch and in the neo-terminal ileum after IRA.

COMMENTS

Background

Inflammatory changes of the distal ileum in ulcerative colitis (UC) may be observed in backwash ileitis and after total proctocolectomy with ileal pouch (“pouchitis”). Although total proctocolectomy with ileal pouch currently represents the most frequently performed surgical procedure in UC, colectomy with ileo-rectal anastomosis (IRA) is still in these patients. The persistence of the diseased rectal stump after IRA requires endoscopic surveillance. Ileal inflammation may be observed in UC patients with pouchitis, being related to changes of the ileal epithelium towards colonic epithelium (“colonic metaplasia”). Whether the ileum above IRA in patients with IRA for UC may develop inflammatory changes as observed in pouchitis is unknown and this observation may be useful for proper follow up of patients after surgery.

Research frontiers

The etiology of pouchitis in patients with UC is unknown. However, the development of changes of the ileal mucosa lining the pouch, including flattening, reduced number and/or complete villar atrophy has been involved in the pathogenesis of pouchitis. These changes of the ileum, becoming similar

to the colonic epithelium ("colonic metaplasia") have been reported more frequently associated with pouchitis. Changes of the resident bacterial flora after proctocolectomy therefore have been involved in the development of both colonic metaplasia and pouchitis. It is conceivable that after total colectomy for UC and related changes of the common bacterial flora, ileal lesions may develop not only after ileal pouch, but also after IRA.

Innovations and breakthroughs

The present study showed that in UC, ileal lesions associated with changes towards colonic epithelium may develop after IRA. Changes of the ileal content after colectomy may contribute to the development of colonic metaplasia, leading to ileal lesions also after IRA.

Applications

Results from our study suggest that patients with IRA for UC need endoscopic assessment not only for cancer surveillance but also for assessing the possible development of ileal lesions above anastomosis. Present findings also add new insights regarding the natural history of UC after IRA.

Terminology

Tropomyosin isoform 5 (hTM5) is a cytoskeletal microfilament protein present in the epithelium from human colon, but not from the ileum. Mucosal and circulating antibodies against hTM5 have been demonstrated in patients with UC. Evidences indicate that bacterial-host interactions may induce the expression of cryptic cytoskeletal proteins on human cells surface. Cytoskeletal proteins include a family of intracytoplasmic proteins (α -actinin, talin, ezrin, villin, F-actin, myosin II, calpactin, gelsolin, laminin, tropomyosin), modulating the structure, shape, and motility of several cell types, including human colonic epithelial cells.

Peer review

This is a clinical and immunohistochemical study supporting the need of continued endoscopic follow up of UC patients after IRA.

REFERENCES

- 1 Abdelrazeq AS, Wilson TR, Leitch DL, Lund JN, Leveson SH. Ileitis in ulcerative colitis: is it a backwash? *Dis Colon Rectum* 2005; **48**: 2038-2046
- 2 Gustavsson S, Weiland LH, Kelly KA. Relationship of backwash ileitis to ileal pouchitis after ileal pouch-anal anastomosis. *Dis Colon Rectum* 1987; **30**: 25-28
- 3 Haskell H, Andrews CW Jr, Reddy SI, Dendrinis 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
- 4 Apel R, Cohen Z, Andrews CW Jr, McLeod R, Steinhart H, Odze RD. Prospective evaluation of early morphological changes in pelvic ileal pouches. *Gastroenterology* 1994; **107**: 435-443
- 5 Arai K, Koganei K, Kimura H, Akatani M, Kitoh F, Sugita A, Fukushima T. Incidence and outcome of complications following restorative proctocolectomy. *Am J Surg* 2005; **190**: 39-42
- 6 Brunel M, Penna C, Turet E, Balladur P, Parc R. Restorative proctocolectomy for distal ulcerative colitis. *Gut* 1999; **45**: 542-545
- 7 Chambers WM, McC Mortensen NJ. Should ileal pouch-anal anastomosis include mucosectomy? *Colorectal Dis* 2007; **9**: 384-392
- 8 Madden MV, Farthing MJ, Nicholls RJ. Inflammation in ileal reservoirs: 'pouchitis'. *Gut* 1990; **31**: 247-249
- 9 Meagher AP, Farouk R, Dozois RR, Kelly KA, Pemberton JH. J ileal pouch-anal anastomosis for chronic ulcerative colitis: complications and long-term outcome in 1310 patients. *Br J Surg* 1998; **85**: 800-803
- 10 Meuwissen SG, Hoitsma H, Boot H, Seldenrijk CA. Pouchitis (pouch ileitis). *Neth J Med* 1989; **35** Suppl 1: S54-S66
- 11 Michelassi F, Lee J, Rubin M, Fichera A, Kasza K, Karrison T, Hurst RD. Long-term functional results after ileal pouch anal restorative proctocolectomy for ulcerative colitis: a prospective observational study. *Ann Surg* 2003; **238**: 433-441; discussion 442-445
- 12 Sagar PM, Pemberton JH. Ileal pouch function and dysfunction. *Dig Dis* 1997; **15**: 172-188
- 13 Salemans JM, Nagengast FM, Lubbers EJ, Kuipers JH. Postoperative and long-term results of ileal pouch-anal anastomosis for ulcerative colitis and familial polyposis coli. *Dig Dis Sci* 1992; **37**: 1882-1889
- 14 Sandborn WJ, Tremaine WJ, Batts KP, Pemberton JH, Phillips SF. Pouchitis after ileal pouch-anal anastomosis: a Pouchitis Disease Activity Index. *Mayo Clin Proc* 1994; **69**: 409-415
- 15 Shen B, Fazio VW, Remzi FH, Lashner BA. Clinical approach to diseases of ileal pouch-anal anastomosis. *Am J Gastroenterol* 2005; **100**: 2796-2807
- 16 Zuccaro G Jr, Fazio VW, Church JM, Lavery IC, Ruderman WB, Farmer RG. Pouch ileitis. *Dig Dis Sci* 1989; **34**: 1505-1510
- 17 Shen B, Fazio VW, Remzi FH, Delaney CP, Bennett AE, Achkar JP, Brzezinski A, Khandwala F, Liu W, Bambrick ML, Bast J, Lashner B. Comprehensive evaluation of inflammatory and noninflammatory sequelae of ileal pouch-anal anastomoses. *Am J Gastroenterol* 2005; **100**: 93-101
- 18 Gionchetti P, Rizzello F, Venturi A, Brigidi P, Matteuzzi D, Bazzocchi G, Poggioli G, Miglioli M, Campieri M. Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. *Gastroenterology* 2000; **119**: 305-309
- 19 Gionchetti P, Rizzello F, Helwig U, Venturi A, Lammers KM, Brigidi P, Vitali B, Poggioli G, Miglioli M, Campieri M. Prophylaxis of pouchitis onset with probiotic therapy: a double-blind, placebo-controlled trial. *Gastroenterology* 2003; **124**: 1202-1209
- 20 Hurst RD, Molinari M, Chung TP, Rubin M, Michelassi F. Prospective study of the incidence, timing and treatment of pouchitis in 104 consecutive patients after restorative proctocolectomy. *Arch Surg* 1996; **131**: 497-500; discussion 501-502
- 21 Mimura T, Rizzello F, Helwig U, Poggioli G, Schreiber S, Talbot IC, Nicholls RJ, Gionchetti P, Campieri M, Kamm MA. Once daily high dose probiotic therapy (VSL#3) for maintaining remission in recurrent or refractory pouchitis. *Gut* 2004; **53**: 108-114
- 22 Sandborn WJ, McLeod R, Jewell DP. Medical therapy for induction and maintenance of remission in pouchitis: a systematic review. *Inflamm Bowel Dis* 1999; **5**: 33-39
- 23 Biancone L, Palmieri G, Lombardi A, Colantoni A, Tonelli F, Das KM, Pallone F. Tropomyosin expression in the ileal pouch: a relationship with the development of pouchitis in ulcerative colitis. *Am J Gastroenterol* 2003; **98**: 2719-2726
- 24 Fruin AB, El-Zammer O, Stucchi AF, O'Brien M, Becker JM. Colonic metaplasia in the ileal pouch is associated with inflammation and is not the result of long-term adaptation. *J Gastrointest Surg* 2003; **7**: 246-253; discussion 253-254
- 25 Kettlewell MGW. Proctocolectomy for ulcerative colitis. In: Allan RN, Keighley MRB, Alexander-Williams J & Hawkins C. *Inflammatory Bowel Diseases*. Edinburgh: Churchill Livingstone, 1991: 439-445
- 26 Brooke BN. Outcome of surgery for ulcerative colitis. *Lancet* 1956; **271**: 532-536
- 27 Larson DW, Pemberton JH. Current concepts and controversies in surgery for IBD. *Gastroenterology* 2004; **126**: 1611-1619
- 28 Bulow C, Vasen H, Jarvinen H, Bjork J, Bisgaard ML, Bulow S. Ileorectal anastomosis is appropriate for a subset of patients with familial adenomatous polyposis. *Gastroenterology* 2000; **119**: 1454-1460
- 29 Lepisto A, Jarvinen HJ. Fate of the rectum after colectomy with ileorectal anastomosis in ulcerative colitis. *Scand J Surg* 2005; **94**: 40-42
- 30 Watts JM, Hughes ES. Ulcerative colitis and Crohn's disease: results after colectomy and ileorectal anastomosis. *Br J Surg* 1977; **64**: 77-83
- 31 Roggo A, Brunner U, Ottinger LW, Largiader F. The

- continuing challenge of aneurysms of the popliteal artery. *Surg Gynecol Obstet* 1993; **177**: 565-572
- 32 **Nicholls RJ**, Holt SD, Lubowski DZ. Restorative proctocolectomy with ileal reservoir. Comparison of two-stage vs. three-stage procedures and analysis of factors that might affect outcome. *Dis Colon Rectum* 1989; **32**: 323-326
- 33 **Kesari KV**, Yoshizaki N, Geng X, Lin JJ, Das KM. Externalization of tropomyosin isoform 5 in colon epithelial cells. *Clin Exp Immunol* 1999; **118**: 219-227
- 34 **Das KM**, Dasgupta A, Mandal A, Geng X. Autoimmunity to cytoskeletal protein tropomyosin. A clue to the pathogenetic mechanism for ulcerative colitis. *J Immunol* 1993; **150**: 2487-2493
- 35 **Lin JJ**, Hegmann TE, Lin JL. Differential localization of tropomyosin isoforms in cultured nonmuscle cells. *J Cell Biol* 1988; **107**: 563-572
- 36 **Takahashi F**, Das KM. Isolation and characterization of a colonic autoantigen specifically recognized by colon tissue-bound immunoglobulin G from idiopathic ulcerative colitis. *J Clin Invest* 1985; **76**: 311-318
- 37 **Tremaine WJ**, Sandborn WJ, Wolff BG, Carpenter HA, Zinsmeister AR, Metzger PP. Bismuth carbomer foam enemas for active chronic pouchitis: a randomized, double-blind, placebo-controlled trial. *Aliment Pharmacol Ther* 1997; **11**: 1041-1046
- 38 **Rutgeerts P**, Geboes K, Vantrappen G, Beyls J, Kerremans R, Hiele M. Predictability of the postoperative course of Crohn's disease. *Gastroenterology* 1990; **99**: 956-963
- 39 **Sandborn WJ**, Feagan BG, Hanauer SB, Lochs H, Lofberg R, Modigliani R, Present DH, Rutgeerts P, Scholmerich J, Stange EF, Sutherland LR. A review of activity indices and efficacy endpoints for clinical trials of medical therapy in adults with Crohn's disease. *Gastroenterology* 2002; **122**: 512-530
- 40 **Best WR**, Beckett JM, Singleton JW, Kern F Jr. Development of a Crohn's disease activity index. National Cooperative Crohn's Disease Study. *Gastroenterology* 1976; **70**: 439-444
- 41 **Costamagna G**, Shah SK, Riccioni ME, Foschia F, Mutignani M, Perri V, Vecchioli A, Brizi MG, Picciocchi A, Marano P. A prospective trial comparing small bowel radiographs and video capsule endoscopy for suspected small bowel disease. *Gastroenterology* 2002; **123**: 999-1005
- 42 **Iddan G**, Meron G, Glukhovskiy A, Swain P. Wireless capsule endoscopy. *Nature* 2000; **405**: 417
- 43 **Biancone L**, Calabrese E, Petruzzello C, Onali S, Caruso A, Palmieri G, Sica GS, Pallone F. Wireless capsule endoscopy and small intestine contrast ultrasonography in recurrence of Crohn's disease. *Inflamm Bowel Dis* 2007; **13**: 1256-1265
- 44 **Vallance BA**, Finlay BB. Exploitation of host cells by enteropathogenic *Escherichia coli*. *Proc Natl Acad Sci USA* 2000; **97**: 8799-8806
- 45 **Luo Y**, Frey EA, Pfuetzner RA, Creagh AL, Knoechel DG, Haynes CA, Finlay BB, Strynadka NC. Crystal structure of enteropathogenic *Escherichia coli* intimin-receptor complex. *Nature* 2000; **405**: 1073-1077

S- Editor Li DL L- Editor Li M E- Editor Zhang WB



Continuous wound infusion of local anaesthetic agents following colorectal surgery: Systematic review and meta-analysis

Alan Karthikesalingam, Stewart R Walsh, Sheraz R Markar, Umar Sadat, Tjun Y Tang, Charles M Malata

Alan Karthikesalingam, Stewart R Walsh, Sheraz R Markar, Umar Sadat, Tjun Y Tang, Department of General Surgery, Addenbrooke's Hospital, Cambridge University Hospitals NHS Foundation Trust, Cambridge CB2 2QQ, United Kingdom

Charles M Malata, Department of Plastic and Reconstructive Surgery, Addenbrooke's Hospital, Cambridge University Hospitals Foundation Trust, Cambridge CB2 2QQ, United Kingdom

Author contributions: Karthikesalingam A and Malata CM designed research; Karthikesalingam A, Markar SR, Walsh SR and Sadat U performed research; Walsh SR, Tang TY and Sadat U analysed data; and Karthikesalingam A, Walsh SR, Sadat U, Markar SR and Malata CM wrote the paper.

Correspondence to: Stewart R Walsh, Department of General Surgery, Addenbrooke's Hospital, Cambridge University Hospitals NHS Foundation Trust, Specialist Registrar in General Surgery, Box 201, Level 7, Hills Road, Cambridge CB2 2QQ, United Kingdom. srwalsh@doctors.org.uk

Telephone: +44-1223-217246 Fax: +44-1223-216015

Received: May 25, 2008 Revised: July 3, 2008

Accepted: July 10, 2008

Published online: September 14, 2008

Abstract

AIM: To provide a specific review and meta-analysis of the available evidence for continuous wound infusion of local anaesthetic agents following midline laparotomy for major colorectal surgery.

METHODS: Medline, Embase, trial registries, conference proceedings and article reference lists were searched to identify randomised, controlled trials of continuous wound infusion of local anaesthetic agents following colorectal surgery. The primary outcomes were opioid consumption, pain visual analogue scores (VASs), return to bowel function and length of hospital stay. Weighted mean difference were calculated for continuous outcomes.

RESULTS: Five trials containing 542 laparotomy wounds were eligible for inclusion. There was a significant decrease in post-operative pain VAS at rest on day 3 (weighted mean difference: -0.43; 95% CI: -0.81 to -0.04; $P = 0.03$) but not on post-operative day 1 and 2. Local anaesthetic infusion was associated with a significant reduction in pain VAS on movement on all three post-operative days (day 1 weighted mean difference: -1.14; 95% CI: -2.24 to -0.041; $P = 0.04$, day 2 weighted mean difference: -0.97, 95% CI: -1.91

to -0.029; $P = 0.04$, day 3 weighted mean difference: -0.61; 95% CI: 1.01 to -0.20; $P = 0.0038$). Local anaesthetic wound infusion was associated with a significant decrease in total opioid consumption (weighted mean difference: -40.13; 95% CI: -76.74 to -3.53; $P = 0.03$). There was no significant decrease in length of stay (weighted mean difference: -20.87; 95% CI: -46.96 to 5.21; $P = 0.12$) or return of bowel function (weighted mean difference: -9.40; 95% CI: -33.98 to 15.17; $P = 0.45$).

CONCLUSION: The results of this systematic review and meta-analysis suggest that local anaesthetic wound infusion following laparotomy for major colorectal surgery is a promising technique but do not provide conclusive evidence of benefit. Further research is required including cost-effectiveness analysis.

© 2008 The WJG Press. All rights reserved.

Key words: Colorectal surgery; Laparotomy; Local anaesthesia; Infusion; Wound healing

Peer reviewer: Conor P Delaney, MD, MCh, PhD, FRCSI, FACS, Professor of Surgery, Case Western Reserve University, Chief, Division of Colorectal Surgery, Vice-Chairman, Department of Surgery, Director, Institute for Surgery and Innovation, University Hospitals, Case Medical Center, 11100 Euclid Avenue Cleveland, Cleveland 44106-5047, United States

Karthikesalingam A, Walsh SR, Markar SR, Sadat U, Tang TY, Malata CM. Continuous wound infusion of local anaesthetic agents following colorectal surgery: Systematic review and meta-analysis. *World J Gastroenterol* 2008; 14(34): 5301-5305 Available from: URL: <http://www.wjgnet.com/1007-9327/14/5301.asp> DOI: <http://dx.doi.org/10.3748/wjg.14.5301>

INTRODUCTION

Open surgery comprising colonic resection and primary bowel anastomosis accounts for up to a third of elective general surgical admissions^[1]. The control of pain following these operations represents a major challenge as highly complex nociceptive pathways are involved^[2-4]. Pain control following abdominal laparotomy and bowel anastomosis is therefore not amenable to

pharmacological monotherapy and modern analgesic strategies following major colorectal surgery involve the combination of many agents including parenteral opiates, nonsteroidal anti-inflammatory drugs (NSAIDs), paracetamol and epidural infusion techniques^[5].

Unfortunately, there is no ideal analgesic regimen - all current techniques have disadvantages in the form of important side-effects, cost, patient compliance, procedural complications and delays in discharge^[6]. Suboptimal post-operative pain control is of great clinical consequence and has been associated with cardiovascular and respiratory complications and increased gastrointestinal paralysis^[5].

A recent systematic review^[7] has revealed the promise of continuous wound infusion of local anaesthetic agents to provide improved pain control following thoracic^[8-10], abdominal^[11-13], gynaecological^[14-16], and orthopaedic^[17-19] operations, but there is a need for a more focused review of the evidence specific to colorectal laparotomy.

MATERIALS AND METHODS

An electronic search was performed using the Embase and Medline databases from 1966 until 2007. The search terms "postoperative pain", "postoperative analgesia", "local anesthetics", "continuous", "infusion", "perfusion", "irrigation", "patient-controlled", and MeSH headings "Colorectal Surgery" (MeSH), "Laparotomy" (MeSH), were used in combination with the Boolean operators AND or OR. Two authors independently performed electronic searches in March 2008. The electronic search was supplemented by a hand search of published abstracts from meetings of the Surgical Research Society, the Society of Academic and Research Surgery, the American Society of Anesthesiologists, the Anaesthetic Research Society and the Association of Surgeons of Great Britain and Ireland from 1980 to 2007. The reference lists of articles obtained were also searched to identify further relevant citations. Finally, the search included the Current Controlled Trials Register (www.controlled-trials.com) and the Cochrane Database of Controlled Trials.

Abstracts of the citations identified by the search were then scrutinised by two observers (SRW and AK) in order to determine eligibility for inclusion in the meta-analysis. Studies were included if they met each of the following criteria: randomised controlled trial, patients undergoing midline laparotomy for colorectal surgery, randomisation to groups with or without continuous wound infusion of local anaesthetic.

The primary outcome measure for the meta-analysis was the opioid consumption in each arm. Data from eligible trials were entered into a computerized spreadsheet for analysis. The quality of each trial was assessed using the Jadad scoring system^[20]. The statistical analysis was performed using Statsdirect 2.5.7 (Statsdirect Ltd., UK). Weighted mean difference were calculated for the effect of local anaesthetic infusion on opioid consumption and linear analogue pain scores on post-operative days 1, 2

and 3. Further pooled outcome measures were duration of hospital stay and time to return of bowel function. All pooled outcome measures were determined using random-effects models as described by Der Simonian and Laird^[21]. Heterogeneity amongst the trials was assessed by Cochran's Q statistic, a null hypothesis test in which $P < 0.05$ is taken to indicate the presence of significant heterogeneity. The Egger test was used to assess the funnel plot for significant asymmetry, indicating possible publication or other biases.

RESULTS

The initial search identified 590 papers. After screening, 5 randomised controlled trials were identified^[22-26]. The five trials included 542 laparotomy wounds, of which 259 were randomised to infusion of local anaesthetic agents.

Outcome measures

Opioid consumption: Four of the five trials reported total opioid consumption with or without local anaesthetic wound infusions^[22-25] (Figure 1A). Local anaesthetic wound infusion was associated with a significant decrease in total opioid consumption (weighted mean difference: -40.13; 95% CI: -76.74 to -3.53; $P = 0.03$). This outcome measure was associated with significant statistical heterogeneity (Cochran's $Q = 45.31$, $P = 0.02$) but not significant bias (Egger Test = -4.69, $P = 0.27$).

Four of the five trials reported separate data for opioid consumption with or without local anaesthetic wound infusion on post-operative day 1^[22,23,25,26] (Figure 1B). Local anaesthetic wound infusion was associated with a significant decrease in opioid consumption on post-operative day 1 (weighted mean difference: -8.34; 95% CI: -16.38 to -0.31; $P = 0.04$). There was significant statistical heterogeneity (Cochran's $Q = 9.98$, $P = 0.019$) but not significant bias (Egger test: -2.11, $P = 0.48$).

Three trials reported opioid consumption on post-operative days 2 and 3^[22,23,26] (Table 1). There was no significant effect on opioid consumption (d 2 weighted mean difference: -9.49; 95% CI: -20.37 to 1.39; $P = 0.087$; day 3 weighted mean difference: -4.80; 95% CI: -11.72 to 2.13; $P = 0.17$). Two trials did not report this outcome measure rendering calculation of statistical heterogeneity or bias impossible.

Visual analogue pain scores at rest

Four of the five trials reported visual analogue scores (VASs) of pain on post-operative days 1, 2 and 3^[22-24,26]. Post-operative pain was reduced with local anaesthetic infusion on d 1 and 2 but the difference was not significant (Table 1) (d 1 weighted mean difference: -0.18; 95% CI: -1.31 to 0.95; $P = 0.75$ and d 2 weighted mean difference: -0.20; 95% CI: -1.06 to 0.66; $P = 0.65$). However, these outcome measures were associated with significant statistical heterogeneity (Cochran's $Q = 18.15$ and 15.42 , $P < 0.05$). The use of local anaesthetic wound infusions was associated with a significant decrease in post-operative pain at rest on d 3 (Figure 1C) (weighted mean

Table 1 Results of meta-analyses

Outcome measure	Weighted mean difference	95% CI	P	Heterogeneity	Bias
Opioid consumption					
Total	-40.13	-76.74 to -3.53	0.03	$P = 0.02$	$P = 0.27$
Postoperative day 1	-8.34	-16.38 to -0.31	0.04	$P = 0.019$	$P = 0.48$
Postoperative day 2	-9.41	-20.37 to 1.39	0.087	NA	NA
Postoperative day 3	-4.8	-11.72 to 2.13	0.17	NA	NA
Visual analogue pain score at rest					
Postoperative day 1	-0.18	-1.31 to 0.95	0.75	$P < 0.05$	$P = 0.80$
Postoperative day 2	-0.20	-1.06 to 0.66	0.65	$P < 0.05$	$P = 0.47$
Postoperative day 3	-0.43	-0.81 to -0.044	0.029	NA	$P = 0.63$
Visual analogue pain score on coughing or movement					
Postoperative day 1	-1.14	-2.24 to -0.041	0.04	NA	NA
Postoperative day 2	-0.97	-1.91 to -0.029	0.04	NA	NA
Postoperative day 3	-0.61	1.01 to -0.20	0.0038	NA	NA
Duration of hospital stay	-20.87	-46.94 to 5.21	0.12	$P = 0.016$	$P = 0.30$
Time to return of bowel function	-9.4	-33.98 to 15.17	0.45	NA	NA

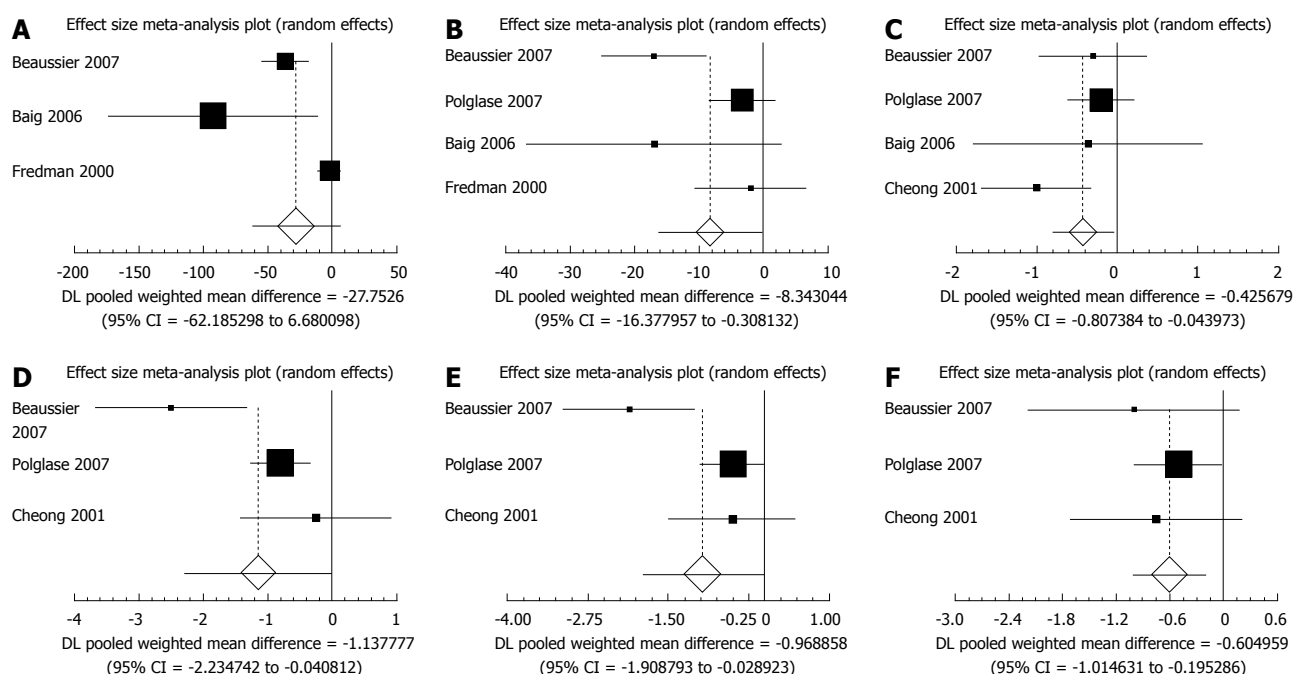


Figure 1 A: Forest plot for total postoperative opioid consumption with or without continuous wound infusion of local anaesthetic agent; B: Forest plot for opioid consumption on postoperative d 1 with or without continuous wound infusion of local anaesthetic agent; C: Forest plot for pain VAS at rest on postoperative d 3 with or without continuous wound infusion of local anaesthetic agent; D: Forest plot for pain VAS on coughing or movement on postoperative d 1 with or without continuous wound infusion of local anaesthetic agent; E: Forest plot for pain VAS on coughing or movement on postoperative d 2 with or without continuous wound infusion of local anaesthetic agent; F: Forest plot for pain VAS on coughing or movement on postoperative d 3 with or without continuous wound infusion of local anaesthetic agent.

difference: -0.43; 95% CI: -0.81 to -0.044; $P = 0.0288$). There was no evidence of bias for days 1, 2 or 3 (day 1 Egger test 0.99, $P = 0.80$; day 2 Egger test 2.75, $P = 0.47$; day 3 Egger test -1.00, $P = 0.63$).

Visual analogue pain scores on coughing or movement

Three of the five trials reported pain VAS on coughing or movement, grouped for this analysis as a composite endpoint^[23,24,26]. Local Anaesthetic infusion was associated with a significant reduction in pain VAS on all three post-operative days (Figures 1D to F) (day 1 weighted mean difference: -1.14; 95% CI: -2.24 to -0.041; $P = 0.04$, day 2 weighted mean difference: -0.97,

95% CI: -1.91 to -0.029; $P = 0.04$, day 3 weighted mean difference: -0.61; 95% CI: 1.01 to -0.20; $P = 0.0038$). Two trials did not report this pain on movement, rendering calculation of statistical heterogeneity or bias impossible.

Duration of hospital stay

All five trials reported length of stay. There was no significant decrease in length of stay (Table 1) (weighted mean difference: -20.87; 95% CI: -46.96 to 5.21; $P = 0.12$). This outcome measure was associated with significant statistical heterogeneity (Cochran's Q: 12.20, $P = 0.016$) without significant bias (Egger test: -1.12, $P = 0.30$).

Time to return of bowel function

Mean time to production of faeces was reported by three trials^[22-24]. There was no significant effect of local anaesthetic wound infusion (Table 1) (weighted mean difference: -9.40; 95% CI: -33.98 to 15.17; $P = 0.45$). Two trials did not report this outcome measure rendering calculation of statistical heterogeneity or bias impossible.

DISCUSSION

The results of our meta-analysis suggest that wound infusions are a promising adjunct to existing analgesic regimens following laparotomy for major colorectal surgery. The results do not, however, provide conclusive evidence of significant benefit conferred by this technique and it is doubtful whether the data gathered are sufficient to support generalisation of this conclusion to routine practice. The number of eligible trials (5) and total abdominal wounds (542) is small, and meta-analyses on small samples may be vulnerable to confounding if one or two of the eligible trials demonstrate a strong trend for or against the intervention under investigation.

For the purpose of this meta-analysis, the outcome measure "opioid consumption" was chosen to reflect opioid-sparing effect provided by local anaesthetic infusions. However, the significant statistical heterogeneity associated with this outcome measure reflects a variety of background analgesic regimens used in both control and treatment groups. Polglase *et al* utilized a multimodal analgesic regimen whereas the other trials studied used only patient controlled opioid analgesia to provide background analgesia. This degree of methodological heterogeneity between the trials may have influenced the meta-analysis.

Analysis of pain VAS may also have been affected by methodological heterogeneity between the trials studied. Furthermore, pain VAS is a non-parametric variable whereas the meta-analysis models used assume parametric distribution of the variables under study. The variable "length of stay" reflects a composite endpoint that may have been affected by several factors other than the presence of local anaesthetic infusions, and therefore it is not possible to draw causative inferences from the results of this pooled outcome measure with great validity. It was not possible to obtain sufficient data for all the trials under study to provide a reliable analysis of return to bowel function.

An economic analysis of local anaesthetic wound infusions is also needed - it seems likely that a greater amount of data is needed to clarify any trends in post-operative complications that may support the use of these infusions. Further large randomised controlled trials are required to investigate the promise of local anaesthetic wound infusions in major colorectal surgery, using standardized local anaesthetic agents, background analgesic regimens, experimental protocols, discharge criteria and anatomical site for wound infusion delivery.

In conclusion, Although suboptimal postoperative pain control is associated with cardiovascular, respira-

tory and gastrointestinal complications, many multimodal regimens for analgesia following major colorectal laparotomy provide inadequate pain relief. Although the number of trials available for meta-analysis is small, the available data demonstrate potential benefit in terms of reduction in opioid consumption following laparotomy for major colorectal surgery. Further large-scale studies will be needed to ascertain if any clear benefit or harm is conferred by the prophylactic use of local anaesthetic wound infusions in major colorectal surgery. Future research on this topic should also address the inaccuracies introduced by the methodological heterogeneity previously addressed in available trials, and provide a cost-effectiveness analysis of the use of continuous wound infusions in colorectal surgery.

COMMENTS

Background

Pain control following abdominal laparotomy and bowel anastomosis in colorectal surgery is a complex challenge not amenable to pharmacological monotherapy. Modern multimodal analgesic regimens may provide suboptimal post-operative pain control, which is associated with cardiovascular and respiratory complications and increased gastrointestinal paralysis.

Research frontiers

Continuous wound infusions of local anaesthetic agents have been suggested to provide improved pain control following a broad range of surgical incisions, both alone and as part of a multimodal analgesic regimen.

Innovations and breakthroughs

There is a need for a focused and quantitative review of the evidence for the analgesic benefit of continuous wound infusion of local anaesthetics specific to colorectal laparotomy, which is provided by this meta-analysis.

Applications

The meta-analysis demonstrates potential benefit in terms of reduction in opioid consumption following laparotomy for major colorectal surgery. The review highlights the need for future research on this topic and identifies that such future research should address the inaccuracies introduced by the methodological heterogeneity identified in available trials, and provides a cost-effectiveness analysis of the use of continuous wound infusions in colorectal surgery.

Terminology

Visual analogue scale (VAS) is a validated research tool used to quantitatively assess the subjective experience of patients' pain perception.

Peer review

The authors present a systematic analysis of local anaesthetics in wounds after open colorectal surgery. This is an area that has not been addressed by systematic analysis previously. It is a well-done and timely review.

REFERENCES

- 1 Gendall KA, Kennedy RR, Watson AJ, Frizelle FA. The effect of epidural analgesia on postoperative outcome after colorectal surgery. *Colorectal Dis* 2007; **9**: 584-598; discussion 598-600
- 2 Kehlet H. Surgical stress: the role of pain and analgesia. *Br J Anaesth* 1989; **63**: 189-195
- 3 Stein C. Peripheral mechanisms of opioid analgesia. *Anesth Analg* 1993; **76**: 182-191
- 4 Dahl JB, Kehlet H. Non-steroidal anti-inflammatory drugs: rationale for use in severe postoperative pain. *Br J Anaesth* 1991; **66**: 703-712
- 5 PROSPECT working group. Procedure specific post-operative pain management: open colonic resection. Available from: URL: <http://www.postoppain.org/frameset.htm> Accessed 28th January 2008
- 6 Kehlet H, Liu SS. Continuous local anesthetic wound infusion to improve postoperative outcome: back to the

- periphery? *Anesthesiology* 2007; **107**: 369-371
- 7 **Liu SS**, Richman JM, Thirlby RC, Wu CL. Efficacy of continuous wound catheters delivering local anesthetic for postoperative analgesia: a quantitative and qualitative systematic review of randomized controlled trials. *J Am Coll Surg* 2006; **203**: 914-932
- 8 **Barron DJ**, Tolan MJ, Lea RE. A randomized controlled trial of continuous extra-pleural analgesia post-thoracotomy: efficacy and choice of local anaesthetic. *Eur J Anaesthesiol* 1999; **16**: 236-245
- 9 **Dowling R**, Thielmeier K, Ghaly A, Barber D, Boice T, Dine A. Improved pain control after cardiac surgery: results of a randomized, double-blind, clinical trial. *J Thorac Cardiovasc Surg* 2003; **126**: 1271-1278
- 10 **Francois T**, Blanloeil Y, Pillet F, Moren J, Mazoit X, Geay G, Douet MC. Effect of interpleural administration of bupivacaine or lidocaine on pain and morphine requirement after esophagectomy with thoracotomy: a randomized, double-blind and controlled study. *Anesth Analg* 1995; **80**: 718-723
- 11 **Chester JF**, Ravindranath K, White BD, Shanahan D, Taylor RS, Lloyd-Williams K. Wound perfusion with bupivacaine: objective evidence for efficacy in postoperative pain relief. *Ann R Coll Surg Engl* 1989; **71**: 394-396
- 12 **Lau H**, Patil NG, Lee F. Randomized clinical trial of postoperative subfascial infusion with bupivacaine following ambulatory open mesh repair of inguinal hernia. *Dig Surg* 2003; **20**: 285-289
- 13 **Levack ID**, Holmes JD, Robertson GS. Abdominal wound perfusion for the relief of postoperative pain. *Br J Anaesth* 1986; **58**: 615-619
- 14 **Fredman B**, Shapiro A, Zohar E, Feldman E, Shorer S, Rawal N, Jedeikin R. The analgesic efficacy of patient-controlled ropivacaine instillation after Cesarean delivery. *Anesth Analg* 2000; **91**: 1436-1440
- 15 **Gupta A**, Perniola A, Axelsson K, Thorn SE, Crafoord K, Rawal N. Postoperative pain after abdominal hysterectomy: a double-blind comparison between placebo and local anesthetic infused intraperitoneally. *Anesth Analg* 2004; **99**: 1173-1179, table of contents
- 16 **Kushner DM**, LaGalbo R, Connor JP, Chappell R, Stewart SL, Hartenbach EM. Use of a bupivacaine continuous wound infusion system in gynecologic oncology: a randomized trial. *Obstet Gynecol* 2005; **106**: 227-233
- 17 **Alford JW**, Fadale PD. Evaluation of postoperative bupivacaine infusion for pain management after anterior cruciate ligament reconstruction. *Arthroscopy* 2003; **19**: 855-861
- 18 **Barber FA**, Herbert MA. The effectiveness of an anesthetic continuous-infusion device on postoperative pain control. *Arthroscopy* 2002; **18**: 76-81
- 19 **Bianconi M**, Ferraro L, Ricci R, Zanoli G, Antonelli T, Giulia B, Guberti A, Massari L. The pharmacokinetics and efficacy of ropivacaine continuous wound instillation after spine fusion surgery. *Anesth Analg* 2004; **98**: 166-172, table of contents
- 20 **Jadad AR**, Moore RA, Carroll D, Jenkinson C, Reynolds DJ, Gavaghan DJ, McQuay HJ. Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control Clin Trials* 1996; **17**: 1-12
- 21 **DerSimonian R**, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986; **7**: 177-188
- 22 **Baig MK**, Zmora O, Derdemezi J, Weiss EG, Nogueras JJ, Wexner SD. Use of the ON-Q pain management system is associated with decreased postoperative analgesic requirement: double blind randomized placebo pilot study. *J Am Coll Surg* 2006; **202**: 297-305
- 23 **Beaussier M**, El'Ayoubi H, Schiffer E, Rollin M, Parc Y, Mazoit JX, Azizi L, Gervaz P, Rohr S, Biermann C, Lienhart A, Eledjam JJ. Continuous preperitoneal infusion of ropivacaine provides effective analgesia and accelerates recovery after colorectal surgery: a randomized, double-blind, placebo-controlled study. *Anesthesiology* 2007; **107**: 461-468
- 24 **Cheong WK**, Seow-Choen F, Eu KW, Tang CL, Heah SM. Randomized clinical trial of local bupivacaine perfusion versus parenteral morphine infusion for pain relief after laparotomy. *Br J Surg* 2001; **88**: 357-359
- 25 **Fredman B**, Zohar E, Tarabykin A, Shapiro A, Mayo A, Klein E, Jedeikin R. Bupivacaine wound instillation via an electronic patient-controlled analgesia device and a double-catheter system does not decrease postoperative pain or opioid requirements after major abdominal surgery. *Anesth Analg* 2001; **92**: 189-193
- 26 **Polglase AL**, McMurrick PJ, Simpson PJ, Wale RJ, Carne PW, Johnson W, Chee J, Ooi CW, Chong JW, Kingsland SR, Buchbinder R. Continuous wound infusion of local anesthetic for the control of pain after elective abdominal colorectal surgery. *Dis Colon Rectum* 2007; **50**: 2158-2167

S- Editor Li DL L- Editor Lutze M E- Editor Ma WH

RAPID COMMUNICATION

mRNA levels of TLR4 and TLR5 are independent of *H pylori*

Elvira Garza-González, Virgilio Bocanegra-García, Francisco Javier Bosques-Padilla,
Juan Pablo Flores-Gutiérrez, Francisco Moreno, Guillermo Ignacio Perez-Perez

Elvira Garza-González, Francisco Moreno, Departamento de Microbiología, Facultad de Medicina, Universidad Autónoma de Nuevo León, Monterrey 64460, Mexico

Virgilio Bocanegra-García, Departamento de Biología Molecular y Bioingeniería, UAM Reynosa Aztlán, Universidad Autónoma de Tamaulipas, Reynosa, Tamaulipas 88740, Mexico
Francisco Javier Bosques-Padilla, Juan Pablo Flores-Gutiérrez, Hospital Universitario "Dr. José Eleuterio González", Universidad Autónoma de Nuevo León, Monterrey 64460, Mexico

Guillermo Ignacio Perez-Perez, Department of Medicine and Microbiology, New York University School of Medicine, New York NY 10010, United States

Author contributions: Garza-González E designed and coordinated the study, optimized PCR conditions, and drafted the manuscript; Bocanegra-García V and Moreno F extracted RNAs and performed molecular assays; Flores-Gutiérrez JP performed histological examination and interpretation of results; Bosques-Padilla FJ recruited patients and carried out endoscopies; Pérez-Perez GI participated in the design, coordination of the study and revised the drafted manuscript.

Correspondence to: Elvira Garza-González, Departamento de Microbiología, Facultad de Medicina, Universidad Autónoma de Nuevo León, Av. Madero y Dr. Aguirre s/n, Colonia Mitras Centro, Monterrey 64460,

Mexico. elvira_garza_gzz@yahoo.com

Telephone: +52-81-83294166 Fax: +52-81-83294166

Received: May 4, 2008 Revised: August 18, 2008

Accepted: August 25, 2008

Published online: September 14, 2008

there were no differences in TLR4 or TLR5 mRNA levels among the different clinical presentations/histological findings ($P > 0.05$). In the *in vitro* assay, the mRNA levels of TLR4 or TLR5 in AGS cells were not influenced by the *vacAs1* status or the clinical condition associated with the strains ($P > 0.05$ for both TLR4 and TLR5).

CONCLUSION: The results of this study show that the mRNA levels of TLR4 and TLR5 in gastric cells, both *in vivo* and *in vitro*, are independent of *H pylori* colonization and suggest that *vacA* may not be a significant player in the first step of innate immune recognition mediated by TLR4 or TLR5.

© 2008 The WJG Press. All rights reserved.

Key words: *H pylori*; Toll-like receptor 4; Toll-like receptor 5; AGS cells; mRNA

Peer reviewer: Dr. Limas Kupcinskas, Professor, Gastroenterology of Kaunas University of Medicine, Mickeviciaus 9, Kaunas LT 44307, Lithuania

Garza-González E, Bocanegra-García V, Bosques-Padilla FJ, Flores-Gutiérrez JP, Moreno F, Perez-Perez GI. mRNA levels of TLR4 and TLR5 are independent of *H pylori*. *World J Gastroenterol* 2008; 14(34): 5306-5310 Available from: URL: <http://www.wjgnet.com/1007-9327/14/5306.asp> DOI: <http://dx.doi.org/10.3748/wjg.14.5306>

Abstract

AIM: To determine if the presence *H pylori* or its virulence affect toll-like receptor 4 (TLR4) and TLR5 mRNA expression levels.

METHODS: For the *in vivo* assays, gastric biopsies were obtained from 40 patients and *H pylori* status was determined. For the *in vitro* assays, human gastric adenocarcinoma mucosal cells (AGS) were cultured in the presence or absence of twelve selected *H pylori* strains. *H pylori* strains isolated from culture-positive patients and selected strains were genotyped for *cagA* and *vacA*. The cDNA was obtained from mRNA extracted from biopsies and from infected AGS cells. TLR4 and TLR5 mRNA levels were examined by real-time PCR.

RESULTS: The presence of *H pylori* did not affect the mRNA levels of TLR4 or TLR5 in gastric biopsies. The mRNA levels of both receptors were not influenced by the *vacA* status ($P > 0.05$ for both receptors) and

INTRODUCTION

H pylori is a Gram-negative, flagellated bacterium that colonizes the gastric mucosa of approximately two-thirds of the world's population and is the primary cause of peptic ulcers and gastric adenocarcinoma. The complex interactions between different *H pylori* strains, the host immune system or environmental factors (or combinations thereof) are responsible for the significant variability in disease presentation associated with *H pylori* infection^[1].

Vacuolating cytotoxin (VacA) and the CagA protein are the two major virulence markers usually associated with *H pylori* pathogenicity. VacA induces the formation of intracellular vacuoles in epithelial cell lines. Aside from its direct cell-damaging effect *in vitro*, VacA also plays a major role in inducing cytoskeletal changes, apoptosis and suppression of epithelial cell prolifera-

tion^[2]. The *vacA* gene is present in all *H pylori* strains and contains at least two variable domains. The s-region, which encodes the signal peptide, exists as either an s1 or s2 isoform. The *vacA* s1 genotype has been linked to increased disease severity^[2,3].

Some *H pylori* strains contain a pathogenicity island, which carries a number of virulence factors, including *cagA*, which is considered to be a marker for this island. A type IV secretory system translocates the CagA protein into host epithelial cells where it is phosphorylated by host-cell kinases^[4]. *H pylori cagA*-positive strains have been associated with more severe inflammation of the gastric mucosa and more severe disease manifestations^[1,2].

Toll-like receptors (TLRs) are a family of mammalian homologs of the *Drosophila* Toll proteins and, in mammalian systems, TLR4 confers responsiveness to Gram-negative lipopolysaccharide (LPS), while TLR5 recognizes flagellin^[5]. Previous studies have shown that gastric epithelia express both TLR4 and TLR5^[6-9]. Here, we studied the mRNA levels of TLR4 and TLR5 in gastric epithelial cells to determine if distinctive changes in the levels of mRNA could be affected by the presence of toxigenic and non-toxigenic (in particular *vacA*+ strains) *H pylori* strains.

MATERIALS AND METHODS

Study population, RNA isolation, and *H pylori* status

Forty patients (mean age, 58.3 years; age range, 18–81 years; F/M, 25/15) with indications of upper gastro-duodenal endoscopy were included and the study was approved by the local ethics committee. Eighteen biopsy specimens were obtained from each patient. Total RNA was extracted from four of the biopsies from each patient (two from the antrum and two from the corpus) using Trizol® reagent (Invitrogen, Carlsbad, CA, USA) according to manufacturer's instructions. Eight biopsies were used for histological evaluation; two from the lesser curvature, two from the greater curvature, two from the incisura angularis, and two from the prepyloric region. Biopsies were fixed, paraffin embedded and examined histologically after hematoxylin-eosin staining.

Patients' *H pylori* status was determined by histology, the rapid urease test performed by an in-house validated test on two biopsies (one from the antrum and one from the corpus)^[10], and culture analysis performed by standard methods on four biopsies (two from the antrum and two from the corpus)^[11]. Bacterial genomic DNA was extracted from *H pylori* strains and typing for *cagA* and *vacA* was performed using primers previously described^[3,12]. Patients were considered *H pylori*-positive when at least two of the diagnostic tests were positive.

Infection of AGS cells and RNA isolation

From our collection, we selected 12 strains that were isolated from patients with gastritis, distal gastric cancer, and peptic ulcer disease (4 from each pathologic/histologic finding). All strains were genotyped for *vacA* and *cagA* as described above. Human gastric epithelial

Table 1 Distribution of *H pylori* infection status and the *H pylori* genotype

Diagnosis	<i>H pylori</i> genotype (n = 20 strains)		
	<i>vacA</i> s1	<i>vacA</i> s2	<i>cagA</i>
Gastritis	5/15	10/15	15/15
Intestinal metaplasia	4/4	0/4	4/4
Antral ulcer	0/1	1/1	1/1
Atrophic gastritis	0/0	0/0	0/0

AGS CRL-1739 cells were grown in RPMI 1640 supplemented with 10% FBS. *H pylori* bacteria were added at a multiplicity of infection (MOI) of 100:1, followed by a phosphate buffered saline (PBS) pH 7.4 wash to remove non-adherent bacteria. After 24 h, total RNA was isolated using the RNA tissue kit (Gentra Systems, Minneapolis, MN). The *H pylori* J99 ATCC 700824 strain was used as a control in all experiments.

Reverse transcription and real-time PCR

Five micrograms of RNA were reverse-transcribed using SuperScript III (Invitrogen) in a 20 µL reaction volume using oligo dT primers. The resulting cDNA was real-time-amplified in a final volume of 25 µL. The mix contained 1 U of Hot-Start *Taq* DNA polymerase (Invitrogen), 1 × reaction buffer, 200 µmol/L of each deoxynucleoside triphosphate (dNTPs), 3 mmol/L MgCl₂, 0.3 µmol/L of each primer and 0.25 × SYBR Green (Molecular Probes, Eugene OR). PCR was performed in glass capillaries using a Light Cycler instrument (Roche Applied Science, Indianapolis, IN). All reactions were performed in duplicate, and the thermal cycling conditions were 1 min at 94°C, followed by 35 cycles of 94°C for 10 s, 59°C for 10 s and 72°C for 10 s with a ramp of 5°C/s. Real time PCR for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was performed as described previously^[7]. The relative amounts of the PCR products were calculated as the ratio of each TLR mRNA to GAPDH mRNA.

Statistical analysis

Comparisons were performed using a non-paired Student's *t* test. Analyses were carried out using the Statistics software 7.0 (Melbourne, Australia).

RESULTS

H pylori infection status and histological findings in the study population

The majority, 57.5 % (n = 23), of the patients examined were *H pylori* positive (F/M, 14/9; 22–81 years; 53.8 ± 20.3). Of the 40 patients in the study, 29 had gastritis confirmed by histology, 5 had intestinal metaplasia (IM) and 4 had antral ulcers. Only two patients had atrophic gastritis. All of the *H pylori* strains that were isolated were *cagA*-positive (Table 1).

TLR4 and TLR5 mRNA levels in gastric biopsies

We plotted the TLR/GADPH ratios for both TLR4

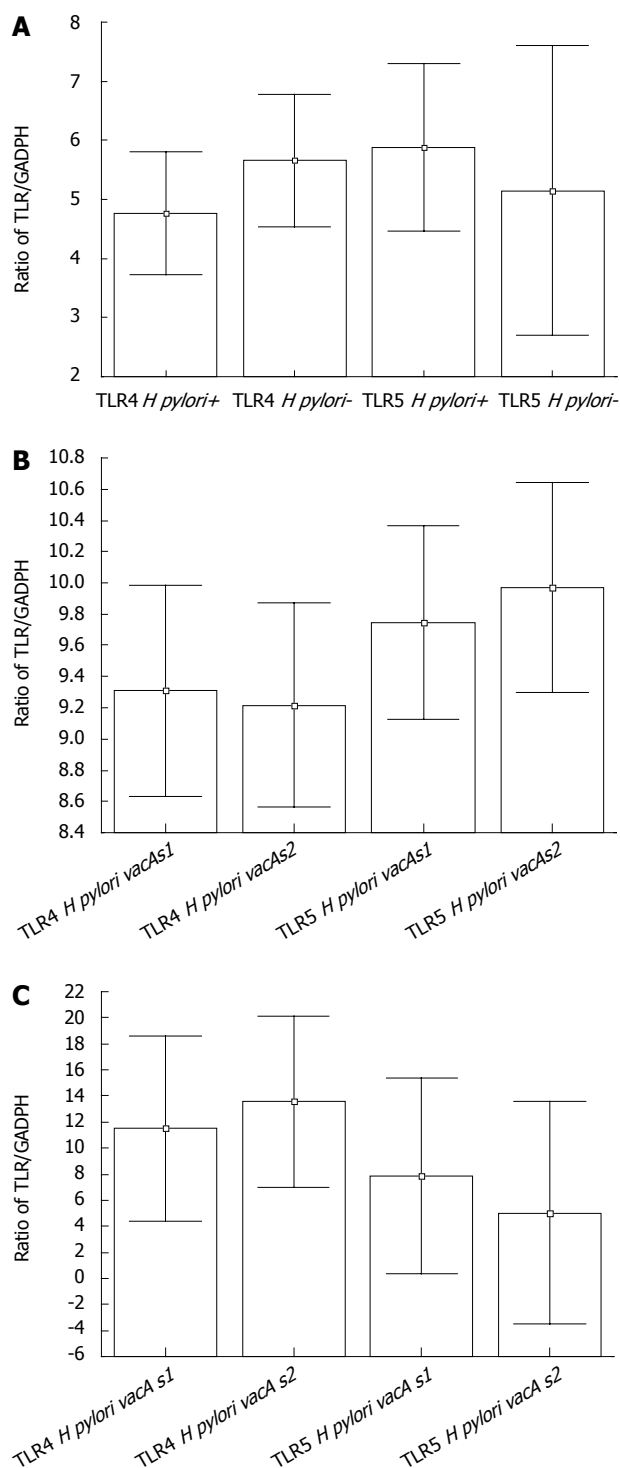


Figure 1 A: Mean (95% CI) ratio of TLR/GADPH mRNA levels for both TLR 4 and TLR5 in *H. pylori*-positive and *H. pylori*-negative patients; B: Mean (95%CI) TLR/GADPH mRNA ratios for both TLR 4 and TLR5 in patients infected with either *H. pylori vacA*s1 or *H. pylori vacA*s2 ($P > 0.05$ for both analysis); C: Mean (95% CI) TLR/GADPH mRNA ratios for both TLR 4 and TLR5 expression in AGS cells infected with *H. pylori vacA*s1 or *H. pylori vacA*s2. All is $P > 0.05$.

and TLR5 in both infected and non-infected patients (Figure 1A). The presence of *H. pylori* did not affect the mRNA levels of either Toll receptor. We did a similar analysis comparing patients infected with either *H. pylori vacA*s1 or *H. pylori vacA*s2 (Figure 1B). These data demonstrated that the mRNA levels of TLR4 and TLR5 were

not influenced by the *vacA* status ($P > 0.05$ for both receptors). There were no differences in TLR4 or TLR5 mRNA levels between patients with different clinical presentations/histological findings ($P > 0.05$, data not shown). We were not able to extract conclusions with respect to *cagA*, because all strains were *cagA* positive.

mRNA levels of TLR4 and TLR5 in AGS cells

Eleven (91.7%) of the selected strains were *cagA*-positive and there was an equal *vacA* s1 and s2 distribution between the strains examined. There were no differences in the mRNA levels of TLR4 or TLR5 regarding the *vacA*s1 status or the clinical condition associated with the infecting strains ($P > 0.05$ for both TLR4 and TLR5, Figure 1C).

DISCUSSION

Several studies have addressed the mRNA levels and protein expression of TLR4 and TLR5 in gastrointestinal cells or in AGS cells^[6-9,13]. In this study, we examined the mRNA levels of TLR4 and TLR5 in gastric epithelial cells (biopsies and AGS cells) in order to determine if significant changes in mRNA levels could be related to the presence of *H. pylori* or differences between the virulence of the strains. Our examination of gastric biopsies from infected and non-infected patients showed that there were no quantitative differences in the mRNA levels of these receptors regardless of whether *H. pylori* was present or of the patient's *H. pylori vacA* status.

Gastritis is thought to precede the development of IM, antral ulcer or atrophic gastritis^[1,2]. In this study, we included biopsies from 40 patients and among them, 29 had gastritis, 5 had IM, 4 had antral ulcers, and 2 had atrophic gastritis. When we compared the mRNA levels of both receptors between patients with gastritis and with each clinical condition/histological findings, we were unable to identify differences, suggesting that mRNA levels for both receptors may not be influenced by the infection process, or at least not at the time points selected for analysis.

Our analysis of TLR4 and TLR5 mRNA levels in AGS cells in the presence or absence of *H. pylori* showed that the amounts of TLR4 and TLR5 mRNA in human gastric epithelial cells were independent of *H. pylori vacA* status. In the *in vitro* assay, the analysis showed no differences in the amounts of TLR4 and TLR5 mRNA linked to different clinical conditions related to the *H. pylori* strains selected.

Our results do not exclude the possibility of differential expression between TLR4 and TLR5 receptors since mRNA levels do not accurately reflect protein expression. In this study, we used quantitative real-time PCR, which is the most commonly used technique for studying mRNA expression levels^[14]. This technique has some advantages, such as accuracy, sensitivity and reproducibility. Also, it allows for high throughput analyses and can be performed on very small samples. However, some problems associated with this

technique must be addressed, such as the effects of different amounts of starting material, especially when analyzing biopsies. To deal with this variation, an internal control (housekeeping gene) must be simultaneously amplified with the gene of interest for normalization purposes^[15]. In this study, even though we included analysis of GAPDH as an internal control we could not assume that protein expression levels of both receptors were equal based on the levels of detected mRNA.

An additional caveat was that the *in vivo* mRNA analysis of pooled biopsies from forty patients minimized the ability to identify biopsy-to-biopsy or patient-to-patient differences. Since RNA was extracted from all four antrum and corpus biopsies in the same vial, different gene expression profiles associated with different tissues would not have been detected. By analyzing four combined biopsies, it was possible that mRNA differences between corpus and antrum mucosal samples could have been missed. However, in the *in vitro* assay the mRNA levels obtained following infections with 12 different *H pylori* strains showed no differences in TLR expression. Although this is a uniform tissue, the observation that at least different *H pylori* strains did not affect TLR mRNA levels suggested that the same results would be observed in biopsy samples.

Some investigators have suggested that the subcellular distribution of receptors, rather than TLR expression level, could be relevant in the pathogenesis of inflammatory diseases because the expression of these receptors seems to be constitutive^[7]. Our results are consistent with this view. Nonetheless, a study examining the cellular distribution of expression in relation to *H pylori vacA* status would be an interesting issue to address.

Schmausser *et al.*^[9], reported that gastric epithelium with intestinal metaplasia and dysplasia expressed TLR4 and TLR5. They demonstrated that 17 out of 22 patients strongly expressed TLR4 (77.27%) and all 22 patients with gastric carcinoma expressed TLR5. Our study confirmed the presence of TLR4 and TLR5 mRNA, which preceded the expression of both receptors.

It is likely that evaluating the roles of other Toll-like receptors would help elucidate differences in disease manifestation and severity of diseases caused by *H pylori* infections. Valuable information regarding the recognition of whole *H pylori* or its LPS by TLR2 has been reported. Smith *et al.*^[16] demonstrated that gastric epithelial cells recognized and responded to *H pylori* infection, at least in part, via TLR2 and that *H pylori* LPS was a TLR2 agonist.

Additionally, Mandell *et al.*^[17] demonstrated that cytokine responses to whole *H pylori* were mediated by TLR2. Based on these investigations, more work examining the role of TLR2 in relation to the *H pylori vacA* status is needed. The results of this study show that the TLR4 and TLR5 mRNA levels in gastric cells both *in vivo* and *in vitro* are independent of *H pylori* and suggest that *vacA* may not be involved in the first steps of innate immune-recognition of *H pylori*.

COMMENTS

Background

H pylori is the primary cause of peptic ulcers and gastric adenocarcinoma. The variability of clinical manifestations is associated with bacterial, host immune responses and environmental factors.

Research frontiers

Gastric epithelia express toll-like receptor 4 (TLR4) and TLR5. We studied the mRNA levels of TLR4 and TLR5 in gastric epithelial cells to determine if distinctive changes in mRNA levels could be influenced by the presence of toxigenic *H pylori* strains.

Innovations and breakthroughs

In this study, we analyzed the mRNA levels of both TLR4 and TLR5 in gastric biopsies from infected and non-infected patients and in AGS cells infected with *H pylori*. We correlated these results with the *vacA* status of the strains. There were no quantitative differences in the mRNA levels of these receptors regardless of *H pylori* presence or the *H pylori vacA* status both in gastric biopsies and in AGS cells.

Applications

The mRNA levels of TLR4 and TLR5 in gastric cells both *in vivo* and *in vitro* are independent of *H pylori* or their *vacA* status.

Peer review

The results show that the mRNA levels of TLR4 and TLR5 in gastric cells are not influenced by *H pylori vacA* status and suggest that *vacA* may not be a significant player in the first step of innate immune recognition mediated by TLR4 or TLR5. It seems innovative and very interesting.

REFERENCES

- 1 Blaser MJ, Berg DE. Helicobacter pylori genetic diversity and risk of human disease. *J Clin Invest* 2001; **107**: 767-773
- 2 Figueiredo C, Machado JC, Yamaoka Y. Pathogenesis of Helicobacter pylori Infection. *Helicobacter* 2005; **10** Suppl 1: 14-20
- 3 Atherton JC, Cao P, Peek RM Jr, Tummuru MK, Blaser MJ, Cover TL. Mosaicism in vacuolating cytotoxin alleles of Helicobacter pylori. Association of specific vacA types with cytotoxin production and peptic ulceration. *J Biol Chem* 1995; **270**: 17771-17777
- 4 Odenbreit S, Puls J, Sedlmaier B, Gerland E, Fischer W, Haas R. Translocation of Helicobacter pylori CagA into gastric epithelial cells by type IV secretion. *Science* 2000; **287**: 1497-1500
- 5 Ferrero RL. Innate immune recognition of the extracellular mucosal pathogen, Helicobacter pylori. *Mol Immunol* 2005; **42**: 879-885
- 6 Su B, Ceponis PJ, Lebel S, Huynh H, Sherman PM. Helicobacter pylori activates Toll-like receptor 4 expression in gastrointestinal epithelial cells. *Infect Immun* 2003; **71**: 3496-3502
- 7 Schmausser B, Andrulis M, Endrich S, Lee SK, Josenhans C, Muller-Hermelink HK, Eck M. Expression and subcellular distribution of toll-like receptors TLR4, TLR5 and TLR9 on the gastric epithelium in Helicobacter pylori infection. *Clin Exp Immunol* 2004; **136**: 521-526
- 8 Backhed F, Rokbi B, Torstensson E, Zhao Y, Nilsson C, Seguin D, Normark S, Buchan AM, Richter-Dahlfors A. Gastric mucosal recognition of Helicobacter pylori is independent of Toll-like receptor 4. *J Infect Dis* 2003; **187**: 829-836
- 9 Schmausser B, Andrulis M, Endrich S, Muller-Hermelink HK, Eck M. Toll-like receptors TLR4, TLR5 and TLR9 on gastric carcinoma cells: an implication for interaction with Helicobacter pylori. *Int J Med Microbiol* 2005; **295**: 179-185
- 10 Flores-Orta D, Bosques-Padilla F, Gomez-Leija G, Frederick F. Comparative study of rapid urease test (Hazzell test) vs CLO-test in the diagnosis of Helicobacter pylori infection. *Gut* 1997; **41** Suppl 3: A160
- 11 Perez-Perez GI. Accurate diagnosis of Helicobacter pylori. Culture, including transport. *Gastroenterol Clin North Am*

- 2000; **29**: 879-884
- 12 **Rugge M**, Busatto G, Cassaro M, Shiao YH, Russo V, Leandro G, Avellini C, Fabiano A, Sidoni A, Covacci A. Patients younger than 40 years with gastric carcinoma: *Helicobacter pylori* genotype and associated gastritis phenotype. *Cancer* 1999; **85**: 2506-2511
- 13 **Su B**, Ceponis PJ, Lebel S, Huynh H, Sherman PM. *Helicobacter pylori* activates Toll-like receptor 4 expression in gastrointestinal epithelial cells. *Infect Immun* 2003; **71**: 3496-3502
- 14 **Bustin SA**, Nolan T. Pitfalls of quantitative real-time reverse-transcription polymerase chain reaction. *J Biomol Tech* 2004; **15**: 155-166
- 15 **Bustin SA**. Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. *J Mol Endocrinol* 2000; **25**: 169-193
- 16 **Smith MF Jr**, Mitchell A, Li G, Ding S, Fitzmaurice AM, Ryan K, Crowe S, Goldberg JB. Toll-like receptor (TLR) 2 and TLR5, but not TLR4, are required for *Helicobacter pylori*-induced NF-kappa B activation and chemokine expression by epithelial cells. *J Biol Chem* 2003; **278**: 32552-32560
- 17 **Mandell L**, Moran AP, Cocchiarella A, Houghton J, Taylor N, Fox JG, Wang TC, Kurt-Jones EA. Intact gram-negative *Helicobacter pylori*, *Helicobacter felis*, and *Helicobacter hepaticus* bacteria activate innate immunity via toll-like receptor 2 but not toll-like receptor 4. *Infect Immun* 2004; **72**: 6446-6454

S- Editor Zhong XY L- Editor Negro F E- Editor Ma WH



Chemoradiotherapy with twice-weekly administration of low-dose gemcitabine for locally advanced pancreatic cancer

Hisato Igarashi, Tetsuhide Ito, Ken Kawabe, Terumasa Hisano, Yoshiyuki Arita, Toyoma Kaku, Ryoichi Takayanagi

Hisato Igarashi, Tetsuhide Ito, Ken Kawabe, Terumasa Hisano, Yoshiyuki Arita, Toyoma Kaku, Ryoichi Takayanagi, Department of Medicine and Bioregulatory Science, Graduate School of Medical Science, Kyushu University, Fukuoka 812-8582, Japan

Author contributions: Igarashi H analyzed the data and wrote the paper; Ito T designed the study and revised the paper; Kawabe K, Hisano T, Arita Y and Kaku T collected and analyzed the data; Takayanagi R revised the paper.

Correspondence to: Hisato Igarashi, MD, PhD, Department of Medicine and Bioregulatory Science, Graduate School of Medical Science, Kyushu University 3-1-1 Maidashi Higashi-ku Fukuoka 812-8582 Japan. igaras@intmed3.med.kyushu-u.ac.jp

Telephone: +81-92-6425285 Fax: +81-92-6425287

Received: May 13, 2008 Revised: July 28, 2008

Accepted: August 4, 2008

Published online: September 14, 2008

Abstract

AIM: To evaluate the chemoradiotherapy for locally advanced pancreatic cancer utilizing low dose gemcitabine as a radiation sensitizer administered twice weekly.

METHODS: We performed a retrospective analysis of chemoradiotherapy utilizing gemcitabine administered twice weekly at a dose of 40 mg/m². After that, maintenance systemic chemotherapy with gemcitabine, at a dose of 1000 mg/m², was administered weekly for 3 wk with 1-wk rest until disease progression or unacceptable toxicity developed.

RESULTS: Eighteen patients with locally advanced unresectable pancreatic cancer were enrolled. Three of those patients could not continue with the therapy; one patient had interstitial pneumonia during radiation therapy and two other patients showed liver metastasis or peritoneal metastasis during an early stage of the therapy. The median survival was 15.0 mo and the overall 1-year survival rate was 60%, while the median progression-free survival was 8.0 mo. The subgroup which showed the reduction of tumor development, more than 50% showed a tendency for a better prognosis; however, other parameters including age, gender and performance status did not correlate with survival. The median survival of the groups that died of liver metastasis and peritoneal metastasis were 13.0 mo and 27.7 mo, respectively.

CONCLUSION: Chemoradiotherapy with low-dose gemcitabine administered twice weekly could be effective to patients with locally advanced pancreatic cancer; however, patients developing liver metastases had a worse prognosis. Another chemoradiotherapy strategy might be needed for those patients, such as administering one or two cycles of chemotherapy initially, followed by chemoradiotherapy for the cases with no distant metastases.

© 2008 The WJG Press. All rights reserved.

Key words: Advanced pancreatic cancer; Chemoradiotherapy; Gemcitabine; Radiosensitizer; Tumor marker

Peer reviewer: Michael E Zenilman, MD, Clarence and Mary Dennis Professor and Chairman, Department of Surgery, SUNY Downstate Medical Center, Box40, 450, Clarkson Avenue, Brooklyn, NY 11202, United States

Igarashi H, Ito T, Kawabe K, Hisano T, Arita Y, Kaku T, Takayanagi R. Chemoradiotherapy with twice-weekly administration of low-dose gemcitabine for locally advanced pancreatic cancer. *World J Gastroenterol* 2008; 14(34): 5311-5315 Available from: URL: <http://www.wjgnet.com/1007-9327/14/5311.asp> DOI: <http://dx.doi.org/10.3748/wjg.14.5311>

INTRODUCTION

Pancreatic cancer is one of the leading causes of cancer death in the world and in most patients the tumor is surgically unresectable at the time of diagnosis^[1]. Even in the patient with complete surgical resection, both distant and local patterns of recurrence are common^[2]. In approximately 50% of resected pancreatic tumors, the surgical margins are involved with tumor cells, so it can be assumed that most patients are harboring occult metastases at the time of diagnosis^[3]. Recently, studies for adjuvant chemotherapy or chemoradiotherapy, and those for neoadjuvant chemotherapy or chemoradiotherapy have been investigated^[3-6].

For patients with locally advanced pancreatic cancer, chemoradiotherapy has been accepted as a standard treatment^[7]. The results of previous randomized trials

have indicated that external-beam radiation therapy and 5-fluorouracil (5-FU) therapy results in a significantly longer survival time than radiotherapy^[8] or chemotherapy alone^[9]. Gemcitabine, a deoxycytidine analog that functions as an antimetabolite, has been approved for use in patients with advanced pancreatic cancer^[10,11]. In a randomized study, gemcitabine improved survival in inoperable pancreatic cancer in comparison with 5-FU^[12]. Gemcitabine has also been shown to exert an effect in 5-FU-refractory pancreatic cancer^[13]. Gemcitabine has also been shown to be a potent radiosensitizer, both *in vivo* and *in vitro*^[14]. The vast majority of the reported phase I-III clinical trials have used gemcitabine as a single agent given weekly in a single dose^[7,15,16] (i.e. 250 mg/m²).

Several preclinical data, including animal studies^[17], would suggest that maximum radiation sensitization with gemcitabine is observed at a lower dose administered twice weekly^[15,17]. Blackstock *et al*^[14,15] and Magnino *et al*^[18] reported on a phase II study of chemoradiotherapy in which the patients were treated with gemcitabine twice weekly at 40 mg/m² and 50 mg/m², respectively, associated with radiotherapy. Therefore, in the present study, we analyzed the results of retrospective analysis of chemoradiotherapy for locally advanced pancreatic cancer, utilizing gemcitabine as a radiation sensitizer administered twice weekly at a dose of 40 mg/m², followed by maintenance systemic chemotherapy with gemcitabine.

MATERIALS AND METHODS

Eligibility criteria included (1) locally advanced unresectable pancreatic cancer confirmed histologically or by imaging techniques including systemic computed tomography; (2) 20-74 years of age; (3) ECOG performance status of 0-2; (4) adequate hematological function, and adequate renal function, and (5) no prior anti-cancer treatment. A total dose of 40-50.4 Gy was delivered using 1.8-2.0 Gy daily fractions. Treatment planning was determined by a three-dimensional treatment planner. The targeted irradiation volume included the tumor, possible surrounding edema, and 1-cm margin. Gemcitabine, at a dose of 40 mg/m², was administered as a 30-min intravenous infusion twice weekly (80 mg/m² per week) for 4-5 wk. Gemcitabine was given within 2 h before radiation treatment. At 2 wk after the completion of chemoradiotherapy, maintenance systemic chemotherapy of gemcitabine at a dose of 1000 mg/m² was administered as a 30-min intravenous infusion weekly for 3 wk with 1-wk rest until disease progression or unacceptable toxicity. Both radiation therapy and chemotherapy were suspended for grade 3 hematological toxicities or grade 2 non-hematological toxicities (according to the National Cancer Institute Common Toxicity Criteria) during the treatment course, and treatment was resumed when toxicity was resolved. The objective tumor response, as defined by the WHO criteria, was assessed every 2 mo or 3 mo by computed tomography scan or earlier if clinically indicated.

The Kaplan-Meier method was used to estimate the distribution of overall survival and progression free

Table 1 Patient characteristics

Number of patients completing the protocol	15
Gender	
Male	9 (60%)
Female	6 (40%)
Age (yr)	
Mean (range)	62.2 (50-73)
Tumor location	
Head	7 (46.7%)
Head-Body	1 (6.6%)
Body-Tail	7 (46.7%)
Total radiation dose	
40.0 Gy	12 (80%)
50.0 Gy	1 (6.6%)
50.4 Gy	2 (13.4%)
Response	
Complete response	1 (6.6%)
Partial response	4 (26.7%)
Stable disease	9 (60%)
Progressive disease	1 (6.6%)
Cause of death	
Liver metastasis	10 (66.7%)
Peritoneal metastasis	3 (20%)

survival. Progression free survival was calculated from the first day of treatment until there was evidence of clinical progression, tumor progression assessed by computed tomography scan measurement or death. Overall survival was calculated from the first day of treatment until the date of death. In this study, there is no control arm to treat the locally advanced pancreatic cancer.

RESULTS

Clinical data

Eighteen patients were enrolled in this study. Three of those patients could not continue with the therapy under this protocol; one patient had interstitial pneumonia during radiation therapy and two other patients showed liver metastasis or peritoneal metastasis in an early stage of this protocol. Fifteen patients, including nine males and six females, completed therapy as planned and patient characteristics are shown in Table 1. The mean age was 62.2 years old (range, 50-73). The mean diameter of the tumor was 4.8-cm and the tumor was located in the pancreatic head in seven patients. Twelve patients received radiotherapy at a total of 40-Gy, two patients at a total dose of 50-Gy and one patient with 50.4-Gy. In general, therapy was well tolerated, one patient suffered AGML and another patient had an eruption. All the patients showed elevation of tumor markers, including CA19-9, Span-1 and DUPAN-2, at the enrollment for this study.

Survival

Regarding overall response, there was one complete response, 4 partial responses, 9 stable diseases and one progressive disease; the response rate was 33%. No patients could undergo tumor resection even after the completion of chemoradiotherapy, because of infiltration

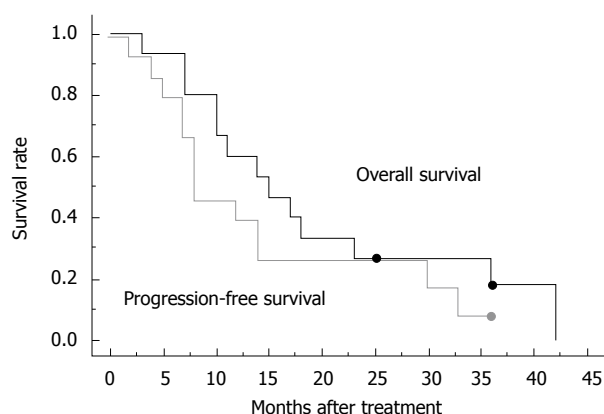


Figure 1 Overall survival curve and progression-free survival curve for 15 patients who received chemoradiotherapy under this study protocol. Dot indicates censored cases.

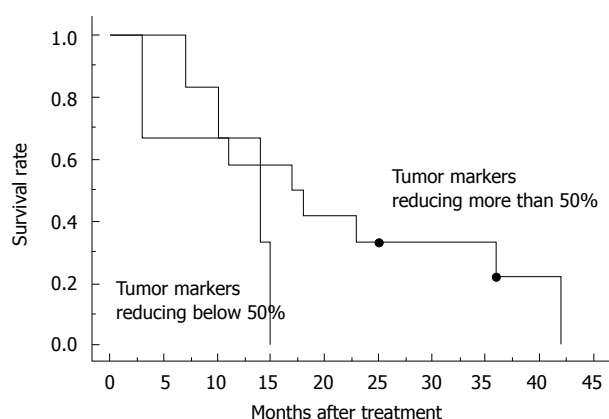


Figure 2 Overall survival curve for patients with tumor markers reducing more than 50% compared to that of pretreatment ($n = 12$) and those reducing below 50% ($n = 3$). Dot indicates censored cases.

of the adjacent large vessels. The median survival was 15.0 mo and the overall 1-year survival rate was 60%, while the median progression-free survival was 8.0 mo, estimated by the Kaplan-Meier method (Figure 1). In 80% of the patients, the level of tumor marker, including CA19-9, Span-1 and DUPAN-2, was reduced more than 50% compared to that of pretreatment. The subgroup where the tumor marker was reduced more than 50% had a tendency for a better prognosis (Figure 2), compared to the group with reduced tumor marker below 50% of pretreatment. Blackstock *et al.*^[15] postulated previously that the extended median survival observed in the CA19-9 responding patients might reflect the impact of the improved local control. However, a recent study demonstrated that pretreatment serum CA19-9 concentration was an independent prognostic factor for survival for advanced pancreatic cancer, but a decrease in concentration during chemotherapy was not significantly associated with lengthened survival compared with those who did not have a corresponding decrease^[19]; therefore, the importance of decreasing in serum tumor marker concentration during therapy requires further discussion. In the subgroup with a tumor size less than 4-cm in diameter, median progression-free survival was 14.0 mo,

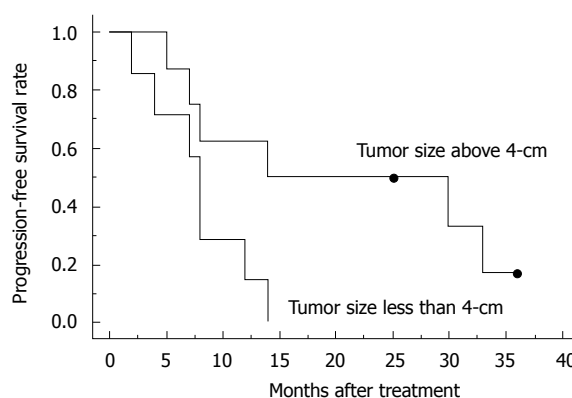


Figure 3 Progression free-survival curve for patients with tumor size less than 4-cm ($n = 6$) and those with a size above 4-cm ($n = 9$). Dot indicates censored cases.

which was better than those above 4-cm in diameter (8.0 mo, Figure 3). Other parameters, including age, gender and performance status, did not correlate with survival. The major causes of death were liver metastasis and peritoneal metastasis. The median survival of the groups that died of liver metastasis and peritoneal metastasis were 13.0 mo and 27.7 mo, respectively.

DISCUSSION

A recent retrospective comparison of the toxicity and efficacy of concurrent gemcitabine-based chemoradiotherapy with that of 5-FU based chemoradiotherapy for the patients with unresectable pancreatic cancer^[20], showed a significantly higher toxicity rate in patients treated with gemcitabine and similar median survival times between the two arms. Investigators in Taiwan^[21] reported favorable results for chemoradiotherapy with concurrent gemcitabine administration (600 mg/m² once a week); however, this needs further confirmation by larger multi-institutional clinical trials.

Although this study, using a twice weekly gemcitabine infusion schedule for locally advanced pancreatic cancer was not a controlled study, the results of the median survival time, median disease free survival time and overall 1-year survival rate was found to be preferable compared to previous studies^[7-9,15]. Okusaka *et al.*^[7] presented data of a phase II study for locally advanced pancreatic cancer treated with external-beam radiation (50.4 Gy) and weekly gemcitabine (250 mg/m² once a week) followed by maintenance chemotherapy using gemcitabine. The median survival time, median progression-free survival time and 1-year survival rate was 9.5 mo, 4.4 mo and 28%, respectively^[7]. An expanded retrospective review of patients receiving gemcitabine-based chemoradiotherapy at the M. D. Anderson Cancer Center reflected the difficulties combining the systemic toxicities of 200-500 mg/m² doses of gemcitabine with the local-regional toxicities associated with chemoradiotherapy to the upper abdomen^[22]. Furthermore, in the original GITSG trial of radiation and 5-FU based chemotherapy, 18% and 21% of the patients randomized into the

40-Gy and the 60-Gy treatment arms, respectively, were unable to complete all planned radiation^[8]. For those patients completing the chemoradiotherapy, almost one-third were unable to initiate the planned maintenance 5-FU chemotherapy^[8]. In this study, 15 of 18 patients could complete the planned protocol which might have come from the treatment with gemcitabine administered *via* a twice weekly infusion with radiation therapy and that most patients received radiation therapy at a total dose of 40-Gy. This might have resulted in the successful initiation in the maintenance of gemcitabine chemotherapy and to obtain a feasible survival rate in this trial. Some investigators did not propose maintenance chemotherapy after chemoradiotherapy^[1]. Several studies of chemoradiotherapy used a therapeutic sequence with prior chemoradiotherapy and then chemotherapy until disease progression, but increased toxicity of chemotherapy after chemoradiotherapy limits this strategy^[23,24], which might partially contribute to the total dose of radiation.

Two of the three patients enrolled initially who did not continue with the therapy under this protocol showed liver metastasis or peritoneal metastasis in the early stage of this protocol. Blackstock *et al*^[15] pointed out in their study that the radiation sensitizing properties of twice weekly gemcitabine were important for improving the local control, and did not impact the survival for patients harboring micrometastatic disease at the initiation treatment. Huguet *et al*^[1] discussed that, an important concern about administering chemoradiotherapy as first-line treatment in patients with locally advanced pancreatic cancer was that approximately 30% of them had occult metastatic disease at diagnosis and thus, they would clearly not benefit from this locoregional treatment. Furthermore, another investigator demonstrated that a fraction of patients with locally advanced pancreatic cancer developed metastases within a few weeks and died very quickly despite the type of treatment^[25]. In this study, the patients who developed liver metastasis had a worse prognosis, which might owe to the miss-diagnosis of the staging of the disease at the initiation of the therapy, because of failure to detect micrometastasis by conventional imaging modalities. In this situation, we might need another strategy for the chemoradiotherapy for locally advanced pancreatic cancer, such as one in which the patients receive one or two cycles of systemic chemotherapy using gemcitabine at a dose of 1000 mg/m² weekly for 3 wk with 1-wk rest, and then re-evaluated the staging of the disease, initiating the chemoradiotherapy under the protocol in this study. A recent study suggested that after control of disease by initial chemotherapy for at least 3 mo using combination of leucovorin, fluorouracil and gemcitabine, or gemcitabine and oxaliplatin, chemoradiotherapy with 5-FU, could significantly improve survival in patients with locally advanced pancreatic cancer compared with chemotherapy alone^[1].

In conclusion, chemoradiotherapy with low-dose gemcitabine given twice weekly could be effective to patients with locally advanced pancreatic cancer; however, patients developing liver metastases had a worse prog-

nosis. We might need another strategy for the chemoradiotherapy for those patients. Further investigations are required in the near future.

COMMENTS

Background

Pancreatic cancer is the fifth most common cause of cancer death in Japan. The prognosis is extremely poor because it is difficult to detect this disease in the early stage and also the postoperative incidence of recurrence is still high. We do not have any effective treatment for inoperable patients. Recently, chemoradiotherapy has been regarded as one of the standard therapies for locally advanced pancreatic cancer and it has improved the survival and presented a clinical benefit.

Research frontiers

In the early 1980s, fluorouracil-based concomitant chemoradiotherapy was shown to be better than radiotherapy alone for patients with locally advanced pancreatic cancer. Gemcitabine has improved the outcome of patients with advanced disease by improving survival with a clinical benefit. Gemcitabine also has been shown to be a potent radiosensitizer, both *in vivo* and *in vitro*. The vast majority of the reported phase I -III clinical trials have used gemcitabine as a single agent given weekly in a single dose (i.e. 250 mg/m²), and there is no consensus of the protocol of the administration of gemcitabine.

Innovations and breakthroughs

Several preclinical data, including animal studies, would suggest that maximum radiation sensitization with gemcitabine is observed at a lower dose administered twice weekly. In this study, we show that we could obtain the feasible results of survival compared to previous studies using our protocol. There existed some patients who could not continue the therapy, because of developing metastases. One reason could be the failure to detect micrometastasis by conventional imaging modalities at the beginning of chemoradiotherapy.

Applications

Chemoradiotherapy, with low-dose gemcitabine given twice weekly, could be effective to patients with locally advanced pancreatic cancer. To improve this survival data, we may need stricter selection of the cases suitable for this chemoradiotherapy; however, using conventional imaging modalities, it seems to be hard to diagnose the micrometastasis, especially in the liver before this chemoradiotherapy. Another strategy that may be useful is where patients receive one or two cycles of systemic chemotherapy using gemcitabine at a dose of 1000 mg/m² weekly for 3 wk with 1-wk rest, and then be re-evaluated for the staging of the disease, and then initiating the chemoradiotherapy under the protocol in this study.

Peer review

This is a nicely written paper that looks at the use of gemcitabine as a radiation sensitizer for pancreatic cancer. They report on twice-weekly doses. This dose contributes new information to the literature.

REFERENCES

- 1 Huguet F, Andre T, Hammel P, Artru P, Balosso J, Selle F, Deniaud-Alexandre E, Ruzsiewicz P, Touboul E, Labianca R, de Gramont A, Louvet C. Impact of chemoradiotherapy after disease control with chemotherapy in locally advanced pancreatic adenocarcinoma in GERCOR phase II and III studies. *J Clin Oncol* 2007; **25**: 326-331
- 2 White RR, Shah AS, Tyler DS. Pancreatic cancer since Halsted: how far have we come and where are we going? *Ann Surg* 2003; **238**: S132-S144; discussion S145-S147
- 3 Takai S, Satoi S, Yanagimoto H, Toyokawa H, Takahashi K, Terakawa N, Araki H, Matsui Y, Sohigawa M, Kamiyama Y. Neoadjuvant chemoradiation in patients with potentially resectable pancreatic cancer. *Pancreas* 2008; **36**: e26-e32
- 4 Oettle H, Post S, Neuhaus P, Gellert K, Langrehr J, Ridwelski K, Schramm H, Fahlke J, Zuelke C, Burkart C, Gutterlet K, Kettner E, Schmalenberg H, Weigang-Koehler K, Bechstein WO, Niedergethmann M, Schmidt-Wolf I, Roll L, Doerken B, Riess H. Adjuvant chemotherapy with gemcitabine vs observation in patients undergoing

- curative-intent resection of pancreatic cancer: a randomized controlled trial. *JAMA* 2007; **297**: 267-277
- 5 **Regine WF**, Winter KA, Abrams RA, Safran H, Hoffman JP, Konski A, Benson AB, Macdonald JS, Kudrimoti MR, Fromm ML, Haddock MG, Schaefer P, Willett CG, Rich TA. Fluorouracil vs gemcitabine chemotherapy before and after fluorouracil-based chemoradiation following resection of pancreatic adenocarcinoma: a randomized controlled trial. *JAMA* 2008; **299**: 1019-1026
 - 6 **Vento P**, Mustonen H, Joensuu T, Karkkainen P, Kivilaakso E, Kiviluoto T. Impact of preoperative chemoradiotherapy on survival in patients with resectable pancreatic cancer. *World J Gastroenterol* 2007; **13**: 2945-2951
 - 7 **Okusaka T**, Ito Y, Ueno H, Ikeda M, Takezako Y, Morizane C, Kagami Y, Ikeda H. Phase II study of radiotherapy combined with gemcitabine for locally advanced pancreatic cancer. *Br J Cancer* 2004; **91**: 673-677
 - 8 **Moertel CG**, Frytak S, Hahn RG, O'Connell MJ, Reitemeier RJ, Rubin J, Schutt AJ, Weiland LH, Childs DS, Holbrook MA, Lavin PT, Livstone E, Spiro H, Knowlton A, Kalsner M, Barkin J, Lessner H, Mann-Kaplan R, Ramming K, Douglas HO Jr, Thomas P, Nave H, Bateman J, Lokich J, Brooks J, Chaffey J, Corson JM, Zarncheck N, Novak JW. Therapy of locally unresectable pancreatic carcinoma: a randomized comparison of high dose (6000 rads) radiation alone, moderate dose radiation (4000 rads + 5-fluorouracil), and high dose radiation + 5-fluorouracil: The Gastrointestinal Tumor Study Group. *Cancer* 1981; **48**: 1705-1710
 - 9 **Treatment of locally unresectable carcinoma of the pancreas: comparison of combined-modality therapy (chemotherapy plus radiotherapy) to chemotherapy alone.** Gastrointestinal Tumor Study Group. *J Natl Cancer Inst* 1988; **80**: 751-755
 - 10 **Berlin JD**, Catalano P, Thomas JP, Kugler JW, Haller DG, Benson AB 3rd. Phase III study of gemcitabine in combination with fluorouracil versus gemcitabine alone in patients with advanced pancreatic carcinoma: Eastern Cooperative Oncology Group Trial E2297. *J Clin Oncol* 2002; **20**: 3270-3275
 - 11 **Sakamoto H**, Kitano M, Suetomi Y, Takeyama Y, Ohyanagi H, Nakai T, Yasuda C, Kudo M. Comparison of standard-dose and low-dose gemcitabine regimens in pancreatic adenocarcinoma patients: a prospective randomized trial. *J Gastroenterol* 2006; **41**: 70-76
 - 12 **Burris HA 3rd**, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, Cripps MC, Portenoy RK, Storniolo AM, Tarassoff P, Nelson R, Dorr FA, Stephens CD, Von Hoff DD. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. *J Clin Oncol* 1997; **15**: 2403-2413
 - 13 **Rothenberg ML**. New developments in chemotherapy for patients with advanced pancreatic cancer. *Oncology (Williston Park)* 1996; **10**: 18-22
 - 14 **Blackstock AW**, Bernard SA, Richards F, Eagle KS, Case LD, Poole ME, Savage PD, Tepper JE. Phase I trial of twice-weekly gemcitabine and concurrent radiation in patients with advanced pancreatic cancer. *J Clin Oncol* 1999; **17**: 2208-2212
 - 15 **Blackstock AW**, Tepper JE, Niedwiecki D, Hollis DR, Mayer RJ, Tempero MA. Cancer and leukemia group B (CALGB) 89805: phase II chemoradiation trial using gemcitabine in patients with locoregional adenocarcinoma of the pancreas. *Int J Gastrointest Cancer* 2003; **34**: 107-116
 - 16 **Crane CH**, Varadhachary G, Pisters PW, Evans DB, Wolff RA. Future chemoradiation strategies in pancreatic cancer. *Semin Oncol* 2007; **34**: 335-346
 - 17 **Fields MT**, Eisbruch A, Normolle D, Orfali A, Davis MA, Pu AT, Lawrence TS. Radiosensitization produced in vivo by once- vs. twice-weekly 2'-difluoro-2'-deoxycytidine (gemcitabine). *Int J Radiat Oncol Biol Phys* 2000; **47**: 785-791
 - 18 **Magnino A**, Gatti M, Massucco P, Sperti E, Faggiuolo R, Regge D, Capussotti L, Gabriele P, Aglietta M. Phase II trial of primary radiation therapy and concurrent chemotherapy for patients with locally advanced pancreatic cancer. *Oncology* 2005; **68**: 493-499
 - 19 **Hess V**, Glimelius B, Grawe P, Dietrich D, Bodoky G, Ruhstaller T, Bajetta E, Saletti P, Figer A, Scheithauer W, Herrmann R. CA 19-9 tumour-marker response to chemotherapy in patients with advanced pancreatic cancer enrolled in a randomised controlled trial. *Lancet Oncol* 2008; **9**: 132-138
 - 20 **Crane CH**, Abbruzzese JL, Evans DB, Wolff RA, Ballo MT, Delclos M, Milas L, Mason K, Charnsangavej C, Pisters PW, Lee JE, Lenzi R, Vauthey JN, Wong AB, Phan T, Nguyen Q, Janjan NA. Is the therapeutic index better with gemcitabine-based chemoradiation than with 5-fluorouracil-based chemoradiation in locally advanced pancreatic cancer? *Int J Radiat Oncol Biol Phys* 2002; **52**: 1293-1302
 - 21 **Li CP**, Chao Y, Chi KH, Chan WK, Teng HC, Lee RC, Chang FY, Lee SD, Yen SH. Concurrent chemoradiotherapy treatment of locally advanced pancreatic cancer: gemcitabine versus 5-fluorouracil, a randomized controlled study. *Int J Radiat Oncol Biol Phys* 2003; **57**: 98-104
 - 22 **Crane CH**, Janjan NA, Evans DB, Wolff RA, Ballo MT, Milas L, Mason K, Charnsangavej C, Pisters PW, Lee JE, Lenzi R, Vauthey JN, Wong A, Phan T, Nguyen Q, Abbruzzese JL. Toxicity and efficacy of concurrent gemcitabine and radiotherapy for locally advanced pancreatic cancer. *Int J Pancreatol* 2001; **29**: 9-18
 - 23 **Epelbaum R**, Rosenblatt E, Nasrallah S, Faraggi D, Gaitini D, Mizrahi S, Kuten A. Phase II study of gemcitabine combined with radiation therapy in patients with localized, unresectable pancreatic cancer. *J Surg Oncol* 2002; **81**: 138-143
 - 24 **Schneider BJ**, Ben-Josef E, McGinn CJ, Chang AE, Colletti LM, Normolle DP, Hejna GF, Lawrence TS, Zalupski MM. Capecitabine and radiation therapy preceded and followed by combination chemotherapy in advanced pancreatic cancer. *Int J Radiat Oncol Biol Phys* 2005; **63**: 1325-1330
 - 25 **Andre T**, Balosso J, Louvet C, Hannoun L, Houry S, Huguier M, Colonna M, Lotz JP, De Gramont A, Bellaiche A, Parc R, Touboul E, Izrael V. Combined radiotherapy and chemotherapy (cisplatin and 5-fluorouracil) as palliative treatment for localized unresectable or adjuvant treatment for resected pancreatic adenocarcinoma: results of a feasibility study. *Int J Radiat Oncol Biol Phys* 2000; **46**: 903-911

S- Editor Zhong XY L- Editor Rippe RA E- Editor Ma WH



RAPID COMMUNICATION

Endoscopic findings can predict the efficacy of leukocytapheresis for steroid-naïve patients with moderately active ulcerative colitis

Yasushi Umehara, Masatoshi Kudo, Masanori Kawasaki

Yasushi Umehara, Masatoshi Kudo, Masanori Kawasaki, Division of Gastroenterology and Hepatology, Department of Internal Medicine Kinki University School of Medicine, 377-2 Ohno-Higashi, Osaka-Sayama 589-8511, Japan

Author contributions: Umehara Y, Kudo M and Kawasaki M contributed equally to this work; Umehara Y and Kudo M designed research; Umehara Y and Kawasaki M performed research; Umehara Y and Kudo M contributed new reagents/analytic tools; Umehara Y and Kudo M analyzed data and Umehara Y wrote the paper.

Correspondence to: Masatoshi Kudo, MD, PhD, Division of Gastroenterology and Hepatology, Department of Internal Medicine Kinki University School of Medicine, 377-2 Ohno-Higashi, Osaka-Sayama 589-8511, Japan. m-kudo@med.kindai.ac.jp

Telephone: +81-723-660221-3525 Fax: +81-723-672880

Received: June 21, 2008 Revised: August 11, 2008

Accepted: August 18, 2008

Published online: September 14, 2008

for steroid-naïve UC patients with moderate disease activity. Moreover, the efficacy of the treatment can be predicted on the basis of endoscopic findings.

© 2008 The WJG Press. All rights reserved.

Key words: Ulcerative colitis; Steroid-naïve; Leukocytapheresis; Efficacy; Endoscopic findings

Peer reviewer: Alastair JM Watson, Professor, Department of Gastroenterology, University of Liverpool, the Henry Wellcome Laboratory, Nuffield Bldg, Crown St, Liverpool L69 3GE, United Kingdom

Umehara Y, Kudo M, Kawasaki M. Endoscopic findings can predict the efficacy of leukocytapheresis for steroid-naïve patients with moderately active ulcerative colitis. *World J Gastroenterol* 2008; 14(34): 5316-5321 Available from: URL: <http://www.wjgnet.com/1007-9327/14/5316.asp> DOI: <http://dx.doi.org/10.3748/wjg.14.5316>

Abstract

AIM: To investigate the therapeutic usefulness of leukocytapheresis (LCAP; Cellsoba) in steroid-naïve patients with moderately active ulcerative colitis (UC).

METHODS: Eighteen steroid-naïve patients with moderately active UC received one LCAP session every week for five consecutive weeks.

RESULTS: The remission rate 8 weeks after the last LCAP session was 61.1% (11/18). All three patients with deep ulcers showed worsening after LCAP. For the remaining 15 patients, who had erosions or geographic ulcers, the average clinical activity index (CAI) score dropped significantly from 9.4 to 3.8 eight weeks after the last LCAP session ($t = 4.89$, $P = 0.001$). The average C-reactive protein (CRP) levels before and after LCAP were 1.2 mg/dL and 1.0 mg/dL, respectively. Of the patients with erosions, geographic ulcers, and deep ulcers, 100% (9/9), 33.3% (2/6), and 0% (0/3) were in remission 8 weeks after the last LCAP session, respectively ($\chi^2 = 7.65$, $P < 0.005$). Forty-eight weeks after the last LCAP session, the remission rates for patients with erosions and geographic ulcers were 44.4% (4/9) and 16.7% (1/6), respectively. Only one patient suffered a mild adverse event after LCAP (nausea).

CONCLUSION: LCAP is a useful and safe therapy

INTRODUCTION

Although the etiology of ulcerative colitis (UC) is still unknown, it is believed that an immune abnormality may be involved in its development^[1,2]. It is characterized by chronic over-activation of the colonic mucosal immune system. Consequently, if remission cannot be achieved by salazosulfapyridine or mesalazine treatment, the second line of treatment has conventionally been to administer steroids^[2-6]. However, steroid administration can increase susceptibility to infections, diabetes mellitus and osteoporosis. Recently, it was reported that steroid-refractory or steroid-dependent patients with UC can be effectively treated by cytapheresis^[7-10]. To determine whether leukocytapheresis (LCAP) may also be useful with other UC patients, we administered LCAP to 18 steroid-naïve UC patients. We also assessed whether the efficacy of LCAP can be predicted on the basis of endoscopic findings.

MATERIALS AND METHODS

From January 2005 to April 2007, 33 UC patients were treated with LCAP at our hospital. All patients were

Table 1 Steroid-naïve UC patient characteristics (mean \pm SD)

Characteristics	Data
Male/Female	11/7
Age (yr)	46.1 \pm 18.4
Duration of disease (yr)	6.0 \pm 8.5
Clinical course	
First attack	4
Relapse-remitting	6
Chronic continuous	8
Extent of disease	
Entire	15
Left sided	2
Rectum	1
Endoscopic findings	
Erosions	9
Geographic ulcers	6
Deep ulcers	3

examined by colonoscopy before treatment and UC was diagnosed on the basis of established endoscopic and histological criteria^[11]. At the time of diagnosis, infectious colitis (*Salmonella*, *Campylobacter*, *Vibrio*, *Yersinia* and *Shigella spp.*) was ruled out by stool culture and *Clostridium difficile* toxin testing. Moreover, we excluded Crohn's disease, ischemic colitis, radiation colitis and intestinal Behçet disease. None of patients were receiving drugs, including non-steroidal anti-inflammatory drugs or antibiotics. Patients with severe cardiovascular disease, severe cerebral disease, severe anemia (hemoglobin; less than 8 g/dL) and hypotension (less than 80 mmHg) were excluded. Of the 33 patients, 15 had severe activity and were treated with steroids along with LCAP. These patients were excluded from the study. The remaining 18 steroid-naïve patients had moderate activity, as defined by a Lichtiger's clinical activity index (CAI) score^[12] of < 12. These patients were enrolled in the study. Their characteristics are summarized in Table 1.

For all patients, LCAP sessions were performed once a week for five consecutive weeks by using Cellsorba (Asahi Medical Co., Ltd, Tokyo, Japan). Leukocyte removal in LCAP is effective because of its adherence to fibers in the filter. The throughput was 2-3 L of whole blood and the flow rate was 30-50 mL/min for approximately 60 min. The access and return lines were connected to cubital veins. Heparin was used as an anticoagulant for the extracorporeal circulation.

Patients were 46.1 \pm 18.4 years old; there were 11 males and 7 females. Their duration of disease was 6.0 \pm 8.5 years. With regard to their clinical course, four patients presented the "first attack" type, six the "relapse-remitting" type, and eight the "chronic continuous" type. We defined patients presenting with an activity phase lasting for 6 mo or longer from the first attack as belonging to the "chronic continuous" type. Fifteen patients had total colitis, two had left-sided colitis and one had proctitis. We performed endoscopy before and after LCAP. For the present study, we classified the patients into three groups on the basis of the endoscopic findings before LCAP treatment, namely, those with erosions, geographic ulcers, or deep

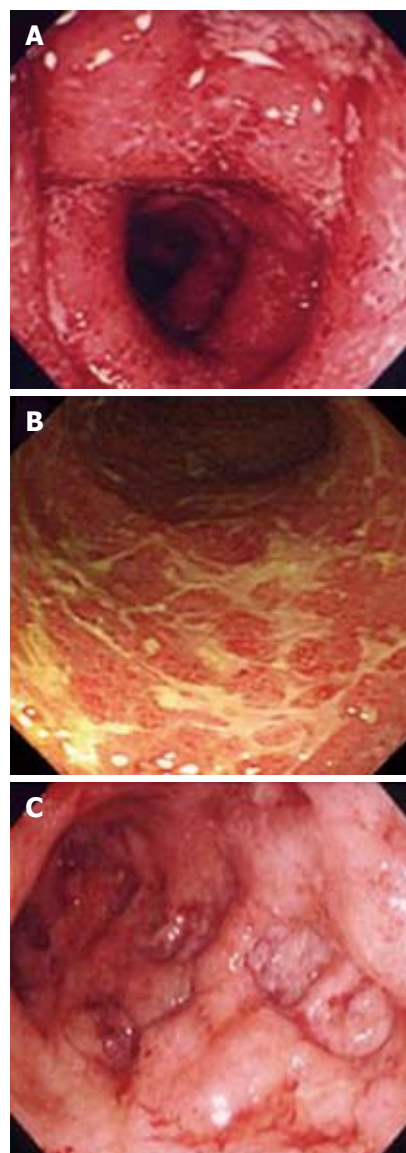


Figure 1 The patients were divided before LCAP into three groups according to whether their endoscopic findings revealed erosions (A), geographic ulcers (B), or deep ulcers (C). Representative findings are shown.

ulcers (Figure 1). Nine patients had erosions in the large intestine, six had geographic ulcers, and three had deep ulcers. All patients were concomitantly treated with mesalazine (2250 mg/d) for at least 4 weeks prior to the initiation of LCAP therapy. There was no change in the dosage of mesalazine. Immunomodulators such as azathioprine, 6-mercaptopurine and cyclosporine were never administered.

Disease activity was evaluated before and after LCAP by measuring the CAI. A CAI less than 4 indicates remission. Relapses were also identified when the patient needed another therapy, such as steroid or cyclosporine treatment and/or LCAP.

The endpoint of this study was to determine the factor related to remission by LCAP.

Statistical analysis

The Wilcoxon test was used to compare CAI scores

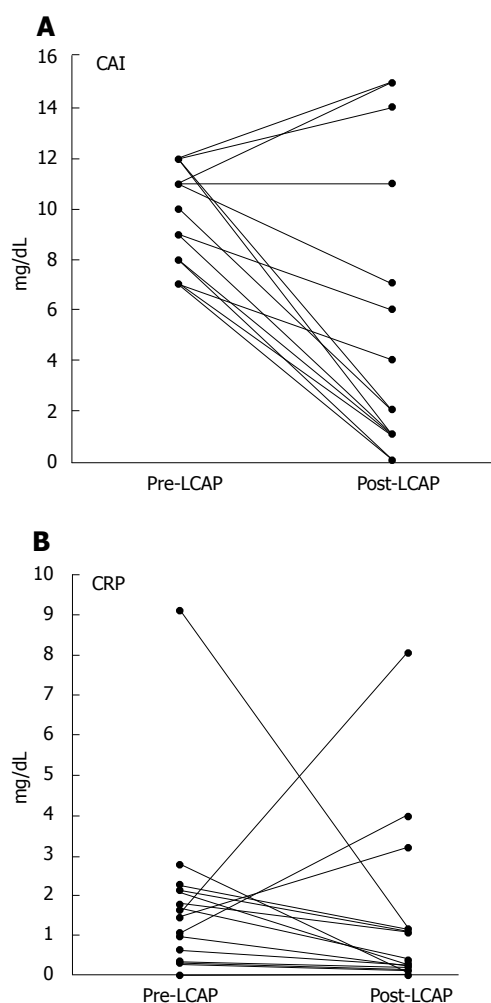


Figure 2 Change in average CAI score (A) and CRP levels (B) 8 wk after the last LCAP session. The data of the three patients with deep ulcers whose conditions worsened during LCAP are not included. The average CAI dropped from 9.4 ± 1.9 to 3.8 ± 4.8 ($P = 0.001$) while the average CRP levels before and after LCAP were 1.2 ± 0.8 mg/dL and 1.0 ± 2.0 mg/dL, respectively, and did not differ significantly.

and C-reactive protein (CRP) levels before and after treatment. The Mann-Whitney U -test was used to compare the age, duration of disease, pre-CAI, post-CAI, pre-CRP and post-CRP levels of two groups, while the χ^2 test was used to test the effect of sex. χ^2 test was used to compare the clinical course, extent of disease and endoscopic findings of two groups. $P < 0.05$ was considered to indicate statistical significance. Results were presented as mean \pm SD.

RESULTS

Efficacy of LCAP

For all 18 patients, the remission rates 8 and 48 wk after the last LCAP session were 61.1% (11/18) and 27.7% (5/18), respectively. At 48 wk after remission, the relapse rate was 54.5% (6/11), and the duration to relapse was 8.7 ± 4.2 mo. Three patients with deep ulcers worsened during LCAP and required additional treatments such as steroids. However, the remaining 15 patients showed a significant drop in the CAI score from 9.4 ± 1.9 to $3.8 \pm$

Table 2 Comparison of responders and non-responders 8 wk after the last LCAP session (mean \pm SD)

	Responders (<i>n</i> = 11)	Non-responders (<i>n</i> = 7)	<i>P</i>
Patient characteristic			
Male/Female	7/4	4/3	NS
Age (yr)	39.7 ± 15.9	56.1 ± 17.5	NS
Duration of disease (yr)	5.7 ± 7.7	6.3 ± 9.7	NS
Pre-CAI	9.0 ± 2.0	11.1 ± 0.9	NS
Pre-CRP	1.0 ± 0.8	2.4 ± 2.7	NS
Clinical course			
First attack	4	0	< 0.001
Relapse-remitting	6	0	
Chronic continuous	1	7	
Extent of disease			
Entire	8	7	NS
Left sided	2	0	
Proctitis	1	0	
Endoscopic findings			
Erosions	9	0	< 0.005
Geographic ulcers	2	4	
Deep ulcers	0	3	

LCAP: Leukocytopheresis; CAI: Clinical activity index; CRP: C-reactive protein levels (mg/dL).

4.8 eight weeks after the last LCAP session ($P = 0.001$) (Figure 2). The CRP levels before (1.2 ± 0.8 mg/dL) and after (1.0 ± 2.0 mg/dL) LCAP did not differ significantly (Figure 2). When the endoscopic findings obtained before LCAP were considered, we found that all nine patients with erosions had entered remission (100%) 8 wk after the last LCAP session. However, only two of six (33%) and none of three of the patients with geographic ulcers and deep ulcers, respectively, had entered remission at this time point ($P < 0.005$) (Table 2). The remission rates dropped over time as of the patients with erosions and geographic ulcers who were in remission at the 8-wk timepoint, 44.4% (4/9) and 16.7% (1/6) remained in remission 48 wk after LCAP.

Clinical characteristics of the patients who entered remission

Table 2 shows how the responders compare to the non-responders 8 wk after LCAP. These two groups did not differ significantly in patient characteristics (i.e., sex, age, duration of disease, pre-CAI levels and pre-CRP levels). However, all first attack and relapse-remitting type patients entered remission while seven of the eight chronic continuous type patients did not ($P < 0.001$). Whether there was total colitis, left-sided colitis, or proctitis was not significantly associated with the ability of the patient to enter remission after LCAP. As indicated above, with regard to the endoscopic findings, all patients with erosions entered remission after LCAP but it was more difficult to induce remission in patients with geographic ulcers or deep ulcers ($P < 0.005$).

Clinical characteristic of the patients who entered remission and then relapsed

Of the 11 patients who entered remission 8 wk after LCAP, six relapsed. Table 3 summarizes the

Table 3 Comparison of the remission and relapse groups 48 wk after the last LCAP session (mean \pm SD)

	Remission (n = 5)	Relapse (n = 6)	P
Patient characteristic			
Male/Female	2/3	5/1	NS
Age (yr)	45.4 \pm 13.5	35.0 \pm 16.1	NS
Duration of disease (yr)	3.5 \pm 3.2	7.6 \pm 9.6	NS
Pre-CAI	8.8 \pm 1.7	9.1 \pm 2.2	NS
Post-CAI	0.6 \pm 0.4	1.8 \pm 1.0	< 0.05
Pre-CRP	1.0 \pm 0.9	1.0 \pm 0.8	NS
Post-CRP	0.18 \pm 0.14	0.32 \pm 0.39	NS
Clinical course			
First attack	3	1	
Relapse-remitting	2	4	
Chronic continuous	0	1	NS
Extent of disease			
Entire	3	5	
Left sided	1	1	
Proctitis	1	0	NS
Endoscopic findings			
Erosions	4	5	
Geographic ulcers	1	1	
Deep ulcers	0	0	NS

CAI: Clinical activity index; CRP: C-reactive protein levels (mg/dL).

characteristics of the five patients who remained in remission 48 wk after LCAP and the six relapsed patients. The two groups only differed significantly in terms of the post-CAI scores ($P < 0.05$). None of the other parameters, namely, the clinical course, extent of disease, or endoscopic findings, correlated with relapse.

Course of the cases who worsened during LCAP

Table 4 summarizes the courses of the three cases with deep ulcers that worsened during LCAP. As a result, two cases were given steroids and one case received cyclosporine. Two patients became infected with cytomegalovirus and received ganciclovir. After a transient improvement, two patients relapsed and underwent surgery. The remaining patient, who developed interstitial pneumonitis, died of aspiration pneumonitis. Aspiration pneumonitis developed 3 mo after LCAP, and therefore the two events were probably unrelated to each other. We think that interstitial pneumonitis was a cause of the development of aspiration pneumonitis.

Adverse effects

None of the patients experienced any severe adverse effects from LCAP. Only one patient reported a mild adverse event (nausea). However, this patient did not suffer from the same problem after subsequent LCAP sessions.

DISCUSSION

When UC patients fail to enter remission after salazosulfapyridine or mesalazine treatment, the conventional second-line therapy involves administration of steroids^[13,14]. However, steroids can cause severe side effects in some patients^[15,16]. When patients with severe

Table 4 Course of cases whose condition worsened during LCAP

	Case 1	Case 2	Case 3
Additional therapy	PSL	PSL	CsA
Complication	-	GCV CMV	GCV CMV
Result	Operation	Operation	Aspiration pneumonitis Dead

PSL: Prednisolone; CsA: Cyclosporine; GCV: Ganciclovir; CMV: Cytomegalovirus.

activity fail to respond to steroids, they must undergo a colectomy, although cyclosporine can sometimes induce remission in these cases^[17-20]. In 1995, Sawada *et al.*^[21] introduced LCAP therapy for patients with UC. This therapy is now a widely used treatment option for UC^[22,23]. LCAP is a method where the blood is passed though a leukocyte removal filter before being returned to the body. On average, 1.6×10^{10} leukocytes are removed during one session. These leukocytes include granulocytes, lymphocytes and monocytes. Almost 100% of granulocytes and monocytes and 60% of lymphocytes are removed by removal filter^[24,25]. It has been reported that 73.3% of steroid-refractory patients with UC enter remission after LCAP^[26]. It is likely that this treatment is effective because it reduces the number of leukocytes available for transmigration and infiltration into the colonic mucosa.

In this study, we found that 61.1% of steroid-naïve UC patients (11/18) had entered remission 8 wk after the last LCAP session. At this time point, the average CAI score had dropped significantly from 9.4 ± 1.9 to 3.8 ± 4.8 ($P = 0.001$) (the three cases who worsened during LCAP were excluded from this calculation). Since steroids can induce remission in 45% to 90% of salazosulfapyridine or mesalazine non-responders^[15,27-29], it appears that LCAP is as efficacious as steroids as a second-line treatment. Given the low rate of adverse events suffered by patients treated with LCAP, we propose that patients with moderately active UC should be treated with LCAP before steroids are considered. It should be noted, however, that 54.5% of the patients in remission (6/11) relapsed 48 wk after the last LCAP session, and that the average duration to relapse was 8.7 mo. Thus, while LCAP is useful for inducing remission in steroid-naïve UC patients, it does not maintain remission.

Analysis of the endoscopic findings of the patients revealed that while all nine patients with erosions had entered remission 8 wk after the last LCAP session, only two of six (33%) and none of three patients with geographic ulcers and deep ulcers, respectively, entered remission. Indeed, the three cases with deep ulcers worsened during LCAP and had to be given steroids (2 cases) or cyclosporine (1 case). Two of these cases became infected with cytomegalovirus and were administered ganciclovir. These observations suggest that patients with geographic ulcers and deep ulcers tend to be refractory to LCAP, particularly the latter. Indeed,

LCAP may not improve the situation for patients with deep ulcers given their higher risk of developing cytomegalovirus infections^[30]. Such patients should perhaps be treated with an intensive therapy such as cyclosporine at a more early stage^[31]. However, since all patients with erosion entered remission 8 wk after LCAP and many (44.4%) remained in remission at the 48-wk time point, LCAP is strongly recommended for patients with erosion.

The post-CAI was the only factor that predicted a relapse. In other words, if the post-CAI could be maintained at < 1 by LCAP, it may be possible to maintain long duration remission.

In conclusion, LCAP is a useful and safe therapy for steroid-naïve UC patients with moderate activity. Moreover, endoscopic findings help to predict the efficacy of this treatment.

COMMENTS

Background

An immune dysfunction is believed to be involved in the development of ulcerative colitis (UC). For a long time, steroids have represented the second line therapy for the induction to remission in UC, if remission cannot be achieved by salazosulfapyridine or mesalazine treatment. However, steroid administration can cause several side effects. Leukocytapheresis (LCAP) have been reported to be effective for steroid-refractory or steroid-dependent patients with UC; however, the data of LCAP for steroid-naïve patient with UC is limited.

Research frontiers

To determine whether LCAP may also be useful in steroid-naïve UC patients, these authors administered LCAP to steroid-naïve UC patients. They also assessed whether the efficacy of LCAP can be predicted on the basis of endoscopic findings.

Innovations and breakthroughs

The authors found that 61.1% of steroid-naïve UC patients (11/18) had entered remission 8 wk after the last LCAP session. Since steroids can induce remission in 45% to 90% of salazosulfapyridine or mesalazine non-responders, it appears that LCAP is as efficacious as steroids as a second-line treatment. Analysis of the endoscopic findings of the patients revealed that the remission rate of the patients with erosion was extremely high after LCAP, compared to the extremely low rate observed in patients with geographic ulcers and deep ulcers. None of the patients experienced any severe adverse effects from LCAP.

Applications

Given the low rate of adverse events suffered by patients treated with LCAP, authors propose that patients with moderately active UC should be treated with LCAP before steroids are considered.

Terminology

LCAP is a treatment procedure where leukocytes are removed through their adherence to fibers in the filter.

Peer review

LCAP is a useful and safe therapy for steroid-naïve UC patients with moderate activity. Moreover, endoscopic findings help to predict the efficacy of this treatment. This is an interesting study.

REFERENCES

- 1 Arai F, Takahashi T, Furukawa K, Matsushima K, Asakura H. Mucosal expression of interleukin-6 and interleukin-8 messenger RNA in ulcerative colitis and in Crohn's disease. *Dig Dis Sci* 1998; **43**: 2071-2079
- 2 Podolsky DK. Inflammatory bowel disease (1). *N Engl J Med* 1991; **325**: 928-937
- 3 Truelove SC, Jewell DP. Intensive intravenous regimen for severe attacks of ulcerative colitis. *Lancet* 1974; **1**: 1067-1070
- 4 Meyers S, Sachar DB, Goldberg JD, Janowitz HD. Corticotropin versus hydrocortisone in the intravenous treatment of ulcerative colitis. A prospective, randomized, double-blind clinical trial. *Gastroenterology* 1983; **85**: 351-357
- 5 Hanauer SB. Inflammatory bowel disease. *N Engl J Med* 1996; **334**: 841-848
- 6 Jarnerot G, Rolny P, Sandberg-Gertzen H. Intensive intravenous treatment of ulcerative colitis. *Gastroenterology* 1985; **89**: 1005-1013
- 7 Naganuma M, Funakoshi S, Sakuraba A, Takagi H, Inoue N, Ogata H, Iwao Y, Ishi H, Hibi T. Granulocytapheresis is useful as an alternative therapy in patients with steroid-refractory or -dependent ulcerative colitis. *Inflamm Bowel Dis* 2004; **10**: 251-257
- 8 Giampaolo B, Giuseppe P, Michele B, Alessandro M, Fabrizio S, Alfonso C. Treatment of active steroid-refractory inflammatory bowel diseases with granulocytapheresis: Our experience with a prospective study. *World J Gastroenterol* 2006; **12**: 2201-2204
- 9 Emmrich J, Petermann S, Nowak D, Beutner I, Brock P, Klingel R, Mausfeld-Lafdihiya P, Liebe S, Ramlow W. Leukocytapheresis (LCAP) in the management of chronic active ulcerative colitis—results of a randomized pilot trial. *Dig Dis Sci* 2007; **52**: 2044-2053
- 10 Domenech E, Hinojosa J, Esteve-Comas M, Gomollon F, Herrera JM, Bastida G, Obrador A, Ruiz R, Saro C, Gassull MA. Granulocytapheresis in steroid-dependent inflammatory bowel disease: a prospective, open, pilot study. *Aliment Pharmacol Ther* 2004; **20**: 1347-1352
- 11 Garland CF, Lilienfeld AM, Mendeloff AI, Markowitz JA, Terrell KB, Garland FC. Incidence rates of ulcerative colitis and Crohn's disease in fifteen areas of the United States. *Gastroenterology* 1981; **81**: 1115-1124
- 12 Lichtiger S, Present DH, Kornbluth A, Gelernt I, Bauer J, Galler G, Michelassi F, Hanauer S. Cyclosporine in severe ulcerative colitis refractory to steroid therapy. *N Engl J Med* 1994; **330**: 1841-1845
- 13 Podolsky DK. Inflammatory bowel disease. *N Engl J Med* 2002; **347**: 417-429
- 14 Kornbluth A, Sachar DB. Ulcerative colitis practice guidelines in adults (update): American College of Gastroenterology, Practice Parameters Committee. *Am J Gastroenterol* 2004; **99**: 1371-1385
- 15 Lennard-Jones JE. Toward optimal use of corticosteroids in ulcerative colitis and Crohn's disease. *Gut* 1983; **24**: 177-181
- 16 Baron JH, Connell AM, Kanaghinis TG, Lennard-Jones JE, Jones AF. Out-patient treatment of ulcerative colitis. Comparison between three doses of oral prednisone. *Br Med J* 1962; **2**: 441-443
- 17 Rowe FA, Walker JH, Karp LC, Vasiliaskas EA, Plevy SE, Targan SR. Factors predictive of response to cyclosporin treatment for severe, steroid-resistant ulcerative colitis. *Am J Gastroenterol* 2000; **95**: 2000-2008
- 18 Stack WA, Long RG, Hawkey CJ. Short- and long-term outcome of patients treated with cyclosporin for severe acute ulcerative colitis. *Aliment Pharmacol Ther* 1998; **12**: 973-978
- 19 Sandborn WJ. A critical review of cyclosporin therapy in inflammatory bowel disease. *Inflamm Bowel Dis* 1995; **1**: 48-63
- 20 Santos J, Baudet S, Casellas F, Guarner L, Vilaseca J, Malagelada JR. Efficacy of intravenous cyclosporine for steroid refractory attacks of ulcerative colitis. *J Clin Gastroenterol* 1995; **20**: 285-289
- 21 Sawada K, Ohnishi K, Fukui S, Yamada K, Yamamura M, Amano K, Amano K, Wada M, Tanida N, Satomi M. Leukocytapheresis therapy, performed with leukocyte removal filter, for inflammatory bowel disease. *J Gastroenterol* 1995; **30**: 322-329
- 22 Sawada K, Shimoyama T. Therapeutic cytappheresis for inflammatory bowel disease. *Ther Apher* 1998; **2**: 90-92
- 23 Sawada K, Muto T, Shimoyama T, Satomi M, Sawada T, Nagawa H, Hiwatashi N, Asakura H, Hibi T. Multicenter

- randomized controlled trial for the treatment of ulcerative colitis with a leukocytapheresis column. *Curr Pharm Des* 2003; **9**: 307-321
- 24 **Shirokaze J**. Leukocytapheresis using a leukocyte removal filter. *Ther Apher* 2002; **6**: 261-266
- 25 **Yamaji K**, Tsuda H, Hashimoto H. Current topics on cytappheresis technologies. *Ther Apher* 2001; **5**: 287-292
- 26 **Kohgo Y**, Hibi H, Chiba T, Shimoyama T, Muto T, Yamamura K, Popovsky MA. Leukocyte apheresis using a centrifugal cell separator in refractory ulcerative colitis: a multicenter open label trial. *Ther Apher* 2002; **6**: 255-260
- 27 **Lennard-Jones JE**, Longmore AJ, Newell AC, Wilson CW, Jones FA. An assessment of prednisone, salazopyrin, and topical hydrocortisone hemisuccinate used as out-patient treatment for ulcerative colitis. *Gut* 1960; **1**: 217-222
- 28 **Powell-Tuck J**, Bown RL, Lennard-Jones JE. A comparison of oral prednisolone given as single or multiple daily doses for active proctocolitis. *Scand J Gastroenterol* 1978; **13**: 833-837
- 29 **Faubion WA Jr**, Loftus EV Jr, Harmsen WS, Zinsmeister AR, Sandborn WJ. The natural history of corticosteroid therapy for inflammatory bowel disease: a population-based study. *Gastroenterology* 2001; **121**: 255-260
- 30 **Nishimoto Y**, Matsumoto T, Suekane H, Shimizu M, Mikami Y, Iida M. Cytomegalovirus infection in a patient with ulcerative colitis: colonoscopic findings. *Gastrointest Endosc* 2001; **53**: 816-818
- 31 **D'Haens G**, Lemmens L, Geboes K, Vandeputte L, Van Acker F, Mortelmans L, Peeters M, Vermeire S, Penninckx F, Nevens F, Hiele M, Rutgeerts P. Intravenous cyclosporine versus intravenous corticosteroids as single therapy for severe attacks of ulcerative colitis. *Gastroenterology* 2001; **120**: 1323-1329

S- Editor Li DL L- Editor Negro F E- Editor Zhang WB



RAPID COMMUNICATION

Halothane hepatitis in Iran: A review of 59 cases

Payam Egtesadi-Araghi, Amir-Ali Sohrabpour, Homayoon Vahedi, Mehdi Saberi-Firoozi

Payam Egtesadi-Araghi, Homayoon Vahedi, Digestive Disease Research Center, Shariati Hospital, Medical Sciences/University of Tehran, Tehran 14117, Iran

Amir-Ali Sohrabpour, Mehdi Saberi-Firoozi, Gastroenterology Research Center, Shiraz University of Medical Sciences, Shiraz 14117, Iran

Author contributions: Egtesadi-Araghi P designed and performed research, analyzed data and wrote the paper; Vahedi H contributed in providing cases and critical review of the project; Sohrabpour A and Saberi-Firoozi M contributed in providing cases.

Supported by A grant from the Digestive Disease Research Center, Tehran University of Medical Sciences, Iran, No. 83.36

Correspondence to: Dr. Payam Egtesadi-Araghi, Digestive Disease Research Center, Shariati Hospital, North Karegar Avenue, Medical Sciences/University of Tehran, Tehran 14117, Iran. payam_egtesadi@yahoo.com

Telephone: +98-21-44454814 Fax: +98-21-44454814

Received: June 11, 2008 Revised: July 27, 2008

Accepted: August 3, 2008

Published online: September 14, 2008

could have been avoided. To lessen occurrence of further cases of HH, the authors suggest that in female patients having a history of surgery (or delivery) with general anesthesia, the use of halothane should be absolutely avoided. Utilization of proper substitutes in adults' anesthesia is advocated.

© 2008 The WJG Press. All rights reserved.

Key words: Anesthesia; Inhalation; Halothane; Hepatitis; Drug-induced

Peer reviewer: Jacques Bernuau, MD, Beaujon Hospital, 100, boulevard du Général Leclerc, Cedex 92118, France

Egtesadi-Araghi P, Sohrabpour A, Vahedi H, Saberi-Firoozi M. Halothane hepatitis in Iran: A review of 59 cases. *World J Gastroenterol* 2008; 14(34): 5322-5326 Available from: URL: <http://www.wjgnet.com/1007-9327/14/5322.asp> DOI: <http://dx.doi.org/10.3748/wjg.14.5322>

Abstract

AIM: To study halothane hepatitis (HH) in Iran and its associated risk factors.

METHODS: We retrospectively studied files of all cases diagnosed with HH referred to three referral hospitals and four private centers in Iran from April 1994 to September 2006. Information on age at surgery, gender, medications history, obesity, history of previous exposure, previous reaction to halothane, familial history, type of surgery, perioperative hypoxia or sepsis, morbidity and mortality were recorded and analyzed.

RESULTS: A total of 59 cases were identified. Forty-eight (81%) were women. The median age at the time of surgery was 44 years (range, 18 to 80 years). Sixty percent of patients were above 40-year-old. Obesity was observed in 22.2%. Previous history of exposures to halothane was noted in 61% of which 50% had history of post-exposure reaction. Coronary artery bypass graft (CABG), cholecystectomy, and cosmetic surgeries (mainly weight reduction) were the most frequent surgeries. The mortality rate was 12.2%. In patients developing encephalopathy, it was as high as 50%.

CONCLUSION: HH remains an important cause of morbidity and mortality in centers still using this anesthetic. However, a large percentage of these cases

INTRODUCTION

Halothane was first introduced to clinical practice in 1956 and was immediately recognized as a great advance in anesthesia^[1]. First reports of postoperative liver necrosis with halothane began to appear in 1958^[2,3] followed by further anecdotal reports^[4-7]. By 1963, 7 years after the introduction of halothane, at least 350 cases of "halothane hepatitis" (HH) had been reported^[8]. These reports led to the National Halothane Study, which estimated fatal hepatic necrosis following halothane anesthesia to be approximately 1 in 35 000 in the US^[9]. Other retrospective studies confirmed that halothane was associated with severe liver dysfunction, with an incidence ranging from 1 in 6000 to 1 in 35 000^[10].

Two major types of hepatotoxicity are associated with halothane administration: type I (mild) and type II (fulminant). Type II hepatotoxicity is associated with massive centrilobular liver cell necrosis that leads to fulminant liver failure and is clinically characterized by fever, jaundice, and grossly elevated serum transaminase levels. It appears to be immune mediated and initiated by oxidative halothane metabolism by cytochrome P450 to an intermediate compound. This compound binds to trifluoroacetyl proteins in the hepatic endoplasmic reticulum, thought to occur in genetically predisposed

individuals^[11]. In this study, type II hepatotoxicity was regarded as HH.

Concerns about its hepatotoxicity have virtually eliminated the use of halothane for adults in the United States and many other countries. Halothane was eventually replaced by safer newer volatile anesthetics such as isoflurane^[12,13]. But in other countries with different medicolegal climates, halothane is still widely used because of its relatively low cost^[11]. There are reports of HH from South Africa^[14], Tunisia^[15], Kenya^[16], India^[17], and Spain^[18]. Halothane is being used as the main anesthetic in more than 80% of hospitals of Iran^[19]. Unfortunately, increasing numbers of HH are being reported in Iran. In this retrospective study, we review cases reported within a 12-year period from 7 centers in Iran.

MATERIALS AND METHODS

Cases were recruited from three referral hospitals (Namazi Hospital of Shiraz University of Medical Sciences, Shariati Hospital of Tehran University of Medical Sciences, Mehr General Hospital of Tehran) and four referral GI clinics in Tehran, from April 1994 to September 2006.

There is no definite test for the diagnosis of HH and it is basically a diagnosis of exclusion. In our study, the diagnosis of HH was confirmed if the patient met the following criteria; clinical findings (jaundice, malaise), paraclinical findings (marked elevation of ALT, AST, total and direct bilirubin), recent exposure to halothane, exclusion of other causes of liver damage (viral hepatitis, *etc*) and confirmation by at least one gastroenterologist as fulminant HH.

Charts were reviewed to determine the age at surgery, weight, gender, medication history (including P450 inducing drugs), preoperative obesity (BMI ≥ 30), history of recurrent exposure to halothane, history of previous reaction to halothane (unexplained post-operative fever, jaundice, abnormal liver enzymes following earlier halothane anesthetics), positive family history (defined as reactions to halothane in first degree relatives), type of surgery, perioperative hypoxia, interval between anesthesia with halothane and symptoms heralding or attesting hepatitis, postoperative sepsis, morbidity and mortality.

Data were presented as simple count and percent, median and range, or mean \pm SD and were compared by Fisher's exact test. Statistical calculations were performed using SPSS version 15.0. $P < 0.05$ was considered significant.

The study was approved by the institutional review board and ethics committee of the Digestive Disease Research Center (DDRC) of Tehran University of Medical Sciences.

RESULTS

Fifty-nine cases of HH were identified, 48 women (81%), 11 men (19%). The median age at the time

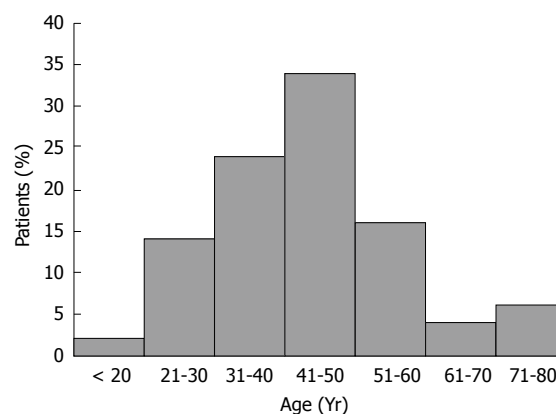


Figure 1 The age distribution of patients with HH.

Table 1 Characteristics and laboratory test results of 59 patients with HH

Variable	n	%
Characteristics		
Female gender	48	81.4
Obesity	6	22.2
Recurrent exposure history	22	61.2
Post-exposure reaction history	10	17
Encephalopathy	8	14.5
Requiring ICU care	7	12.5
Mortality	6	12.2
Laboratory test results		
ALT rise	48	100
AST rise	47	97.9
Total bilirubin rise	41	97.6
Direct bilirubin rise	37	100
Alkaline phosphates rise	35	100
PT rise	21	61.8

Percentages are relative to patients for whom data was available.

of surgery was 44 years (range, 18 to 80 years). Sixty percent of patients were above 40 years old. The age distribution of patients is given in Figure 1.

The mean of interval between anesthesia and hepatitis symptoms was 15.2 ± 13.6 d. The mean weight of patients was 76.1 ± 18.7 kg and 22.2% of patients were obese. Data on clinical findings, morbidity, and mortality are given in Table 1. No cases had previous history of liver disease or sepsis. One case had documented perioperative hypoxia, another one had a positive family history of HH, and two others had positive drug history (Phenobarbital). Table 1 shows the results of routine laboratory investigations including biochemistry and coagulation tests.

Coronary artery bypass graft (CABG), cholecystectomy and cosmetic operations (mainly weight reducing surgeries including partial gastrectomy and liposuction) were the most common operations. Information about different types of surgery is given in Table 2. The mortality rate was 12.2%. In patients who developed encephalopathy, it was as high as 50%. The mortality rate in male patients was higher than females (20% *vs* 12%), although this did not reach statistical significance.

Table 2 Surgery types and the percentage of cases of HH regarding each type of surgery

Variable	n	%
Cholecystectomy	5	8.5
CABG surgery	4	6.8
Aesthetic surgeries		
Partial gastrectomy	1	1.7
Liposuction	1	1.7
Face lifting	1	1.7
Mandible reconstruction	1	1.7
Others	3	3.4
General Surgeries		
Intra-abdominal surgeries	2	3.4
Extra-abdominal surgeries	4	6.8
Obstetrics & Gynecologic surgeries		
Cesarean section	2	3.4
Others	9	3.4
Eye surgeries	3	5.1
Orthopedic surgeries	2	3.4
Urologic surgeries	2	3.4
Other types of surgery	5	8.5

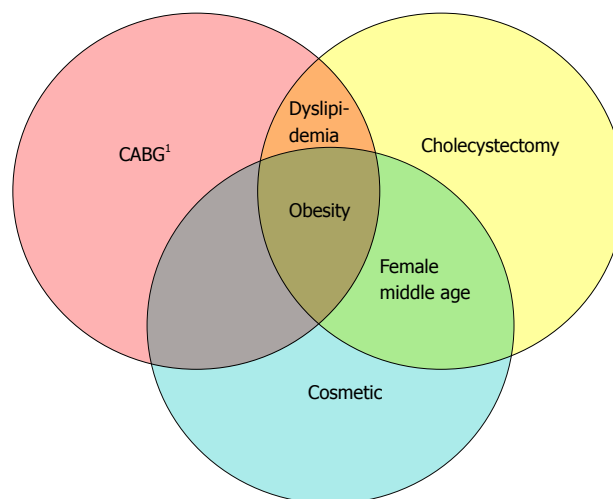
Percentages are relative to patients for whom data was available.

DISCUSSION

Because of the retrospective nature of the study, confirmation of the diagnosis with antibodies to halothane-altered protein antigens was not possible.

It has been shown that HH is more frequent in females, male-to-female ratio ranging from 1:1.6^[11] to 1:2^[20,21]. In our series, this ratio was 1:4.3. Additionally, it has been revealed that middle-aged patients have a greater propensity to develop liver damage than the young or elderly^[11,21-24]. Over 70% of patients are more than 40 years old with peak age of 50-60^[10]. We also observed a greater prevalence among middle-aged patients, yet it appears that the patients in our series are younger compared to other series^[14]. The younger age of our patients could be due to the fact that the most common surgery in our series, cholecystectomy, is frequently performed in young to middle-aged women. Obesity appears to be the common factor between most frequent operations associated with HH in our series. CABG, cholecystectomy and weight reducing-cosmetic surgeries are all linked with obesity (Figure 2). It has been showed that hepatic dysfunction is more common in obese than in non-obese patients^[23-25]. As halothane accumulates in adipose tissue, this could delay its excretion and, theoretically, prolong exposure to potentially reactive halothane metabolites, resulting in increased risk in obese patients. In addition, obese patients metabolize halothane more extensively than do non-obese patients^[26], further predisposing them to liver injury.

Other possible relationships between the most common surgeries are shown in Figure 2. Female gender and middle age are common risk factors for gall stone formation, cholecystitis, and coronary artery disease^[27-30]. Dyslipidemia is also a risk factor for coronary artery disease and plays an important role in the pathogenesis of gallstones^[27,28]. Furthermore, hypoperfusion is the possible explanation of hepatic injury in CABG and cholecystectomy surgeries. Imbalance between oxygen

**Figure 2** Common risk factors between three most common surgeries among patients with HH. ¹Coronary Artery Bypass Graft Surgery.

supply and demand predisposes the patient to halothane-induced liver damage^[10,31]. Halothane has been shown to decrease both portal blood flow and hepatic arterial blood flow^[32-35]. Furthermore, surgical manipulation of the splanchnic bed may reduce hepatic blood flow^[36]. It has been demonstrated that CABG is accompanied with splanchnic hypoperfusion and hypoxia^[37,38]. The ischemia is probably caused by hypoperfusion due to low cardiac output, hypotension due to blood loss, and intra-abdominal atheroemboli. As a result, ischemia may be the common mechanism of predisposing the patients to the HH in CABG and cholecystectomy surgeries.

Multiple exposures to halothane are the single greatest risk factor for HH^[11]. The association between hepatotoxicity and repeated exposure to halothane might be explained by the fact that halothane anesthesia itself induces drug metabolizing enzymes^[39-41]. The risk of HH is increased greatly when repeated halothane anesthetics are given over a short period^[11,22,42,43], especially at intervals of < 6 wk^[11]. In the present study, previous history of exposure to halothane was noted in 22 of 36 (61.1%) and obvious post-exposure reaction history in 10 of 22 (45.5%). Considering the fact that guidelines clearly elucidated that patients with previous reaction to halothane are among the high-risk groups^[44] and should have not received halothane again, in addition to the results of this study, it is concluded that a large percentage of these cases could have been avoided. To lessen further occurrence of cases of HH, we suggest that the use of halothane in patients who are at risk of HH should be absolutely avoided. This group of patients consists of female patients having a history of post-anesthesia reactions following exposure to halogenated anesthetics. On the other hand, the item of "post-exposure reaction history" is often quite difficult to obtain with a reasonable certainty; therefore, its usefulness in preventing HH should be weak. As a result, a second set of criteria, which is female gender patients with a history of surgery (or delivery) with general anesthesia, is recommended. This is likely easier

to obtain and can be simply defined pre-operatively by the anesthesiologist *via* reviewing the “anesthesia sheet” of the previous general anesthesia. Strict adherence to this set of criteria will reduce, but not totally prevent, further cases from occurring in countries still utilizing halothane in adults’ anesthesia.

Furthermore, the incidence of liver injury was greater in subjects treated with phenobarbitone before halothane anesthesia than in those not taking enzyme inducing medication^[11,45]. There were two cases of chronic Phenobarbital use in our series among the 16 for which data was available.

Susceptibility to HH may be heritable and positive family history must be taken into consideration^[11,46]. We had one case with positive family history of HH out of 7 cases that had data.

Type II hepatotoxicity has a mortality rate of approximately 50%, which rises to 80% when hepatic encephalopathy is present^[10,11,31,47]. Our data confirms this since the mortality was 50% in our patients with encephalopathy. Such patients have a very poor prognosis and should be referred to specialist centers where orthotopic liver transplantation is available^[48]. Patients who survive the acute illness usually make a complete recovery^[11]. Considering that none of our patients underwent liver transplantation, our mortality rate is considerably lower than other reports^[14]. Improved intensive care, faster diagnosis and initiation of medical care may be involved. In consistence with former reports^[22,24,49], mortality in male patients was higher than females in our series (20% *vs* 12.1%), although it did not reach statistical significance.

Although halothane is rarely associated with fulminant hepatic failure^[48], it occupies the fifth place of suspected hepatic adverse drug reactions with a fatal outcome received by the WHO Collaborating Centre for International Drug Monitoring in Uppsala Sweden from 1968 to 2003^[50]. Consequently, its usage was restricted in adults’ anesthesia; however, it is still widely used in many countries, including Iran, mainly due to economic reasons^[11].

Guidelines have been developed to reduce the probability of a patient developing HH; still, adverse reactions continue to occur. Even though it was clearly noted in guidelines that patients thought to be sensitized to halothane must never be re-exposed to the drug, we found ten cases of known previous reaction that were re-exposed to halothane. Based on the results of this study, to lessen further cases of HH to occur, the authors suggest that in female patients having a history of surgery (or delivery) with general anesthesia, the use of halothane should be absolutely avoided. However, the most effective preventive tool is to avoid the use of halothane in adults’ anesthesia. Utilization of proper substitutes is advocated.

ACKNOWLEDGMENTS

This study was the postdoctoral project of Dr. Payam Eghtesadi-Araghi and supported by a grant

from Digestive Disease Research Center of Tehran University of Medical Sciences. The authors wish to thank Professor Malekzadeh, Dr. Merat, Dr. Nasser-Moghaddam, Dr. Ganji, and Dr. Mortezaei for their invaluable assistance.

COMMENTS

Background

Halothane is a volatile anesthetic, which was first introduced to clinical practice in 1956. After several years, concerns about its hepatotoxicity have virtually eliminated the use of halothane for adults in the United States and many other countries. It was replaced by safer newer volatile anesthetics such as isoflurane. However, in some countries with different medicolegal climates, halothane is still widely used because of its relatively low cost. In Iran, halothane is being used as the main anesthetic in more than 80% of hospitals. Unfortunately, increasing numbers of halothane hepatitis (HH) are being reported in Iran.

Research frontiers

Although the use of halothane was restricted in many countries, there are reports of HH occurring in South Africa, Tunisia, Kenya, India, and Spain in recent years.

Innovations and breakthroughs

In previous studies, guidelines have been developed to reduce the probability of a patient developing HH; however, the results of this study revealed that they are largely ignored in Iran. Furthermore, the results showed that a large percentage of these cases could have been avoided. In this study, we aimed to suggest a set of criteria that can easily define the high risk group of patients pre-operatively.

Applications

To reduce, but not totally prevent, further cases of HH from occurring in countries still utilizing halothane in adults’ anesthesia, the authors suggest that the use of halothane should be absolutely avoided in female patients with a history of surgery (or delivery) with general anesthesia. However, the most effective preventive tool is to avoid the use of halothane in adults’ anesthesia. Utilization of proper substitutes is advocated.

Terminology

Two major types of hepatotoxicity are associated with halothane administration: type I (mild) and type II (fulminant). Type II hepatotoxicity is associated with massive centrilobular liver cell necrosis that leads to fulminant liver failure and is clinically characterized by fever, jaundice, and grossly elevated serum transaminase levels. In this study, type II hepatotoxicity was regarded as HH.

Peer review

This series of 59 cases of HH collected in 12 years in a country with health security conditions lower than those found in the US or Western Europe is of high medical interest for the Iranian population and populations of other countries in the Middle East and, most likely, also in Africa and Asia. Therefore, the topic of the present paper is quite pertinent and extremely interesting for many physicians around the world.

REFERENCES

- 1 **Johnstone M.** The human cardiovascular response to fluothane anaesthesia. 1956. *Br J Anaesth* 1998; **80**: 396-405; discussion 395
- 2 **Burnap TK, Galla SJ, Vandam LD.** Anesthetic, circulatory and respiratory effects of fluothane. *Anesthesiology* 1958; **19**: 307-320
- 3 **Postoperative death after fluothane.** *Anesthesiology* 1958; **19**: 562-563
- 4 **Brody GL, Sweet RB.** Halothane anesthesia as a possible cause of massive hepatic necrosis. *Anesthesiology* 1963; **24**: 29-37
- 5 **Bunker JP, Blumenfeld CM.** Liver necrosis after halothane anesthesia. Cause or coincidence? *N Engl J Med* 1963; **268**: 531-534
- 6 **Lindenbaum J, Leifer E.** Hepatic necrosis associated with halothane anesthesia. *N Engl J Med* 1963; **268**: 525-530

- 7 **Temple RL**, Cote RA, Gorens SW. Massive hepatic necrosis following general anesthesia. *Anesth Analg* 1962; **41**: 586-592
- 8 **Brown BR Jr**, Gandolfi AJ. Adverse effects of volatile anaesthetics. *Br J Anaesth* 1987; **59**: 14-23
- 9 **Summary of the national Halothane Study**. Possible association between halothane anesthesia and postoperative hepatic necrosis. *JAMA* 1966; **197**: 775-788
- 10 **Ray DC**, Drummond GB. Halothane hepatitis. *Br J Anaesth* 1991; **67**: 84-99
- 11 **Peralta R**, Poterack KA, Guzowski S. Halothane Hepatotoxicity. Last modified date: May 25, 2006. Available from URL: <http://www.emedicine.com>
- 12 **Allan LG**, Hussey AJ, Howie J, Beckett GJ, Smith AF, Hayes JD, Drummond GB. Hepatic glutathione S-transferase release after halothane anaesthesia: open randomised comparison with isoflurane. *Lancet* 1987; **1**: 771-774
- 13 **Hussey AJ**, Aldridge LM, Paul D, Ray DC, Beckett GJ, Allan LG. Plasma glutathione S-transferase concentration as a measure of hepatocellular integrity following a single general anaesthetic with halothane, enflurane or isoflurane. *Br J Anaesth* 1988; **60**: 130-135
- 14 **Voigt MD**, Workman B, Lombard C, Kirsch RE. Halothane hepatitis in a South African population--frequency and the influence of gender and ethnicity. *S Afr Med J* 1997; **87**: 882-885
- 15 **Daghfous R**, el Aidli S, Sfaxi M, Daghfous M, Kastalli S, Srairi S, Loueslati MH, Belkahia C. [Halothane-induced hepatitis. 8 case reports] *Tunis Med* 2003; **81**: 874-878
- 16 **Otedo AE**. Halothane induced hepatitis: case report. *East Afr Med J* 2004; **81**: 538-539
- 17 **Kumar GP**, Bhat VJ, Sowdi V. Fulminant hepatic failure following halothane anaesthesia. *J Clin Forensic Med* 2005; **12**: 271-273
- 18 **Rodes J**, Bruguera M. The uses of error: iatrogenic hepatitis. *Lancet* 2001; **357**: 791
- 19 **The "Halothane" will be replaced with newer anesthetic drugs in Iran**. Date Accessed: July 17, 2008. Available from URL: <http://www.reporter.ir/archives/86/10/005402.php>
- 20 **Benjamin SB**, Goodman ZD, Ishak KG, Zimmerman HJ, Irey NS. The morphologic spectrum of halothane-induced hepatic injury: analysis of 77 cases. *Hepatology* 1985; **5**: 1163-1171
- 21 **Neuberger J**, Williams R. Halothane anaesthesia and liver damage. *Br Med J (Clin Res Ed)* 1984; **289**: 1136-1139
- 22 **Bottiger LE**, Dalen E, Hallen B. Halothane-induced liver damage: an analysis of the material reported to the Swedish Adverse Drug Reaction Committee, 1966-1973. *Acta Anaesthesiol Scand* 1976; **20**: 40-46
- 23 **Carney FM**, Van Dyke RA. Halothane hepatitis: a critical review. *Anesth Analg* 1972; **51**: 135-160
- 24 **Walton B**, Simpson BR, Strunin L, Doniach D, Perrin J, Appleyard AJ. Unexplained hepatitis following halothane. *Br Med J* 1976; **1**: 1171-1176
- 25 **Peters RL**, Edmondson HA, Reynolds TB, Meister JC, Curpey TJ. Hepatic necrosis associated with halothane anesthesia. *Am J Med* 1969; **47**: 748-764
- 26 **Bentley JB**, Vaughan RW, Gandolfi AJ, Cork RC. Halothane biotransformation in obese and nonobese patients. *Anesthesiology* 1982; **57**: 94-97
- 27 **Boland LL**, Folsom AR, Rosamond WD. Hyperinsulinemia, dyslipidemia, and obesity as risk factors for hospitalized gallbladder disease. A prospective study. *Ann Epidemiol* 2002; **12**: 131-140
- 28 **Grunhage F**, Lammert F. Gallstone disease. Pathogenesis of gallstones: A genetic perspective. *Best Pract Res Clin Gastroenterol* 2006; **20**: 997-1015
- 29 **Wild SH**, Byrne CD. ABC of obesity. Risk factors for diabetes and coronary heart disease. *BMJ* 2006; **333**: 1009-1011
- 30 **Franzosi MG**. Should we continue to use BMI as a cardiovascular risk factor? *Lancet* 2006; **368**: 624-625
- 31 **Cousins MJ**, Plummer JL, Hall PD. Risk factors for halothane hepatitis. *Aust N Z J Surg* 1989; **59**: 5-14
- 32 **Gelman S**, Fowler KC, Smith LR. Liver circulation and function during isoflurane and halothane anesthesia. *Anesthesiology* 1984; **61**: 726-730
- 33 **Hursh D**, Gelman S, Bradley EL Jr. Hepatic oxygen supply during halothane or isoflurane anesthesia in guinea pigs. *Anesthesiology* 1987; **67**: 701-706
- 34 **Gelman S**, Rimerman V, Fowler KC, Bishop SP, Bradley EL Jr. The effect of halothane, isoflurane, and blood loss on hepatotoxicity and hepatic oxygen availability in phenobarbital-pretreated hypoxic rats. *Anesth Analg* 1984; **63**: 965-972
- 35 **Hughes RL**, Campbell D, Fitch W. Effects of enflurane and halothane on liver blood flow and oxygen consumption in the greyhound. *Br J Anaesth* 1980; **52**: 1079-1086
- 36 **Seltzer JL**, Goldberg ME, Larijani GE, Ritter DE, Starsnic MA, Stahl GL, Lefer AM. Prostacyclin mediation of vasodilation following mesenteric traction. *Anesthesiology* 1988; **68**: 514-518
- 37 **D'Ancona G**, Baillot R, Poirier B, Dagenais F, de Ibarra JJ, Bauset R, Mathieu P, Doyle D. Determinants of gastrointestinal complications in cardiac surgery. *Tex Heart Inst J* 2003; **30**: 280-285
- 38 **Zacharias A**, Schwann TA, Parenteau GL, Riordan CJ, Durham SJ, Engoren M, Fenn-Buderer N, Habib RH. Predictors of gastrointestinal complications in cardiac surgery. *Tex Heart Inst J* 2000; **27**: 93-99
- 39 **Nimmo WS**, Thompson PG, Prescott LF. Microsomal enzyme induction after halothane anaesthesia. *Br J Clin Pharmacol* 1981; **12**: 433-434
- 40 **St Haxholdt O**, Loft S, Clemmensen A, Hjortso E. Increased hepatic microsomal activity after halothane anaesthesia in children. *Anaesthesia* 1986; **41**: 579-581
- 41 **Neuberger JM**. Halothane and hepatitis. Incidence, predisposing factors and exposure guidelines. *Drug Saf* 1990; **5**: 28-38
- 42 **Inman WH**, Mushin WW. Jaundice after repeated exposure to halothane: an analysis of Reports to the Committee on Safety of Medicines. *Br Med J* 1974; **1**: 5-10
- 43 **Inman WH**, Mushin WW. Jaundice after repeated exposure to halothane: a further analysis of reports to the Committee on Safety of Medicines. *Br Med J* 1978; **2**: 1455-1456
- 44 **Neuberger J**, Williams R. Halothane hepatitis. *Dig Dis* 1988; **6**: 52-64
- 45 **Nomura F**, Hatano H, Ohnishi K, Akikusa B, Okuda K. Effects of anticonvulsant agents on halothane-induced liver injury in human subjects and experimental animals. *Hepatology* 1986; **6**: 952-956
- 46 **Hoft RH**, Bunker JP, Goodman HI, Gregory PB. Halothane hepatitis in three pairs of closely related women. *N Engl J Med* 1981; **304**: 1023-1024
- 47 **Bjornsson E**, Jerlstad P, Bergqvist A, Olsson R. Fulminant drug-induced hepatic failure leading to death or liver transplantation in Sweden. *Scand J Gastroenterol* 2005; **40**: 1095-1101
- 48 **Neuberger J**. Halothane hepatitis. *Eur J Gastroenterol Hepatol* 1998; **10**: 631-633
- 49 **Moult PJ**, Sherlock S. Halothane-related hepatitis. A clinical study of twenty-six cases. *Q J Med* 1975; **44**: 99-114
- 50 **Bjornsson E**, Olsson R. Suspected drug-induced liver fatalities reported to the WHO database. *Dig Liver Dis* 2006; **38**: 33-38

S- Editor Li DL L- Editor Li M E- Editor Yin DH



Anti-HBc screening in Indian blood donors: Still an unresolved issue

Hari Krishan Dhawan, Neelam Marwaha, Ratti Ram Sharma, Yogesh Chawla, Beenu Thakral, Karan Saluja, Sanjeev Kumar Sharma, Manish K Thakur, Ashish Jain

Hari Krishan Dhawan, Neelam Marwaha, Ratti Ram Sharma, Beenu Thakral, Karan Saluja, Manish K Thakur, Ashish Jain, Department of Transfusion Medicine PGIMER, Chandigarh 160012, India

Yogesh Chawla, Sanjeev Kumar Sharma, Department of Hepatology PGIMER, Chandigarh 160012, India

Author contributions: Marwaha N, Sharma RR and Chawla Y designed the research; Dhawan HK and Sharma SK performed the research; Thakral B, Saluja K, Thakur MK and Jain A analyzed data; Dhawan HK wrote the paper.

Correspondence to: Hari Krishan Dhawan, Department of Transfusion Medicine PGIMER, Chandigarh 160012, India. hkdpgimer@gmail.com

Telephone: + 91-172-2756481 Fax: + 91-172-2744401

Received: June 20, 2008 Revised: July 14, 2008

Accepted: July 21, 2008

Published online: September 14, 2008

anti-HBc screening and discarding large number of blood units *versus* considering ID NAT (Individual donor nucleic acid testing) needs to be assessed.

© 2008 The WJG Press. All rights reserved.

Key words: Hepatitis B core antigen; Hepatitis B surface antigen; Hepatitis B virus; Transfusion-associated hepatitis B virus; Blood donors

Peer reviewer: Dr. Deepak Narayan Amarapurkar, Department Of Gastroenterology, Bombay Hospital & Medical Research Centre, D 401 Ameya Soc, New Prabhadevi Road, Prabhadevi, Mumbai 400025, India

Dhawan HK, Marwaha N, Sharma RR, Chawla Y, Thakral B, Saluja K, Sharma SK, Thakur MK, Jain A. Anti-HBc screening in Indian blood donors: Still an unresolved issue. *World J Gastroenterol* 2008; 14(34): 5327-5330 Available from: URL: <http://www.wjgnet.com/1007-9327/14/5327.asp> DOI: <http://dx.doi.org/10.3748/wjg.14.5327>

Abstract

AIM: To study the seroprevalence of antibody to hepatitis B core antigen (anti-HBc) in healthy blood donors negative for HBsAg and to evaluate whether anti-HBc detection could be adopted in India as a screening assay for HBV in addition to HBsAg.

METHODS: A total of 1700 serum samples collected from HBsAg-negative healthy blood donors were tested for the presence of anti-HBc antibody (IgM + IgG). All samples reactive for anti-HBc antibody were then investigated for presence of anti-HBs and for liver function tests (LFTs). One hundred serum samples reactive for anti-HBc were tested for HBV DNA by PCR method.

RESULTS: Out of 1700 samples tested, 142 (8.4%) blood samples were found to be reactive for anti-HBc. It was significantly lower in voluntary (6.9%) as compared to replacement donors (10.4%, $P = 0.011$). Seventy-two (50.7%) anti-HBc reactive samples were also reactive for anti-HBs with levels > 10 mIU/mL and 70 (49.3%) samples were non-reactive for anti-HBs, these units were labeled as anti-HBc-only. These 142 anti-HBc reactive units were also tested for liver function test. HBV DNA was detected in only 1 of 100 samples tested.

CONCLUSION: Keeping in view that 8%-18% of donor population in India is anti-HBc reactive, inclusion of anti-HBc testing will lead to high discard rate. Anti-HBs as proposed previously does not seem to predict clearance of the virus. Cost effectiveness of introducing universal

INTRODUCTION

Hepatitis B virus (HBV) infection is a serious global health problem affecting two billion people worldwide, and 350 million people suffer from chronic HBV infection^[1]. Despite mandatory screening for HBsAg by ELISA for over 20 years, transfusion-associated HBV (TAHBV) continues to be a major problem in India, more so in patients receiving repeated transfusions^[2]. The incidence of transfusion-associated hepatitis (TAH) after cardiac surgery (4 + 2.4 units transfusion) was estimated at 6.9%, of which TAHBV constituted 20%^[2]. The incidence of TAHBV in patients receiving multiple transfusions, such as thalassemia, ranged from 17.9% in the first year to 69.2% by the third year. Patients on renal dialysis showed similar rates of infection with HBV^[2]. It has been demonstrated that some HBsAg-negative individuals and those reactive for anti-HBc continue to replicate HBV^[3,4]. Thus the absence of HBsAg in the blood of apparently healthy individuals may not be sufficient to ensure lack of circulating HBV. Blood containing anti-HBc with or without detectable presence of HBsAg might be infectious; therefore, routine blood donor screening for anti-HBc has been implemented

in some countries resulting in a decrease in the risk of post-transfusion HBV infection^[5].

These findings suggest that recovery from acute hepatitis B virus infection may not result in complete virus elimination, but rather the immune system keeps the virus at a very low level called occult hepatitis B infection. Occult hepatitis B infection (OBI) is defined as the presence of HBV DNA in blood or tissues without detectable HBsAg, with or without antibody to hepatitis B core antigen (anti-HBc) or hepatitis B surface antigen (anti-HBs)^[6]. Such occult hepatitis B infection may be detected in (1) individuals with resolving HBV infection reactive for both anti-HBc and anti-HBs, (2) “anti-HBc-only” carriers in a window period of infection who are seronegative for HBsAg, and (3) carriers in whom HBsAg is not detectable due to presence of escape mutants^[7].

As of today, some countries have retained or adopted anti-HBc testing to decrease HBV transfusion risk, while others have not. Anti-HBc testing is still not mandatory in blood banks in India and only HBsAg testing by ELISA is used as screening test for HBV^[8]. In this study, we aimed to evaluate whether anti-HBc detection could be adopted in India as a screening assay for HBV in addition to HBsAg to improve the safety of blood transfusion.

MATERIALS AND METHODS

Materials

This study was conducted by the Department of Transfusion Medicine in collaboration with Department of Hepatology, PGIMER, Chandigarh, India from July 2005 to December 2006, after obtaining an approval from the Ethics Committee of the Institute. Seventeen hundred HBsAg ELISA non-reactive blood donors were included, 998 of them were voluntary donors and 702 were replacement donors. These samples were then tested for anti-HBc (IgM + IgG), anti-HBs, LFTs and HBV DNA in the following sequence: (1) Blood units non-reactive for HBsAg and reactive for anti-HBc were tested for antibody to surface antigen (anti-HBs) and for liver function tests (LFTs); (2) the blood units non-reactive for HBsAg but reactive for anti-HBc with or without anti-HBs were tested for HBV DNA by PCR method.

Serological tests

HBsAg was tested by using commercial ELISA kit SURASE B-96 (TMB) (GBC, Taiwan, ROC); anti-HBc antibodies were tested using commercial ELISA kit HBcAb two-step incubation (MBS-SRL, Milano, Italy); anti-HBs antibodies were tested using commercial ELISA kit MONOLISA anti-HBs 3.0 (BIORAD).

HBV DNA detection

The DNA from serum was extracted using QiaAmp (Qiagen, Hilden, Germany), followed by in-house nested PCR, amplifying two different regions of the HBV genome using two sets of primers shown in Table 1. The procedure was standardized by positive control obtained from National Institute of Virology Pune, India.

Table 1 Primers used for HBV PCR

First PCR

GP1 5'-YCCTGCTGGTGGCTCCAGTTC-3': sense nt 3144-3164
GP2 5'-AAGCCANACARTGGGGGAAAGC-3': antisense nt 583-604

Second PCR (nt 120-604)

GP3 5'-GICTAGACTCGTGGTGGACTTCTCTC-3': sense nt 120-145
GP4 5'-AAGCCANACARTGGGGGAAAGC-3': antisense nt 583-604

First PCR was carried out in 20 µL volume containing 1 × Taq buffer [100 mmol/L Tris HCL (pH 8.4), 25 mmol/L MgCl₂ and 500 mmol/L KCL], 20 pmol of reverse and forward primers (Table 1), 1 unit of Taq polymerase and 200 ng viral DNA. Thirty-five amplification cycles were performed in PCR machine (Stratagene, La Jolla, USA). Each cycle consisted of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 30 s. A second PCR was performed using the first PCR product as a template using the primers GP-3 and GP-4 which amplified a 485-bp product.

For the detection of PCR product, the second PCR product was run on 20 g/L agarose in TBE buffer containing 0.5 µg/mL ethidium bromide at 50 V for about 1 h and finally visualized under a UV transilluminator (UVP, Upland, USA) and then photographed. A 100-bp DNA ladder (MBI Fermentas, Opelstrasse, Germany) was also run in parallel and the predicted size of the PCR product, which was found to be 485 bp.

Biochemical tests

Liver function tests, including serum bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), were carried out by enzymatic method.

RESULTS

A total of 1700 HBsAg non-reactive blood donors (998 voluntary and 702 replacement donors) were screened for anti-HBc (IgM + IgG) using competitive ELISA, of them 142 turned out to be reactive, giving an overall seropositivity of 8.4%. It was markedly lower in voluntary (6.9%) as compared to replacement donors (10.4%, $P = 0.011$). Donors with age 18-30 years had minimum seropositivity (6.1%) with 50% donors contributing from this group. No significant difference was found in the seropositivity of first time *versus* repeat donors, male *versus* female donors and student *versus* non-student donors, although the seropositivity was slightly less in female donors and student donors.

The anti-HBc reactive donors were tested for anti-HBs. Seventy-two (50.7%) anti-HBc reactive samples were also positive for anti-HBs with levels > 10 mIU/mL and 70 (49.3%) samples were non-reactive for anti-HBs, these units were labeled as anti-HBc-only. The anti-HBc-only reactivity was significantly lower in voluntary (34/998, 3.4%) than in replacement donors (36/702, 5.1%; $P = 0.38$) (Table 2). The anti-HBc reactive units were also tested for LFTs. All the samples had normal

Table 2 Prevalence of anti-HBc and anti-HBs in study population

Donor category	Number	Anti-HBc total	Anti-HBc-only	Anti-HBc and anti-HBs
Voluntary (%)	998 (58.7)	69 (6.9)	34 (3.4)	35 (3.5)
Replacement (%)	702 (41.3)	73 (10.4)	36 (5.1)	37 (5.3)
Total (%)	1700	142 (8.4)	70 (4.1)	72 (4.2)
P value		0.011	0.038	0.03

serum bilirubin levels, and 25 (18%) samples showed enzyme elevation. Out of these 25 samples with elevated enzyme levels, 14 (56%) were positive for anti-HBc-only and 11 (44%) were positive for anti-HBc + anti-HBs ($P = 0.460$).

One hundred samples from these 142 anti-HBc reactive (45 anti-HBc-only and 55 anti-HBc with anti-HBs) samples were tested for HBV DNA by using PCR. Only one sample was positive for HBV DNA. This sample had an anti-HBs level > 150 mIU/mL, LFTs for this sample were within normal limits. This was a 22-year-old male repeat replacement blood donor.

DISCUSSION

Despite mandatory screening of donor blood for HBsAg, transfusion-associated HBV (TAHBV) continues to be a major problem in India, more so in patients receiving repeated transfusions^[2].

Literature worldwide shows presence of anti-HBc in HBsAg-negative blood donors. The incidence of anti-HBc in blood donors varies from 0.07% to 18%, and 0.3%-38% of these donors show presence of HBV DNA in their blood, depending on the type of blood donors and the endemicity of disease in the study population^[2,4,6,9-12].

This study was conducted on 1700 HBsAg ELISA non-reactive blood donors. The study population belonged to Chandigarh and states of Punjab, Haryana, Himachal Pradesh, Jammu and Kashmir. The present study showed 8.4% anti-HBc positivity. Prevalence of anti-HBc was 6.9% in voluntary donors and 10.4% in replacement donors. A study reported from New Delhi (Northern India) by Chaudhuri *et al*^[2] revealed that the prevalence of anti-HBc was 10.82% with distribution of 6.92% in voluntary donors and 12.53% in replacement donors. In contrast, a study from West Bengal (Eastern India) by Bhattacharya *et al*^[7] showed an anti-HBc positivity as high as 18.3% in voluntary blood donors. Prevalence of anti-HBc reported by Behzad-Behbahani *et al*^[10] in Iran was 6.55%, in this study only voluntary donors were included. High prevalence of anti-HBc (17.28%) was reported by Bhatti *et al*^[6] from Pakistan, and all the donors in this study were replacement donors. The prevalence of anti-HBc in Europe and North America is quite low, an anti-HBc prevalence of 0.07% in the UK and 1.5% in Germany has been reported^[6]. A study from Japan^[4] reported anti-HBc prevalence of as 1.1%. The enormous variation in global seroprevalence of anti-HBc among blood donors is a

reflection of difference in the type of blood donors and HBV endemicity of the study population. The low seroprevalence in US, UK and German blood donors may be due to 100% voluntary donor base, stringent donor screening, high literacy rates and self exclusion by high- risk donors. In our study, a significantly low seropositivity (6.1%) was seen in donors with age 18-30 years as compared to donors with age 31-40 years (11.6%, $P = 0.003$). Donors with age 18-30 years were 50% of the study population and were largely composed of young college students. The results are comparable with study from our department in 2004 by Sharma *et al*^[13] that student donors have significantly lower incidence of all the markers for transfusion-transmitted infections. Therefore, efforts should be made to increase and retain these young motivated voluntary donors to maintain safe blood supply.

Our study revealed similar prevalence of anti-HBc positivity in first time donors (8.4%) and repeat donors (8.3%, $P = 0.94$), suggesting that lack of education among both our donor groups regarding minor modes of HBV transmission like tattooing, ear/nose piercing, sharing of shaving kits or a visit to a road side barber. It highlights the uniformity of donor behavior between the two groups. Our study is in accordance with recent three studies by Retrovirus Epidemiology Donor Study (REDS)^[14-16] groups which showed that the incidence rate of viral infection is not lower in repeat blood donors than first time donors and abbreviated screening for repeat blood donors is not advisable.

Prevalence of anti-HBc-only was 4.1% in our study, out of which 3.2% was in voluntary and 5.1% in the replacement donors ($P = 0.038$). In a study from New Delhi (Northern India)^[2], the reported prevalence of anti-HBc-only was 4.2%, out of which 2.72% was in voluntary and 4.85% in replacement donors^[2]. Similarly, a study from Iran^[10] reported anti-HBc-only prevalence of 2.3%.

Anti-HBc reactive samples were tested for LFTs. All the samples had normal serum bilirubin levels, and 25 (18%) samples showed elevation of liver enzymes. Elevated levels of liver enzymes in our donors indicates underlying hepatitis or some kind of liver injury which can be ruled out by additional investigations, such as hepatic ultrasound, liver biopsy and genetic testing which were not an objective of the present study.

HBV DNA was detected in 1 of 100 anti-HBc reactive donors tested. None of the anti-HBc-only samples were positive for HBV DNA. One sample was positive for HBV DNA and sample also contained anti-HBs levels > 150 mIU/mL, LFTs for this sample were within normal limits. Other studies from India^[2,7] reported HBV DNA positivity of 20.87% in New Delhi (Northern India) and 21% in West Bengal (Eastern India). HBV DNA was detected among 12.2% of anti-HBc reactive donors in Iran, 2.8% in Lebanon, 2.9% in Pakistan^[7]. A study from Japan^[4] reported that HBV DNA was detected in 19 (38%) of 50 anti-HBc reactive samples. The viral load in these samples is usually low and their detection requires sensitive DNA amplification techniques.

In our study, low incidence of HBV DNA in anti-HBc reactive samples may be due to limitation of sensitivity of HBV DNA amplification technique. Another reason of low HBV DNA positivity in our study could be due to type of blood donors and the endemicity of disease in the study population, in our study 50% of donors were between 18-30 years, largely belong to young college students. This group being better educated can understand and cooperate in answering to the risk factors in acquiring HBV infection during pre-donation questionnaire. Another study from Chandigarh (Northwestern India) by Duseja *et al*^[17] showed 0% prevalence of HBV DNA in 100 adult healthy blood donors, non-reactive for HBsAg.

Keeping in view that 8% to 18% of donor population in India is anti-HBc reactive, inclusion of anti-HBc testing will lead to high discard rate. Anti-HBs as proposed previously does not seem to predict clearance of the virus as the single donor, who tested reactive for HBV DNA in our study, had high anti-HBs titers. Cost effectiveness of introducing universal anti-HBc screening and discarding large number of blood units *versus* considering ID NAT (Individual donor nucleic acid testing) needs to be assessed. Awareness and education of donors is required regarding minor modes of HBV transmission, modification of the donor questionnaire to eliminate all donors with a history of jaundice in adult life and more stringent one-to-one donor screening to elicit such information should be implemented.

COMMENTS

Background

Despite mandatory screening of donor blood for HBsAg, transfusion-associated HBV (TAHBV) continues to be a major problem in India, more so in patients receiving repeated transfusions.

Research frontiers

The present study was undertaken to assess the seroprevalence of antibody to hepatitis B core antigen (anti-HBc) in serum samples of healthy blood donors negative for HBsAg and to evaluate whether anti-HBc detection could be adopted in India as a screening assay for HBV in addition to HBsAg.

Innovations and breakthroughs

There is high prevalence of anti-HBc in Indian blood donors. Voluntary donors have lower incidence of anti-HBc than replacement donors. Anti-HBs does not seem to predict clearance of the virus in the blood donors.

Applications

Keeping in view that 8%-18% of donor population in India is anti-HBc reactive, inclusion of anti-HBc testing will lead to high discard rate. Cost effectiveness of introducing universal anti-HBc screening and discarding large number of blood units *versus* considering ID NAT needs to be assessed.

Peer review

This is an important and timely paper assessing the role of anti-HBc testing to detect occult HBV infectivity for addressing the important issue of TAHBV in Indian blood donors. The research was done at PGIMER Chandigarh, India, one of the premier institutes in the country. This paper deserves publication.

REFERENCES

- Schmidt M, Nubling CM, Scheiblaue H, Chudy M, Walch LA, Seifried E, Roth WK, Hourfar MK. Anti-HBc screening

- of blood donors: a comparison of nine anti-HBc tests. *Vox Sang* 2006; **91**: 237-243
- Chaudhuri V, Nanu A, Panda SK, Chand P. Evaluation of serologic screening of blood donors in India reveals a lack of correlation between anti-HBc titer and PCR-amplified HBV DNA. *Transfusion* 2003; **43**: 1442-1448
- Nanu A, Sharma SP, Chatterjee K, Jyoti P. Markers for transfusion-transmissible infections in north Indian voluntary and replacement blood donors: prevalence and trends 1989-1996. *Vox Sang* 1997; **73**: 70-73
- Yotsuyanagi H, Yasuda K, Moriya K, Shintani Y, Fujie H, Tsutsumi T, Nojiri N, Fuji T, Hoshino H, Shimoda K, Hino K, Kimura S, Iino S, Koike K. Frequent presence of HBV in the sera of HBsAg-negative, anti-HBc-positive blood donors. *Transfusion* 2001; **41**: 1093-1099
- Kleinman SH, Kuhns MC, Todd DS, Glynn SA, McNamara A, DiMarco A, Busch MP. Frequency of HBV DNA detection in US blood donors testing positive for the presence of anti-HBc: implications for transfusion transmission and donor screening. *Transfusion* 2003; **43**: 696-704
- Bhatti FA, Ullah Z, Salamat N, Ayub M, Ghani E. Anti-hepatitis B core antigen testing, viral markers, and occult hepatitis B virus infection in Pakistani blood donors: implications for transfusion practice. *Transfusion* 2007; **47**: 74-79
- Bhattacharya P, Chandra PK, Datta S, Banerjee A, Chakraborty S, Rajendran K, Basu SK, Bhattacharya SK, Chakravarty R. Significant increase in HBV, HCV, HIV and syphilis infections among blood donors in West Bengal, Eastern India 2004-2005: exploratory screening reveals high frequency of occult HBV infection. *World J Gastroenterol* 2007; **13**: 3730-3733
- Malik V. *Drugs and Cosmetics Act, 1940*. 16th ed. Lucknow: Eastern Book Company, 2003: 279-303
- Kleinman SH, Busch MP. HBV: amplified and back in the blood safety spotlight. *Transfusion* 2001; **41**: 1081-1085
- Behzad-Behbahani A, Mafi-Nejad A, Tabei SZ, Lankarani KB, Torab A, Moaddeb A. Anti-HBc & HBV-DNA detection in blood donors negative for hepatitis B virus surface antigen in reducing risk of transfusion associated HBV infection. *Indian J Med Res* 2006; **123**: 37-42
- Allain JP. Occult hepatitis B virus infection: implications in transfusion. *Vox Sang* 2004; **86**: 83-91
- Comanor L, Holland P. Hepatitis B virus blood screening: unfinished agendas. *Vox Sang* 2006; **91**: 1-12
- Sharma RR, Cheema R, Vajpayee M, Rao U, Kumar S, Marwaha N, Agnihotri SK. Prevalence of markers of transfusion transmissible diseases in voluntary and replacement blood donors. *Natl Med J India* 2004; **17**: 19-21
- Glynn SA, Schreiber GB, Busch MP, Kleinman SH, Williams AE, Nass CC, Ownby HE, Smith JW. Demographic characteristics, unreported risk behaviors, and the prevalence and incidence of viral infections: a comparison of apheresis and whole-blood donors. The Retrovirus Epidemiology Donor Study. *Transfusion* 1998; **38**: 350-358
- Williams AE, Thomson RA, Schreiber GB, Watanabe K, Bethel J, Lo A, Kleinman SH, Hollingsworth CG, Nemo GJ. Estimates of infectious disease risk factors in US blood donors. Retrovirus Epidemiology Donor Study. *JAMA* 1997; **277**: 967-972
- Schreiber GB, Glynn SA, Busch MP, Sharma UK, Wright DJ, Kleinman SH. Incidence rates of viral infections among repeat donors: are frequent donors safer? *Transfusion* 2001; **41**: 730-735
- Duseja A, Sharma S, Subramanian PG, Agnihotri SK, Chakraborti A, Chawla Y. Occult hepatitis B virus (HBV) infection in healthy blood donors. *Indian J Pathol Microbiol* 2003; **46**: 690-692

S- Editor Zhong XY L- Editor Kumar M E- Editor Ma WH



Characteristics of paraesophageal varices: A study with 64-row multidetector computed tomography portal venography

Li-Qin Zhao, Wen He, Guang Chen

Li-Qin Zhao, Wen He, Guang Chen, Department of Radiology, Beijing Friendship Hospital, Capital Medical University, Beijing 100050, China

Author contributions: He W and Zhao LQ designed the research; Zhao LQ, Chen G performed the data collection; Zhao LQ and He W performed the post-processing of the images and analyzed data; Zhao LQ wrote the manuscript; He W revised the paper.

Supported by The Science Technology Program of Beijing Education Committee, No. KM200810025002

Correspondence to: Wen He, MD, Department of Radiology, Beijing Friendship Hospital, Capital Medical University, 95 Yong'an Road, Xuan Wu District, Beijing 100050, China. hewen1724@sina.com

Telephone: +86-10-63138470 Fax: +86-10-63134411

Received: June 19, 2008 Revised: July 27, 2008

Accepted: August 3, 2008

Published online: September 14, 2008

Abstract

AIM: To identify the characteristics of morphology, location and collateral circulation involved in paraesophageal varices (para-EV) of portal hypertension patients with 64-row multidetector computed tomography (MDCT).

METHODS: Fifty-two of 501 patients with portal hypertensive cirrhosis accompanied with esophageal varices were selected for 64-row MDCT examination after the observation of para-EV. The CT protocol included unenhanced, arterial and portal phases with a slice thickness of 0.625 mm and a scanning field of 2 cm above the bifurcation to the lower edge of kidney. The CT portal venography (CTPV) was reformatted on AW4.3 workstation. The characteristics of origination, location, morphology and collateral circulation in para-EV were observed.

RESULTS: Among the 52 cases of para-EV, 50 showed the originations from the posterior branch of left gastric vein, while the others from the anterior branch. Fifty cases demonstrated their locations close to the esophageal-gastric junction, and the other two cases were extended to the inferior bifurcation of the trachea. The circuitous pattern was observed in 16 cases, while reticulated pattern was seen in 36 cases. Collateral circulation identified 4 cases of single periesophageal varices (peri-EV) communication, 3 cases of single hemiazygous vein, one case of single inferior vena cava, 41 cases of mixed type (collateral communica-

tions of at least 2 of above mentioned types) and 3 cases of undetermined communications. Among all the cases, 43 patients showed the communications between para-EV and peri-EV, while hemiazygous vein (43 cases) and inferior vena cava (5 cases) were also involved.

CONCLUSION: Sixty-four-row multidetector computed tomography portal venography could display the location, morphology, origin, and collateral types of para-EV, which provides important and referable information for clinical management and disease prognosis.

© 2008 The WJG Press. All rights reserved.

Key words: Computer tomography; Portal venography; Paraesophageal varices; Hepatic cirrhosis; Portal hypertension

Peer reviewers: Juan G Abalde, MD, Hepatic Hemodynamic Laboratory, Liver Unit, Hospital Clinic, University of Barcelona, Villarroel 170, Barcelona 08036, Spain; Aydin Karabacakoglu, PhD, Assistant Professor, Department of Radiology, Meram Medical Faculty, Selcuk University, Konya 42080, Turkey

Zhao LQ, He W, Chen G. Characteristics of paraesophageal varices: A study with 64-row multidetector computed tomography portal venography. *World J Gastroenterol* 2008; 14(34): 5331-5335 Available from: URL: <http://www.wjgnet.com/1007-9327/14/5331.asp> DOI: <http://dx.doi.org/10.3748/wjg.14.5331>

INTRODUCTION

Esophageal varice is a common collateral circulation manifestation in portal hypertension, and may cause a severe complication if ruptured^[1-3]. Endoscopic therapy is an effective approach that is being applied worldwide^[4,5], although the recurrences have been reported in many cases^[6]. Some authors reported that the existence of perforating veins, the communicating branches between periesophageal varices (peri-EV) and paraesophageal varices (para-EV), and their blood flow found by endoscopic color Doppler ultrasonography (ECDUS) affected the recurrences of hemorrhage caused by rupturing^[7]. However, ECDUS is an invasive technique, requires specialized equipment

and technology, and is incapable of demonstrating the drainage types and morphologic features of esophageal varices. Multidetector computed tomography portal venography (MDCTPV) has been used widely in collateral circulation studies of esophageal and gastric varices^[8-11]. The drainage veins of esophageal varices can be clearly displayed on MDCTPV^[10]. Our study focuses on the utilization of MDCTPV in observing para-EV drainage types to provide referable information for clinical management selection and prognosis evaluation.

MATERIALS AND METHODS

Patient population

A total of 501 portal hypertensive cirrhotic patients with esophageal varices were investigated from April 2007 to May 2008. Among them, 52 patients with presence of para-EV were selected for this study, including 35 male and 17 female, aged 21-71 years, averaging 45.2 years. There were 27 cases of hepatitis B, 3 hepatitis C, 16 alcoholic, 2 primary biliary and 4 cases of cryptogenic cirrhosis. Based on Child's grading, 9 cases were Grade A, 28 cases Grade B and 15 cases of Grade C. Among the 52 patients, 50 were accompanied with gastric varices. Fifty patients were not treated with esophageal varice ligation (EVL), esophageal varice sclerotherapy (EVS) or any other endoscopic therapies, while 1 case received post-EVL therapy and 1 case received post-EVS therapy. Liver cirrhosis was confirmed in all patients through general consideration from clinical histories, laboratory findings, as well as CT, sonography or MRI examinations. Informed consent for CT scans and the use of contrast media were obtained from all patients before scanning procedures.

Imaging acquisition

A GE VCT 64-row MDCT scanner was applied to perform unenhanced, arterial and portal vein phase enhanced scans on all patients. The scanning range covers from 2 cm above the tracheal bifurcation to the lower edge of the kidney. One hundred mL of non-ionic contrast medium (Omnipaque 350) was introduced with an infusion rate of 4.0 mL/s. The arterial phase scanning time delay was determined with Smart technology, and portal phase scanning was initiated at 25 s after the beginning of arterial phase. Slice thickness was set at 5.0 mm, and reconstitution thickness was 0.625 mm.

Image analysis

Post-processing methods: vertebrae, costal bones and other structures with bony densities were removed using GE AW4.3 workstation, and then maximum intensity projection (MIP), multi-planner reformation (MPR) technique were applied to reveal the origin, location, morphology and communicating veins of para-EV. The 3D structures of esophageal varices were displayed with volume rendering (VR) technique.

Observation item and indications

Origin of para-EV: The relationship between para-EV

and anterior or posterior branches of the left gastric vein were examined and determined.

Morphology and location of para-EV: The morphological patterns of para-EV were categorized into circuitous pattern and reticulated pattern, in which circuitous pattern was denoted as para-EV linear, near-parallel pathways, while reticulated pattern was denoted as prominent para-EV winding and distortion, forming meshwork or loops. In case of the presence of both circuitous and reticulated patterns, the categorization was determined according to their diameter ratios and then was defined as "mainly circuitous pattern" or "mainly reticulated pattern". Large-diameter vessels with a ratio of over 1 were defined as dominant type. The para-EV was divided into 3 sections according to the extent, including the upper section located at a level superior to the tracheal bifurcation; the middle section located at or near the tracheal bifurcation level, and the lower section located at the abdominal as well as the lower thoracic segment of the esophageal periphery.

Communicating veins of para-EV: The communications between para-EV and peri-EV, hemiazygous vein, subphrenic vein, inferior vena cava and other vessels were investigated. The criteria set for CTPV judgment on the communication of para-EV and its collaterals were as follows: (1) Para-EV was directly conjoint with the above-mentioned vessels; (2) Para-EV and the vessels mentioned above were conjoint with their peripheral circuitous and reticulated blood vessels, respectively.

RESULTS

Origination of para-EV

There were 50 patients with their varices originating from the posterior branch of the left gastric vein (50/52, 96.15%) (Figure 1A) while the varices of the other 2 patients originated from the anterior branch of the left gastric vein (2/52, 3.85%), in which the posterior branch of the left gastric vein was absent and the anterior branch of left gastric vein routes up and enters the fundus through the gastroesophageal junction to form gastric fundal varices. The latter routes up to form peri-EV, then penetrate through esophageal wall to form para-EV.

Morphology and location of para-EV

Sixteen cases of circuitous morphological pattern (16/52, 36.54%) (Figure 1B) and 36 cases of reticulated morphological pattern (36/52, 63.46%) (Figure 1C) were observed. The locations of para-EV were identified as follows: 50 cases in lower section (50/52, 96.15%) (Figure 1C), 2 cases in middle section (2/52, 3.85%), and none in upper section.

Communicating veins of para-EV

Among the 52 cases, 3 showed undetermined communicating vessels (3/52, 5.77%) with 1 of circuitous morphological pattern, while the others were of reticulated

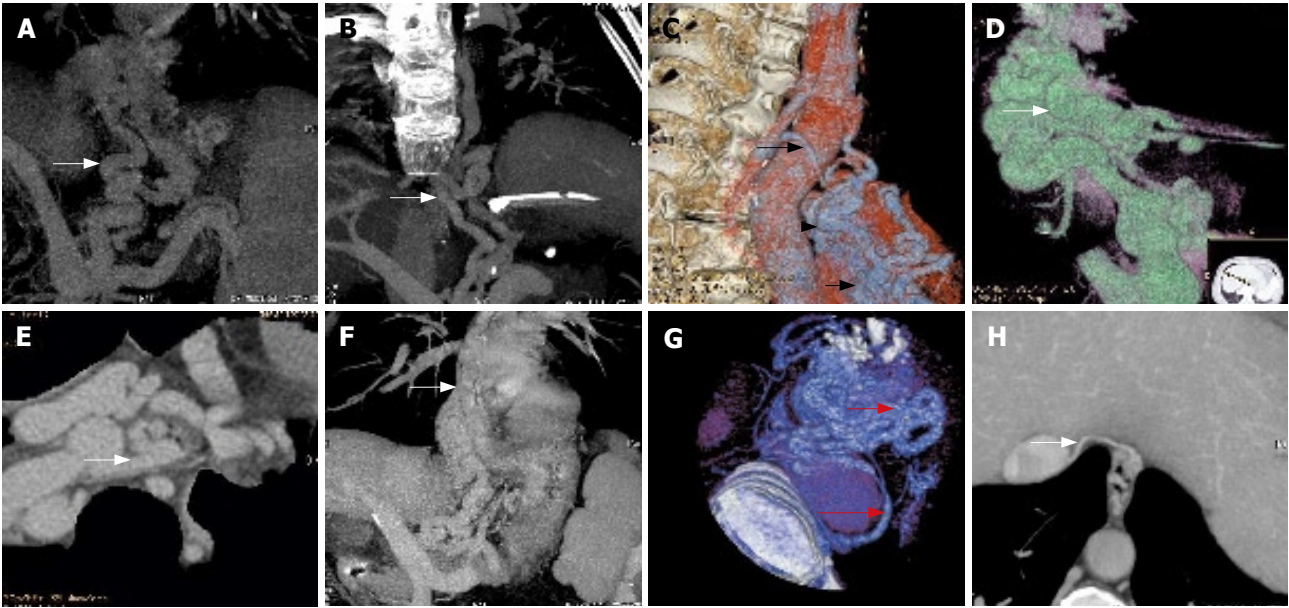


Figure 1 A: Para-EV originated from the posterior branch of the left gastric varices (arrow); B: Circuitous morphological para-EV (arrow); C: Para-EV in reticulatus pattern (short arrow), located at lower section of the esophagus (arrowhead), communicating to hemiazygous vein (long arrow); D: Para-EV communicating to peri-EV (arrow); E: Para-EV communicating to peri-EV directly (arrow); F: Para-EV communicating to peri-EV with peripheral circuitous appearance (arrow); G: Para-EV communicating to peri-EV (short arrow) and the hemiazygous vein (long arrow); H: Para-EV communicating to the subphrenic vein (arrow).

Table 1 Communicating varices and para-EV shapes				
Drainage vein		Shape	Cases	Total
Single	Peri-EV	Reti	4	8
	Hemi	Cir	3	
	Sub	Cir	1	
	Peri-EV + Hemi	Cir	8	
	Peri-EV + Hemi	Reti	29	
Mixed	Sub + Peri-EV	Cir	1	41
	Sub + Hemi	Cir	2	
	Sub + Peri-EV + Hemi	Reti	1	
Uncertain		Cir	1	3
		Reti	2	
Total				52

Hemi: Hemiazygos vein; Sub: Subphrenic vein; Reti: Reticular; Cir: Circuitous.

morphological pattern. In the other 49 cases, 43 cases involved the peri-EV (43/49, 87.76%) (Figure 1D-G), and a similar number of patients also demonstrated the hemiazygous vein (Figure 1B, C and G). Inferior vena cava was included in 5 cases (5/49, 10.20%) (Figure 1H).

The specific communicating patterns found in this study were listed as follows (Table 1): 8 cases with single communication, 41 with mixed communications and, in 3 cases, the drainage veins of the para-EV remained uncertain. Among the 8 cases of single communication, the para-EV connecting to peri-EV (Figure 1E and F) with reticulatus shape was found in 4 cases (4/49, 8.16%), connecting to Hemiazygos vein of circuitous appearance in 3 cases (3/49, 5.77%), and to the subphrenic vein draining circuitously in 1 case (1/49, 1.92%).

In the 41 cases of mixed type, the para-EV was found connecting to peri-EV and hemiazygous vein in 37 cases (37/49, 71.15%) (Figure 1G), with circuitous

shape in 8 cases and reticulatus in 29 cases. Three cases presented para-EV communicating to subphrenic vein: 1 to peri-EV (1/49, 1.92%) and 2 to Hemiazygos vein circuitously (2/49, 3.84%). In 1 case (1/49, 1.92%), all the drainage mentioned above was observed with reticulatus appearance. The para-EV was circuitous in 1 case and reticulatus in 2 cases of uncertain drainage.

DISCUSSION

The prevalence rate of esophageal varices is high in patients with liver cirrhosis, and the mortality rate is very high imposed by massive hemorrhage due to rupture^[2,12]. It is reported that more than 70% of portal hypertension patients with a variceal bleeding history may suffer from recurrent bleeding^[13]. The esophageal varice was divided into three groups: peri-EV, para-EV and perforating veins^[14]. The factors associated with the rupture of esophageal varices included the extent and collateral circulation of peri-EV and para-EV^[15,16]. Therefore, the acquisition of reliable images for the above mentioned factors will provide valuable information for further treatment implementation and prognosis of the patients.

Angiography is one of the earliest investigations applied in hemodynamic evaluation of portal hypertension-induced esophageal varices by displaying esophageal varices and their drainage vessels through arterial portography^[17]. However, the invasive procedure and the incapability to differentiate peri-EV and para-EV prevent it to be applied in a general clinical setting.

Color Doppler ultrasound has been widely used to investigate the relationship between EV and hemodynamics associated with portal hypertension and liver cirrhosis. But it could not show the para-EV and peri-EV clearly^[18].

Endoscopy is a more popular approach for the observation of morphological patterns, extension and severity of esophageal submucotic varices with the limitations to intramural and para-EV^[19].

ECDUS is gradually becoming a useful tool for esophageal varicose inspection, which is able to display peri-EV, para-EV, perforating veins and the blood flow directions of perforating veins^[7,14,20]. However, it is unable to clearly display the pathway and collaterals of para-EV. On the other hand, endoscopic sonography is costly, and also an invasive procedure that requires a skillful operator, which limited its application in clinical settings.

MDCTPV has already been a matured investigation technique used to examine esophageal and gastric varices. It is competent in displaying the types of morphological pattern, origin and collateral circulations of esophageal and gastric varices^[10,11]. Sixty-four-row MDCT consists of thin slice of 0.625 mm, and a high spatial resolution, which makes it capable of displaying the communicating patterns of para-EV and peri-EV. Among the 52 cases in our study, this pattern of collateral circulation was shown in 49 (94.23%) cases. Generally, it is a non-invasive technique, and hence, it can be used to evaluate para-EV conveniently. However, displaying of this sort of collaterals is relatively rough and unsatisfactory in detail if compared with EUS. In addition, it is incapable to assess the blood flow directions of paraesophageal-periesophageal collateral circulation due to the characters of CTPV.

According to our study, vast majority of para-EV originate from the posterior branch of left gastric vein, and only 2 cases originated from the anterior branch of left gastric vein. The latter is highly associated with gastric fundal varices. A previous report suggested that^[21], in cases of coexisting gastric fundal varices and esophageal varices in lower esophageal segment, the extent of esophageal varices could be aggravated after imposing obliteration therapy for gastric fundal varices. In our study, there was one patient who experienced alleviation of gastric varices with aggravated para-EV and peri-EV after receiving gastroesophageal varices treatment. The origination of para-EV may be related directly to its anatomical formation.

In our study, para-EV was found more abundant near the superior part of the gastroesophageal junction, which is also the lower esophageal segment (96.15%) than in the middle segment (3.85%). There was no para-EV involved in the upper segment. This suggests that para-EV usually occurs in the middle and lower esophageal segment and drains into the vena cava system through collateral vessels. The involved drainage vessels that we have noted were peri-EV, hemiazygous veins and subphrenic veins.

The communicating branches of para-EV and hemiazygous vein manifest as drainage “detour” veins bypassing along the anterior descending aorta to the hemiazygous vein, and subsequently drain into the superior vena cava. Ibukuro *et al*^[22] described the above anatomic changes as preaortic vein using CT and CTAP

application. This “detour” is a commonly seen drainage route, and has an occurrence rate of 87.76%.

The hemodynamics of para-EV communicating peri-EV is relatively complicated. The communicating vessels of para-EV and peri-EV are mostly situated close to the top of the cardia level. Sato *et al*^[7] observed the presence of perforating veins between peri-EV and para-EV with Levovist ECDUS, and meanwhile, he found the bidirectional blood flow of peri-EV and para-EV, which included the directions from para-EV to peri-EV and from peri-EV to para-EV, as well as mixed type that presented both blood flow directions. This may explain why previous para-EV studies about the recurrence risk of post-esophageal varices treatment remain controversial^[20,23,24].

Para-EV can still drain into the inferior vena cava through the subphrenic vein, or directly communicate with the inferior vena cava. The subphrenic veins are the bilateral vessels which end up at inferior vena cava at the diaphragm level^[25]. The communication between para-EV and subphrenic veins shown on CTPV was consistent to the precaval route and morphological pattern as reported by Ibukuro *et al*^[25] from their CTAP and autopsy findings.

The degree of varices will be more severe if the morphological patterns of para-EV are reticulated. Under such condition, para-EV and peri-EV are individually communicated, or may be accompanied with hemiazygous vein drainage. They will mostly drain into the hemiazygous veins or subphrenic veins if the collaterals of para-EV are circuitous. Explanations about this correlation and its pathological significance require further studies.

Even though CTPV is incapable of displaying the blood flow directions of para-EV, it has a certain guidance value for EUS application. If para-EV is detected on CTPV, EUS should be further implemented to confirm the presence of specific communicating branches between peri-EV and para-EV as well as the location and hemodynamic characteristics of their communicating branches so as to provide relevant referable information for treatment selections.

COMMENTS

Background

The existence of para-EV and perforating veins and the direction of their blood flow can affect the recurrence of hemorrhage caused by rupturing.

Research frontiers

The morphology, location and collateral circulation characteristics of para-EV in portal hypertensive patients were studied with 64-row multidetector computer tomography portal venography (MDCTPV), which is a non-invasive method.

Innovations and breakthroughs

In majority cases of this study, the collateral circulation pattern of para-EV and the morphological characteristics of para-EV were revealed thanks to the high spatial resolution images of the advanced MDCT and the appropriate image post-processing.

Applications

MDCTPV is a noninvasive method to display the morphological characteristics of para-EV, which is useful in clinical management and disease prognosis.

Terminology

Paraesophageal varices are the varices that exist outside the esophagus. Peri-

EV is the varices that exist in the esophageal wall or the submucotic varices. Perforating vein is the communicating branch between peri-EV and para-EV. The subphrenic vein is the bilateral vessel that ends up at inferior vena cava at the diaphragm level. CTPV is the abbreviation of CT portal venography.

Peer review

This is a good review with excellent images. MDCTPV is a convenient method to display the morphological and collateral circulations of the para-EV. It is of important value in the selection of clinical therapy and evaluation of prognosis.

REFERENCES

- 1 **Bhasin DK**, Malhi NJ. Variceal bleeding and portal hypertension: much to learn, much to explore. *Endoscopy* 2002; **34**: 119-128
- 2 **Bratovic I**, Lacevic N. Management of esophageal varices. *Med Arh* 2002; **56**: 11-12
- 3 **Brandenburger LA**, Regenstein FG. Variceal Hemorrhage. *Curr Treat Options Gastroenterol* 2002; **5**: 73-80
- 4 **Gotoh Y**, Iwakiri R, Sakata Y, Koyama T, Noda T, Matsunaga C, Ogata SI, Ishibashi S, Sakata H, Tsunada S, Fujimoto K. Evaluation of endoscopic variceal ligation in prophylactic therapy for bleeding of oesophageal varices: a prospective, controlled trial compared with endoscopic injection sclerotherapy. *J Gastroenterol Hepatol* 1999; **14**: 241-244
- 5 **Mizumoto H**, Matsutani S, Fukuzawa T, Ishii H, Sato G, Maruyama H, Saisho H. Hemodynamics in the left gastric vein after endoscopic ligation of esophageal varices combined with sclerotherapy. *J Gastroenterol Hepatol* 2001; **16**: 495-500
- 6 **Grace ND**, Groszmann RJ, Garcia-Tsao G, Burroughs AK, Pagliaro L, Makuch RW, Bosch J, Stiegmann GV, Henderson JM, de Franchis R, Wagner JL, Conn HO, Rodes J. Portal hypertension and variceal bleeding: an AASLD single topic symposium. *Hepatology* 1998; **28**: 868-880
- 7 **Sato T**, Yamazaki K, Toyota J, Karino Y, Ohmura T, Akaike J, Kuwata Y, Suga T. Perforating veins in recurrent esophageal varices evaluated by endoscopic color Doppler ultrasonography with a galactose-based contrast agent. *J Gastroenterol* 2004; **39**: 422-428
- 8 **Kang HK**, Jeong YY, Choi JH, Choi S, Chung TW, Seo JJ, Kim JK, Yoon W, Park JG. Three-dimensional multi-detector row CT portal venography in the evaluation of portosystemic collateral vessels in liver cirrhosis. *Radiographics* 2002; **22**: 1053-1061
- 9 **Agarwal A**, Jain M. Multidetector CT portal venography in evaluation of portosystemic collateral vessels. *J Med Imaging Radiat Oncol* 2008; **52**: 4-9
- 10 **Zhang LQ**, He W. CT portal venography of collateral veins in esophageal varices. *Zhongguo Yixue Yingxiang Jishu* 2007; **23**: 242-245
- 11 **Zhao LQ**, He W, Zhao H, Yu YZ. The value of CT portalvenography in the diagnosis of collateral veins in patients with gastric varices. *Zhonghua Fangshexue Zazhi* 2006; **40**: 1175-1178
- 12 **Seewald S**, Seitz U, Yang AM, Soehendra N. Variceal bleeding and portal hypertension: still a therapeutic challenge? *Endoscopy* 2001; **33**: 126-139
- 13 **Grace ND**, Groszmann RJ, Garcia-Tsao G, Burroughs AK, Pagliaro L, Makuch RW, Bosch J, Stiegmann GV, Henderson JM, de Franchis R, Wagner JL, Conn HO, Rodes J. Portal hypertension and variceal bleeding: an AASLD single topic symposium. *Hepatology* 1998; **28**: 868-880
- 14 **Irisawa A**, Shibukawa G, Obara K, Saito A, Takagi T, Shishido H, Odajima H, Abe M, Sugino T, Suzuki T, Kasukawa R, Sato Y. Collateral vessels around the esophageal wall in patients with portal hypertension: comparison of EUS imaging and microscopic findings at autopsy. *Gastrointest Endosc* 2002; **56**: 249-253
- 15 **Irisawa A**, Obara K, Sato Y, Saito A, Takiguchi F, Shishido H, Sakamoto H, Kasukawa R. EUS analysis of collateral veins inside and outside the esophageal wall in portal hypertension. *Gastrointest Endosc* 1999; **50**: 374-380
- 16 **Irisawa A**, Saito A, Obara K, Shibukawa G, Takagi T, Yamamoto G, Sakamoto H, Takiguchi F, Shishido H, Hikichi T, Oyama H, Sato N, Katakura K, Kasukawa R, Sato Y. Usefulness of endoscopic ultrasonographic analysis of variceal hemodynamics for the treatment of esophageal varices. *Fukushima J Med Sci* 2001; **47**: 39-50
- 17 **Watanabe K**, Kimura K, Matsutani S, Ohto M, Okuda K. Portal hemodynamics in patients with gastric varices. A study in 230 patients with esophageal and/or gastric varices using portal vein catheterization. *Gastroenterology* 1988; **95**: 434-440
- 18 **Li FH**, Hao J, Xia JG, Li HL, Fang H. Hemodynamic analysis of esophageal varices in patients with liver cirrhosis using color Doppler ultrasound. *World J Gastroenterol* 2005; **11**: 4560-4565
- 19 **Chikamori F**, Kuniyoshi N, Shibuya S, Takase Y. Correlation between endoscopic and angiographic findings in patients with esophageal and isolated gastric varices. *Dig Surg* 2001; **18**: 176-181
- 20 **Choudhuri G**, Dhiman RK, Agarwal DK. Endosonographic evaluation of the venous anatomy around the gastro-esophageal junction in patients with portal hypertension. *Hepatogastroenterology* 1996; **43**: 1250-1255
- 21 **Cho SK**, Shin SW, Lee IH, Do YS, Choo SW, Park KB, Yoo BC. Balloon-occluded retrograde transvenous obliteration of gastric varices: outcomes and complications in 49 patients. *AJR Am J Roentgenol* 2007; **189**: W365-W372
- 22 **Ibukuro K**, Tsukiyama T, Mori K, Inoue Y. Preaortic esophageal veins: CT appearance. *AJR Am J Roentgenol* 1998; **170**: 1535-1538
- 23 **Dhiman RK**, Choudhuri G, Saraswat VA, Agarwal DK, Naik SR. Role of paraesophageal collaterals and perforating veins on outcome of endoscopic sclerotherapy for oesophageal varices: an endosonographic study. *Gut* 1996; **38**: 759-764
- 24 **Hino S**, Kakutani H, Ikeda K, Uchiyama Y, Sumiyama K, Kuramochi A, Kitamura Y, Matsuda K, Arakawa H, Kawamura M, Masuda K, Suzuki H. Hemodynamic assessment of the left gastric vein in patients with esophageal varices with color Doppler EUS: factors affecting development of esophageal varices. *Gastrointest Endosc* 2002; **55**: 512-517
- 25 **Ibukuro K**, Tsukiyama T, Mori K, Inoue Y. Precaval draining vein from paraesophageal varices: radiologic-anatomic correlation. *AJR Am J Roentgenol* 1999; **172**: 651-654

S- Editor Li DL L- Editor Ma JY E- Editor Yin DH



RAPID COMMUNICATION

Effect of music on procedure time and sedation during colonoscopy: A meta-analysis

Wilson WS Tam, Eliza LY Wong, Sheila F Twinn

Wilson WS Tam, Sheila F Twinn, Nethersole School of Nursing, 8/F Esther Lee Building, The Chinese University of Hong Kong, Shatin, Hong Kong, China
Eliza LY Wong, School of Public Health, 4/F Postgraduate Education Centre, Prince of Wales Hospital, Shatin, Hong Kong, China

Author contributions: Tam WWS, Wong ELY and Twinn SF designed the study; Tam WWS and Wong ELY performed the search and data (papers) collection; Tam WWS performed the analysis; Tam WWS, Wong ELY and Twinn SF wrote the paper.

Correspondence to: Wilson WS Tam, Nethersole School of Nursing, Room 830, Esther Lee Building, The Chinese University of Hong Kong, Shatin, Hong Kong, China. wilsontam@graduate.hku.hk

Telephone: +86-852-26098172 Fax: +86-852-26035935

Received: July 10, 2008 Revised: August 25, 2008

Accepted: September 2, 2008

Published online: September 14, 2008

© 2008 The WJG Press. All rights reserved.

Key words: Colonoscopy; Colon cancer; Meta-analysis; Music therapy; Screening

Peer reviewer: Yoshiharu Motoo, MD, PhD, FACP, FACG, Professor and Chairman, Department of Medical Oncology, Kanazawa Medical University, 1-1 Daigaku, Uchinada, Ishikawa 920-0293, Japan

Tam WWS, Wong ELY, Twinn SF. Effect of music on procedure time and sedation during colonoscopy: A meta-analysis. *World J Gastroenterol* 2008; 14(34): 5336-5343 Available from: URL: <http://www.wjgnet.com/1007-9327/14/5336.asp> DOI: <http://dx.doi.org/10.3748/wjg.14.5336>

Abstract

AIM: To integrate results from different studies in examining the effectiveness of music in reducing the procedure time and the amount of sedation used during colonoscopic procedure.

METHODS: An electronic search in various databases was performed to identify related articles. Study quality was evaluated by the Jadad's scale. The random effect model was used to pool the effect from individual trials and the Cohen Q-statistic was used to determine heterogeneity. Egger's regression was used to detect publication bias.

RESULTS: Eight studies with 722 subjects were included in this meta-analysis. The combined mean difference for the time taken for the colonoscopy procedure between the music and control groups was -2.84 with 95% CI (-5.61 to -0.08), implying a short time for the music group. The combined mean difference for the use of sedation was -0.46 with 95%CI (-0.91 to -0.01), showing a significant reduction in the use of sedation in the music group. Heterogeneity was observed in both analyses but no publication bias was detected.

CONCLUSION: Listening to music is effective in reducing procedure time and amount of sedation during colonoscopy and should be promoted.

INTRODUCTION

In 2005, colorectal cancer was the fourth most frequent cancer type worldwide for both sexes and colon cancer accounted for about 655 000 deaths per year^[1]. Most colon cancers develop from polyps that grow abnormally in the colon. If polyps grow unnoticed and are not removed, they may become cancerous. Indeed, colorectal cancer is among the most preventable and curable cancers and with early detection 75%-90% of them can be prevented^[2]. The aim of screening is to find precancerous polyps so they can be removed before they become cancerous. Screening for colon cancer has been shown to be effective in reducing mortality^[3].

Colonoscopy is now the recommended method for screening colon cancer^[4]. During a colonoscopy procedure, physicians insert a colonoscope into the rectum of a patient from the anus and slowly guide it into the colon for direct visualization and diagnosis^[5]. As can be imagined, it is not a comfortable experience. In fact, many people refuse to undergo colonoscopy because of discomfort and anxiety^[6], some feel out of control with what would be happening during the procedure and were fearful of looking foolish^[7]. Even those who agree to undergo a colonoscopy feel frightened and anxious before the procedure^[8]. After it, they express it was an unpleasant and stressful experience^[9]. Therefore, most physicians prefer to

perform colonoscopy with conscious sedation^[110] although some still start the procedure without sedation, particularly in non-anxious patients^[111].

Since the use of sedation is risky-it may contribute to the occurrence of cardiovascular events and is associated with the risk of cardio-respiratory complication^[112,113] especially for elderly patients^[114]-and costly, non-pharmacological methods for alleviating patients' discomfort and anxiety have been developed and music therapy is one of them. The use of music therapy to promote relaxation has a long history in medicine^[115,116]. The ancient Chinese medical reference, Yellow Emperor's Classics of Internal Medicine, mentioned the use of music for treatment^[117], while ancient Indian treatises, like Samaveda, stated the therapeutic utility of music^[118]. Earlier studies show the effectiveness of music on patients with acute myocardial infarctions^[119] and receiving intensive medical/surgical care^[120]. The effects of music on different screening procedures were examined during the early 1990s. For example, Fullhart^[121] and Palakanis^[6] studied anxiety in patients undergoing flexible sigmoidoscopy and Bampton^[122] assessed the role of relaxation music on patient tolerance of gastrointestinal endoscopic procedures. Since then, more and more studies have examined the effect of music during colonoscopy, but no conclusion has yet been reached about its effectiveness. Recently, a meta-analysis^[23] focusing on the general endoscopic procedure was published and the authors reported significantly lower anxiety levels and shorter procedure times. The aim of this meta-analysis is to integrate results from different studies in examining the effectiveness of music in reducing procedure time and amount of sedation used during colonoscopy.

MATERIALS AND METHODS

Search strategies

We identified studies to evaluate the effect of listening to music during colonoscopy. The electronic search was conducted on six databases, namely, AMED-Allied and Complementary Medicine (1985 to 2007), ACP journal club (1991 to 2007), CINAHL (1982 to March 2007), Cochrane Central Register of Controlled trials (up to 1st quarter of 2007), EMBASE (1980 to March 2007), and MEDLINE (1966 to March 2007). Only two sets of keywords, i.e. Colonoscopy and Music/Music, were used to include as many articles as possible. We also attempted to identify any potential unpublished studies such as theses and dissertations using the above keywords through the ProQuest Dissertations and Theses database, and the web using Internet search engines, Google and Yahoo, with thesis or dissertation as additional keywords. Reference lists of the collected papers were also checked for any potential articles.

Inclusion criteria

Only randomized controlled studies reported in English

were included. At least one of the comparison groups included listening to music during colonoscopy as part of the intervention.

Data extraction

For each eligible study, we extracted information on author/s, year of publication, age distribution, gender proportion, other demographic data, intervention, outcome measures, and results. The data extraction form was modified from the data extraction form of van Tulder^[24]. Two investigators (WT and EW) of the study independently extracted the data, which was then summarized by one of them. Agreement was reached on the extracted data before proceeding to data analysis.

Methodological quality

The methodological quality of the included studies was assessed using the Jadad's scale^[25]. The quality variables recorded in the criteria list included the procedure of patient allocation, information regarding withdrawals and dropouts, blinding of patients, and outcome assessment. A maximum of five points can be obtained using the scale. However, as blinding of patients is difficult while assessing behavioral interventions^[26], only blinding of outcome assessors was considered in the scale. Furthermore, as withdrawals and attrition of patients were unlikely in such a short period, such item in the Jadad's scale was also ignored. Therefore, the maximum points obtainable for each study became four. Besides the Jadad's scale, one additional question was extracted from van Tulder^[24] for assessing the comparability of the two comparison groups.

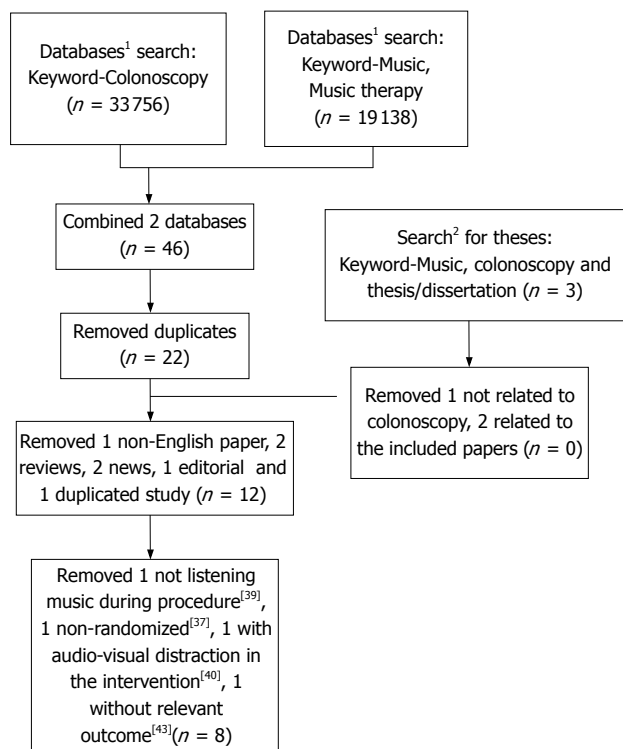
Statistical analysis

Random-effect models were used to combine the outcome effect, i.e. the (standardized) difference between treated and control groups, for each study^[27]. Heterogeneity was examined using the Q-test and I²^[27]. All analyses were conducted using RevMan^[27]. Publication bias was examined by funnel plot and Egger's regression^[28].

RESULTS

Search strategies

The electronic search was conducted in May 2007 and 19 records were found. The title and abstracts of these papers were examined and it was found that one was written in Portuguese^[29], one was a systematic review focus on pain^[30], one was a discussion paper on the use of sedation^[31], one was an editorial^[32], and two were news^[33,34]. One paper^[35] occurred twice in our search as the surname of the first author was recorded differently in two electronic databases (EBASE and MEDLINE), so one was removed. Hence, only 12 studies^[35-46] were included and the full articles were obtained for further examination. A study^[37] was found to be a non-randomized study



¹Databases included CINAHL (1982 to 2007 March), Cochrane Central Register of Controlled trials (up to 1st quarter of 2007), EMBASE (1980 to 2007 March) and MEDLINE (1966 to 2006 January); ²Search in ProQuest Dissertations and Theses database (1861 to 2007) and through search engine yahoo and google (only the first 20 pages of results were checked for detail).

Figure 1 Flowchart of the search history.

and was excluded and another study^[43] not examining the procedure time nor the amount of sedation was also excluded. One study^[39] required their subjects in the intervention group to listen to music before the colonoscopy procedure on a voluntary basis. The author was contacted to see how many subjects in the intervention group listened to music during the procedure. Although the author implied that most of the subjects continued to listen to music during the procedure, the study was excluded because not all participants in the intervention group listened to music during the procedure. Another study^[40] provided audiovisual distraction to patients instead on listening to music alone and the effect may have been enhanced; therefore, it was also excluded from the analysis.

As to the search of potential theses or dissertations, one doctoral dissertation^[47] and two master theses^[48,49] were identified and their abstracts were obtained. However, one^[48] was not related to colonoscopy and the others^[47,49] related to the papers from previous search^[40,45]. A flowchart is provided to show the search history (Figure 1).

A total of 722 subjects were involved in 8 studies (Table 1), four from Europe^[35,36,42,44], two from the US^[36,45], and three from Asia^[38,41,46]. Two studies

provided different types of music for their patients to choose from. Four studies broadcasted the music through headphones/earphones^[35,38,41,45], three broadcasted as background music^[36,42,44], and one did not specify the media method^[46]. Seven studies mentioned that all colonoscopy procedures were conducted by an experienced colonoscopist^[42,46] or a group of experienced colonoscopists^[35,36,38,41] for both comparison groups.

Quality of studies

All of the included studies were randomized controlled studies and only five of them reported the method of randomization^[35,36,38,41,42]. No information on attrition was reported in any study probably because most of the procedures were completed in a relatively short period of time. Incomplete procedures were reported in two studies but no significant incomplete rates were detected^[35,41]. Although it would be impossible to blind the subjects, outcomes assessor was blinded in two studies^[35,38] by requesting the patients in the control group to take the earphones. Six studies^[35,36,41,42,45,46] showed that baseline characteristics between control and treatment groups were comparable. One study did not provide the test statistics^[44] and one did not report the baseline characteristics^[38] (Table 2). Overall, five studies^[35,36,38,41,42] got 2 points (half) or above in our modified Jadad's scale.

Procedure time (Figure 2)

The total time taken for the procedure between music and control groups was measured in seven studies^[35,36,38,41,44-46]. Six of them^[36,38,41,44-46] showed a reduction of time in the music group but only in one case was this significant^[44]. Since two studies^[36,38] did not provide the means or standard deviations, the corresponding authors of the studies were contacted to obtain this information. Authors from both studies^[36,38] generously provided the means and standard deviations of the respective parameters and therefore their results could be included in the analysis. The combined mean difference between the treated and control groups was -2.84 with 95% CI (-5.61 to -0.08). The Q-value and the I² were 13.46 ($P = 0.04$) and 55%, suggesting possible heterogeneity among the studies. Publication bias was not detected using Egger's regression method ($P = 0.9133$).

Sedation (Figure 2)

Six studies^[36,38,41,42,44,45] examined the use of sedation, i.e. midazolam in mg, and four^[38,41,42,45] showed a reduction in the music group. Sedation was given or added based on patients' request^[38,41,44] or colonoscopists' decision^[36,42]. The means and standard deviations of the 2 studies^[36,38] were requested from the authors. The combined mean difference for the six trials was -0.46 with 95% CI (-0.91 to -0.01), showing a marginally significant reduction of the use of sedation in the

Table 1 Characteristics of each of the included studies

Study	Design	Baseline characteristic	Comparison group	Outcome
Andrada <i>et al</i> (2004) ^[35]	RCT	Intervention- 31 males 32 females, mean age: 46 (14.22) Control- 28 males 27 females, mean age: 49 (13.88)	Intervention- Listening music until the end of procedure (classical tracts) Control- Usual colonoscopy screening	Blood pressure, capillary and oxygen saturation, heart rate level of anxiety
Bechtold <i>et al</i> (2006) ^[36]	RCT	Intervention- 41 males and 44 females, age: 58.5 Control- 42 males 39 females, age: 54.1	Intervention- Playing music upon the entrance the patient (same music for all patients) Control- 50 mg of meperidine and 1 or 2 mg of midazolam	Dose consumed, time to time, reach cecum, total procedure insertion difficulty scale, experience scale, pain scale, want music next time
Harikumar <i>et al</i> (2006) ^[38]	RCT	Intervention- 38 (male + female), age 15 - 60 Control- 40 (male + female), age 15-60	Intervention- Listening music (popular film songs based on classical rages, classical music, devotional songs, folk songs, soft instrumental music, and bioacoustics) Control- 2 mg intravenous boluses of midazolam on demand; Colonoscopic procedure performed by endoscopists with experience of performing at least 200 full-length colonoscopic procedure	Pain score, discomfort score, procedure time, recovery time, dose consumed
Lee <i>et al</i> (2002) ^[41]	RCT	Intervention 1- 33 male 22 female Inter-quartile age range: 46-68 mean age: 54 Intervention 2- 29 male 26 female, Inter-quartile age range: 39-67 mean age: 47 Intervention 3- 27 males 28 females, Inter-quartile age range: 40-65 mean age: 51	Intervention 1- Patient-controlled sedation Intervention 2- Patient-controlled sedation+ music1 Intervention 3- Music1 Could request intravenous administration of diazemuls (0.1 mg/kg) and meperidine (0.5 mg/kg) Nasal oxygen (2 L/min) to all patients in the study 1Type of music: classical, jazz, popular (Chinese and English) and Chinese opera Examination performed endoscopists having more than 300 similar procedures before	Dose of propofol, pain score, satisfactory score, willingness to repeat the same mode of sedation.
Ovayolu <i>et al</i> (2006) ^[42]	RCT	Intervention- 14 males 16 females, age: 20-39 (<i>n</i> = 10), 40-59 (<i>n</i> = 11), 60 or above (<i>n</i> = 9) Control- 14 males 16 females, age: 20-39 (<i>n</i> = 5), 40-59 (<i>n</i> = 11), 60 or above (<i>n</i> = 14)	Intervention- Listening music (classical Turkish music, a slow and relaxing music) Control- Usual colonoscopy screening; procedure performed by an expert endoscopist with experience of performing at least 200 full-length procedure previously	Dose of sedation consumed, anxiety score, pain score, VAS score, willingness to the procedure score and satisfaction score.
Schimann <i>et al</i> (2002) ^[44]	RCT	Intervention- 25 males 34 females, mean age: 52.3 (13.9), Control- 33 males 27 females, mean age: 55.8 (13.5)	Intervention- Music from radio Control- Conventional procedure	Request of sedation (midazolam), oxygen supplement, procedure time
Smolen <i>et al</i> (2002) ^[45]	RCT	Intervention- 10 male 6 females, mean age: 58.83 (13.64) Control- 7 males 9 females, mean age: 61.06 (9.48)	Intervention- Listening music (classical, jazz, pop rock and easy listening) Control- Undergo standard colonoscopy procedure including explanation of the procedure by the nurse; receive a standard pre-procedure sedation consisting of a slow intravenous injection of 1 mg of midazolam in combination with 50 mg of meperidine	Sedation, anxiety and heart rate
Uedo <i>et al</i> (2004) ^[46]	RCT	Intervention- 7 males 7 females, mean age: 54 (6) Control- 11 males 4 females, mean age: 54 (8)	Intervention- Listen the music (easy listening style) from the beginning and during procedure Control- Usual procedure for undergoing colonoscopy, no anxiolytic medications, no antisecretory agents	Salivart cortisol levels

Table 2 Quality assessment by Jadad's scale and baseline comparison

Study (year of publication)	Is the study randomized?	Is the procedure appropriate & reported?	Is the study blind to the assessors?	Is the blinding method appropriate & reported?	Were the groups similar at baseline?
Andrada <i>et al</i> (2004) ^[35]	C	C	C	C	C
Bechtold <i>et al</i> (2006) ^[36]	C	C	N	NA	C
Harikumar <i>et al</i> (2006) ^[38]	C	C	C	C	P ¹
Lee <i>et al</i> (2002) ^[41]	C	P	C	N	C
Ovayolu <i>et al</i> (2006) ^[42]	C	C	N	NA	C
Schiemann <i>et al</i> (2002) ^[44]	C	N	N	NA	P ²
Smolen <i>et al</i> (2002) ^[45]	C	N	N	NA	C
Uedo <i>et al</i> (2004) ^[46]	C	N	N	NA	C

Remark: C, correct; N, not correct; P, partly correct; NA, not applicable. ¹No result provided; ²No test result provided.

music group. The Q-test and I^2 were respectively 34.83 ($P < 0.001$) and 86% suggesting strong heterogeneity. Publication bias was not detected using Egger's regression method ($P = 0.1150$).

DISCUSSION

Colon cancer is the fourth leading cause of death among all cancers^[1] but remains one of the most preventable and curable cancers if detected early^[2]. Screening for colon cancer has been shown to be an effective method of reducing the risk of mortality, but the compliance rate is still low probably due to the unpleasant feeling of patients during the procedure^[9]. Non-pharmacological methods for alleviating patients discomfort and anxiety have been developed and, in the early 1990s, Palakanis^[6] demonstrated that listening to music before and during sigmoidoscopy was effective in reducing one's anxiety. Colonoscopy has been the recommended procedure for screening colon cancer^[4] and more studies have been conducted in examining the effect of listening to music during this procedure.

Our results show that listening to music during the colonoscopy would effectively reduce the mean procedure time and the amount of sedation used. One possible explanation for the reduction of sedation is that patients in the music group are more relaxed and with less anxiety. Therefore, the physician can complete the procedure in a shorter period of time and use less sedation^[45]. The reduction of procedure time implies a reduction of the anxious, frightening, and unpleasant time spent while undergoing the procedure and may be useful in enhancing the compliance rate.

It was reported that conscious sedation with midazolam contributed to the occurrence of cardiovascular events during colonoscopy^[12] and was associated with the risk of cardio-respiratory complication^[14]. Avoidance of sedation may provide a quicker patient discharge, less need for monitoring, and overall cost savings^[50]. Our results also found a significant reduction in anxiety score, but only weak evidence was observed for pain score, blood pressure, and mean recovery time.

Besides the above-mentioned beneficial effects

to patients, two advantages of listening to music during colonoscopy are cheapness and ease of implementation^[51]. Although cassette players and compact disc players were used in most of the included studies, digital players, like MP3 players, may be a better choice in the future^[52]. With advanced technology, a thumb-sized MP3 player can store hundreds of songs at a much lower cost. Therefore, more choices can be given to patients, which is important as personal preference has a strong impact on one's responses to music^[53].

No harmful effects from listening to music were reported in any study in the meta-analysis and other references that we read. Only one shortcoming about patients listening to music through headphone/earphone was the isolation of verbal communication between patients and the medical staff during the procedure. However, broadcasting the music as background music might disturb the staff conducting the procedure probably because an imposed choice of musical selection can be annoying to the listener^[53].

Recently, a meta-analysis was published on a similar topic^[23] but there are several differences between that study and the present one. First of all, colonoscopy was the focus of this paper. Second, this study's search strategy was more comprehensive, meaning that more databases were included and theses/dissertations were also identified. Third, besides the numerically combined results, the characteristics of all included studies were presented and discussed in the text or in the table.

Although our findings confirm the effectiveness of listening to music during the colonoscopy procedure, several areas are worth further investigation. These include the choice of music, the mode of broadcasting music (earphone, background, or both), the possibility of using placebo to the patients in the control group, the possibility of blinding to the colonoscopist/s or medical staff involved in the procedure, the interaction of the medical staff to background music as well as the effect of playing audiovisual materials. Finally, it was suggested that the role of music should be considered whenever applicable^[54].

Our results confirm that patient listening to music during colonoscopy is an effective way in reducing

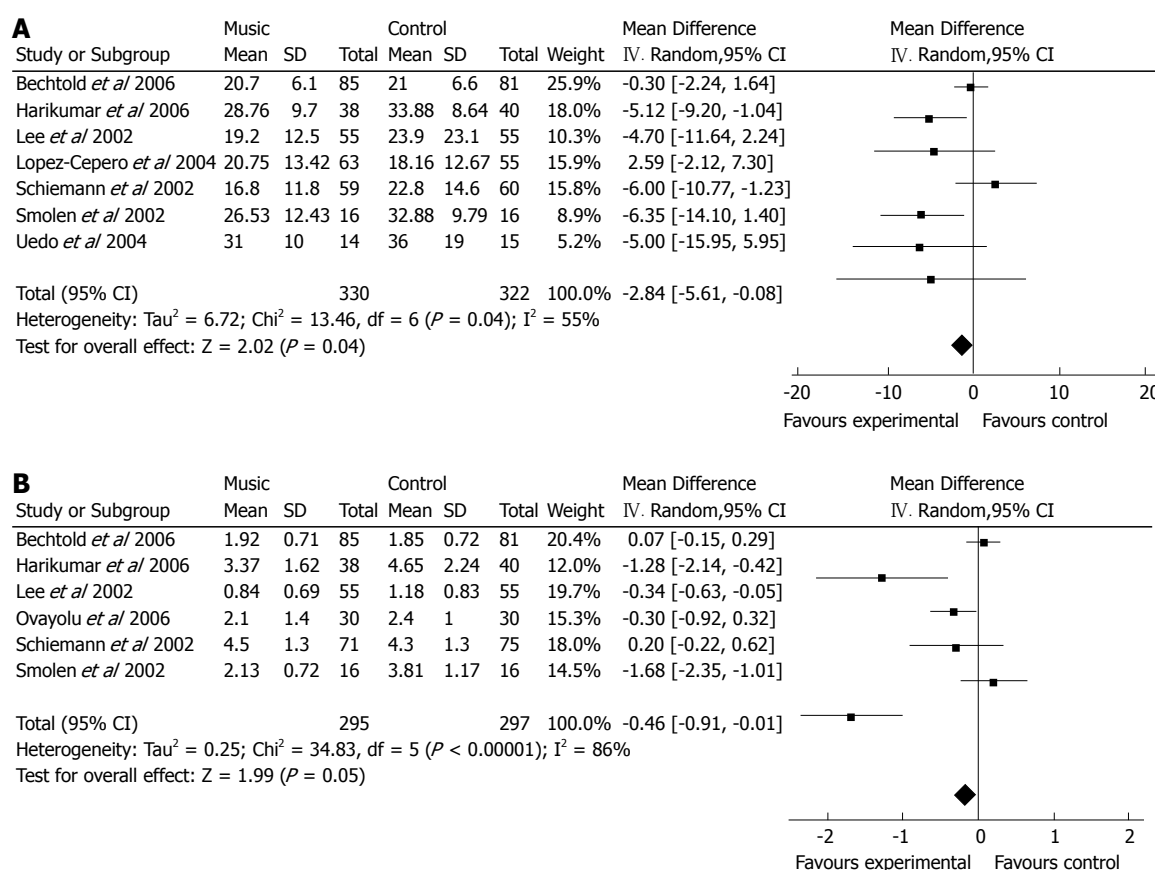


Figure 2 A: Forrest plot of the effect of music in procedure time; B: Forrest plot of the effect of music in sedation.

procedure time, anxiety, and the amount of sedation. More importantly, no harmful effects were observed for all the studies. Therefore, listening to music during colonoscopy should be recommended.

ACKNOWLEDGMENTS

The authors would like to thank Prof. Matthew Bechtold, Prof. Nair Harikumar, Prof. Ann Hayes and Prof. John Marshall for providing the information in their studies.

COMMENTS

Background

Colonoscopy is the recommended method for screening colon cancer but many people are unwilling to receive it because of fearing the pain, anxiety and other reasons. The use of music in medical research has long history and the beneficial effects of listening music during colonoscopy have been widely reported.

Research frontiers

A meta-analysis was conducted to integrate results from different studies in examining the effectiveness of music in reducing the procedure time and the amount of sedation during colonoscopy.

Innovations and breakthroughs

Our results show that listening to music during colonoscopy may effectively reduce the mean procedure time and the amount of sedation used.

Applications

Listening to music during colonoscopy should be promoted because of its beneficial effect and negligible cost.

Peer review

This is a research on a kind of a complementary and alternative medicine, a "music therapy".

REFERENCES

- 1 WHO. Facts sheets, Cancer, WHO 2006. Available from: <http://www.who.int/mediacentre/factsheets/fs297/en/index.html>
- 2 Winawer SJ, Fletcher RH, Miller L, Godlee F, Stolar MH, Mulrow CD, Woolf SH, Glick SN, Ganiats TG, Bond JH, Rosen L, Zapka JG, Olsen SJ, Giardiello FM, Sisk JE, Van Antwerp R, Brown-Davis C, Marciniak DA, Mayer RJ. Colorectal cancer screening: clinical guidelines and rationale. *Gastroenterology* 1997; **112**: 594-642
- 3 Selby JV, Friedman GD, Quesenberry CP Jr, Weiss NS. A case-control study of screening sigmoidoscopy and mortality from colorectal cancer. *N Engl J Med* 1992; **326**: 653-657
- 4 Davila RE, Rajan E, Baron TH, Adler DG, Egan JV, Faigel DO, Gan SI, Hirota WK, Leighton JA, Lichtenstein D, Qureshi WA, Shen B, Zuckerman MJ, VanGuilder T, Fanelli RD. ASGE guideline: colorectal cancer screening and surveillance. *Gastrointest Endosc* 2006; **63**: 546-557
- 5 Waye JD, Rex DK, Williams CB. Colonoscopy: Principles and practice. 1st ed. Malden, Mass. Oxford: Blackwell Sci Pub, 2003: 655
- 6 Palakanis KC, DeNobile JW, Sweeney WB, Blankenship CL. Effect of music therapy on state anxiety in patients undergoing flexible sigmoidoscopy. *Dis Colon Rectum* 1994; **37**: 478-481
- 7 Salmon P, Shah R, Berg S, Williams C. Evaluating customer satisfaction with colonoscopy. *Endoscopy* 1994;

- 26: 342-346
- 8 **Salmore RG**, Nelson JP. The effect of preprocedure teaching, relaxation instruction, and music on anxiety as measured by blood pressures in an outpatient gastrointestinal endoscopy laboratory. *Gastroenterol Nurs* 2000; **23**: 102-110
- 9 **Kim LS**, Koch J, Yee J, Halvorsen R, Cello JP, Rockey DC. Comparison of patients' experiences during imaging tests of the colon. *Gastrointest Endosc* 2001; **54**: 67-74
- 10 **Balsells F**, Wyllie R, Kay M, Steffen R. Use of conscious sedation for lower and upper gastrointestinal endoscopic examinations in children, adolescents, and young adults: a twelve-year review. *Gastrointest Endosc* 1997; **45**: 375-380
- 11 **Fisher NC**, Bailey S, Gibson JA. A prospective, randomized controlled trial of sedation vs. no sedation in outpatient diagnostic upper gastrointestinal endoscopy. *Endoscopy* 1998; **30**: 21-24
- 12 **Ristikankare M**, Julkunen R, Laitinen T, Wang SX, Heikkinen M, Janatuinen E, Hartikainen J. Effect of conscious sedation on cardiac autonomic regulation during colonoscopy. *Scand J Gastroenterol* 2000; **35**: 990-996
- 13 **Bell GD**. Premedication, preparation, and surveillance. *Endoscopy* 2002; **34**: 2-12
- 14 **Yuno K**, Iishi H, Tatsuta M, Hifumi K, Omori M. Intravenous midazolam as a sedative for colonoscopy: a randomized, double-blind clinical trial. *Aliment Pharmacol Ther* 1996; **10**: 981-984
- 15 **Rorke MA**. Music therapy in the age of enlightenment. *J Music Ther* 2001; **38**: 66-73
- 16 **West M**. Music Therapy in Antiquity. Horden P, editor. Music as medicine: the history of music therapy since antiquity. Aldershot; Brookfield, Vt: Ashgate, 2000: 51-68
- 17 **Ni MS**. The Yellow Emperor's Classic of internal medicine: A new translation of the Neijing Suwen with commentary. Boston: Shambhala Pub, 1995: 14-17
- 18 **Katz JB**. Music Therapy: Some Possibilities in the Indian Tradition. Horden P, editor. Music as medicine: the history of music therapy since antiquity. Aldershot; Brookfield, Vt: Ashgate, 2000: 84-102
- 19 **White JM**. Music therapy: an intervention to reduce anxiety in the myocardial infarction patient. *Clin Nurse Spec* 1992; **6**: 58-63
- 20 **Updike P**. Music therapy results for ICU patients. *Dimens Crit Care Nurs* 1990; **9**: 39-45
- 21 **Fullhart JW**. Preparatory information and anxiety before sigmoidoscopy: a comparative study. *Gastroenterol Nurs* 1992; **14**: 286-290
- 22 **Bampton P**, Draper B. Effect of relaxation music on patient tolerance of gastrointestinal endoscopic procedures. *J Clin Gastroenterol* 1997; **25**: 343-345
- 23 **Rudin D**, Kiss A, Wetz RV, Sottile VM. Music in the endoscopy suite: a meta-analysis of randomized controlled studies. *Endoscopy* 2007; **39**: 507-510
- 24 **van Tulder M**, Furlan A, Bombardier C, Bouter L. Updated method guidelines for systematic reviews in the cochrane collaboration back review group. *Spine* 2003; **28**: 1290-1299
- 25 **Jadad AR**, Moore RA, Carroll D, Jenkinson C, Reynolds DJ, Gavaghan DJ, McQuay HJ. Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control Clin Trials* 1996; **17**: 1-12
- 26 **Monninkhof E**, van der Valk P, van der Palen J, van Herwaarden C, Partridge MR, Zielhuis G. Self-management education for patients with chronic obstructive pulmonary disease: a systematic review. *Thorax* 2003; **58**: 394-398
- 27 **Review Manager (RevMan)** [Computer program] Version 5.0. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2008
- 28 **Egger M**, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; **315**: 629-634
- 29 **Arruda Alves PR**, Roncaratti E, Habr Gama A. Reduction in salivary levels of cortisol by music therapy during colonoscopy. *GED-Gastrenterologia Endoscopia Digestiva* 2004; **23**: 83-84
- 30 **Cepeda MS**, Carr DB, Lau J, Alvarez H. Music for pain relief. *Cochrane Database Syst Rev* 2006; CD004843
- 31 **Lazzaroni M**, Bianchi Porro G. Preparation, premedication, and surveillance. *Endoscopy* 2005; **37**: 101-109
- 32 **Lotufo PA**. The noise stops you from hearing good music: the possibilities for a mortality reduction program for cancer of the colon and rectum in Sao Paulo. *Sao Paulo Med J* 2003; **121**: 95-96
- 33 **Kurth T**, Krevvsky B, Chiu C. Late-breaking news. *Bottom Line Health* 2007; **21**: 1
- 34 **Anonymous**. Tuned up for colon test. *Nur Standard* 2006; **21**: 8
- 35 **Lopez-Cepero Andrada JM**, Amaya Vidal A, Castro Aguilar-Tablada T, Garcia Reina I, Silva L, Ruiz Guinaldo A, Larrauri De la Rosa J, Herrero Cibaja I, Ferre Alamo A, Benitez Roldan A. Anxiety during the performance of colonoscopies: modification using music therapy. *Eur J Gastroenterol Hepatol* 2004; **16**: 1381-1386
- 36 **Bechtold ML**, Perez RA, Puli SR, Marshall JB. Effect of music on patients undergoing outpatient colonoscopy. *World J Gastroenterol* 2006; **12**: 7309-7312
- 37 **Binek J**, Sagmeister M, Borovicka J, Knierim M, Magdeburg B, Meyenberger C. Perception of gastrointestinal endoscopy by patients and examiners with and without background music. *Digestion* 2003; **68**: 5-8
- 38 **Harikumar R**, Raj M, Paul A, Harish K, Kumar SK, Sandesh K, Asharaf S, Thomas V. Listening to music decreases need for sedative medication during colonoscopy: a randomized, controlled trial. *Indian J Gastroenterol* 2006; **25**: 3-5
- 39 **Hayes A**, Buffum M, Lanier E, Rodahl E, Sasso C. A music intervention to reduce anxiety prior to gastrointestinal procedures. *Gastroenterol Nurs* 2003; **26**: 145-149
- 40 **Lee DW**, Chan AC, Wong SK, Fung TM, Li AC, Chan SK, Mui LM, Ng EK, Chung SC. Can visual distraction decrease the dose of patient-controlled sedation required during colonoscopy? A prospective randomized controlled trial. *Endoscopy* 2004; **36**: 197-201
- 41 **Lee DW**, Chan KW, Poon CM, Ko CW, Chan KH, Sin KS, Sze TS, Chan AC. Relaxation music decreases the dose of patient-controlled sedation during colonoscopy: a prospective randomized controlled trial. *Gastrointest Endosc* 2002; **55**: 33-36
- 42 **Ovayolu N**, Ucan O, Pehlivan S, Pehlivan Y, Buyukhatipoglu H, Savas MC, Gulsen MT. Listening to Turkish classical music decreases patients' anxiety, pain, dissatisfaction and the dose of sedative and analgesic drugs during colonoscopy: a prospective randomized controlled trial. *World J Gastroenterol* 2006; **12**: 7532-7536
- 43 **Salmore RG**, Nelson JP. The effect of preprocedure teaching, relaxation instruction, and music on anxiety as measured by blood pressures in an outpatient gastrointestinal endoscopy laboratory. *Gastroenterol Nurs* 2000; **23**: 102-110
- 44 **Schiemann U**, Gross M, Reuter R, Kellner H. Improved procedure of colonoscopy under accompanying music therapy. *Eur J Med Res* 2002; **7**: 131-134
- 45 **Smolen D**, Topp R, Singer L. The effect of self-selected music during colonoscopy on anxiety, heart rate, and blood pressure. *Appl Nurs Res* 2002; **15**: 126-136
- 46 **Uedo N**, Ishikawa H, Morimoto K, Ishihara R, Narahara H, Akedo I, Ioka T, Kaji I, Fukuda S. Reduction in salivary cortisol level by music therapy during colonoscopic examination. *Hepatogastroenterology* 2004;

- 51: 451-453
- 47 **Lee DWH.** The use of patient-controlled and adjunct sedation for colonoscopy. Thesis. Chinese University of Hong Kong 2005
- 48 **Parker DB.** The effect of Music Therapy for pain and anxiety versus literature on the immediate and future perceptions of cardiac patients. Thesis. The Florida State University, 2004
- 49 **Smolen D,** Topp R, Singer L. The effect of self-selected music during colonoscopy on anxiety, heart rate, and blood pressure. *Appl Nurs Res* 2002; **15**: 126-136
- 50 **Takahashi Y,** Tanaka H, Kinjo M, Sakumoto K. Sedation-free colonoscopy. *Dis Colon Rectum* 2005; **48**: 855-859
- 51 **Kemper KJ,** Danhauer SC. Music as therapy. *South Med J* 2005; **98**: 282-288
- 52 **Maag M.** Podcasting and MP3 players: emerging education technologies. *Comput Inform Nurs* 2006; **24**: 9-13
- 53 **Bonny HL.** Music and healing. *Music Ther* 1986; **6A**: 3-12.
- 54 **Thaut MH.** The future of music in therapy and medicine. *Ann N Y Acad Sci* 2005; **1060**: 303-308

S- Editor Zhong XY **L- Editor** Negro F **E- Editor** Zhang WB



RAPID COMMUNICATION

Effect of mutant p27^{kip1} gene on human cholangiocarcinoma cell line, QBC₉₃₉

Jian Luo, Yong-Jun Chen, Wei-Yu Wang, Sheng-Quan Zou

Jian Luo, Yong-Jun Chen, Wei-Yu Wang, Sheng-Quan Zou, Department of General Surgery, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei Province, China

Author contributions: Luo J, Chen YJ, Wang WY and Zou SQ designed the research; Luo J and Chen YJ performed the research; Wang WY contributed to the new reagents/analytic tools; Wang WY analyzed the data; Luo J wrote the paper.

Supported by The National High Technology Research and Development Program of China (863 Program), No. Z2002AA214061

Correspondence to: Sheng-Quan Zou, Department of General Surgery, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei Province, China. sqzou@tjh.tjmu.edu.cn

Telephone: +86-27-83662398 **Fax:** +86-27-83662851

Received: December 16, 2007 **Revised:** August 8, 2008

Accepted: August 15, 2008

Published online: September 14, 2008

Peer reviewer: Gustav Paumgartner, Professor, University of Munich, Klinikum Grosshadern, Marchioninstr. 15, Munich, D-81377, Germany

Luo J, Chen YJ, Wang WY, Zou SQ. Effect of mutant p27^{kip1} gene on human cholangiocarcinoma cell line, QBC₉₃₉. *World J Gastroenterol* 2008; 14(34): 5344-5348 Available from: URL: <http://www.wjgnet.com/1007-9327/14/5344.asp> DOI: <http://dx.doi.org/10.3748/wjg.14.5344>

Abstract

AIM: To investigate the effects of exogenously mutated p27^{kip1} (p27) on proliferation and apoptosis of human cholangiocarcinoma cell line, QBC₉₃₉ *in vivo*.

METHODS: Adenoviral vectors were used to transfect mutated p27 cDNA into human QBC₉₃₉ cell line. Expression of p27 was detected by RT-PCR. Western blot. Cell growth, morphological change, cell cycle, apoptosis and cloning formation were determined by MTT assay and flow cytometry.

RESULTS: The expression of p27 protein and mRNA was increased significantly in QBC₉₃₉ cell line transfected with Ad-p27mt. The transfer of Ad-p27mt could significantly inhibit the growth of QBC₉₃₉ cells, decrease the cloning formation rate and induce apoptosis. p27 over expression caused cell cycle arrest at G₀/G₁ phase 72 h after infection with Ad-p27mt.

CONCLUSION: p27 may cause cell cycle arrest at G₀/G₁ phase and subsequently lead to apoptosis. Recombinant adenovirus expressing mutant p27 may be potentially useful in gene therapy for cholangiocarcinoma.

INTRODUCTION

It is well known that cell cycle progression is governed by cyclin-dependent kinases (CDKs). P27^{kip1} (p27), a key inhibitor of CDKs, can directly inhibit the entry of cell cycle from G₁ phase into S phase. A major mechanism underlying the regulation of p27 is proteolysis by the ubiquitin-proteasome pathway. Phosphorylation of p27 on threonine 187 (T187) by Cdk2 creates a binding site for a Skp2-containing E3 ubiquitin-protein ligase, SCF. Ubiquitylation of p27 by SCF results in degradation of p27 by the proteasome.

In this study, a replication-deficient adenovirus vector encoding a mutated p27 at the Thr-187/pyrophosphorylation site was constructed and transfected into the cultured human cholangiocarcinoma cell line QBC₉₃₉, in order to investigate the effects of adenovirus-mediated p27 on proliferation and apoptosis of cholangiocarcinoma cells.

MATERIALS AND METHODS

Materials

Human cholangiocarcinoma cell line QBC₉₃₉ was kindly donated by Professor Wang Shu-Guang of the Hepatobiliary Department of Xinan Hospital, Third Military Medical University. Tetramethyl-azo-zole-cyan (MTT) and iodized-dine (PI) were purchased from Sigma Ltd. Human-source rat anti-p27 monoclonal antibody was purchased from Beijing Zhongshan Ltd. Sense and anti-sense primers of p27 were synthesized by Shanghai Sangon Bioengineering and Technology Service Co. Ltd. Recombinant adenovirus vehicle Ad-p27mt and adenovirus control vehicle Ad-LacZ were kindly donated by Professor Xu Shao-Yong at Digestive Medical Department and Doctor Wang Jia-Ning at

© 2008 The WJG Press. All rights reserved.

Key words: Adenovirus; Cholangiocarcinoma; Gene therapy; Cell cycle; Apoptosis

Cardiovascular Department, Yunyang Medical College. CO₂ gas incubator (Binder, Germany), inverted phase contrast microscope (Olympus, Japan), FACsort flow cytometry (USA BD Ltd.) were used in this study. Cells used in experiments were divided into control group (QBC₉₃₉ group), Ad-LacZ group and Ad-p27mt group.

Cell culture and transfection

Human cholangiocarcinoma cell line QBC₉₃₉ was incubated in 10% FCS-containing RPMI 1640 culture medium at 37°C at an atmosphere containing 50 mL/L CO₂, and infected with Ad-LacZ or with Ad-p27mt at multiplicity of infection (MOI) of 50 as the density reached to 40%-50%.

Transduction efficacy of recombinant adenovirus

Ad-LacZ was used to infect QBC₉₃₉ cholangiocarcinoma cells when the MOI was set at 25, 50, 100 and 200. X-gal staining was performed after 48 h culture. Blue-staining cells were counted and the percentage was calculated to confirm recombinant adenovirus infection efficacy. Results demonstrated that as MOI ≥ 50, recombinant adenovirus was able to implement an approximately 100% transduction efficacy rate on the two types of cells.

Cell growth inhibition test (MTT assay)

Cells (4000-6000 cells/well) were inoculated in 96-well plates. The culture fluid was discarded after 48 h of grouping, and 150 µL/well (0.5 mg/mL) MTT solution was supplemented at 37°C for 4 h followed by 150 µL/well DMSO, and shaken for 10 min. Absorbance (A) value was detected with an autokinetic enzyme scaling meter at 492 nm wavelength. Cell growth suppressive rate = (1-A value of experimental group/A value of the same titre QBC₉₃₉ group) × 100%.

Clone formation

The cells infected with Ad-LacZ or with Ad-p27mt at a MOI of 50 were transferred into a 12-well plate (500 cells/well) in triplicate and cultured for 3, 6, 9, and 12 d, respectively, then fixed with methanol and stained with 0.4% crystal violet. Clones containing at least 50 cells were counted under inverse microscope. Clone formation ratio (%) = cell clone amounts/500 × 100%.

Extraction of total RNA and RT-PCR

Total cellular RNA was extracted from QBC₉₃₉ cells transfected with Ad-p27mt and Ad-LacZ for 48 h using the Trizol method. PCR was performed after reverse transcription. The sequences of P27mt gene are upstream primer: 5'-CCTAGAGGGCAAGTACGAGTG-3', downstream primer: 5'-GAAGAATCGTCGGTTGCAGGTCGCT-3'. Reaction parameters were pre-degenerated at 95°C for 5 min, degenerated at 94°C for 30 s, 39 cycles of annealing at 56.3°C for 35 s, extension at 72°C for 35 s, a final extension at 72°C for 10 min. Electrophoresis was performed for the PCR products on a 2% agarose gel.

Western blot

Cellular protein disposed for 72 h was extracted with the

Table 1 In different testing groups QBC₉₃₉ cells

Group	Cell clone count (ratio, cell clone amounts/500)			
	3 d after transfect	6 d after transfect	9 d after transfect	12 d after transfect
Ad-p27mt group	9 (1.8)	14 (2.8)	15 (3.0)	21 (4.2)
Ad-LacZ group (n = 3)	16 (3.2)	27 (5.4)	49 (9.8)	56 (11.2)
QBC ₉₃₉ group (n = 3)	18 (3.6)	31 (6.2)	46 (9.2)	62 (12.4)

F = 10.361, P = 0.011 (cell clone ratio, Ad-p27m vs AdLacZ group).

same method as described above. The proteins electro-transferred onto nitrocellulose membranes and blocked by confining liquid were bound to p27 monoclonal antibody and secondary antibody, and colored by enhanced chemiluminescence (ECL).

Cell cycle and apoptosis counting analysis by flow cytometry

Cells of each group (above 10⁶ cells in each group) were harvested at different time points. RNA enzyme was added at 37°C and reacted for 1 h after cells were fixed in 70% alcohol at 4°C for 24 h (final concentration 50 µg/mL). After 20-30 min of PI solution (concentration 100 µg/mL) staining, cells were counted by monochromatic fluorescence flow cytometry to observe the apoptosis rate.

Statistical analysis

All data were expressed as mean ± SD. The data were analyzed with SPSS 10.0 software. Variance analysis SNK method was employed in comparison of multi-groups. P < 0.05 was considered statistically significant.

RESULTS

Titre of recombinant adenovirus

Ultraviolet spectrophotometry showed that the titre of recombinant adenovirus after multiplication, amplification, and purification was up to 7.95 × 10¹² CFU/mL.

Transduction efficacy of recombinant adenovirus

Ad-LacZ was used to infect QBC₉₃₉ cholangiocarcinoma cells. The multiplicity of infection (MOI) was 25, 50, 100 and 200. X-gal staining was performed after 48 h culture. Blue-staining cells were counted and the percentage was calculated to confirm the recombinant adenovirus infection efficacy. Results demonstrated that as MOI ≥ 50, recombinant adenovirus was able to implement an approximately transduction efficacy rate of 100% in the two types of cells.

Growth suppression of QBC₉₃₉ cells by introduction of mutated p27 gene

Clone formation test: The number and ratio of cellular clones in different groups are shown in Table 1 and Figure 1. The transfer of Ad-p27mt significantly inhibited the growth of QBC₉₃₉ cells, decreased the clone formation, which was significantly different from the Ad-LacZ-infected and uninfected groups (F = 10.361, P = 0.011) with no statistical difference.

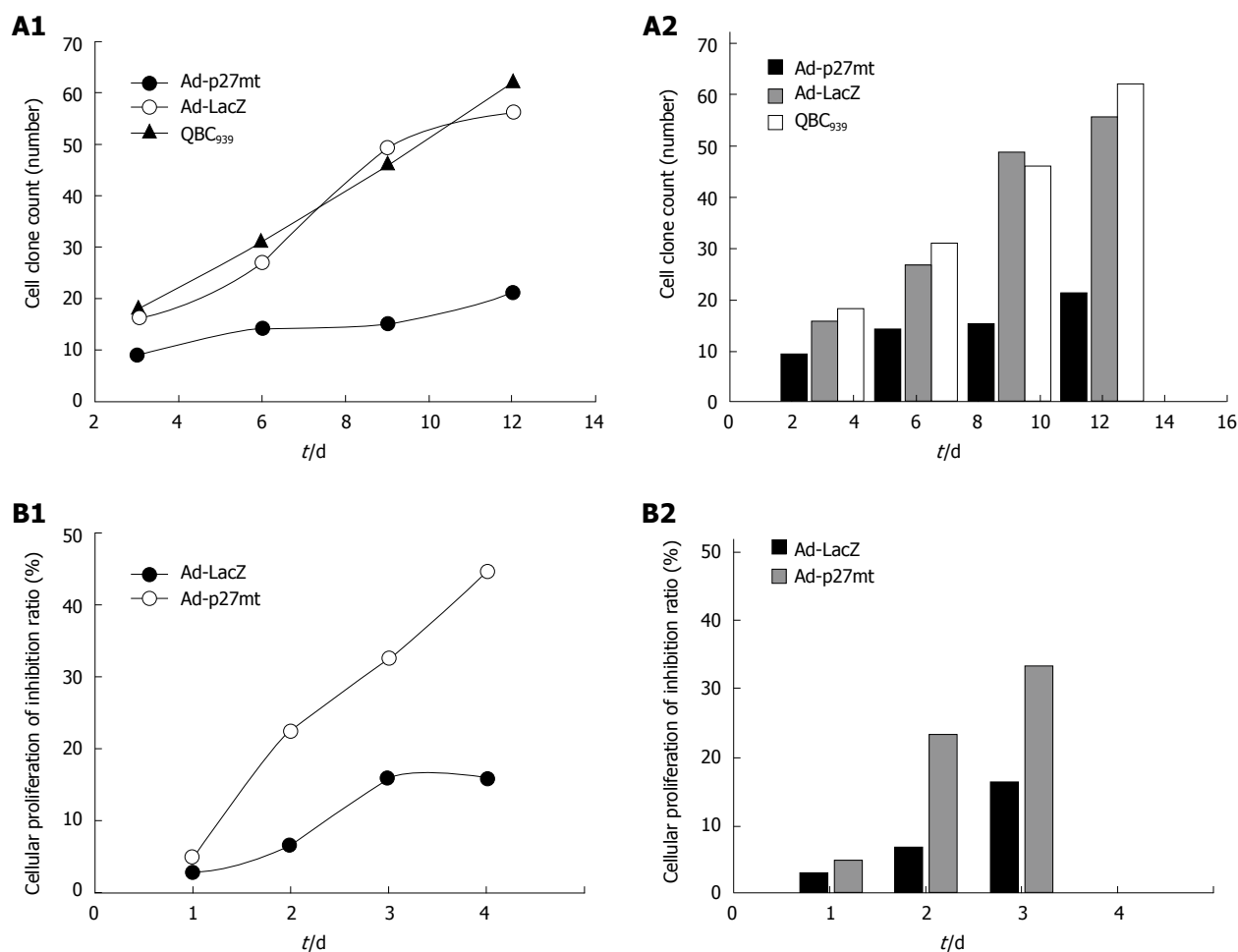


Figure 1 Clone formation test and MTT assay showing effect of Ad-p27mt and Ad-LacZ on growth curve of QBC₉₃₉ cells (**A1**, **B1**) and on the proliferation of QBC₉₃₉ cells (**A2**, **B2**).

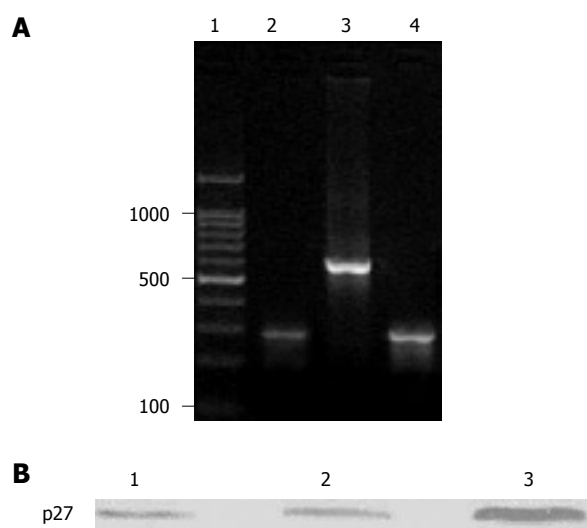


Figure 2 p27 expression at mRNA (**A**) and protein level (**B**). Lane 1: Marker; Lane 2: QBC₉₃₉; Lane 3: β -actin; Lane 4: QBC₉₃₉/Ad-p27mt in Figure 2A; Lane 1: QBC₉₃₉; Lane 2: QBC₉₃₉/Ad-LacZ; Lane 3: QBC₉₃₉/Ad-p27mt in Figure 2B.

MTT assay for cell growth and viability: MTT assays also indicated that the proliferation of QBC₉₃₉ cells was significantly inhibited after Adp27 infection, with its inhibitory effect peaked at 72 h. The transfer

of Ad-p27mt could significantly inhibit the growth of QBC₉₃₉ cells and decrease clone formation. After 24, 48 and 72 h of Adp27 infection, the average CD value was remarkably lower in Adp27-infected group than in Ad LacZ-infected and uninfected groups, revealing that introduction of exogenously mutated p27 gene *via* a recombinant adenovirus vector could significantly suppress the growth of QBC₉₃₉ cells in a non time-dependent manner within 72 h.

Expression of p27 in cholangiocarcinoma QBC₉₃₉ cells at mRNA level

Gel electrophoresis for the RT-PCR products displayed that the expression of p27 was decreased in normal control group, but the expression of QBC₉₃₉ cells was significantly elevated with a distinct 275bp objective gene strap (Figure 2A).

Expression of p27 in cholangiocarcinoma QBC₉₃₉ cells at protein level

The expression of p27 was significantly increased in Ad-p27mt-transfected QBC₉₃₉ cells. However, the expression of p27 could be detected in a small number of Ad-LacZ-transfected QBC₉₃₉ cells (endogenous) and in the control group (Figure 2B).

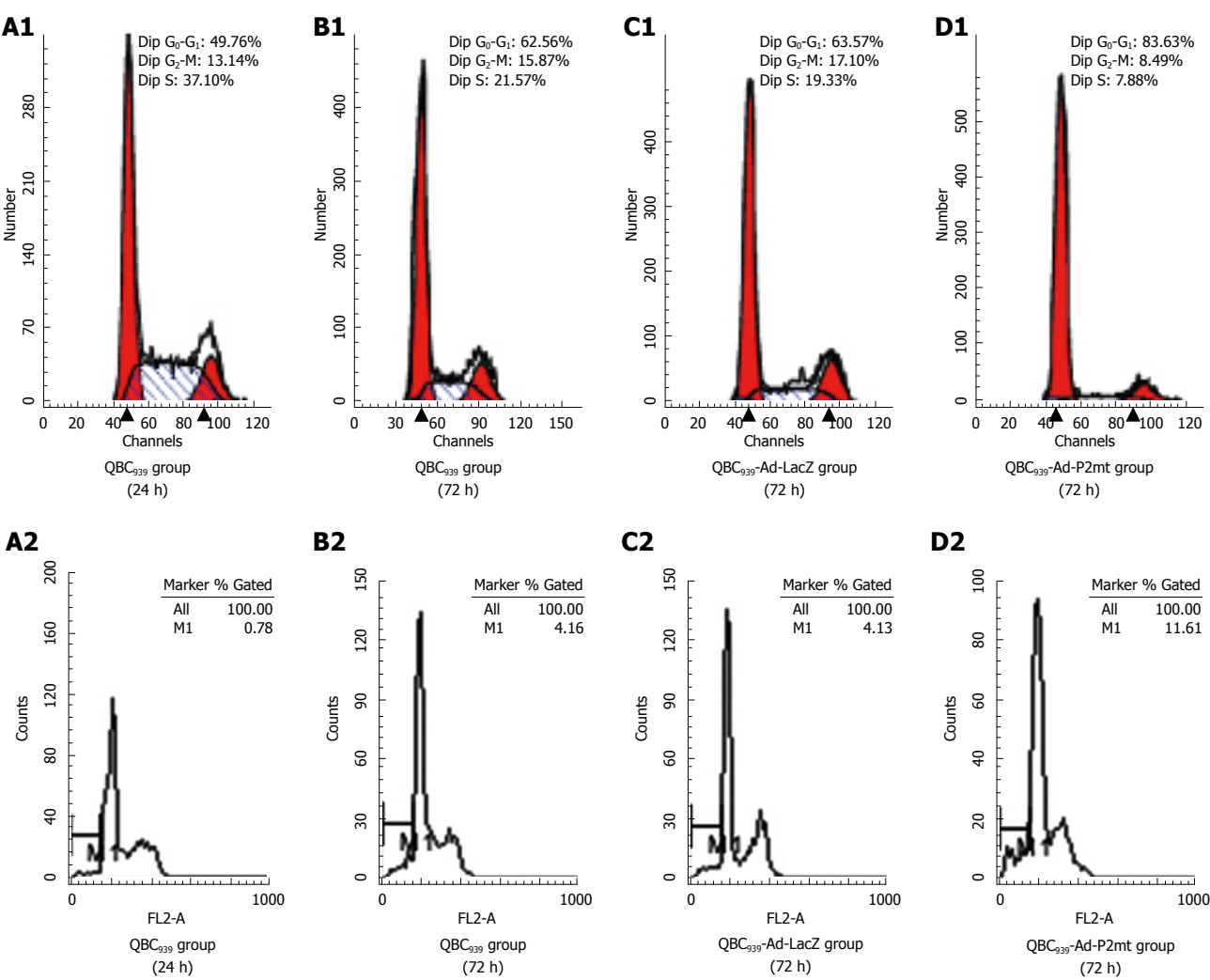


Figure 3 Impact of exogenous p27 gene on QBC₉₃₉ cell cycle and apoptosis.

Table 2 Influence of gene transfact on QBC ₉₃₉ cells at G ₀ /G ₁ phase and apoptosis in different testing groups (mean ± SD)				
Group	G ₀ /G ₁ (%) /apoptosis (%)			
	24 h after transfact	48 h after transfact	72 h after transfact	
Ad-p27mt group (n = 3)	61.02 ± 1.03/ 0.81 ± 0.052	73.32 ± 2.99/ 5.27 ± 0.030	83.63 ± 2.10/ 11.61 ± 1.23	
Ad-LacZgroup (n = 3)	54.91 ± 2.32/ 0.76 ± 0.031	62.56 ± 2.71/ 1.28 ± 0.043	63.57 ± 2.32/ 4.16 ± 0.230	
QBC ₉₃₉ group (n = 3)	49.76 ± 1.97/ 0.78 ± 0.041	56.95 ± 1.06/ 1.10 ± 0.071	62.56 ± 2.88/ 4.13 ± 0.454	

F = 15.954, P = 0.012 (G₀/G₁ cell ratio, Ad-p27m vs AdLacZ group); F = 3.236, P > 0.05 (apoptosis rates, Ad-p27m vs AdLacZ group).

Impact of exogenous p27 gene on QBC₉₃₉ cell cycle and apoptosis

A high expression level of exogenous p27 protein in QBC₉₃₉ cells evoked a strong cell cycle arrest at G₀/G₁ phase in a time-dependent manner within 72 h. The cell ratio was stabilized at about 83.63% ± 2.10% in a non time-dependent manner after 72 h, which was significantly different from that in the Ad-LacZ-infected and uninfected groups (F = 15.954, P = 0.012; Table 2

and Figure 3). The apoptosis rate was 11.61% ± 1.23% when the cells were infected with Ad-p27mt for 72 h. The sub-G₁ apoptosis was more significant in Ad-p27mt group than in AdLacZ and control groups (Table 2 and Figure 3). All results were obtained from experiments performed in triplicate.

DISCUSSION

Cholangiocarcinoma remains one of the most difficult tumors to treat in clinical practice. Currently, there is no effective chemotherapy for this disease. Surgery offers the only opportunity to cure it. However, the majority of patients fail to qualify for such a treatment. Therefore, new therapeutic modalities are needed. Gene therapy is regard as one of the most important and potential new modalities for this disease.

The CDK inhibitor p27 plays a major role in controlling the cell cycle, which negatively regulates the transition from the G₁ into the S phase. Moreover, p27^{kip1} is also a tumor suppressor. Loss of p27 function weakens the control of G₁/S checkpoint, thus accelerating cell cycle progression and predisposing cells to malignant transformation^[1,2]. Ganoth *et al*^[3]

and Troncone *et al*^[4] reported that the degradation of p27 is mainly regulated by post-translational ubiquitin-proteasome-mediated proteolysis of phosphorylation in threonine (Thr) 187. In order to inhibit the degradation of p27 and restore the function of G₁/S checkpoint, we transfected mutated p27 into cholangiocarcinoma QBC₉₃₉ cell line, which has a mutation of Thr-187/Pro-188 (ACGCCC) to Met-187/Ile-188(ATGATC).

Western blot analysis showed that 72 h after infection with Ad-p27mt, p27 in the QBC₉₃₉ cells expressed a strong band, whereas Ad-LacZ-infected QBC₉₃₉ cells showed a faint p27 protein product in the uninfected groups, suggesting that transgenes can be successfully induced and expressed. The elevated level of p27 expression demonstrated that mutated p27 was resistant to degradation and more stable than wild p27, indicating that phosphorylation of threonine (Thr) 187 can trigger degradation of wild p27. The transfer of Ad-p27mt significantly inhibited the proliferation of QBC₉₃₉ cells, decreased clone formation, strongly induced cell cycle arrest and apoptosis at G₁/S phase within 72 h after infection, which is consistent with the previous findings^[5].

It has been well documented that over expression of wild p27 *via* adenoviral gene transfer on p27-deficient tumor cells could strongly inhibit cell cycle arrest and even lead to significantly apoptosis in disparate types of human cancers, such as spongicytoma, lung cancer, leukaemia. It is a common phenomenon that recombinant adenovirus-mediated p27 can eliminate carcinoma cells through apoptosis. Although there was no significant difference in apoptosis between Adp27mt- and AdLacZ-infected cells, uninfected cells at any time point, our data show that 72 h after infection with Adp27mt, the typical sub-G₁ apoptotic peak could be observed by flow cytometry, which was more apparent than in AdLacZ-infected and uninfected cells. Although the precise mechanism by which p27 induces apoptosis is unclear, transfer of p27 is associated with a moderate level of apoptosis as shown by FACS analysis. Since QBC₉₃₉ cells have mutated p53, the mechanism underlying apoptosis induced by transfer of p27 must be p53-independent. Further investigation is needed on how p27 regulates and induces apoptosis.

In conclusion, Ad-p27mt at Thr-187 can be used as a novel, potent, tumor-suppressing gene therapy tool in the treatment of cholangiocarcinoma.

ACKNOWLEDGMENTS

The authors thank Doctors Chen YJ and Wang WY for their long, concerted, and fruitful efforts, Professor Wang SG for kindly donation of human cholangiocarcinoma cell line QBC₉₃₉, Professors Xu SY and Wang JN for their recombinant adenovirus vehicle Ad-p27mt and adenovirus control vehicle Ad-LacZ, Professor Zou SQ for his useful suggestions, and a great debt of gratitude to its organizer, Professor Zou SQ.

COMMENTS

Background

As a cyclin-dependent kinase inhibitor, p27^{Kip1} (p27) regulates cell cycle progression by transcriptional, translational and proteolytic mechanisms. G₁/S cell cycle progression requires p27 proteolysis, which is triggered by its phosphorylation of threonine (Thr) 187. Increased p27 causes proliferating cells to exit from the cell cycle, while decreased p27 is required for quiescent cells to resume cell division. Low levels of p27 are associated with excessive cell proliferation in pathological conditions such as inflammation and cancers. High levels of p27 are observed in such conditions of diminished cell proliferation as in late stages of arterial wound repair in atherosclerosis. Interestingly, in many types of tumors such as gastric, prostate and breast carcinomas, the expression of p27 gene is down-regulated. Loss of p27 expression may result in tumor development and/or progression.

Research frontiers

The research involved cell morphology oncology, cell morphology, molecular biology and gene therapy for cholangiocarcinoma.

Innovations and breakthroughs

It is well known that the degradation of p27 is mainly regulated by post-translational ubiquitin-proteasome-mediated proteolysis during phosphorylation of threonine (Thr) 187. In order to inhibit the degradation of p27 and restore the function of G₁/S checkpoint, we transfected mutated p27 into cholangiocarcinoma QBC₉₃₉ cell line, which can mutate from Thr-187/Pro-188 (ACGCCC) to Met-187/Ile-188(ATGATC). The recombinant adenoviral vector cannot replicate in target cells because it lacks the E1 gene, thus only expressing the inserted gene. Because the foreign gene fragment is not incorporated into the genome of target cells, the danger of mutations affecting treatment is reduced. Meanwhile, adenoviral vectors are stable and easy to purify. This technique can effectively affect both proliferating and quiescent cells *ex vivo*. The potential for gene therapy by using the recombinant adenovirus is worthy of extensive attention. The results of our study suggest that adenovirus-mediated p27 gene transfection can be used as a novel gene therapy for cholangiocarcinoma.

Applications

The prognosis of cholangiocarcinoma is extremely poor although aggressive multidisciplinary cancer therapies have been used in clinical practice. Thus, it is imperative to develop new and effective treatment modalities for cholangiocarcinoma, such as gene therapy.

Peer review

The authors showed that transfection of a human cholangiocarcinoma cell line (QBC₉₃₉) could cause cell cycle arrest and apoptosis, which are of interest in developing new treatment modalities for cholangiocarcinoma. The methods used were well described. The results are of scientific interest.

REFERENCES

- 1 Milde-Langosch K, Hagen M, Bamberger AM, Loning T. Expression and prognostic value of the cell-cycle regulatory proteins, Rb, p16MTS1, p21WAF1, p27KIP1, cyclin E, and cyclin D2, in ovarian cancer. *Int J Gynecol Pathol* 2003; **22**: 168-174
- 2 Fukunaga M. Immunohistochemical characterization of cyclin E and p27KIP1 expression in early hydatidiform moles. *Int J Gynecol Pathol* 2004; **23**: 259-264
- 3 Ganoh D, Bornstein G, Ko TK, Larsen B, Tyers M, Pagano M, Herskho A. The cell-cycle regulatory protein Cks1 is required for SCF(Skp2)-mediated ubiquitinylation of p27. *Nat Cell Biol* 2001; **3**: 321-324
- 4 Troncone G, Martinez JC, Iaccarino A, Zeppa P, Caleo A, Russo M, Migliaccio I, Motti ML, Califano D, Palmieri EA, Palombini L. p27Kip1 is expressed in proliferating cells in its form phosphorylated on threonine 187. *BMC Clin Pathol* 2005; **5**: 3
- 5 Katner AL, Gootam P, Hoang QB, Gnarr JR, Rayford W. A recombinant adenovirus expressing p7(Kip1) induces cell cycle arrest and apoptosis in human 786-0 renal carcinoma cells. *J Urol* 2002; **168**: 766-773

S- Editor Zhong XY L- Editor Wang XL E- Editor Lin YP



A "false positive" octreoscan in ileal Crohn's disease

Alberto Fernandez, Olga Tabuenca, Angeles Peteiro

Alberto Fernandez, Gastroenterology Department, POVISA Hospital, Vigo 36211, Spain

Olga Tabuenca, Nuclear Medicine Department, POVISA Hospital, Vigo 36211, Spain

Angeles Peteiro, Pathology Department, POVISA Hospital, Vigo 36211, Spain

Author contributions: Fernandez A, Tabuenca O and Peteiro A contributed equally to this work and wrote the paper.

Correspondence to: Alberto Fernandez, Servicio de Aparato Digestivo, Hospital POVISA, c/ Salamanca 5, Vigo (Pontevedra) 36211. Spain. afvillaverde@gmail.com

Telephone: +34-98-6413144 Fax: +34-98-6421439

Received: May 24, 2008 Revised: July 28, 2008

Accepted: August 4, 2008

Published online: September 14, 2008

Abstract

We present a case report of a patient with a suspicious ileal carcinoid tumour. Clinical examination as well as computer tomography (CT) scan suggested a tumour. Octeotide scan showed uptake in the same bowel loop reported as pathological in CT. The patient underwent surgery and biopsy which reported Crohn's disease (CD). The interest in the case is due to the fact that this is, to the best of our knowledge, the second report of Crohn's disease as a cause of false positive octeotide scan. Unfortunately, no somatostatin receptors could be found in the sample, so further studies should be performed.

© 2008 The WJG Press. All rights reserved.

Key words: Crohn's disease; Carcinoid tumour; Octreoscan; Somatostatin receptor scintigraphy; ^{111}In -DTPA- octreotide

Peer reviewer: Gert De Hertogh, Ph.D, Department of Morphology and Molecular Pathology, University Hospitals KU-Leuven, Minderbroedersstraat 12, Leuven 3000, Belgium

Fernandez A, Tabuenca O, Peteiro A. A "false positive" octreoscan in ileal Crohn's disease. *World J Gastroenterol* 2008; 14(34): 5349-5352 Available from: URL: <http://www.wjgnet.com/1007-9327/14/5349.asp> DOI: <http://dx.doi.org/10.3748/wjg.14.5349>

INTRODUCTION

Somatostatin receptors (SS-Rs) are membrane

glycoproteins spreading over a large number of body tissues and can be found in normal and pathological conditions^[1]. Many benign and malignant tumours over express SS-R in their membranes especially in neuroendocrine tumours (NETs) but there are other benign conditions showing an increase number of SS-Rs, such as granulomatous or inflammatory disease^[2].

Octeotide is an analogue whose molecule is a shortened version of somatostatin's, from 8 to 14 aminoacides, sharing its active nuclei and allowing the ^{111}In -DTPA molecule to bind to the N-terminal (d-phe).

Thus, ^{111}In -DTPA-D-Phe1- octeotide (^{111}In -DTPAOC) is the labelled form of octeotide. There are 5 subtypes of SS-R, and somatostatin shows a high affinity for all of them. However, octeotide has a high affinity for only SS-R 2 subtype and a low affinity for SS-R 3 and SS-R 5 subtypes. Therefore, pathological conditions over expressing these receptors are able to be imaged in ^{111}In -DTPAOC scans.

The sensitivity of ^{111}In -DTPAOC in detecting these pathological conditions is variable being very high in carcinoid tumour (86%)^[3]. Uptake in other tissues can lead to false-positive findings when studying suspected NETs. Only one case of Crohn's disease (CD) has been recently reported as a cause of false positive scan^[4].

We report a case of a false positive ^{111}In -DTPAOC scintigraphy in a patient with CD mimicking an ileal carcinoid tumour. A discussion about SS-R potential alterations in CD was included.

CASE REPORT

A 40-year-old man was admitted to our outpatient clinic with an 8-mo history of dull abdominal pain and weight loss of 20 kg, but no other gastrointestinal symptoms. In terms of past medical or surgery history, the patient denied of smoking or other diseases. Physical examination only revealed a painful site in the right lower quadrant of the abdomen and a body temperature of 37.3°C. Laboratory test only revealed an increased erythrocyte sedimentation rate of 35 mm/h (normal range: 0-25 mm/h) and C-reactive protein rate of 2.37 mg/dL (normal range: 0-0.5 mg/dL). A computer tomography (CT) scan was performed (Figure 1) and showed thickening of the ileum distal wall and the presence of a 4 cm solid mass with irregular border adjacent to the affected ileum, conditioning retraction, with prominent mesenteric lymph nodes. All these findings suggested a carcinoid tumour.



Figure 1 CT-scan showing thickening of the distal ileum (long arrow) with an adjacent solid mass (short arrow), suggesting a carcinoid tumour.

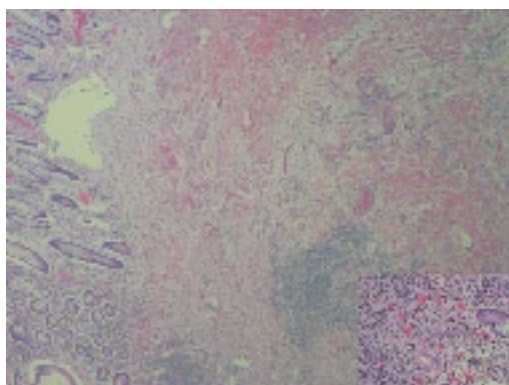


Figure 3 CD showing marked transmural inflammatory changes (involving the walls of veins and arteries): edema, lymphatic dilatation, hyperplasia of the muscularis mucosae, fibrosis ("obliterative muscularization"), and epithelioid granuloma.

Further explorations were performed. Colonoscopy with ileoscopy (at least 15 cm of the distal ileum was explored) showed an irregular ileal mucosa with erythema, irregular nodular areas and marked stiffness, suggesting an infiltrative process. Ten ileal biopsies were taken and showed unspecific inflammatory changes without evidence of malignancy, granulomas or histological features suggestive of carcinoid tumour or CD. A 24-h urine collection for 5-hydroxyindolacetic acid (5-HIAA) showed a normal result of 3.3 mg/24 h (normal range < 10 mg/24 h).

Due to the low sensitivity of the 24-h 5-HIAA test and the high suspicion of carcinoid tumour, an ^{111}In -DTPAOC scan was performed. The patient was injected with 3mCi ^{111}In -DTPAOC. Total body images were obtained at 4 and 24 h, and an abdominal SPECT was also performed at 14 h. The images showed pathological uptake in central and right pelvic fossae at 4 and 24 h (Figure 2). SPECT showed a C-shape uptake in the sagittal plane that matches with the thickened loop on CT. No uptake was observed in the solid mass. The scan was reported as a highly suspicious carcinoid tumour.

With all these results, laparoscopy with curative intention was performed. An inflammatory mass involving the distal ileon was found at surgery, and a distal small bowel resection and right hemicolectomy with lateral anastomosis were performed. Histological analysis

TUMOR SPECT ***** OCTREOTIDO Hospital povisa
Three plane spots collection ***** 5/30/2007 12:13

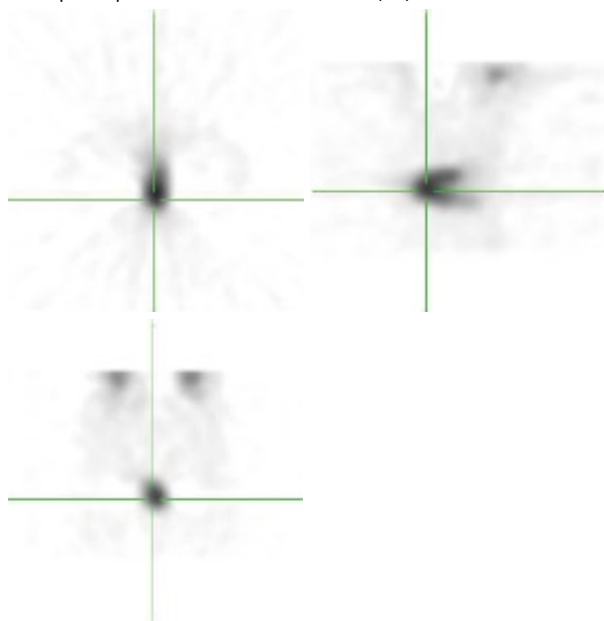


Figure 2 Octeotide-SPECT images showing pathological uptake in the ileal thickened loop at CT-scan.

of macroscopically-affected segments and specimens of non-macroscopically-involved segments was carried out. Thickened serosa and shortened mesentery could be grossly observed in the involved terminal ileum (20 cm), resulting in a corrugated bowel contour in the terminal portion of the small bowel. These changes could probably mimic the impression on CT-scan to an abdominal mass. The mesenteric lymph nodes were enlarged measuring 8 mm in the larger diameter. The detailed macroscopic examination failed to show any tumour mass. Photo of gross specimen neither was nor included. Other gross findings of significance included stricture formation, fissuring, cobblestone appearance (discontinuous involvement with transmural spread) with an intervening normal or edematous mucosa. The main microscopic features were ulceration, fissure, non caseating sarcoid-like granuloma and lymphoid aggregates. The regional lymph nodes showed sinus dilatation and lymphoid hyperplasia. Chromogranin stain was not performed.

Intestinal tissue sections were routinely fixed in 10% neutral formalin and embedded in paraffin. Immunohistochemical staining for Dako® polyclonal antibodies against human somatostatin protein was performed with the standard avidin-biotin method. The slide-mounted tissue sections were allowed to reach room temperature and incubated for 60 min at a solution of 1:900, using saponic to antigenic recuperation. Somatostatin immunohistochemical expression was undetectable in the veins or arteries of inflamed and non inflamed control intestinal tissue sections. No concordance was reached in immunohistochemistry/scintigraphy somatostatin receptor. No areas of dysplasia or carcinoma were identified.

In the absence of features other than the typical appearance of CD, the pathologic diagnosis of Crohn's ileitis was made (Figure 3).

After surgery, azathioprine was started for prevention of postoperative recurrence. The patient underwent a complete clinical recovery and 6 mo later he was asymptomatic. A surveillance/follow-up colonoscopy did not show any abnormality and inflammatory signs of recurrence.

DISCUSSION

There are many cases of carcinoid tumour misdiagnosed as CD only discovered when treatment is not effective or surgery is performed. It was reported that approximately 2.3% of patients with ileal carcinoid are first diagnosed and treated as CD^[5]. However, the reverse is a very uncommon situation.

In our case, the clinic features as well as the image of CT-scan strongly suggested a carcinoid tumour. In order to confirm the suspected disease, ¹¹¹In- DTPAOC scan was performed and showed a C-shape uptake in the bowel loop that corresponded to that reported as pathologic in CT, but there was no uptake in the solid mass. The patient underwent surgery and the final pathologic report was CD. The mass that did not show uptake was reported as inflammatory.

¹¹¹In- DTPAOC is a radio-labelled octreotide analogue that binds to SS-R expressed in cell membranes. Many benign and malignant diseases overexpress SS-R and thus, can be imaged with this radioligand^[6]. Carcinoid tumour, one of the malignant tumours, shows more uptakes in the bowel loop due to its high density of SS-R. Reported values for the detection of known carcinoid tumour localizations vary from 80% to nearly 100%^[1]. Uptake is related to SS-R density and even tumours smaller than 1 cm in diameter can be detected. This is the reason why somatostatin receptor scintigraphy (SRS) plays a central role in locating and assessing the primary gastroenteropancreatic NET^[7] and has a marked effect in the clinical management of these malignancies^[8,9]. However, 12% of SRS examinations result in false-positive localization of NET, understanding a false-positive as an uptake not related with the tested pathology. Renal parapelvic cysts, accessory spleens, ventral hernias, thyroid or breast disease are the most frequent cases of false positive localizations^[10]. SS-R is over expressed in activated peripheral lymphocytes and macrophages in granulomatous and inflammatory diseases, allowing obtaining images^[11,12].

In normal bowel, SS-R is expressed in gastrointestinal mucosa, peripheral nervous system and lymphoid tissue. Several tumours, such as carcinoid tumour, express SS-R in peritumoral veins^[13]. In intestinal inflammatory disease, a high density of SS-R is detected by autoradiography in intestinal intramural veins but not in normal tissues. SS-R is seen in small muscular veins of submucosa, tunica muscularis and subserosa, and the thickness of veins is labelled. These veins are histologically normal and seldom have a minimal lymphocyte infiltration although label intensity does not seem to depend on them as the surrounding

tissues are infiltrated with these cells and do not show labelling^[14-16].

Usually no ¹¹¹In- DTPAOC uptake is found in inflammatory bowel disease. There is only one recent report of ¹¹¹In- DTPAOC uptake in Crohn's disease in literature although there is no evidence that SS-R is determined^[4]. In our case, the samples were studied to assess the existence of SS-R. Immunohistochemical staining of somatostatin receptors was performed and somatostatin immunohistochemical expression was undetectable in the veins or arteries of inflamed and non inflamed control intestines.

Unfortunately, SS-R could not be found in the tissues studied, so we could not explain this abnormal uptake. No concordance was reached in immunohistochemistry/scintigraphy somatostatin receptor, possibly as a consequence of somatostatin receptor heterogeneity. Further studies should be made to assess the possible causes of uptake in CD.

However, there are several reports of small carcinoid tumours (a few millimetres in diameter) found in patients with CD, suggesting a correlation between the pathogenesis of both disorders^[17]. It has been theorized that inflammation creates a favourable environment for the development of carcinoid tumour, although most of the carcinoid tumours reported are found in non-inflamed bowel. Other theories include the hyperstimulation of enteroendocrine cells by inflammation^[18] and the role of proinflammatory cytokines such as PTHrP or IL-6^[19].

We would like to encourage physicians to be aware of CD as a cause of false positive results when performing ¹¹¹In- DTPAOC scans.

REFERENCES

- 1 **Kwekkeboom D**, Krenning EP, de Jong M. Peptide receptor imaging and therapy. *J Nucl Med* 2000; **41**: 1704-1713
- 2 **van Hagen PM**. Somatostatin receptor expression in clinical immunology. *Metabolism* 1996; **45**: 86-87
- 3 **Kwekkeboom DJ**, Krenning EP. Somatostatin receptor imaging. *Semin Nucl Med* 2002; **32**: 84-91
- 4 **Marko J**, Lamba R, Miller F, Buchman A, Spies S, Nikolaidis P. OctreoScan positive Crohn's disease mimicking an ileal carcinoid tumor. *J Clin Gastroenterol* 2008; **42**: 66-68
- 5 **Hsu EY**, Feldman JM, Lichtenstein GR. Ileal carcinoid tumors stimulating Crohn's disease: incidence among 176 consecutive cases of ileal carcinoid. *Am J Gastroenterol* 1997; **92**: 2062-2065
- 6 **Warner RR**, O'dorisio TM. Radiolabeled peptides in diagnosis and tumor imaging: clinical overview. *Semin Nucl Med* 2002; **32**: 79-83
- 7 **Ramage JK**, Davies AH, Ardill J, Bax N, Caplin M, Grossman A, Hawkins R, McNicol AM, Reed N, Sutton R, Thakker R, Aylwin S, Breen D, Britton K, Buchanan K, Corrie P, Gillams A, Lewington V, McCance D, Meeran K, Watkinson A. Guidelines for the management of gastroenteropancreatic neuroendocrine (including carcinoid) tumours. *Gut* 2005; **54** Suppl 4: iv1-iv16
- 8 **Lebtahi R**, Cadiot G, Sarda L, Daou D, Faraggi M, Petegnief Y, Mignon M, le Guludec D. Clinical impact of somatostatin receptor scintigraphy in the management of patients with neuroendocrine gastroenteropancreatic tumors. *J Nucl Med* 1997; **38**: 853-858
- 9 **Termanini B**, Gibril F, Reynolds JC, Doppman JL, Chen CC,

- Stewart CA, Sutliff VE, Jensen RT. Value of somatostatin receptor scintigraphy: a prospective study in gastrinoma of its effect on clinical management. *Gastroenterology* 1997; **112**: 335-347
- 10 **Gibril F**, Reynolds JC, Chen CC, Yu F, Goebel SU, Serrano J, Doppman JL, Jensen RT. Specificity of somatostatin receptor scintigraphy: a prospective study and effects of false-positive localizations on management in patients with gastrinomas. *J Nucl Med* 1999; **40**: 539-553
- 11 **Vanhagen PM**, Krenning EP, Reubi JC, Kwekkeboom DJ, Bakker WH, Mulder AH, Laissue J, Hoogstede HC, Lamberts SW. Somatostatin analogue scintigraphy in granulomatous diseases. *Eur J Nucl Med* 1994; **21**: 497-502
- 12 **Tabuenca Dopico O**, Gutierrez Mendiguchia C, Rego Iraeta A. [Incidental diagnosis of lymph node tuberculosis in an 111-indium-octreotide scintigraphy during a study of acromegaly] *Rev Esp Med Nucl* 2006; **25**: 193-197
- 13 **Reubi JC**, Horisberger U, Laissue J. High density of somatostatin receptors in veins surrounding human cancer tissue: role in tumor-host interaction? *Int J Cancer* 1994; **56**: 681-688
- 14 **Reubi JC**, Mazzucchelli L, Laissue JA. Intestinal vessels express a high density of somatostatin receptors in human inflammatory bowel disease. *Gastroenterology* 1994; **106**: 951-959
- 15 **Denzler B**, Reubi JC. Expression of somatostatin receptors in peritumoral veins of human tumors. *Cancer* 1999; **85**: 188-198
- 16 **Reubi JC**, Waser B, Schaer JC, Laissue JA. Somatostatin receptor sst1-sst5 expression in normal and neoplastic human tissues using receptor autoradiography with subtype-selective ligands. *Eur J Nucl Med* 2001; **28**: 836-846
- 17 **West NE**, Wise PE, Herline AJ, Muldoon RL, Chopp WV, Schwartz DA. Carcinoid tumors are 15 times more common in patients with Crohn's disease. *Inflamm Bowel Dis* 2007; **13**: 1129-1134
- 18 **Le Marc'hadour F**, Bost F, Peoc'h M, Roux JJ, Pasquier D, Pasquier B. Carcinoid tumour complicating inflammatory bowel disease. A study of two cases with review of the literature. *Pathol Res Pract* 1994; **190**: 1185-1192; discussion 1193-1200
- 19 **Barhoum M**, Hutchins L, Fonseca VA. Intractable hypercalcemia due to a metastatic carcinoid secreting parathyroid hormone-related peptide and interleukin-6: response to octreotide. *Am J Med Sci* 1999; **318**: 203-205

S- Editor Li DL **L- Editor** Wang XL **E- Editor** Zhang WB

Inflammatory myoglandular polyp causing hematochezia

Shoji Hirasaki, Masato Okuda, Kenichiro Kudo, Seiyuu Suzuki, Atsuko Shirakawa

Shoji Hirasaki, Masato Okuda, Kenichiro Kudo, Seiyuu Suzuki, Department of Internal Medicine, Sumitomo Besshi Hospital, Niihama 7928543, Japan

Atsuko Shirakawa, Department of Pathology, Sumitomo Besshi Hospital, Niihama 7928543, Japan

Author contributions: Hirasaki S, Okuda M, Kudo K and Suzuki S were involved in the care of the patient; Shirakawa A studied the specimen; Hirasaki S performed the endoscopic resection; Hirasaki S wrote the paper.

Correspondence to: Shoji Hirasaki, Department of Internal Medicine, Sumitomo Besshi Hospital, 3-1 Ohji-cho, Niihama 7928543, Japan. shoji_hirasaki@ni.sbh.gr.jp

Telephone: +81-897-377111 Fax: +81-897-377121

Received: April 18, 2008 Revised: August 11, 2008

Accepted: August 18, 2008

Published online: September 14, 2008

<http://www.wjgnet.com/1007-9327/14/5353.asp> DOI: <http://dx.doi.org/10.3748/wjg.14.5353>

INTRODUCTION

Inflammatory myoglandular polyp (IMGP) is characterized by inflammatory granulation tissue in the lamina propria^[1], proliferation of smooth muscle^[2], and hyperplastic glands with variable cystic changes^[3-7]. Only a small number of cases have been reported and the pathogenesis and natural history remain unclear^[8-12]. Herein, we describe a relatively rare case of inflammatory myoglandular polyp causing hematochezia.

CASE REPORT

A 33-year-old man presented with the symptom of hematochezia. He was in good health with no specific family or past medical history. His body temperature was 36.7°C, blood pressure was 148/82 mmHg, and radial pulse rate was 70 beats/min and regular. He had neither anemia nor jaundice. Neurological examination revealed no abnormal findings. Abdominal palpation revealed tenderness in the left lower quadrant. Routine hematological examination and biochemical tests were within normal limits. Colonoscopy revealed a red, hard, spherical peduncular polyp with erosion and mucous exudation, about 20 mm in diameter, in the descending colon (Figure 1). With conventional colonoscopy, the lesion did not show type III or IV pit pattern although magnifying colonoscopy was not performed. We speculated that this polyp was non-neoplastic. It was suspected to be an inflammatory polyp from endoscopic findings although it should be distinguished from a juvenile polyp. An air contrast barium enema also revealed a pedunculated polyp in the descending colon (Figure 2). Excluding the polyp, there was no lesion in the colorectum. We speculated that the polyp in the descending colon was the causative lesion of hematochezia. Endoscopic polypectomy was performed. At polypectomy, polyp erosion was healed (Figure 3). Histological examination of the specimen revealed inflammatory granulation tissue in the lamina propria, proliferation of smooth muscle, and hyperplastic glands with variable cystic changes (Figure 4). The lesion was diagnosed as an IMGP. After endoscopic polypectomy, the symptom of hematochezia was resolved.

Abstract

A case of inflammatory myoglandular polyp (IMGP) causing hematochezia is reported. The patient was a 33-year-old man who visited our hospital for further evaluation of hematochezia. Colonoscopy revealed a red, hard, spherical peduncular polyp with erosion and mucous exudation, about 20 mm in diameter, in the descending colon. Excluding the polyp, there was no lesion in the colorectum. Endoscopic polypectomy was performed. Histological examination of the specimen revealed inflammatory granulation tissue in the lamina propria, proliferation of smooth muscle, and hyperplastic glands with variable cystic changes. This polyp was diagnosed as an IMGP. The symptom of hematochezia was resolved after endoscopic resection. Our case shows that treatment is necessary for IMGP if intestinal bleeding occurs and endoscopists should be aware of the endoscopic characteristics of IMGP.

© 2008 The WJG Press. All rights reserved.

Key words: Inflammatory myoglandular polyp; Colonoscopy; Endoscopic polypectomy; Gastrointestinal bleeding

Peer reviewer: Dr. James E East, Department of Gastroenterology, St. Mary's Hospital, Clarence Wing, Praed Street, London W2 1NY, United Kingdom

Hirasaki S, Okuda M, Kudo K, Suzuki S, Shirakawa A. Inflammatory myoglandular polyp causing hematochezia. *World J Gastroenterol* 2008; 14(34): 5353-5355 Available from: URL:



Figure 1 Endoscopy showing a red, hard and spherical peduncular polyp, about 20 mm in diameter, in the descending colon.

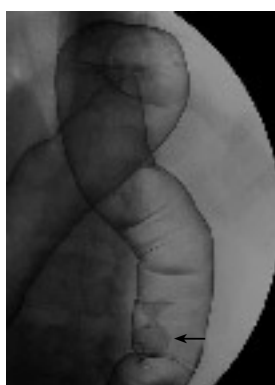


Figure 2 Double contrast radiograph of descending colon showing an about 20 mm peduncular polyp.

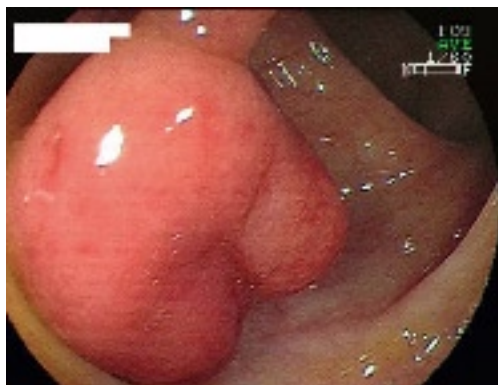


Figure 3 The second colonoscopy showing a healed polyp.

DISCUSSION

IMGP is a non-neoplastic colorectal polyp, first described by Nakamura *et al*^[1]. IMGP is solitary, pedunculated and rarely, covered by a fibrin cap, and follows a benign course. Also, IMGP has no association with inflammatory bowel diseases and is located not only in the rectosigmoid, but also in the descending and transverse colon^[3]. In the present case, it was located in the descending colon. Although the pathogenesis of IMGP remains unknown, Nakamura^[1] proposed that chronic trauma from intestinal peristalsis may contribute to the pathogenesis of IMGP.

Only a small number of IMGP cases have been reported. According to Fujino *et al*^[8], a review of

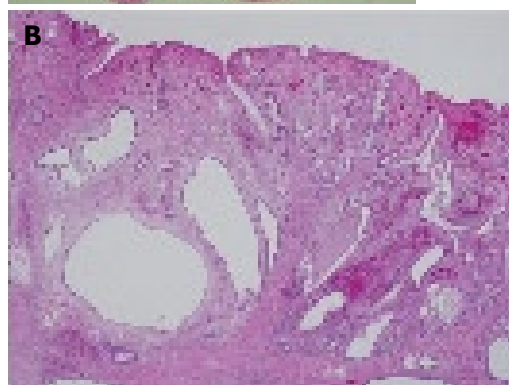
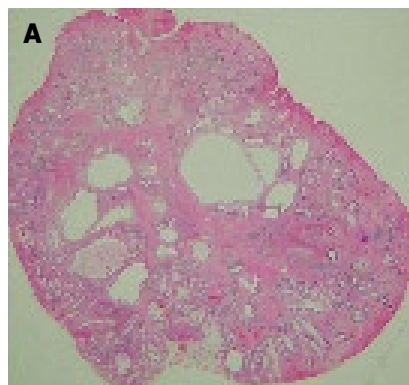


Figure 4 Microscopic findings of the polypectomy specimen. Low-power view of a cross section showing a stalked polyp containing numerous cystically dilated glands (A) and inflammatory granulation tissue in the lamina propria mucosae and proliferation of smooth muscle (B).

the literature revealed 48 cases of IMGP in the large intestine up to 2001. However, recent advances in diagnostic techniques, especially the widespread use of colonoscopy for colorectal tumors, have enabled us to identify small and asymptomatic polyps, and reports on IMGP of the colon have been increasing^[9,10]. Fujino *et al*^[8] described that the macroscopic appearance is the pedunculated type in 83.3% of cases. In that report, the sites of IMGP in the large intestine were studied and 47 of 48 cases (97.9%) had lesions in the rectum to transverse colon. Thus, IMGPs of the large intestine are predominantly in the distal colon^[8,9]. IMGPs in the colon are usually asymptomatic and often detected incidentally on barium enema or endoscopy^[8-10]. Another review of the literature revealed that the main clinical feature of colorectal IMGPs is hematochezia^[10,11]. Endoscopic characteristic findings include (1) pedunculated or semipedunculated, (2) red and (3) smooth, spherical and hyperemic surface with patchy mucous exudation and erosion^[8,9]. In the present case, the endoscopic findings of the polyp were compatible with those of IMGP.

As to therapy, IMGP of the large intestine can best be removed endoscopically, because it is thought to be clinically and histologically benign. Most Japanese cases have been treated with polypectomy or endoscopic mucosal resection (EMR)^[5,7-11]. If we could confirm the histological diagnosis of small colonic IMGPs by endoscopic biopsy, endoscopic resection of IMGP might be unnecessary because IMGP follows a benign course.

However, the diagnosis of colorectal IMGPs could seldom be made by endoscopic biopsy and the final diagnosis of colonic IMGP depends on the pathological findings of EMR or endoscopic polypectomy specimens. Endoscopic or surgical treatment is necessary if gastrointestinal bleeding^[9] or colonic intussusception occurs. Local excision of the polyp is curative. Kayhan *et al*^[12] have reported a case of large IMGP (> 6 cm) that was too large to be removed endoscopically, and was thus treated with surgical resection. We consider that the percentage of patients with colonic IMGP who undergo surgical resection will decrease and endoscopic resection will increase in the future because of recent advances in diagnostic technologies such as improved endoscopic images.

In conclusion, we report a case of IMGP causing hematochezia. IMGP should generally be taken into consideration as a differential diagnosis of peduncular polyp of the colon. IMGP of the large intestine is not fatal and patients remain asymptomatic in their daily lives except for gastrointestinal bleeding or bowel obstruction. Therefore, it is likely that there are many latent patients with IMGP who might be incidentally discovered in the future. Endoscopists should be aware of that IMGP may exhibit the aforementioned endoscopic characteristics and may cause hematochezia. The causes of IMGP are still obscure, and further accumulation of cases may disclose their pathogenesis.

REFERENCES

- 1 **Nakamura S**, Kino I, Akagi T. Inflammatory myoglandular polyps of the colon and rectum. A clinicopathological study of 32 pedunculated polyps, distinct from other types of polyps. *Am J Surg Pathol* 1992; **16**: 772-779
- 2 **Griffiths AP**, Hopkinson JM, Dixon MF. Inflammatory myoglandular polyp causing ileo-ileal intussusception. *Histopathology* 1993; **23**: 596-598
- 3 **Bhathal PS**, Chetty R, Slavin JL. Myoglandular polyps. *Am J Surg Pathol* 1993; **17**: 852-853
- 4 **Gomez Navarro E**, del Rio Martin JV, Sarasa Corral JL, Melero Calleja E. [Myoglandular inflammatory polyp located in the distal end of the rectum] *Rev Esp Enferm Dig* 1994; **85**: 45-46
- 5 **Nagata S**, Sumioka M, Sato O, Miyamoto M, Watanabe C, Yamada H, Hirata K, Imagawa M, Haruma K, Kajiyama G. [Five cases of inflammatory myoglandular polyp] *Nippon Shokakibyo Gakkai Zasshi* 1998; **95**: 145-150
- 6 **Bhardwaj K**, Mohan H, Chopra R, Bhardwaj S, Sachdev A. Inflammatory myoglandular polyp of rectum. *Indian J Gastroenterol* 1998; **17**: 63-64
- 7 **Harada N**, Chijiwa Y, Yao T, Koyanagi M, Ono Y, Motomura S. Inflammatory myoglandular polyp. *J Clin Gastroenterol* 1999; **29**: 104-105
- 8 **Fujino Y**, Orii S, Nakamura S, Sugai T, Saito S, Yamaguchi T, Noro S, Ishii M, Inomata M, Suzuki K. Five cases of colorectal inflammatory myoglandular polyps. *Gastroenterological Endoscopy* 2001; **43**: 1281-1286
- 9 **Moriyama T**, Matsumoto T, Hizawa K, Tada S, Fuchigami T, Iwai K, Yao T, Iida M. Inflammatory myoglandular colorectal polyps: a case series of nine patients. *Endoscopy* 2003; **35**: 363-365
- 10 **Tashiro M**, Yoshikawa I, Matsushashi T, Yamasaki T, Nishikawa S, Taguchi M, Yamasaki M, Kume K, Otsuki M. Images of interest. Gastrointestinal: inflammatory myoglandular polyp of the colon. *J Gastroenterol Hepatol* 2005; **20**: 1123
- 11 **Becheanu G**, Stamm B. Inflammatory myoglandular polyp-a rare but distinct type of colorectal polyps. *Pathol Res Pract* 2003; **199**: 837-839
- 12 **Kayhan B**, Kucukel F, Akdogan M, Ozaslan E, Kucukbas TA, Atoglu O. Inflammatory myoglandular polyp: a rare cause of hematochezia. *Turk J Gastroenterol* 2004; **15**: 117-119

S- Editor Zhong XY L- Editor Wang XL E- Editor Zhang WB

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.

Arno J Dormann, PD, MED

Habil, Medizinische Klinik, Krankenhaus Holweide, Kliniken der Stadt Köln gGmbH, Neufelder St. 32, 51067 Köln, Germany

Yik-Hong Ho, Professor

Department of Surgery, School of Medicine, James Cook University, Townsville 4811, Australia

Dr. Bernardino Rampone

Department of General Surgery and Surgical Oncology, University of Siena, viale Bracci, Siena 53100, Italy

Taku Aoki, MD

Division of Hepato-Biliary-Pancreatic and Transplantation Surgery, Department of Surgery, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-8655, Japan

Tadashi Shimoyama, MD

Hiroaki University, 5 Zaifu-cho, Hiroaki 036-8562, Japan

Yoshio Yamaoka, MD, PhD, Associate Professor

Department of Medicine/Gastroenterology, Baylor College of Medicine and VA Medical Center (111D), 2002 Holcombe Blvd, Houston, Texas 77030, United States

Rakesh Aggarwal, Additional Professor

Department of Gastroenterology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow 226014, India

Rosemar Joyce Burnett, PhD

Department of Epidemiology National School of Public Health, University of Limpopo, Medunsa Campus PO Box 173, MEDUNSA, Pretoria 0204, South Africa

Seyed A Taghavi, Associate Professor

Department of Internal Medicine, Nemazee Hospital, No.23, 59th Alley, Ghasrodasht St., Shiraz 71838-95453, Iran

Yik-Hong Ho, Professor

Department of Surgery, School of Medicine, James Cook University, Townsville 4811, Australia

Alastair John Watson, Professor

The Henry Wellcome Laboratory, Nuffield Building, University of Liverpool, Crown St., Liverpool, L69 3GE, United Kingdom

Satoshi Osawa, MD

First Department of Medicine, Hamamatsu University School of Medicine, 1-20-1 Handayama, Hamamatsu, 431-3192, Japan

Elke Cario, MD

Division of Gastroenterology and Hepatology, University Hospital of Essen, Institutsgruppe I, Virchowstr. 171, Essen D-45147, Germany

Yoshio Yamaoka, MD, PhD, Associate Professor

Department of Medicine/Gastroenterology, Baylor College of Medicine and VA Medical Center (111D), 2002 Holcombe Blvd, Houston, Texas 77030, United States

Walter E Longo, Professor

Department of Surgery, Yale University School of Medicine, 205 Cedar Street, New Haven 06510, United States

Conor P Delaney, MD, MCh, PhD, FRCSI, FACS, Professor of Surgery

Case Western Reserve University, Chief, Division of Colorectal Surgery, Vice-Chairman, Department of Surgery, Director, Institute for Surgery and Innovation, University Hospitals, Case Medical Center, 11100 Euclid Avenue Cleveland, OH 44106-5047, United States

Yuan Yuan, Professor

Cancer Institute of China Medical University, 155 North Nanjing Street, Heping District, Shenyang 110001, Liaoning Province, China

Jose JG Marin, Professor

Head of the Departamento Physiology and Pharmacology, University of Salamanca, CIBERhd, Campus Miguel de Unamuno, ED-S09, Salamanca 37007, Spain

Mercedes Susan Mandell, MD, PhD

Department of Anesthesiology, University of Colorado Health Sciences Ctr., 12401 E. 17th Ave, B113 Aurora, CO 80045, United States

Dr. Martin Hennenberg

Dipl-Biol, Medizinische Klinik & Poliklinik I, Uni-Klinik Bonn, Sigmund-Freud Str. 25, 53105 Bonn, Germany

Kurt Lenz, Professor

Department of Internal Medicine, Konventhospital Barmherzige Brüder, A-4020 Linz, Austria

Atsushi Nakajima, Professor

Division of Gastroenterology, Yokohama City University Graduate School of Medicine, 3-9 Fuku-ura, Kanazawa-ku, Yokohama 236-0004, Japan

Ana Cristina Simões e Silva, MD, PhD, Professor

Faculdade de Medicina UFMG, Departamento de Pediatria, sala 267, Avenida Professor Alfredo Balena, 190, Bairro Santa Efigênia, Belo Horizonte, Minas Gerais 30130-100, Brazil

Oliviero Riggio, Professor

Dipartimento di Medicina Clinica, Sapienza Università di Roma, Viale dell'Università 37, 00185 Roma, Italy

Robert J Korst, MD

Department of Cardiothoracic Surgery, Weill Medical College of Cornell University, Room M404, 525 East 68th Street, New York 10032, United States

Zsuzsa Szondy, Professor

Department of Biochemistry and Molecular Biol, University of Debrecen, Debrecen H-4012, Hungary

Leonidas G Koniaris, Professor

Alan Livingstone Chair in Surgical Oncology, 3550 Sylvester Comprehensive Cancer Center (310T), 1475 NW 12th Ave., Miami, FL 33136, United States

D Mark Pritchard, PhD, FRCP

Gastroenterology of University of Liverpool, 5th Floor UCD Building, Daulby St, Liverpool L69 3GA, United Kingdom

Dr. Bijan Eghtesad, Associate Professor

Department of General Surgery, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland OH 44195, United States

Katsutoshi Yoshizato, PhD

Academic Advisor, Phoenix Bio Co., Ltd., 3-4-1 Kagamiyama, Higashihiroshima, 739-0046, Japan

Mitsuo Shimada, Professor

Department of Digestive and Pediatric Surgery, Tokushima University, Kuramoto 3-18-15, Tokushima 770-8503, Japan

Peter L Lakatos, MD, PhD, Assistant Professor

1st Department of Medicine, Semmelweis University, Koranyi S 2A, Budapest H1083, Hungary

Lesley A Anderson, PhD

MPHe, BSc (Hons), Academic Fellow in Cancer Prevention, Cancer Epidemiology and Prevention Research Group, Centre for Clinical and Population Sciences, Mulhouse Building Grosvenor Road, Belfast BT12 6BJ, United Kingdom

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@aes-eur.org

June 13-14, Amsterdam, Netherlands
 Falk Symposium 165: XX International Bile Acid Meeting. Bile 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 Pancreatologists (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.ilsts.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

APDW 2008
 September 13-16, New Delhi, India
 Organized: Indian Society of Gastroenterology

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.



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 1208 experts in gastroenterology and hepatology from 60 countries. The aim of the journal is to deliver the most clinically relevant original and commentary articles to readers, and to make the full text publicly available to all clinicians, scientists, patients and biomedical students on an unrestricted platform, so that they can access and learn about the most recent key advances in the field.

In addition to the open access nature, another key characteristic of *WJG* is its reading guidance for each article which includes background, research frontier, related reports, breakthroughs, applications, terminology, and comments of peer reviewers for the general readers.

WJG publishes articles on esophageal, gastrointestinal, hepatobiliary and pancreatic tumors, and other esophageal, gastrointestinal, hepatic-biliary and pancreatic diseases in relation to epidemiology, immunology, microbiology, motility & nerve-gut interaction, endocrinology, nutrition & obesity, endoscopy, imaging and advanced hi-technology.

The main goal of *WJG* is to publish high quality commentary articles contributed by leading experts in gastroenterology and hepatology and original articles that combine the clinical practice and advanced basic research, to provide an interactive platform for clinicians and researchers in internal medicine, surgery, infectious diseases, traditional Chinese medicine, oncology, integrated Chinese and Western medicine, imaging, endoscopy, interventional therapy, pathology and other basic medical specialties, and thus eventually improving the clinical practice and healthcare for patients.

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

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 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 case that other registers become available you will be duly notified. A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

Authors should retain one copy of the text, tables, photographs and illustrations because rejected manuscripts will not be returned to the author(s) and the editors will not be responsible for loss or damage to photographs and illustrations sustained during mailing.

Online submissions

Manuscripts should be submitted through the Online Submission System at: <http://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

Full manuscript title, running title, all author(s) name(s), affiliations, institution(s) and/or department(s) where the work was carried out; author contributions; disclosure of any financial support for the research; and the name, full address, telephone and fax numbers and email address of the corresponding author should be included. Titles should be concise and informative (remove all unnecessary words), emphasize what is new, and avoid abbreviations. A short running title of less than 40 letters should be provided. List the author(s)' name(s) as follows: initial and/or first name, middle name or initial(s), and full family name.

Author contributions: The format of this section should be like this: 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 research; Wang CL, Zou CC, Hong F and Wu XM performed research; Xue JZ and Lu JR contributed new reagents/analytic tools; Wang CL, Liang L and Fu JF analyzed data; and Wang CL, Liang L and Fu JF wrote the paper.

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

An informative, structured abstract of no more than 350 words should accompany each manuscript. Abstracts for original contributions should be structured into the following sections: AIM: Only the purpose should be included. METHODS: The materials, techniques, instruments and equipment, and the experimental procedures should be included. RESULTS: The observed and experimental results, including data, effects, outcome, *etc.* should be included. Authors should present *P* value where necessary, and also include any significant data. CONCLUSION: Accurate view and the value of the results should be included.

The format for structured abstracts can be found at: <http://www.wjgnet.com/wjg/help/11.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 body 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, should 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 ¹F, ²F, ³F; 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 manuscripts 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 requirement

PMID roots in the abstract serial number indexed by PubMed (<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed>). The author should supply the PMID for journal citation. For those references that have not been indexed by PubMed, a printed copy of the first page of the full reference should be submitted.

The accuracy of the information for journal citations is very important. Using the reference testing system, the authors and editor should check the authors name, title, journal title, publication date, volume number, start page, and end page. We will interlink all references with PubMed in an ASP file so that the readers can immediately access the abstract of the citations online.

DOI requirement

A CrossRef DOI® (Digital Object Identifier) name is a unique string created to identify a piece of scholarly content in the online environment. The author should supply the DOIs for journal citation (doi:10.3748/wjg.13.6458). This link (<http://www.crossref.org/SimpleTextQuery/>) allows you to retrieve Digital Object Identifiers (DOIs) for journal articles, books, and chapters by simply cutting and pasting the reference list into the box. You may use the form with any reference style, although the tool works most reliably if references are formatted in a standard style such as shown in this example: Assimakopoulos SF, Scopa CD, Vagianos CE. Pathophysiology of increased intestinal permeability in obstructive jaundice. *World J Gastroenterol* 2007; 13(48): 6458-6464

The accuracy of the information of journal citations is very important. We will interlink all references with DOI in ASP file so that readers can access the abstracts of cited articles online immediately.

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]

Both personal authors and an organization as author

- 5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764]

No author given

- 6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303]

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]

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]

No volume or issue

- 9 Outreach: Bringing HIV-positive individuals into care. *HRS/A Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

- 10 **Sherlock S**, Dooley J. Diseases of the liver and biliary 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**, Schorffheide AM. Adolescent pregnancy. 2nd ed. Wiczorek 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

Inappropriate references

Authors should always cite references that are relevant to their article, and avoid any inappropriate references. Inappropriate references include those linked with a hyphen when the difference between the two numbers is greater than five. For example, [1-6], [2-14] and [1, 3, 4-10, 22] are all considered inappropriate references. Authors should not cite their own unrelated published articles.

Statistical data

Write as mean \pm SD or mean \pm SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as *v* (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 \pm 2.1 mmol/L; blood CEA mass concentration, *p* (CEA) = 8.6 24.5 μ g/L; CO₂ volume fraction, 50 mL/L CO₂, not 5% CO₂; 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/15.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: *EcoRI*, *HindIII*, *BamHI*, *KhoI*, *KpnI*, 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

Ocean International Center, Building D

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/9.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/10.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.